Setting up Cows for First Postpartum Artificial Insemination

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Introduction

A long standing goal of reproductive physiologists was to develop a hormonal synchronization program that could overcome the problems and limitations associated with visual detection of estrus in dairy cattle. This goal was realized in 1995 with the publication of a hormonal synchronization protocol that combined GnRH and $PGF_{2\alpha}$ to control ovarian physiology and is now commonly referred to as the Ovsynch protocol (Pursley et al., 1995). The Ovsynch protocol synchronizes follicular development, luteal regression and ovulation such that artificial insemination can be conducted at a fixed-time without the need for estrus detection, commonly referred to as timed artificial insemination (TAI). Subsequent studies that repeated this work soon verified the results of the original publication (Burke et al., 1996; Pursley et al., 1997a,b), and dairy producers and veterinarians began to implement the Ovsynch protocol as a tool for reproductive management on commercial dairies. In the nearly 15 years since this first publication, today many dairy farms in the U.S. and around the world have adopted systematic synchronization protocols as a routine strategy for submitting cows for first and greater postpartum AI service (Caraviello et al., 2006).

Publication of the original Ovsynch protocol represented a paradigm shift for both the industry that began to implement it as well as for reproductive physiologists who began to focus efforts to modify the original Ovsynch protocol to improve fertility to TAI. This active area of research has resulted in a proliferation of synchronization protocols referred to using nonstandard nomenclature such as Ovsynch-56, Presynch, Cosynch, Double-Ovsynch, G6G, and Resynch-32. It takes time for researchers to sift and winnow ideas and data to reach a consensus on which protocols should be recommended for use on production dairy farms. At certain points in time, a consensus among scientists, farmers, veterinarians, and consultants cannot be reached. Furthermore, because this is an active area of research, new data hold the potential to change longstanding recommendations. In the mean time, dairy producers and their advisors are left with the task of deciding which protocols to implement on their dairies.

The purpose of this report is to overview current protocols for synchronizing ovulation in lactating dairy cows. While many options exist for synchronizing ovulation, this list of protocols was developed based on published research data and use in the industry by the Dairy Cattle Reproduction Council. This group comprises representatives from academia, the AI and pharmaceutical industries, bovine practitioners, and dairy farmers.

Timing of AI Relative to Synchronized Ovulations in Lactating Dairy Cows

For a recent review of timing of AI relative to behavioral estrus and synchronized ovulations in lactating dairy cows see Fricke (2008). Because most cows submitted for

TAI after hormonal synchronization show little or no outward signs of estrous behavior upon which to base timing of insemination, a new line of research has arisen to optimize the timing of induced ovulation (accomplished using GnRH analogs) after induction of luteal regression (accomplished using $PGF_{2\alpha}$ or its analogs) within an Ovsynch protocol as well as timing of AI in relation to the induced ovulation.

It is common for farms to choose to adopt a TAI schedule that represents a variation in which the TAI is performed during the same cow-handling period as the second GnRH (i.e., Cosynch), thereby eliminating one cow-handling period compared with the first reported Ovsynch protocol (Pursley et al., 1995). An initial field trial conducted on two dairies in Kansas comparef various combinations of Presynch + Ovsynch and Presynch + Cosynch (Portaluppi and Stevenson, 2005). Although results from this experiment indicated a conception rate advantage for the 72 h Cosynch protocol, several subsequent experiments have not supported these results (Brusveen et al., 2008; Nebel et al., 2008).

Although the timing of insemination in a Cosynch protocol may not maximize conception rate to TAI (Pursley et al, 1998; Dalton et al., 2001; Brusveen et al., 2008), use of Cosynch allows for cows to be handled at the same time of the day on different days, thereby allowing for cows to be restrained in self-locking head gates or a palpation rail after a specified milking in 3X milking systems in which cow-handling periods are dictated by the milking routine rather than by pre-selected protocol intervals. Simplification of reproductive management protocols may also improve overall compliance to the protocol, a major determinant of the overall effectiveness of a synchronized breeding program (Fricke et al., 2003). Nonetheless, protocols resulting in superior fertility lead to improved reproductive performance that may easily justify the increased level of management required to comply with the optimized protocol.

Ovsynch56

The optimal timing of the second GnRH injection and TAI in an Ovsynch protocol was tested by Brusveen et al. (2008) by comparing two Cosynch protocols (i.e., Cosynch-48 and Cosynch-72 vs. Ovsynch-56. Lactating Holstein cows (n = 927 cows; n = 1,507 TAI) were blocked by pen on a commercial dairy, and pens were rotated among the three treatments. All cows received GnRH followed 7 d later by $PGF_{2\alpha}$ and then received one of the following treatments: 1) GnRH + timed AI 48 h after $PGF_{2\alpha}$ (Cosynch-48); 2) GnRH 56 h after $PGF_{2\alpha}$ + timed AI 72 h after $PGF_{2\alpha}$ (Ovsynch-56); or 3) GnRH + timed AI 72 h after $PGF_{2\alpha}$ (Cosynch-72). Overall fertility was similar for the Cosynch-48 (27%) and Cosynch-72 (23%) treatments, whereas cows receiving the Ovsynch-56 treatment had a greater fertility (36%) compared to Cosynch-48 or Cosynch-72 cows. A subsequent experiment conducted in 3 herds of lactating cows (n=739) confirmed the results of Brusveen et al. (2008). Cows receiving Cosynch-72 had lower fertility than cows receiving Ovsynch-56 (Nebel et al., 2008).

Based on results from these two experiments as well as an understanding of timing of AI in relation to ovulation, Ovsynch-56 (Table 1) is strongly recommended over Cosynch protocols which do not optimize the timing of AI in relation to ovulation.

The Ovsynch portion of all subsequent protocols in this report should include the timing of the Ovsynch-56 protocol for the second GnRH injection and TAI.

Table 1. One possible hormone injection and timed artificial insemination schedule for the Ovsynch-56 protocol based on the results of Brusveen et al. 2008.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	GnRH - AM					
	PGF - AM		GnRH - PM	TAI - AM		

PGF = prostaglandin $F_{2\alpha}$; GnRH = gonadotropin-releasing hormone; TAI = timed artificial insemination; AM = ante meridiem; PM = post meridiem.

Setting up Lactating Dairy Cows for First Postpartum Timed AI

Presynch-Ovsynch

The first results with Ovsynch (Pursley et al., 1995) indicated that all nonpregnant cows could be enrolled into the protocol regardless of their stage during the estrous cycle. Subsequent results from Vasconcelos et al. (1999) using lactating dairy cows, and those of Moreira et al. (2000a) using dairy heifers showed that initiation of Ovsynch between days 5 to 12 of the estrous cycle may result in improved conception rate over the original Ovsynch protocol. Presynchronization of cows to group randomly cycling cows to initiate Ovsynch between days 5 to 12 of the estrous cycle can be accomplished using two injections of $PGF_{2\alpha}$ administered 14 days apart before initiation of the first GnRH injection of Ovsynch. A presynchronization strategy in which two injections of $PGF_{2\alpha}$ administered 14 days apart preceded initiation of Ovsynch by 12 days improved conception rates in lactating dairy cows compared to Ovsynch alone (Moreira et al., 2000b). This presynchronization strategy has become known as Presynch-Ovsynch (Table 2).

Table 2. Possible hormone injection and timed artificial insemination schedule for the Presynch-Ovsynch protocol based on the results of Moreira et al., 2000b.

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

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

Lactating dairy cows were randomly assigned to receive Ovsynch (n=262) or Presynch (n=264) for their first postpartum TAI, which was conducted 16 h after the second GnRH injection. The first and second $PGF_{2\alpha}$ injections for Presynch cows were administered at 37 and 51 days in milk, respectively, and all cows received a TAI at 73 days in milk. One possible hormone injection and timed AI schedule based on this research is shown in Table 1. For cycling cows, conception rate increased from 29% for Ovsynch to 43% for Presynch cows; however, no statistical treatment difference was detected when all cows (cycling and anovular) were included in the analysis). Thus, use of Presynch for programming lactating dairy cows to receive their first postpartum TAI can improve first service conception rate in a dairy herd.

A common question regarding the original Presynch data from Moreira et al. (2000b) pertains to the importance of the 12-day interval between the second $PGF_{2\alpha}$ injection and the first GnRH injection. If this interval could be extended to 14 rather than 12 days, the first four injections could be scheduled to occur on the same day during successive weeks. Although Navanukraw et al. (2004) showed that a 14-day interval between the second $PGF_{2\alpha}$ injection and the first GnRH injection increased fertility compared to Ovsynch alone, Galvao et al. (2007) directly compared Presynch protocols using an 11-day vs. a 14-day interval between the second $PGF_{2\alpha}$ injection and the first GnRH injection in a Presynch-Ovsynch protocol (Table 3). Reducing the interval from 14 to 11 days increased ovulatory response to the first GnRH injection of Ovsynch and improved fertility by about 6%.

Taken together, these results support a shortened interval (i.e., 10-12 rather than 14 days) between the second $PGF_{2\alpha}$ injection and the first GnRH injection of a Presynch-Ovsynch protocol. Based on data from Galvao et al. (2007), farms implementing this change can expect an increase in conception rate of about 5%.

Table 3. Possible hormone injection and timed artificial insemination schedule for the Presynch-Ovsynch protocol based on the results of Galvao et al., 2007.

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

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

Presynch-Ovsynch with detection of estrus and CIDR devices

Despite the widespread adoption of synchronization protocols, accurate detection of estrus behavior continues to play an important role as a part of the overall reproductive management program on most dairies today (Caraviello et al., 2006). By contrast, recent large field trials have reported that 20 to 30 % of high producing lactating Holstein cows are anovular at 60 to 75 DIM (Gümen et al., 2003; Silva et al., 2007), a time coinciding with the end of the voluntary waiting period and onset of AI breeding to detected estrus and/or TAI after synchronization of ovulation in many herds. Because anovular cows represent a substantial population of cows within a herd that cannot be inseminated based on detection of estrus, studies have evaluated methods for differentially treating anovular cows in an attempt to improve their fertility to TAI. One such strategy is to expose anovular cows to exogenous progesterone (P4) to try to resolve the anovular condition before TAI.

The objective of this experiment was to determine the effect of exogenous P4 during an Ovsynch protocol on pregnancies per AI (P/AI) in dairy cows not previously observed in estrus. Lactating cows (n = 3,338) from 7 commercial dairy herds were submitted to a presynchronization protocol (2 injections of $PGF_{2\alpha}$ 14 d apart; Presynch), and cows in estrus after the second $PGF_{2\alpha}$ received AI (EDAI; n = 1,652). Cows not inseminated by 12 to 14 d after the second $PGF_{2\alpha}$ injection were submitted to a TAI protocol (GnRH on d 0, $PGF_{2\alpha}$ on d 7, GnRH 48 to 72 h after $PGF_{2\alpha}$, and TAI 0 to 24 h after GnRH). At onset of the TAI protocol, cows were assigned randomly to receive no exogenous P4 (control, n = 815) or a controlled internal drug releasing (CIDR) insert containing 1.38 g of P4 from d 0 to 7 (CIDR, n = 871). Although cyclic cows had greater fertiltiy at 40 (38.3 vs. 28.9%) and 65 (35.2 vs. 25.8%) days after AI compared to anovular cows, inclusion of a CIDR insert increased fertility for both anovular as well as cyclic cows by about 6% (Chebel et al., 2010).

For herds wanting to incorporate heat detection into their reproductive management strategy, inseminating cows to a detected estrus after the second $PGF_{2\alpha}$ injection of Presynch followed by Ovsynch-56 with inclusion of a CIDR insert between the first GnRH and PGF injections is a viable alternative (Table 4). Based on data from Chebel et al. (2010), farms can expect about a 5% increase in conception rate for cows not detected in estrus after the second $PGF_{2\alpha}$ injection of Presynch and receiving a CIDR insert during the Ovsynch-56 portion of the protocol. .

Table 4. Possible hormone injection and timed artificial insemination schedule for the Presynch-Ovsynch protocol based on the results of Chebel et al., 2010.

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

PGF = prostaglandin $F_{2\alpha}$; GnRH = gonadotropin-releasing hormone; TAI = timed artificial insemination; ED = estrous detection. Shaded cells denote the period of CIDR insertion.

New Presynchronization Strategies

Two limitations of the currently-used used prostaglandin-based presynchronization strategy (i.e., Presynch-Ovsynch) are that 1) $PGF_{2\alpha}$ alone does not likely benefit anovular cows or resolve the anovular condition before cows initiate the first GnRH injection of the Ovsynch-56 portion of the protocol (Moriera et al., 2000b; Souza et al., 2008), and 2) follicular growth is not precisely synchronized after treatment with $PGF_{2\alpha}$ alone (Souza et al., 2008). New presynchronization strategies are now being developed and tested to address these two limitations in an attempt to improve fertility to TAI. Although there are only a few published studies at this point, data from large field trials which directly compare these newer presynchronization strategies with a standard Presynch-Ovsynch protocol are underway.

G6G

A recent study by Bello et al. (2006) used a presynchronization strategy which replaced the two PGF injections of Presynch with a $PGF_{2\alpha}$ injection followed 2 days later by a GnRH injection. The Ovsynch protocol was then initiated 4, 5, or 6 days thereafter to determine which interval resulted in the highest ovulatory response to the first GnRH injection of the Ovsynch-56 protocol. Ovulation to the first GnRH injection of the Ovsynch-56 protocol increased circulating progesterone at the time of the $PGF_{2\alpha}$ injection, reduced variation in the size of the ovulatory follicle, and increased synchronization rates during the Ovsynch-56 protocol (Bello et al., 2006). Of the three treatments compared, the 6-day interval resulted in the highest ovulatory response to the first GnRH injection, the highest luteal regression rate to the $PGF_{2\alpha}$ injection, and the highest conception rate to TAI after the Ovsynch-56 protocol based on about 100 cows per treatment. This protocol has therefore been referred to as the G6G protocol (Table 5). Although a large field trial was not conducted as a part of this initial experiment, the results support the concept that increased synchronization of follicular waves and luteal function may improve outcomes during the Ovsynch protocol. While this protocol has been adopted by some farms, large scale, controlled field trials are needed to compare the G6G protocol with other presynchronization protocols (i.e., Presynch-Ovsynch and/or Double-Ovsych).

Table 5. Possible hormone injection and timed artificial insemination schedule for the G6G protocol based on the results of Bello et al., 2006.

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

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

Double-Ovsynch

A recent study by Souza et al. (2008) evaluated using an Ovsynch protocol as a presynchronization strategy before an Ovsynch-56 protocol. This novel presynchronization strategy has been termed Double-Ovsynch (Table 6). The first Ovsynch protocol within Double-Ovsynch has been termed the “pre-Ovsynch” part of the protocol and incorporates a 72-hour interval between the $PGF_{2\alpha}$ and second GnRH injections. The second Ovsynch protocol within Double-Ovsynch has been termed the “breeding Ovsynch” part of the protocol and is an Ovsynch-56 protocol as described previously.

A field trial was conducted on two commercial dairies in Wisconsin to compare a Double-Ovsynch protocol with a Presynch-Ovsynch protocol. Cows receiving the Double-Ovsynch protocol had increased fertility compared to cows receiving a Presynch-Ovsynch protocol (50% vs. 42%, P = 0.03). Interestingly, there was a treatment by parity group interaction in which the Double-Ovsynch protocol increased fertility only in primiparous (65% vs. 45%; P = 0.02) and not in multiparous (38% vs. 39%) cows. Souza et al. (2008) concluded that future studies using a larger number of cows are needed to further test the hypothesis of higher fertility when using a Double-Ovsynch protocol and to elucidate the physiological mechanisms that underlie the apparent increases in fertility (Souza et al., 2008).

Table 6. Hormone injection and timed artificial insemination schedule for the Double-Ovsynch protocol based on the results of Souza et al., 2008

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

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

Taken together, G6G and Double-Ovsynch protocols offer new strategies to improve fertility to first postpartum TAI in lactating dairy cows. Both strategies aim to improve fertility by resolving the anovular condition before the TAI, more tightly controlling follicular development and luteal regression, and initiating Ovsynch on approximately Day 6 or 7 of the estrous cycle when an ovulatory follicle is present and when P4 is increasing during growth of the new follicular wave. Although some dairies have already adopted these protocols for managing first postpartum TAI, more data is needed to make strong recommendations for their use. Based on data from Souza et al. (2008) farms adopting a Double-Ovsynch protocol can conservatively expect a 5% increase in conception rate compared to a Presynch-Ovsynch protocol.

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