Theriogenology 114 (2018) 165–172

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Development of fertility programs to achieve high 21-day pregnancy rates in high-producing dairy cows

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ARTICLE INFO

Article history:
Received 13 October 2017
Received in revised form
19 March 2018
Accepted 28 March 2018
Available online 31 March 2018

Keywords:
Fertility program
Timed-AI
Dairy cattle

ABSTRACT

Our objective is to overview the research that lead to the development of fertility programs for high-producing lactating dairy cows using only GnRH and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), such as Ovsynch with a focus on the role of progesterone in fertility. A key factor affecting fertility to timed-AI is the response to each hormonal treatment of the Ovsynch protocol. Although not required for fertility, cows ovulating to the first GnRH treatment of the Ovsynch protocol (G1) have greater P/AI than cows failing to ovulate. The association between progesterone concentrations at each treatment during the Ovsynch protocol and fertility is reviewed, and data from 7792 cows from 14 studies are presented. Overall, medium P4 (between 0.5 and 6 ng/mL) at G1, high P4 concentrations (>1.0 ng/mL) at the $PGF_{2\alpha}$, and low P4 concentrations (<0.4 ng/mL) at final GnRH (G2) are associated with greater P/AI. The use of presynchronization treatments that increase the percentage of cows initiating the Ovsynch protocol during early diestrous increases the percentage of cows with medium P4 concentrations at G1 and with high P4 concentration at $PGF_{2\alpha}$. Some cows, however, fail to completely undergo luteal regression after a single $PGF_{2\alpha}$ treatment, particularly cows that initiate the Ovsynch protocol in a low P4 environment and cows with a young (d 6) CL at the time of treatment with $PGF_{2\alpha}$. Addition of a second $PGF_{2\alpha}$ treatment increased the percentage of cows with complete luteal regression and P/AI. The use of fertility programs that include the concepts described in this review have resulted in more P/AI than inseminating cows after an induced estrus at first insemination. In addition, extending these concepts to strategies for resynchronization of ovulation and the implementation of an aggressive reproductive management program for first and subsequent inseminations results in reproductive performance that is unprecedented for high-producing Holstein dairy cows.

1. Introduction

Hormonal synchronization protocols have been incorporated widely into reproductive management programs by dairy farmers [1,2]. The initial impact of TAI protocols on 21-day pregnancy rates in U.S. dairy herds was to increase the AI submission rate [2,3]; 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 [4].

Progesterone (P4) is the most biologically active progestogen in cattle and is primarily produced and secreted into circulation by the corpus luteum (CL) during the estrous cycle and the placenta during pregnancy [5–7]. Much of the research published in the scientific literature has focused on the role of P4 during an Ovsynch protocol (Fig. 1) or at various time points during an Ovsynch protocol on fertility as measured by pregnancies per artificial insemination (P/AI) 32 d after TAI [8–14]. 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 (Fig. 1).

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https://doi.org/10.1016/j.theriogenology.2018.03.037
0093-691X/

Fig. 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.

2. Response to each treatment during an ovsynch protocol determines fertility to Timed-AI

The effect of synchronization to each treatment during an Ovsynch protocol on fertility is illustrated in an analysis conducted as part of an experiment to compare two resynchronization strategies [15]. Lactating Holstein cows (n = 956) diagnosed not pregnant were assigned randomly to a day 32 Resynch protocol or a Double-Ovsynch Resynch protocol. The Day 32 Resynch protocol was initiated 32 days after AI, whereas the Double-Ovsynch Resynch protocol was initiated 22 days after AI. As expected, P/AI 29 days after TAI was greater for cows submitted to the Double-Ovsynch protocol than for cows submitted to the Day 32 protocol (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). There were three criteria that were used to determine synchronization: high progesterone at the time of $PGF_{2\alpha}$ treatment (>1.0 ng/mL), low progesterone at G2 (<0.4 ng/mL), and ovulation in response to G2. Cows that were not synchronized based on any of these three criteria had few P/AI: Low P4 at PGF (8.1%; 9/111), high P4 at G2 (11.4%), or lack of ovulation to G2 (0%; 0/21). Overall, synchronized cows had more P/AI than cows that failed to synchronize based on these three criteria; 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 a Double-Ovsynch protocol increased fertility of Holstein dairy cows during a resynchronization program primarily by increasing synchronization of cows to each treatment during the Ovsynch protocol. Similar analyses have been conducted comparing two Resynch protocols [16] or Double-Ovsynch at first AI [4], and the results and conclusions were similar. The primary factor associated with fertility to TAI, regardless of the treatment protocols, was synchronization to the sequential treatments during the Ovsynch protocol.

Interestingly, researchers using protocols that synchronize ovulation using estradiol benzoate and intravaginal progesterone inserts have reached similar conclusions [17]. Cows that were correctly synchronized by the protocol, based on estradiol benzoate at the start of the protocol, complete regression of the CL at the end of the protocol, and ovulation at the end of the protocol had dramatically greater fertility (61.3%) than non-synchronized cows (15.7%; P < 0.01). Thus, even though synchronization may be measured in different ways using distinct protocols, the fundamental factor that seems to underlie fertility to TAI protocols is correct synchronization during the protocol.

3. Effect of ovulatory response to G1 on P/AI

Ovulatory response to G1 is positively associated with P/AI [18]; however, ovulatory response to G1 is less than optimal, with 50% or fewer dairy cows ovulating when GnRH was administered at a random stage of the estrous cycle [8,15,16,19]. Ovulatory response to GnRH treatment differs based on stage of the estrous cycle, with cows having a dominant follicle of the first follicular wave (i.e., Days 5–8 of the cycle) mostly ovulating in response to GnRH treatment, whereas, mid-cycle cows have much lower responses to GnRH treatment [20]. Reduction in ovulatory response to GnRH could be due to lack of a follicle with ovulatory capacity or an inadequate LH surge in response to GnRH. Circulating P4 concentrations attenuated a GnRH-induced LH surge apparently through a direct inhibitory effect of P4 on the pituitary gland [14], [21]. 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 [22]. Ovulatory response was increased (57.5%, n = 325 vs. 66.6%, n = 326; P < 0.01) for the higher GnRH dose and, combining treatments, cows that ovulated had more P/AI (52.2%, 211/404 vs. 38.5%, 95/247; P < 0.001). The resulting increase in P/AI (47.7% 263/551 vs. 46.2%, 246/533), however, was not statistically significant but was consistent with the ~10% increase in ovulatory response and ~15% increase in P/AI for cows that ovulated [22]. In a subsequent study, we tested a novel approach to increasing ovulatory response to G1 by temporarily decreasing circulating P4 concentrations at G1. This strategy was based on the observation that treatment of cows with an early CL with $PGF_{2\alpha}$ does not fully regress the CL but rather causes a dramatic but temporary decrease in P4 which then slowly rebounds as the CL recovers [23]. Thus, in this study cows were treated with a lower dose of $PGF_{2\alpha}$ at a time that the CL was refractory to $PGF_{2\alpha}$ (i.e., Day 5 of the estrous cycle), to temporarily decrease P4 at G1 thereby increasing the magnitude of the LH surge [11]. 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 approximately 16 h after G2. Cows were assigned randomly to receive 12.5 mg $PGF_{2\alpha}$ (i.e., a half-dose of dinoprost tromethamine) 2 days

Table 1
Synchronization rate for Holstein dairy cows resynchronized using an Ovsynch protocol initiated 32 d after a previous TAI (D32) or a Double Ovsynch (DO) protocol. Adapted from Giordano et al., [15].

	Treatment		*P*-value
	D32% (n)	DO % (n)	
P4 at PGF[a]			
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[b]			
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 - cows that did not ovulate	0 (8)	0 (13)	1.00
P/AI for cows that ovulated	43 (106)	43 (160)	0.97
Synchronization Rate[c]			
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

[a] Cut-off for low vs. high P4 was 1.0 ng/mL.
[b] G2 = Final GnRH treatment. Cut-off for low vs. high P4 was 0.4 ng/mL.
[c] 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.

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 (56.3% vs. 52.9%, for Low-P4 vs. High-P4 cows, respectively) or 67 days after AI (50.5% vs. 47.6%, 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 due to treatment (21%; Low-P4 − High-P4) resulted in the expected numerical increase in P/AI of 3% in Low-P4 vs. High-P4 cows observed in this experiment. Thus, ovulatory response to G1 can be increased during a Double-Ovsynch protocol by either increasing the GnRH dose (~10%) or by decreasing circulating P4 (~20%). Although there was an increase in fertility for the cows that ovulate (~15%), the observed differences in P/AI, although almost exactly what would be expected (~1.5% for higher dose; ~3% for reduced P4), were not statistically significant in these experiments.

To determine the effect of ovulatory response to G1 and the presence of a functional CL at G1 on P/AI, we analyzed synchronization rates of Holstein cows randomized to two Resynch protocols [15]. 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.

4. Effect of P4 concentrations 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 7792 cows from 14 experiments in which P4 was measured at the three hormonal treatments during an Ovsynch protocol [24]. 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 6144 cows were stratified into nine P4 categories from 0 to ≥7 ng/mL using 0.5 ng/mL increments (Fig. 2, upper panel). Overall, P/AI differed ($P < 0.01$) among P4 categories at G1 with fewer P/AI for cows with low P4 (<0.5 ng/mL) or high P4 (≥7.0 ng/mL) compared with cows having intermediate P4. At the $PGF_{2\alpha}$ treatment, cows (n = 3383) were stratified into nine P4 categories from 0 to ≥8 ng/mL using 1.0 ng/mL increments (Fig. 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. Based on this large dataset, suboptimal P4

Table 2
Synchronization rate for resynchronized Holstein dairy cows classified based on the presence or absence of a functional CL (≥0.5 ng/mL of P4) at G1 and ovulatory response to the first GnRH treatment of an Ovsynch protocol (G1). Adapted from Giordano et al., [15].

Item	Cows lacking a functional CL		Cows with a functional CL	
Ovulation to G1	No	Yes	No	Yes
	% (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 ov. to G2	10 (10)	0 (30)	8 (166)	9 (76)
Synchronization rate	21^{c} (44)	49^{b} (61)	66^{a} (231)	77^{a} (90)

[a,b,c]Within a row, proportions with different superscripts differ ($P < 0.01$).

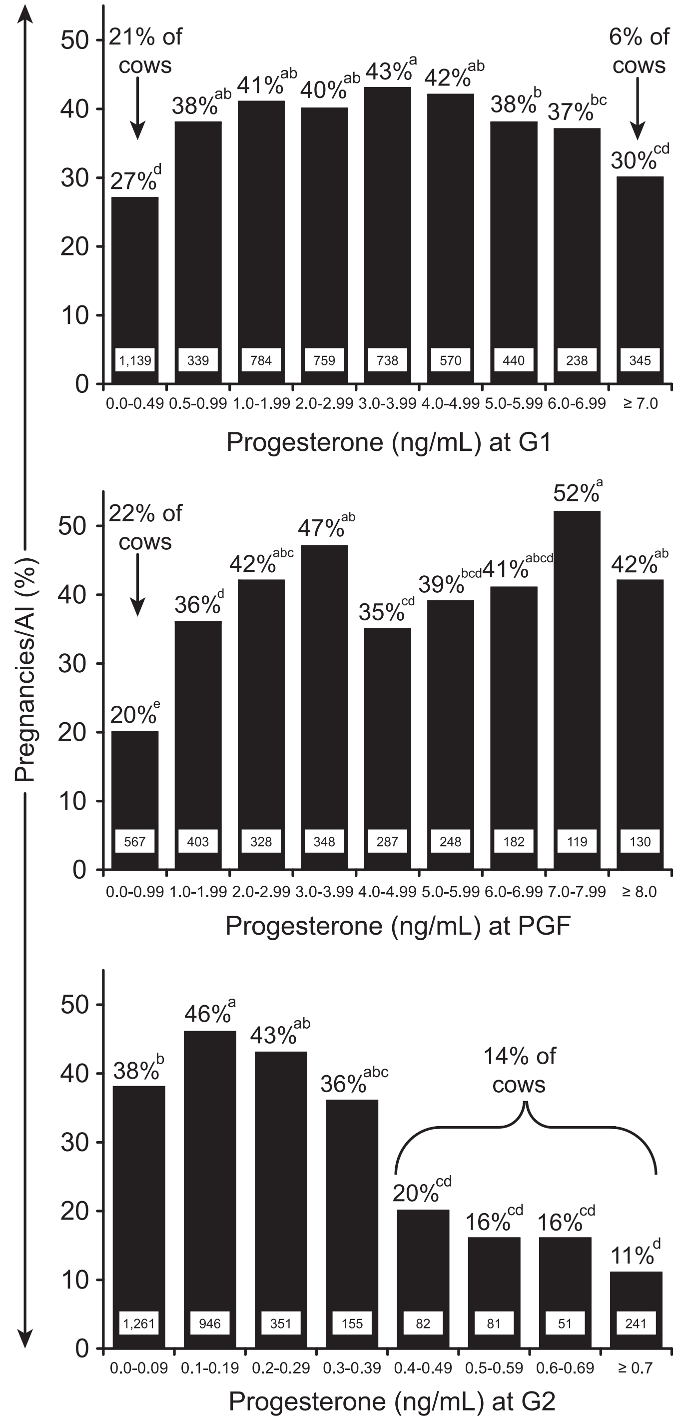

Fig. 2. Effect of progesterone at each treatment of an Ovsynch protocol on pregnancies per AI in lactating Holstein cows. At G1, concentrations of progesterone in 6,144 cows were stratified into nine P4 categories from 0 to ≥7 ng/mL using 0.5 ng/mL increments (upper panel). At the $PGF2\alpha$ treatment, 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. [24].

concentrations could be identified at G1 in 21% of cows (26% lower P/AI) and at the $PGF_{2\alpha}$ treatment in 22% 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. In general, the objective of presynchronization protocols is to increase the percentage of cows that are near days 5–8 of the estrous cycle so that intermediate P4 concentrations are present with a functional dominant follicle that can potentially ovulate to G1. This will result in a greater percentage of cows with P4 at G1 and decrease inappropriate regression of the CL (i.e., before $PGF_{2\alpha}$ treatment) and thereby increase the percentage of cows with elevated P4 at $PGF_{2\alpha}$. Presynchronization strategies tested include one $PGF_{2\alpha}$ treatment administered 10 days [25] or 14 days [26,27], before initiation of an Ovsynch protocol, two $PGF_{2\alpha}$ treatments administered 14 days apart with the second treatment administered 11–14 days before initiation of an Ovsynch protocol (i.e. Presynch-Ovsynch; [28–31]); a single GnRH treatment 7 days before Ovsynch (i.e., GGPG; [16,32–34]), a combination of GnRH and $PGF_{2\alpha}$ 6–7 days before initiation of an Ovsynch protocol (i.e., G6G, Double-Ovsynch, and PG-3-G; [35–37]). Independent of the presynchronization strategy tested, an increase in P/AI occurred when P4 concentrations were increased during and at the time of the $PGF_{2\alpha}$ treatment of the Ovsynch protocol [10,12,22,33,35,38–40].

5. 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, a critical factor associated with P/AI to TAI is P4 concentration at G2. At G2, concentrations of P4 in cows (n = 3148) were stratified into eight P4 categories from 0 to $\geq$0.7 ng/mL using 0.1 ng/mL increments (Fig. 2, lower panel). Overall, P/AI differed ($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 the CL(s) fails to regress fully in a subset of cows thereby 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 [41] or decreased uterine thickness at TAI associated with decreased fertility to TAI in cows [42].

6. Addition of a second $PGF_{2\alpha}$ treatment increases P/AI

Since incomplete luteal regression before TAI, measured as P4 $\geq$0.4 ng/mL at G2, was associated with decreased P/AI at first and Resynch TAI, protocols are now being modified to increase efficiency of CL regression during the protocol. One important observation was that incomplete luteal regression to a single $PGF_{2\alpha}$ treatment and the associated decrease in P/AI is particularly apparent in cows in which an Ovsynch protocol is initiated in a low-P4 environment [10,15,43]. This result is consistent with early results reporting that only 66% of cows on Day 7 of the estrous cycle completely regressed their CL in response to a single $PGF_{2\alpha}$ treatment (as measured by detection of estrus), whereas by Day 8 a total of 91% of cows had complete CL regression after a single $PGF_{2\alpha}$ treatment [44]. This is likely because cows with a young CL (Day 7 of the estrous cycle) at the $PGF_{2\alpha}$ treatment fail to fully regress the CL after a single $PGF_{2\alpha}$ treatment [23]. Alternatively, it is perhaps more surprising that only 14% of cows did not have complete CL regression at G2 of an Ovsynch protocol.

To help understand the luteal dynamics in cows that regressed or did not regress the CL after a single $PGF_{2\alpha}$ treatment, we analyzed data from an experiment in which cows were resynchronized using a Double-Ovsynch protocol [15]. 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, whereas 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, almost identical to the 66% regression in the early study with cows on Day 7 of the estrous cycle [44]. Thus, if an older CL and a younger CL are present at the time of PGF_{2a} treatment, there is a greater percentage of cows that have complete CL regression than if only a younger CL is present. Importantly, 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.

To optimize regression of the CL at the end of TAI protocols researchers have either added a second $PGF_{2\alpha}$ treatment or a greater dose of $PGF_{2\alpha}$ treatment at either first AI or during rebreeding TAI protocols (Resynch). In one experiment, 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 administered 24 h after the first during the breeding week [45]. Cows receiving two $PGF_{2\alpha}$ treatments during the 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 significantly between cows receiving two vs. one $PGF_{2\alpha}$ treatments (53 vs. 47%, respectively). The 6% point difference in P/AI would be expected based on the 12% point increase in luteal regression and a change from 50% P/AI in cows with complete CL regression and 0% P/AI in cows without CL regression. This experiment utilized dinoprost, and there were no observed differences due to parity.

In another experiment, lactating dairy cows (n = 2521 from 12 different farms) were synchronized by Ovsynch or Double-Ovsynch for first AI and randomly assigned to receive 1 or 2 $PGF_{2\alpha}$ treatments at the end of the protocol [46]. Overall, cows treated with 1 $PGF_{2\alpha}$ had fewer P/AI than cows treated with 2 $PGF_{2\alpha}$ treatments (34.4%, 436/1266 vs. 37.6%, 471/1251). Thus, there was an absolute increase of 3.2% in P/AI but a relative improvement of 9.5% (3.2/34.4) by adding the second $PGF_{2\alpha}$ treatment. Interestingly, the second $PGF_{2\alpha}$ treatment resulted in no increase in P/AI for primiparous cows (39.3% vs. 36.5%; $P = 0.39$) but a substantial increase in multiparous cows (32.5% vs. 36.5%; $P < 0.05$; +12.3% increase). This experiment utilized cloprostenol.

Whereas presynchronization strategies have resulted in 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 of Resynch [47–49]. 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 [50]. 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 h after the first $PGF_{2\alpha}$

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 undergoing complete luteal regression by G2 (P4 < 0.4 ng/mL). Adapted from Giordano et al., [15].

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)

treatment in an Ovsynch protocol on P/AI to TAI after a Resynch protocol [10]. 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 low P4 at G1, however, 30% of cows did not undergo complete CL regression, whereas, for cows with high P4 at G1 only 11% of cows failed to undergo complete CL regression. By contrast, almost all cows had complete CL regression (98%) after 2 $PGF_{2\alpha}$ treatments, regardless of low or high P4 at G1. 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 (+13% absolute or +39% relative increase). This is the increase that would be expected based on 30% without complete regression and increase in P/AI from 0 to 45%. By contrast, cows with P4 concentrations ≥1.0 ng/mL at G1, did not differ for P/AI (+4% absolute; $P = 0.46$) between cows receiving one vs. two $PGF_{2\alpha}$ treatments (Table 4). Thus, a second $PGF_{2\alpha}$ treatment increases P/AI due to increased efficiency of CL regression to the TAI protocol. The positive effects, however, are dramatically greater for cows with low P4 than high P4 at G1 due to inefficiency of CL regression after a single PGF treatment in cows with a single young CL.

7. 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 [51]. 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 [52]. 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. [52] 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 decreased period of follicle dominance for cows in the 5-day Ovsynch protocol. Colazo and Ambrose [53] 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 [43]. 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 at G1 of the resynchronization protocol which was removed at the first $PGF_{2\alpha}$ treatment, and all cows received a TAI approximately 16 h 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 4
Effect of 1 vs. 2 $PGF_{2\alpha}$ 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 of an Ovsynch protocol (G1). Adapted from Carvalho et al., [10].

Item	Treatment	
	1 $PGF_{2\alpha}$	2 $PGF_{2\alpha}$
	% (n)	
Cows undergoing 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 d 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 ($P < 0.05$).

8. Conclusions about TAI protocols that optimize fertility in lactating dairy cows

Lack of complete and correct synchronization is a major problem in TAI protocols. For example, with a basic Ovsynch program, only about 50% of cows are correctly synchronized resulting in high fertility for correctly synchronized cows (40–60% P/AI) but low fertility (~10% P/AI) for cows that have some problem with synchronization such as low P4 at $PGF_{2\alpha}$, slightly increased P4 at G2, or lack of ovulation to G2. Based on our analysis of the large dataset of P4 profiles during an Ovsynch protocol, suboptimal P4 concentrations were identified at G1 in 26% of cows (26% lower P/AI), at $PGF_{2\alpha}$ 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 and synchronization during an Ovsynch protocol may allow for a dramatic increase in fertility in lactating dairy cows by optimizing the hormonal environment and ovarian dynamics

Table 5
Effect of presence of a corpus luteum (CL) at Day 0 on pregnancies per AI (P/AI) in Holstein dairy cows 32 d after TAI. Adapted from Santos et al., [43].

P/AI	Treatment			*P*-value		
	7D1PGF	7D2PGF	5D2PGF	Trt	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

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

during the protocol. Some of the most successful strategies that have been employed to improve fertility during TAI protocols are: 1) Presynchronization before Ovsynch, 2) Increasing ovulation to G1, and 3) increasing CL regression prior to G2.

Use of effective presynchronization procedures such as Presynch-11-Ovsynch or Double-Ovsynch can optimize the P4 concentrations, stage of the cycle, and stage of the follicular wave at the time of G1. These strategies will increase percentage of cows at G1 and G2 with optimal P4 concentrations. Effective presynchronization programs have resulted in absolute improvements of 10–20% in P/AI at first AI. These improvements translate into an increase in percentage of cows pregnant to first AI of 30–50% (relative increase). Use of these strategies were the first and most effective method for developing a fertility program (increased P/AI) and not just a service rate program, such as Ovsynch (increased service rate but no change in P/AI).

Treatments that increased ovulation to G1, such as greater GnRH dose and reduced P4 at G1, have given indications of improved physiology during the protocol but any improvement in fertility were not significant due to relatively small improvements in P/AI. Increased ovulation to G1 of 20% would be expected to increase fertility by only 3%, an increase that may be economically important but difficult to detect statistically. Studies will need to be designed with over 2000 cows per treatment group to have sufficient statistical power to test whether these treatments can be used to improve fertility.

Finally, treatments that increase CL regression before TAI are being effectively used to increase P/AI in both first AI and resynch protocols. In general, adding a second $PGF_{2\alpha}$ treatment or an increased dose of $PGF_{2\alpha}$ to a Double-Ovsynch protocol has increased CL regression about 10% and P/AI about 5% in cows synchronized with a 7d-protocol [22,45]. Much greater improvements, however, have been observed during Resynch protocols, particularly for cows that have low P4 or no CL at G1 [10,43]. Interestingly, for cows receiving a 5d-protocol, a double dose of $PGF_{2\alpha}$ was not as effective as two separate doses to induce complete luteal regression [54].

9. Fertility to Timed-AI vs. AI after estrus

The idea that fertility programs and TAI can yield greater fertility than AI to estrus at first insemination in high-producing dairy cows has not been definitively tested. Several experiments compared P/AI of cows inseminated after TAI with cows inseminated after a detected estrus at first AI, with some studies reporting no differences in P/AI [3,55,56], whereas others reported more P/AI for cows receiving TAI [57–59]. In all of these studies, however, DIM at first insemination differed between cows submitted to TAI and cows submitted to AI after a detected estrus.

We recently conducted an experiment to compare AI submission rate and P/AI between cows submitted to a fertility program versus cows inseminated after an induced estrus [4]. Lactating Holstein cows (n = 578) were assigned randomly to receive TAI after a Double Ovsynch protocol (DO) that included a second $PGF_{2\alpha}$ treatment or to receive AI if detected in estrus after receiving 2 $PGF_{2\alpha}$ treatments administered 14 d apart (Estrus). Cows in the Estrus group were induced to cyclicity by treatment with GnRH 7 days prior to the first $PGF_{2\alpha}$ treatments. Treatments were administered to allow cows to be inseminated at similar DIM in both treatments (77 ± 3 DIM).

Results from this experiment are summarized in Table 6. By design, DIM at first insemination did not differ between treatments (76.9 ± 0.2 vs. 76.7 ± 0.3 for DO vs. Estrus cows, respectively), but more DO cows were inseminated within 7 d after the end of the voluntary waiting period than Estrus cows (100.0% vs. 77.5%). Overall, DO cows had more P/AI than Estrus cows at both 33 d (49.0% vs. 38.6%) and 63 d (44.6% vs. 36.4%) after insemination, but pregnancy loss from 33 to 63 d after insemination did not differ between treatments. Thus, the relative increase in P/AI was more than 25% (10.4/38.6 = 26.9%). Primiparous cows had more P/AI than multiparous cows 33 and 63 d after insemination, but the treatment by parity interaction was not significant. Synchronization rate to the hormonal protocols was 85.3%, which did not differ between treatments; however, synchronized DO cows had more P/AI 33 d after insemination than synchronized Estrus cows (54.7% vs. 44.5%).

In summary, 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 VWP 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. We concluded that, because the proportion of synchronized cows did not differ between treatments, DO cows had more P/AI than Estrus cows because of an intrinsic increase in fertility after submission to a fertility program. Thus, use of a higher fertility program like Double-Ovsynch with a second $PGF_{2\alpha}$ treatment can increase service rate and can also increase fertility due to optimization of hormonal concentrations and ovarian dynamics during the protocol.

10. Achieving a 30% 21-day pregnancy rate in a 13,000 KG dairy herd

Many dairy herds have incorporated fertility TAI programs into a complete reproductive management program. For example, 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 University of Wisconsin-Madison Dairy Teaching Herd, which consists of approximately 780 Holstein cows. This herd constitutes 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. Average daily milk production is 45 Kg and average 305ME for the cows is 13,752 kg. All cows are submitted for first TAI between 76 and 82 DIM after a Double Ovsynch protocol as described by Souza et al. [36]. The breeding Ovsynch is conducted as an Ovsynch-56 protocol as described by Brusveen et al. [60] with the addition of a second $PGF_{2\alpha}$ treatment 24 h after the first $PGF_{2\alpha}$ treatment. For Resynch TAI, 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.

Table 6
Effect of treatment on submission rate, pregnancies per AI (P/AI) 33 and 63 d after insemination, and percentage of pregnant cows at 33 and 63 d after insemination in lactating Holstein cows. Adapted from Santos et al., [4].

Item	Treatment[a]		Difference, [b]% (P-Value)
	DO	EST	
n	294	284	
Submission rate, % (no./no.)	100.0 (294/294)	77.5 (220/284)	29 (<0.01)
P/AI at 33 d, % (no./no.)	49.0 (144/294)	38.6 (85/220)	27 (0.02)
Pregnant cows at 33 d, % (no./no.)	49.0 (144/294)	29.9 (85/284)	64 (<0.01)
P/AI at 66 d, % (no./no.)	44.6 (131/294)	36.4 (80/220)	23 (0.05)
Pregnant cows at 66 d, % (no./no.)	44.6 (131/294)	28.2 (80/284)	58 (<0.01)

[a] Each week, lactating Holstein cows at 50 ± 3 DIM (d 0) were stratified by parity (primiparous vs. multiparous) and were randomized to receive first insemination as a timed AI after a Double-Ovsynch (DO) protocol or AI to a detected estrus after a hormonal protocol that synchronized estrus (EST).
[b] Relative difference due to treatment for each item was calculated as the difference between DO and EST cows divided by EST cows.

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 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. [10]. Intravaginal P4 inserts (one per cow) 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 [8,9]. The reproductive performance during the last 3 years has been analyzed (August 2014 to August 2017; Fricke et al., unpublished), 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 (proportion of eligible cows that were inseminated) averaged 69%, and overall conception risk (proportion of inseminated cows that conceived) averaged 50% (n = 4308). Conception risk to first TAI averaged 51% (n = 2087), conception risk to second TAI averaged 50% (n = 1090), and conception risk to third TAI averaged 47% (n = 582). The first three TAI occurred from 76 to 170 DIM, and 88% of cows became pregnant after the first three TAI. Many other herds have had similar types of success using fertility programs to dramatically improve their reproductive management programs.

11. Conclusions

Use of physiological principles has allowed for development of TAI protocols that not only allow for 100% of cows to be inseminated at the end of the voluntary waiting period but also resulted in fertility that can exceed fertility to standing estrus. Use of an optimized fertility protocol, however, is only one part of an overall program for achieving high 21-day pregnancy rates, including a number of management, environmental, nutritional, and health factors. For example, many cow health factors can decrease P/AI to fertility programs for TAI including the incidence of mastitis between TAI and the first pregnancy diagnosis [61], a decrease in body condition score during the first 21 day after calving [62], and uterine or other diseases [63–65]. This research is providing scientific insights into the factors that are limiting fertility in lactating dairy cows and practical tools that can be used by dairy producers and veterinarians to effectively manage reproduction in dairy farms.

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