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

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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 timed artificial insemination (TAI). As the title of this paper 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 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. 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 F2α treatment; G2 = last GnRH treatment; TAI = timed artificial insemination.

Effect of Progesterone at G1 and PGF on Fertility to Timed 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 (Figure 2; Carvalho et al., 2015b). The association between P4 during the Ovsynch protocol and P/AI to TAI was analyzed independently because P4 was not measured for all cows at all hormonal treatments during the Ovsynch protocol in all experiments.

At G1, cows (n = 6,144) were stratified into 9 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 than for cows with intermediate P4. At the PGF2α treatment, cows (n = 3,383) were stratified into 9 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 PGF2α with a 51% relative decrease in P/AI for cows with P4 < 1.0 ng/mL than for 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 PGF2α 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 PGF2α in most cows resulting in more P/AI than for cows submitted to an Ovsynch protocol with no presynchronization. Presynchronization strategies tested thus far have used one PGF2α treatment 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 PGF2α treatments administered 14 days apart with the second treatment administered 10 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., G6G, Double-Ovsynch, and PGF-3-G; Bello et al., 2006; Souza et al., 2008; Stevenson and Pulley, 2012). Independent of the presynchronization strategy tested, there was an increase in P/AI when P4 concentrations were increased at the time of the PGFα treatment of the Ovsynch protocol (Bello et al., 2006, Bisino et al., 2010, Denicol et al., 2012, Stevenson et al., 2012; Martins 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 PGF2α 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. (2015b).

**Effect of Progesterone at G2 on Fertility to Timed AI**

Based on our analysis of cows from 14 different studies in which P4 was measured at the various treatments during an Ovsynch protocol (Figure 2; Carvalho et al., 2015b), a critical factor associated with P/AI to TAI is P4 at G2. At G2, cows (n = 3,148) were stratified into 8 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 than for cows with P4 < 0.4 ng/mL. Based on these data, a major problem with current TAI protocols is that a subset of cows fails to fully regress their CL resulting in P4 levels at G2 that limit fertility. The underlying physiology by which slightly increased P4 levels at G2 cause this decreased fertility to TAI is not clear. Some possibilities include a negative association between P4 during the estrous cycle and oviducal 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).

**Addition of a Second PGF2α Treatment Increases Fertility to Timed AI**

Based on the analysis of the large dataset of P4 profiles during an Ovsynch protocol (Carvalho et al., 2015b), 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 this analysis was that achieving optimal P4 during an Ovsynch protocol may allow for a dramatic 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., 2015a; Santos et al. 2015). This is likely because cows with one young CL (~6d) at the PGF2α treatment during an Ovsynch protocol fail to fully regress to a single PGF2α 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 rate of complete luteal regression (Table 1). Cows with a single CL ~13 days of age had a 97% luteal regression rate, and cows with a CL ~13 days of age and a CL ~6 days of age had a 92% luteal regression rate. By contrast, cows with a single CL ~6 days of age had only a 64% luteal regression rate. Cows with a CL ~13 days of age had a 97% luteal regression rate, and cows with a CL ~6 days of age and a CL ~13 days of age had a 32% luteal regression rate. 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.

Table 1. 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)$^a$.

<table>
<thead>
<tr>
<th>Age and number of CL at PGF$_{2\alpha}$ treatment</th>
<th>Proportion of cows with complete luteolysis, % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 6 CL</td>
<td>64 (59)</td>
</tr>
<tr>
<td>Day 6 and Day 13 CL</td>
<td>92 (74)</td>
</tr>
<tr>
<td>Day 13 CL</td>
<td>97 (166)</td>
</tr>
</tbody>
</table>

$^a$Adapted from Giordano et al., 2012c

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.

**First TAI.** Lactating Holstein cows were randomly assigned to a Double Ovsynch protocol (control) or a Double Ovsynch protocol that included a second PGF$_{2\alpha}$ treatment 24 hours after the first (Brusveen et al., 2009). Cows receiving 2 PGF$_{2\alpha}$ treatments during the Ovsynch protocol had a greater incidence of luteal regression than cows receiving 1 PGF$_{2\alpha}$ treatment (98% vs. 86%); however, P/AI to first TAI did not differ between cows receiving 2 vs. 1 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 PGF2α treatment during a Double Ovsynch protocol may be limited because a Double Ovsynch protocol results in most cows having a CL ~13 days of age, or a CL ~13 days of age and a CL ~6 days of age at the PGF$_{2\alpha}$ treatment and avoids setting up cows with a young CL ~6 days of age at the PGF$_{2\alpha}$ treatment that fail to fully regress (Table 3).

**Resynch TAI.** Whereas resynchronization 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 (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 was measured until a pregnancy diagnosis 32 days later, 19% of cows diagnosed not pregnant lacked a CL > 10 mm in diameter (Ricci et al., 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 PGF2α treatment 24 hours after the first within 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 1 PGF$_{2\alpha}$ treatment had incomplete luteal regression ($\geq$ 0.4 ng/mL) than cows receiving 2 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 2 PGF$_{2\alpha}$ treatments had more ($P = 0.03$) P/AI than cows receiving 1 PGF2α treatment, whereas for cows with P4 concentrations $\geq$ 1.0 ng/mL at G1, P/AI did not differ ($P = 0.46$) between cows receiving 1 vs. 2 PGF$_{2\alpha}$ treatments (Table 2).
Table 2. 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).1

<table>
<thead>
<tr>
<th>Item</th>
<th>1 PGF$_{2\alpha}$</th>
<th>2 PGF$_{2\alpha}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows undergoing complete luteal regression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low P4 (&lt;1.0 ng/mL) at G1</td>
<td>70$^a$ (76)</td>
<td>96$^b$ (74)</td>
</tr>
<tr>
<td>High P4 (&gt;1.0 ng/mL) at G1</td>
<td>89$^a$ (236)</td>
<td>98$^b$ (214)</td>
</tr>
<tr>
<td>Overall</td>
<td>83$^a$ (312)</td>
<td>98$^b$ (288)</td>
</tr>
<tr>
<td>P/AI 32 days after TAI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low P4 (&lt;1.0 ng/mL) at G1</td>
<td>33$^c$ (107)</td>
<td>46$^d$ (110)</td>
</tr>
<tr>
<td>High P4 (&gt;1.0 ng/mL) at G1</td>
<td>33 (312)</td>
<td>37 (289)</td>
</tr>
<tr>
<td>Overall</td>
<td>33$^c$ (419)</td>
<td>39$^d$ (399)</td>
</tr>
</tbody>
</table>

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

Achieving High Fertility in High-Producing Dairy Herds

Reproductive Management

All cows are submitted for first TAI between 77 to 83 DIM after a Double-Ovsynch protocol as described by Souza et al. (2008; Figure 8, lower panel). The second Ovsynch of the Double-Ovsynch protocol is conducted as an Ovsynch-56 protocol as described by Brusveen et al. (2008) with the addition of a second PGF$_{2\alpha}$ treatment 24 h after the first PGF$_{2\alpha}$ treatment (Wiltbank et al., 2015). For second and subsequent TAI, all cows are treated with GnRH 25 d after TAI, and few cows are detected in estrus to receive AI after first TAI. Pregnancy diagnosis is conducted using transrectal ultrasonography 32 d after TAI, and cows diagnosed not pregnant are classified as having or lacking a CL > 10 mm in diameter. Nonpregnant cows with a CL continue an Ovsynch-56 protocol by receiving a PGF$_{2\alpha}$ treatment 32 d after TAI with the addition a second PGF$_{2\alpha}$ treatment 24 h after the first PGF$_{2\alpha}$ treatment. Nonpregnant cows lacking a CL restart an Ovsynch-56 protocol that includes a second PGF$_{2\alpha}$ treatment 24 h after the first as described by Carvalho et al. (2015b). Intravaginal P4 inserts (i.e., CIDR inserts) are included within the Ovsynch protocol for cows lacking a CL. This strategy was designed based on studies in which exogenous P4 increased fertility for cows lacking a CL at initiation of an Ovsynch protocol (Bilby et al., 2013; Bisinotto et al., 2015).

Reproductive Performance

During a one-year period (January 2015 to January 2016), The non-adjusted 21-day pregnancy rate (based on a 50-day VWP) was 25%, whereas the adjusted 21-day pregnancy rate (based on a 76 day VWP) was 33%. The 21-day service rate averaged 68%, and overall fertility for all TAI averaged 52% (n = 1,093). Overall, fertility to first TAI averaged 56% (n = 563), fertility to second TAI averaged 50% (n = 264), and fertility to third TAI averaged 45% (n = 129). The first three TAI occur from 77 to 180 DIM (i.e., a 100-d period), and 90% of cows became pregnant after the first three TAI. Over 95% of the inseminations in the herd are based on TAI. Although not conducted in this herd, detection of estrus after first TAI for cows that return to estrus after failing to conceive to TAI could further drive the 21-d pregnancy rate but would also require AI to occur every day of the week rather than on a prescheduled day of the week.

The intensive reproductive management protocol based on the concepts presented in this chapter integrates the latest information on technologies for synchronization of ovulation and TAI and pregnancy diagnosis and results in reproductive performance that is heretofore unprecedented for a herd of high-producing Holstein cows. Although use of an aggressive 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 fertility to TAI includ-
ing 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 days after calving (Carvalho et al., 2014b), and poor uterine health (Lima et al., 2013).

Conclusion

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 days after calving (Carvalho et al., 2014a), and poor uterine health (Lima et al., 2013).

References


Cartmill, J. A., S. Z. El-Zarkouny, B. A. Hensley, G. C. Lamb, and J. S. Stevenson. 2001. Stage of cycle, body weight in lactation, and poor uterine 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 days after calving (Carvalho et al., 2014b), and poor uterine health (Lima et al., 2013).
Carvalho, P. D., M. C. Wiltbank, and P. M. Fricke. 2015b. Progesterone concentration at each treatment during an Ovsynch protocol affects fertility to timed AI in Holstein cows. J. Dairy Sci. 98(Suppl. 2):92.


