Fertility Programs to Achieve High 21-day Pregnancy Rates in High-Producing Holstein Dairy Herds

Paul M. Fricke, Milo C. Wiltbank, Paulo D. Carvalho, and Julio O. Giordano
Department of Dairy Science, University of Wisconsin - Madison
1675 Observatory Drive, Madison, WI 53706
Email: pmfricke@wisc.edu

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- The key factor affecting fertility in an Ovsynch protocol is the response to each of the three sequential hormonal treatments that can be defined using progesterone profiles.
- Cows with the greatest fertility to timed AI have mid-level progesterone concentrations at the first GnRH treatment, high progesterone at the $PGF_{2\alpha}$ treatment, and low progesterone at the last GnRH treatment of the Ovsynch protocol.
- Presynchronization strategies that incorporate a combination of GnRH and $PGF_{2\alpha}$ to tightly control ovarian function optimize progesterone concentrations at the first GnRH and $PGF_{2\alpha}$ treatments of the Ovsynch protocol, thereby increasing fertility.
- Cows that initiate an Ovsynch protocol in a low-progesterone environment ovulate to the first GnRH treatment at a high rate, but fail to undergo complete luteal regression by the last GnRH treatment resulting in dramatically decreased fertility to timed AI (**TAI**).
- Addition of a second $PGF_{2\alpha}$ treatment 24 hours after the first in an Ovsynch protocol decreases progesterone concentrations at the last GnRH treatment, thereby increasing fertility, particularly for cows that initiate Ovsynch in a low-progesterone environment.
- An aggressive reproductive management strategy that incorporates these concepts can result in an annualized 21-day pregnancy rate that exceeds 30% in healthy, high-producing dairy herds.

INTRODUCTION

Hormonal synchronization protocols have been incorporated widely into reproductive management programs by dairy farmers (Caraviello et al., 2006; Norman et al., 2009). The initial impact of TAI protocols on 21-day pregnancy rates in U.S. dairy herds has been to increase the AI service rate (Norman et al., 2009); however, a deeper understanding of the physiology underlying the Ovsynch protocol has allowed for a dramatic increase in fertility to TAI. As the title of this review suggests, perhaps it is now more appropriate to refer to the latest iteration of hormonal synchronization protocols as fertility programs for lactating dairy cows.

Progesterone (**P4**) is the most biologically active progestagen in cattle and is primarily produced and secreted into circulation by the corpus luteum (**CL**) during the estrous cycle and the placenta during pregnancy. Much of the recent research published in the scientific literature has focused on the role of P4 during an Ovsynch protocol (Figure 1) or at various time points during an Ovsynch protocol on fertility as measured by pregnancies per artificial insemination (**P/AI**) 32 days after TAI. For the purposes of this review, the initial GnRH treatment of an Ovsynch protocol to which TAI occurs will be referred to as **G1** and the final GnRH treatment of an Ovsynch protocol immediately preceding TAI will be referred to as **G2** (Figure 1).

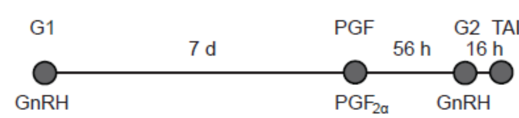

Figure 1. Schematic diagram of an Ovsynch protocol. G1 = first GnRH treatment; PGF = prostaglandin $F_{2\alpha}$ treatment; G2 = last GnRH treatment; TAI = timed artificial insemination.

Response to Each Treatment during an Ovsynch Protocol Determines Fertility to Timed AI

The effect of synchronization to each treatment injection during an Ovsynch protocol on fertility is illustrated in an analysis conducted as part of an experiment to compare two resynchronization strategies (Giordano et al., 2012c). Lactating Holstein cows (n = 956) diagnosed not pregnant were assigned randomly to a day 32 Resynch protocol or a Double Ovsynch Resynch protocol. As expected, P/AI 29 days after TAI was greater for Double Ovsynch than day 32 cows (39 vs. 30%). Synchronization rate by treatment and P/AI of synchronized and nonsynchronized cows were calculated for a subset of cows (n = 433) that had complete information including P4 concentration at $PGF_{2\alpha}$, and G2, as well as ovulatory response to G2 (Table 1). Cows were considered synchronized if they had high progesterone at the time of $PGF_{2\alpha}$ treatment, low progesterone at G2, and ovulated in response to G2.

Table 1. Synchronization risk for Holstein dairy cows resynchronized using an Ovsynch protocol initiated 32 days after a previous TAI (D32) or a Double Ovsynch (DO) protocol

	Treatment		
	D32 % (n)	DO % (n)	*P*-value
P4 at PGF[2]			
Cows with low P4	35 (210)	17 (223)	<0.0001
P/AI for cows with low P4	10 73)	5 (38)	0.44
P/AI for cows with high P4	36 (137)	38 (185)	0.70
P4 at G2[3]			
Cows with high P4	17 137)	7 (185)	0.005
P/AI for cows with high P4	13 (23)	8 (12)	0.68
P/AI for cows with low P4	40 (114)	40 (173)	0.94
Ovulation after G2			
Cows that did not ovulate	7 (114)	8 (173)	0.87
P/AI for cows that did not ovulate	0 (8)	0 (13)	1.00
P/AI for cows that ovulated	43 (106)	43 (160)	0.97
Synchronization risk			
Synchronized cows	51 (210)	72 (223)	0.0001
P/AI for non-synchronized cows	10 (104)	5 (63)	0.27
P/AI for synchronized cows	43 (106)	43 (160)	0.97

[1]Adapted from Giordano et al., 2012c.
[2]Cut-off for low vs. high P4 was 1.0 ng/mL.
[3]GnRH2 = the second GnRH injection of the D32 protocol and the second GnRH injection of the Breeding-Resynch portion of the Double-Ovsynch protocol. Cut-off for low vs. high P4 was 0.4 ng/mL. Cows were considered synchronized when they had high progesterone before PGF treatment, low progesterone after PGF treatment (i.e., at G2), and responded to G2 by ovulating a follicle.

Overall, synchronized cows had more P/AI than cows failing to synchronize; however, P/AI for synchronized cows was identical for cows in both treatments. Thus, the effect of treatment on P/AI resulted from a greater percentage of Double Ovsynch cows than day 32 cows that synchronized (72 vs. 51%), primarily because more day 32 than Double Ovsynch cows did not have a functional CL at the $PGF_{2\alpha}$ treatment of the Ovsynch protocol (35% vs. 17%) or had incomplete CL regression at G2 (17% vs. 7%). We concluded that Double Ovsynch increased fertility of Holstein dairy cows during a resynchronization program primarily by increasing synchronization of cows to each treatment during the Ovsynch protocol. A similar analysis was conducted in another experiment which compared two Resynch protocols (Lopez et al., 2013), and the results and conclusions were similar. The primary factor associated with fertility to TAI regardless of the treatment protocols compared was synchronization to the sequential treatments during the Ovsynch protocol.

Effect of Ovulatory Response to G1 on P/AI

Ovulatory response to G1 increases P/AI as ovulatory response to G1 increases (Bisinotto and Santos, 2012). Furthermore, circulating P4 concentrations attenuated a GnRH-induced LH surge through an inhibitory effect of P4 at the hypothalamic-pituitary axis (Giordano et al., 2012a). Attenuation of the LH surge may in turn decrease ovulatory response to G1 thereby limiting fertility to TAI. We attempted to overcome the negative inhibition of P4 on the GnRH-induced LH surge by increasing the dose of GnRH (200 vs. 100 μg) administered at G1 during a Double Ovsynch protocol (Giordano et al., 2013). Although ovulatory response was slightly increased (58%, n=325 vs. 67%, n=326), the resulting increase in P/AI was not statistically significant (Giordano et al., 2013).

Because the early CL is refractory to $PGF_{2\alpha}$-induced luteolysis until about 7 days after ovulation (Nascimento et al., 2014) unless a mature CL also is present (Howard and Britt, 1990), treatment with $PGF_{2\alpha}$ 5 days after ovulation causes a dramatic decrease in P4 which then slowly rebounds as the CL recovers (Nascimento et al., 2014). We tested a novel approach to increase ovulatory response to G1 by using the refractoriness of the early CL to exogenous $PGF_{2\alpha}$ to temporarily decrease P4 at G1 thereby increasing the magnitude of the LH surge (Carvalho et al., 2015b). Lactating Holstein cows (n = 800) were synchronized for first TAI using a modified Double Ovsynch protocol [Pre-Ovsynch protocol (day 0, GnRH; day 7, $PGF_{2\alpha}$; day 10, GnRH) followed 7 days later by an Ovsynch-56 protocol (day 0, G1; day 7, $PGF_{2\alpha}$; day 8, $PGF_{2\alpha}$; day 9.5, GnRH)] with TAI occurring approximately16 hours after G2. Cows were assigned randomly to receive 12.5 mg $PGF_{2\alpha}$ (a half-dose of dinoprost tromethamine) 2 days before G1 (Low-P4) or serve as untreated controls (High-P4). As expected, High-P4 cows had greater P4 concentrations at G1 than Low-P4 cows (3.0 vs. 1.3 ng/mL, respectively), and ovulatory response to G1 was increased for Low-P4 vs. High-P4 cows (81.1 vs. 60.3%, respectively). Overall, P/AI did not differ between treatments 32 days after TAI (56% vs. 53%, for Low-P4 vs. High-P4 cows, respectively) or 67 days after AI (51% vs. 48%, for Low-P4 vs. High-P4 cows, respectively). The increase in P/AI for cows that ovulated to G1 (16%) combined with the observed increase in ovulation to G1 resulting from treatment (21%; Low-P4 – High-P4) resulted in an expected numerical increase in P/AI of 3% in Low-P4 vs. High-P4 cows observed in this experiment. Thus, although we increased ovulatory response to G1 during a Double Ovsynch protocol (20 percentage points), the increase in P/AI did not reach a level of statistical significance.

To determine the effect of ovulatory response to G1 and the presence of a functional CL at G1 affected P/AI, we analyzed synchronization rates of Holstein cows randomized to two Resynch protocols (Giordano et al., 2012c). Regardless of treatment, cows that ovulated to G1 had more P/AI when cows lacked a functional CL at G1; however, there was no difference in P/AI based on ovulatory response to

G1 when cows had a functional CL at G1 (Table 2). Furthermore, cows with a functional CL at G1 had more P/AI than cows lacking a functional CL at G1 regardless of their ovulatory response to G1. Results from these experiments support that, although ovulatory response to G1 of an Ovsynch protocol can affect P/AI to TAI, P4 at G1 has a greater effect on P/AI than ovulatory response to G1.

Table 2. Synchronization risk for resynchronized Holstein dairy cows classified based on the presence or absence of a functional CL (≥0.5 ng/mL of P4) and ovulatory response to the first GnRH treatment (G1) of an Ovsynch protocol

Item	Cows lacking a functional CL		Cows with a functional CL	
Ovulation to G1	No	Yes	No	Yes
	--- % (n) ---		--- % (n) ---	
Cows at G1	10 (426)	14 (426)	54 (426)	21 (426)
P/AI	11^{b} (44)	23^{ab} (61)	32^{a} (231)	37^{a} (90)
Cows with low P4 at PGF	73^{c} (44)	18^{ab} (61)	26^{b} (231)	8^{a} (90)
Cows with high P4 at G2	17^{ab} (12)	40^{a} (50)	4^{b} (172)	8^{b} (83)
Cows with no ovulation to G2	10 (10)	0 (30)	8 (166)	9 (76)
Synchronization risk	21^{c} (44)	49^{b} (61)	66^{a} (231)	77^{a} (90)

[a,b,c]Within a row, proportions with different superscripts differ ($P < 0.01$).
[1]Adapted from Giordano et al. (2012c).

Effect of Progesterone at G1 and $PGF_{2\alpha}$ on P/AI

To assess the association between P4 concentrations at each treatment of an Ovsynch protocol and P/AI to TAI in lactating Holstein cows, we analyzed data from 7,792 cows from 14 experiments in which P4 was measured at the three hormonal treatments during an Ovsynch protocol (Carvalho et al., 2015 c). The association between P4 during the Ovsynch protocol and P/AI to TAI was analyzed independently because P4 was not measured in all cows at all hormonal treatments during the Ovsynch protocol in all experiments.

At G1, concentrations of P4 in 6,144 cows were stratified into nine P4 categories from 0 to ≥7 ng/mL using 0.5 ng/mL increments (Figure 2, upper panel). Overall, P/AI differed ($P < 0.01$) among P4 categories at G1 with fewer P/AI for cows with P4 <0.5 ng/mL or P4 >7.0 ng/mL compared with cows having intermediate P4. At the $PGF_{2\alpha}$ treatment, cows (n = 3,383) were stratified into nine P4 categories from 0 to ≥8 ng/mL using 1.0 ng/mL increments (Figure 2, middle panel). Overall, P/AI differed ($P < 0.01$) among P4 categories at $PGF_{2\alpha}$ with a 51% relative decrease in P/AI for cows with P4 <1.0 ng/mL compared with cows with P4 >1.0 ng/mL. Based on this large dataset, suboptimal P4 concentrations could be identified at G1 in 26% of cows (26% lower P/AI) and at the $PGF_{2\alpha}$ treatment in 21% of cows (51% lower P/AI).

Presynchronization strategies before initiation of an Ovsynch protocol at first TAI or Resynch TAI can optimize P4 at G1 and $PGF_{2\alpha}$ in most cows resulting in more P/AI than for cows submitted to an Ovsynch protocol with no presynchronization. Presynchronization strategies tested include one $PGF_{2\alpha}$ administered 10 days (Cartmill et al., 2001) or 14 days (Silva et al., 2007; Bruno et al., 2013) before initiation of an Ovsynch protocol, two $PGF_{2\alpha}$ treatments administered 14 days apart with the second treatment administered 11 to 14 days before initiation of an Ovsynch protocol (i.e., Presynch Ovsynch; Moreira et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al., 2004; Galvão et al., 2007), a single GnRH treatment 7 days before Ovsynch (i.e., GGPG;

Giordano et al., 2012b; Lopes et al., 2013; Bruno et al., 2014; Carvalho et al., 2014a), a combination of GnRH and $PGF_{2\alpha}$ 6 to 7 days before initiation of an Ovsynch protocol (i.e., G6G, Double-Ovsynch, and PG-3-G; Bello et al., 2006; Souza et al., 2008; Stevenson and Pulley, 2012, respectively). Independent of the presynchronization strategy tested, an increase in P/AI occurred when P4 concentrations were increased at the time of the $PGF_{2\alpha}$ treatment of the Ovsynch protocol (Bello et al., 2006; Bisinotto et al., 2010; Denicol et al., 2012; Stevenson et al., 2012; Martins et al., 2011).

Effect of Progesterone at G2 on P/AI

Based on our analysis of cows from 14 different studies in which P4 was measured at the various treatments during an Ovsynch protocol (Carvalho et al., 2015c), a critical factor associated with P/AI to TAI is P4 concentration at G2. At G2, concentrations of P4 in cows (n = 3,148) were stratified into eight P4 categories from 0 to ≥0.7 ng/mL using 0.1 ng/mL increments (Figure 2, lower panel). Overall, P/AI differed ($P < 0.01$) among P4 categories at G2 with a 66% relative decrease in P/AI for cows with P4 >0.4 ng/mL compared with cows with P4 <0.4 ng/mL. Based on these data, a major weakness with current TAI protocols is that in a subset of cows the CL(s) fails to regress fully resulting in P4 at G2 that limits fertility. The underlying physiology by which slightly increased P4 concentrations at G2 cause this decreased fertility to TAI is not clear. Some possibilities include a negative association between P4 during the estrous cycle and oviductal and uterine motility thereby decreasing gamete transport and fertilization rate (Bennett et al., 1988) or decreased uterine thickness at TAI associated with decreased fertility to TAI in cows (Souza et al., 2011).

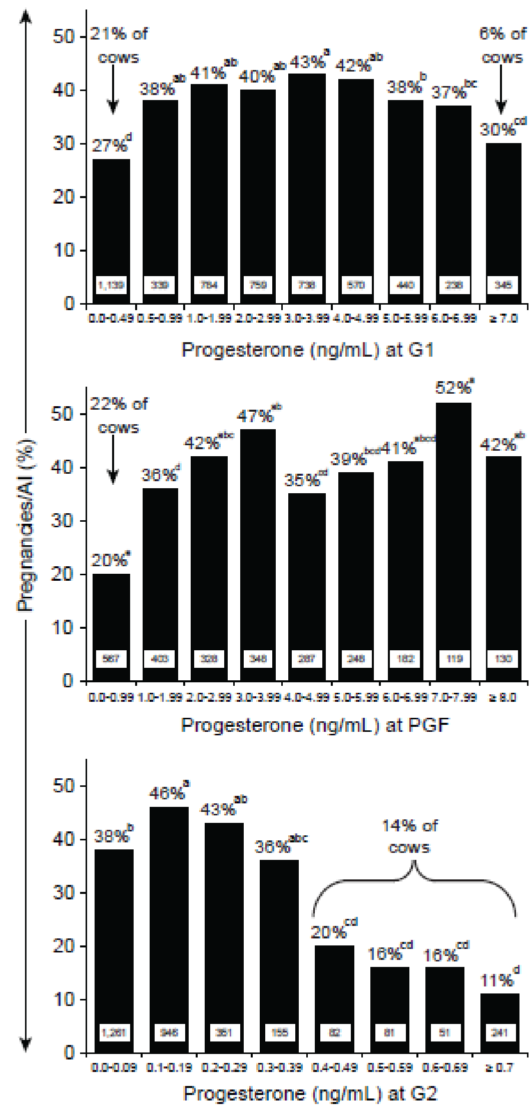

Figure 2. Effect of progesterone at each treatment of an Ovsynch protocol on pregnancies per AI in lactating Holstein cows. At G1, concentrations of progesterone in 6,144 cows were stratified into nine P4 categories from 0 to ≥7 ng/mL using 0.5 ng/mL increments (upper panel). At the $PGF_{2\alpha}$ treatment, concentrations of progesterone in 3,383 cows were stratified into nine P4 categories from 0 to ≥8 ng/mL using 1.0 ng/mL increments (middle panel). At G2, concentrations of progesterone in 3,148 cows were stratified into eight P4 categories from 0 to ≥0.7 ng/mL using 0.1 ng/mL increments (lower panel). Numbers within bars denote number of cows in each progesterone category. Adapted from Carvalho et al. (2015c).

Addition of a Second $PGF_{2\alpha}$ Treatment Increases P/AI

Based on the analysis of the large dataset of P4 profiles during an Ovsynch protocol (Carvalho et al., 2015c), suboptimal P4 concentrations were identified at G1 in 26% of cows (26% lower P/AI), at PGF in 21% of cows (51% lower P/AI), and at G2 in 14% of cows (66% lower P/AI). Our conclusion based on these analyses was that achieving optimal P4 during an Ovsynch protocol may allow for an increase in fertility in lactating dairy cows. Incomplete luteal regression measured as P4 ≥0.4 ng/mL at G2 has been associated with decreased P/AI at first and Resynch TAI. Decreased P/AI associated with incomplete luteal regression is particularly manifested in cows in which an Ovsynch protocol is initiated in a low-P4 environment (Giordano et al., 2012c; Carvalho et al., 2015b; Santos et al., 2015). This is likely because cows with a young CL (approximately 6 days old) at the $PGF_{2\alpha}$ treatment during an Ovsynch protocol fail to fully regress to a single $PGF_{2\alpha}$ treatment because some cows have young CL that have not fully acquired luteolytic capacity (Nascimento et al., 2014).

Based on an analysis of data from an experiment in which cows were resynchronized using a Double Ovsynch protocol (Giordano et al., 2012c), we classified cows based on the age and number of CL present at the $PGF_{2\alpha}$ treatment of an Ovsynch protocol and assessed the risk of complete luteal regression (Table 3). Cows with a single CL approximately 13 days of age had a 97% luteal regression risk, and cows with a CL approximately 13 days of age and a CL approximately 6 days of age had a 92% luteal regression risk. By contrast, cows with a single CL approximately 6 days of age had only a 64% luteal regression risk. Cows that initiate an Ovsynch protocol in a low P4 environment (whether anovular or cyclic and lacking a CL) have a high ovulatory response to G1 resulting in a single CL of 6 days of age present at the $PGF_{2\alpha}$ treatment of the Ovsynch protocol. Approximately one-third of these cows fail to fully regress this young CL resulting in slightly elevated P4 levels at G2 which dramatically decrease P/AI.

Several experiments have assessed the effect of adding a second $PGF_{2\alpha}$ treatment during an Ovsynch protocol to decrease P4 at G2 on fertility to TAI at first TAI as well as at Resynch TAI.

Table 3. Effect of age and number of CL at the final $PGF_{2\alpha}$ treatment during a Double Ovsynch protocol on the proportion of Holstein dairy cows with complete luteal regression by G2 (P4 <0.4 ng/mL)[1]

Age and number of CL at $PGF_{2\alpha}$ treatment	Proportion of cows with complete luteolysis, % (n)
Day 6 CL	64 (59)
Day 6 and Day 13 CL	92 (74)
Day 13 CL	97 (166)

[1]Adapted from Giordano et al. (2012c).

First Timed AI

Lactating Holstein cows were assigned randomly to a Double Ovsynch protocol (control) or a Double Ovsynch protocol that included a second $PGF_{2\alpha}$ treatment 24 hours after the first one during the breeding week (Brusveen et al., 2009). Cows receiving two $PGF_{2\alpha}$ treatments during the Ovsynch protocol had a greater incidence of luteal regression than cows receiving one $PGF_{2\alpha}$ treatment (98% vs. 86%). In contrast, P/AI to first TAI did not differ between cows receiving two vs. one $PGF_{2\alpha}$ treatments (53 vs. 47%, respectively). The 6 percentage point difference in P/AI would be expected based on the 12 percentage point increase in luteal regression combined with a 50% conception rate to TAI in this experiment. Further, the physiological impact of adding a second $PGF_{2\alpha}$ treatment during a Double Ovsynch protocol may be limited because a Double Ovsynch protocol results in most cows having a CL that is approximately13 days of age, or a 13-day old CL and a CL that is approximately 6 days of age at the $PGF_{2\alpha}$ treatment. Therefore, Double Ovsynch treatment precludes setting up cows with a young CL of 6 days of age at the $PGF_{2\alpha}$ treatment that may fail to fully regress.

Resynch TAI

Whereas presynchronization strategies have yielded significant increases in P/AI to first TAI, many herds struggle with poor fertility to an Ovsynch protocol used for Resynch TAI. In several studies, 16%, 22%, and 35% of cows diagnosed not pregnant 32 days after TAI, and that did not receive a GnRH treatment 7 days before pregnancy diagnosis, lacked a CL at G1 (Fricke et al., 2003; Sterry et al., 2006; Giordano et al., 2015). When cows were synchronized for first TAI and P4 profiles and CL diameter were measured weekly until a pregnancy diagnosis occurred 32 days after TAI, 19% of cows diagnosed not pregnant lacked a CL >10 mm in diameter (Ricci et al., 2014). Thus, up to one-third of nonpregnant cows initiate a Resynch protocol in a low P4 environment, which leads to a lack of luteal regression and low fertility to Resynch TAI. We conducted an experiment to determine the effect of adding a second $PGF_{2\alpha}$ treatment 24 hours after the first $PGF_{2\alpha}$ treatment in an Ovsynch protocol would increase P/AI to TAI after a Resynch protocol (Carvalho et al., 2015a). A greater ($P < 0.01$) proportion of cows receiving one $PGF_{2\alpha}$ treatment had incomplete luteal regression (≥0.4 ng/mL) than cows receiving two $PGF_{2\alpha}$ treatments regardless of P4 concentrations at G1 (Table 4). For cows with P4 concentrations <1.0 ng/mL at G1, cows receiving two $PGF_{2\alpha}$ treatments had more ($P = 0.03$) P/AI than cows receiving one $PGF_{2\alpha}$ treatment, whereas for cows with P4 concentrations ≥1.0 ng/mL at G1, P/AI did not differ ($P = 0.46$) between cows receiving one vs. two $PGF_{2\alpha}$ treatments (Table 4).

Table 4. Effect of one vs. two $PGF_{2\alpha}$ treatments during an Ovsynch protocol on luteal regression and pregnancies per AI (P/AI) for Holstein dairy cows with low vs. high progesterone (P4) concentrations at the first GnRH treatment (G1) of an Ovsynch protocol

	$PGF_{2\alpha}$ treatment	
Item	One	Two
	% (n)	
Cows with complete luteal regression		
Low P4 (<1.0 ng/mL) at G1	70[a] (76)	96[b] (74)
High P4 (>1.0 ng/mL) at G1	89[a] (236)	98[b] (214)
Overall	83[a] (312)	98[b] (288)
P/AI 32 days after TAI		
Low P4 (<1.0 ng/mL) at G1	33[c] (107)	46[d] (110)
High P4 (>1.0 ng/mL) at G1	33 (312)	37 (289)
Overall	33[c] (419)	39[d] (399)

[a,b]Proportions differ ($P < 0.01$).
[c,d]Proportions differ ($P < 0.05$).
[1]Adapted from Carvalho et al. (2015a).

Five-Day vs. Seven-Day Ovsynch Protocols

Decreasing the interval between G1 and the $PGF_{2\alpha}$ treatment from 7 (7-day protocol) to 5 (5-day protocol) days in an Ovsynch protocol was first described in a series of experiments with 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, more cows in the 5-day protocol than the 7-day protocol become pregnant to TAI in two experiments (80 vs. 67%, respectively and 65 vs. 56%, respectively). In 2010, a 5-day Ovsynch protocol was compared with a 7-day Cosynch72 protocol in lactating Holstein cows (Santos et al., 2010). In that study, cows in the 5-day protocol received two $PGF_{2\alpha}$ treatments whereas cows in the 7-day protocol received only one $PGF_{2\alpha}$ treatment. Overall, cows in the 5-day protocol had more P/AI than cows in the 7-day protocol (38 vs. 31%). Santos et al. (2010) 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 AI, and P/AI was again greater for the 5-day than the 7-day protocol (39 vs. 34%). The authors attributed this treatment effect to a reduced period of follicle dominance for cows in the 5-day Ovsynch protocol. Colazo and Ambrose (2015) also compared a 5-day protocol with two $PGF_{2\alpha}$ treatments to a 7-day protocol with one $PGF_{2\alpha}$ treatment; however, P/AI did not differ statistically between treatments (39 vs. 34%).

We conducted an experiment to directly compare 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 day on P4 concentrations and P/AI after resynchronization of ovulation and TAI (Santos et al., 2015). Lactating Holstein cows (n = 821) were assigned randomly at a nonpregnancy diagnosis (d 0 = 32 days after AI) to three Resynch protocols: 1) 7D1PGF (GnRH, day 0; $PGF_{2\alpha}$, day 7; GnRH, day 9.5); 2) 7D2PGF (GnRH, day 0; $PGF_{2\alpha}$, day 7; $PGF_{2\alpha}$, day 8; GnRH, day 9.5); and 3) 5D2PGF (GnRH, day 2; $PGF_{2\alpha}$, day 7; $PGF_{2\alpha}$, day 8; GnRH, day 9.5). All cows received an intravaginal P4 insert (PRID Delta; Ceva Santé Animale, Libourne, France) at G1 of the resynchronization protocol which was removed at the first $PGF_{2\alpha}$ treatment, and all cows received a TAI approximately 16 hours after G2.

Overall, no treatment effect was detected for P/AI (Table 5). When the 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 duration of the protocol, whereas no treatment effect was detected for cows that had a CL at G1 (Table 5). We concluded that addition of a second $PGF_{2\alpha}$ treatment to a Resynch protocol increased the proportion of cows with complete luteal regression, particularly for cows with low P4 at G1, thereby increasing P/AI, whereas decreasing the duration of the Ovsynch protocol did not affect P/AI. Thus, a 5-day Ovsynch protocol with two $PGF_{2\alpha}$ treatments results in similar, but not increased, fertility to TAI than a 7-day Ovsynch protocol when two $PGF_{2\alpha}$ treatments also are administered.

Table 5. 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

	Treatment (T)			*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. (2015).
[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.

Achieving a 30% 21-Day Pregnancy Rate in a 30,000 lb Dairy Herd

In 2014, we implemented an aggressive reproductive management system for first and Resynch TAI based on the concepts presented in this overview to manage the Allenstein Dairy Teaching Herd, which consists of approximately 550 Holstein cows located at the Emmons Blaine Dairy Cattle Research Center in Arlington, WI. This facility is one of three locations constituting the Integrated Dairy Facilities that serves the research, teaching, and outreach needs of the Department of Dairy Science and the School of Veterinary Medicine at the University of Wisconsin-Madison. Cows are milked twice daily and fed a TMR that meets or exceeds NRC requirements for high-producing dairy cows. Only 23% of the cows at this location are primiparous because 100 primiparous cows are housed at the Marshfield Agricultural Research Station. Multiparous cows are treated with rbST as per label recommendation, whereas primiparous cows do not receive rbST. Average daily milk production is 98 lb and average ME305 for the cows at this location is 31,116 lb.

First Timed AI

All cows are submitted for first TAI between 76 and 82 DIM after a Double Ovsynch protocol as described by Souza et al. (2008). The breeding Ovsynch is conducted as an Ovsynch-56 protocol as described by Brusveen et al. (2008) with the addition of a second PGF treatment 24 hours after the first $PGF_{2\alpha}$ treatment.

Resynch Timed AI

All cows are treated with GnRH 25 days after TAI. Pregnancy diagnosis is conducted using transrectal ultrasonography 32 days after TAI, and cows diagnosed not pregnant are classified as having or lacking a CL >10 mm in diameter. Nonpregnant cows with a CL continue on with an Ovsynch-56 protocol by receiving a $PGF_{2\alpha}$ treatment 32 days after TAI with the addition 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. (2015a). Intravaginal P4 inserts are included within the Ovsynch protocol for cows lacking a CL based on studies in which 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).

Reproductive Performance

During a 1-year period (August 2014 to August 2015), the adjusted 21-day pregnancy rate (based on a 76-day VWP) in the UW Arlington dairy herd averaged 34%. The 21-day service risk averaged 68%, and overall conception risk averaged 52% (n = 1,093). Conception risk to first TAI averaged 56% (n = 563), conception risk to second TAI averaged 50% (n = 264), and conception risk to third TAI averaged 45% (n = 129). The first three TAI occurred from 76 to 170 DIM, and 88% of cows became pregnant after the first three TAI. More than 95% of cows in the herd received a TAI during this time period.

CONCLUSIONS

This intensive reproductive management protocol based on the concepts presented in this review has resulted in reproductive performance that is unprecedented for a herd of high-producing Holstein dairy cows. Although use of an ideal fertility program is important for achieving a high 21-day pregnancy rate, cows must be healthy to achieve high fertility. Many cow health factors have been reported to decrease P/AI to TAI including the incidence of mastitis between TAI and the first pregnancy diagnosis (Fuenzalida et al., 2015), a decrease in body condition score during the first 21 day after calving (Carvalho et al., 2014a), and poor uterine health (Lima et al., 2013).

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