Reproductive Technologies: Do's and Don'ts

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Introduction

Reproductive management programs for lactating dairy cows that maximize 21-day pregnancy rates integrate technologies for submission of cows for first AI, for early nonpregnancy diagnosis, and for resubmission of nonpregnant cows for second and greater AI. Technologies for submission of cows for first insemination include systems for detection of estrus/activity and hormonal protocols for timed AI. Technologies for nonpregnancy diagnosis include transrectal ultrasonography and measurement of pregnancy-associated glycoproteins in blood or milk. Technologies for second and greater insemination include detection of cows that fail to conceive and return to estrus and hormonal protocols for resynchronization of ovulation. Many new reproductive technologies have the potential to increase reproductive performance if incorporated into a reproductive management program correctly; conversely, they have the potential to decrease reproductive performance if incorporated into a reproductive management program incorrectly. The purpose of this paper is to overview new technologies for submission of cows for first AI, for early nonpregnancy diagnosis, and for resubmission of nonpregnant cows for second and greater AI, and provide some do's and don'ts for incorporation of these technologies into a reproductive management program based on research in these areas.

Technologies for First Insemination

DO consider using a fertility program for first AI
DON'T rely on 100% detection of estrus for first AI

Detection of Estrus/Activity. Despite the widespread adoption of hormonal synchronization protocols that allow for timed AI, detection of behavioral estrus continues to play an important role in the overall reproductive management program on most dairy farms in the U.S. (Caraviello et al., 2006; Miller et al., 2007). Use of detection of estrus alone for submitting lactating dairy cows for first AI generally results in poor reproductive performance. The many challenges of estrous detection on farms include: cows with anovular conditions (Wiltbank et al., 2002); attenuation of the duration of estrous behavior associated with increased milk production near the time of estrus resulting in shorter periods of time in which to visually detect estrous behavior (Lopez et al., 2004); few cows expressing standing estrus at any given time (Roelofs et al., 2005; Palmer et al., 2010); silent ovulations (Palmer et al., 2010; Ranasinghe et al., 2010; Valenza et al., 2012); and reduced expression of estrus due to confinement housing systems (Palmer et al., 2010) with concrete flooring (Britt et al., 1986). Whatever the cause, the low accuracy and efficiency of detection of estrus not only increases time from calving to first artificial insemination (**AI**) but increases the average interval between AI services (Stevenson and Call, 1983) thereby limiting the rate at which cows become pregnant.

Because of the impact of AI service rate on reproductive performance and the problems associated with visual detection of estrus on farms, technologies have been developed and marketed to dairy farmers to enhance detection of estrus by providing continuous surveillance of behavior in the absence or in addition to visual observation of estrus. Increased physical activity is a secondary sign of estrus in cattle, and pedometer systems that detect changes in the number of steps per unit time have been available for many years with some adoption by the dairy industry in the U.S. A new generation of electronic systems that continuously monitor physical activity in cattle (Holman et al., 2011; Jónsson et al., 2011) have been developed and marketed to the dairy industry, and there has been rapid adoption of this new technology in the U.S. over the past several years.

Because of the biological challenges associated with detection of estrus, a combined approach in which AI is based both on activity detected by an activity monitoring system followed by submission of cows not detected with activity to timed AI after synchronization of ovulation may be an effective and economical strategy to submit lactating dairy cows for first AI. We conducted a field trial to compare reproductive performance of lactating dairy cows managed for first AI using timed AI with or without detection of estrus using an activity monitoring system (Fricke et al., 2014). Cows were submitted to a Presynch-Ovsynch protocol for first AI, and activity was monitored in all cows using a commercial activity monitoring system (Heatime, SCR Engineers Ltd., Netanya, Israel) beginning at 24 ± 3 DIM. Cows in treatment 1 with increased activity after the second $PGF_{2α}$ treatment were inseminated based on activity, whereas cows without increased activity were submitted to an Ovsynch protocol beginning 12 d after the second $PGF_{2α}$ treatment of the Presynch protocol and received a timed AI at 75 ± 3 days in milk. Cows in treatment 2 with increased activity after the second $PGF_{2α}$ injection were recorded by the activity monitoring system software but were not inseminated so that all cows in treatment 2 completed the Presynch-Ovsynch protocol and received a timed AI at 75 ± 3 days in milk regardless of whether or not they were detected with increased activity after the second $PGF_{2α}$ injection.

The activity monitoring system detected increased activity in 69% and 70% of cows after the second $PGF_{2α}$ treatment in treatments 1 and 2, respectively (Table 1) which is about 10 to 15 percentage points greater than that reported in studies using tail chalk after the second $PGF_{2α}$ injection of a Presynch-Ovsynch protocol (Stevenson and Phatak, 2005; Chebel and Santos, 2010). Overall, cows in treatment 1 in which inseminations occurred as a combination between AI to activity and timed AI had fewer P/AI compared to cows in treatment 2 in which all cows received timed AI after completing the Presynch-Ovsynch protocol (Table 1). The decrease in P/AI due to inseminating cows with increased activity after the second $PGF_{2α}$ injection was expected because the increase in P/AI due to presynchronization with $PGF_{2α}$ likely results from synchronizing estrus after the second $PGF_{2α}$ injection (Navanukraw et al., 2004) so most cows initiate the Ovsynch protocol on days 5 to 9 of the ensuing estrous cycle thereby increasing P/AI to TAI (Vasconcelos et al., 1999). Inseminating 70% of cows based on activity after the second $PGF_{2α}$ treatment removed the presynchronized cows from the protocol thereby negating the increase in P/AI due to presynchronization. Cows without increased activity after the second $PGF_{2α}$ treatment and submitted to an Ovsynch protocol had P/AI of 33% and 35% for treatments 1 and 2, respectively (Table 1). Pregnancy outcomes of anovular cows subjected to an Ovsynch protocol is generally about 20% compared to about 35% for cycling cows starting an Ovsynch protocol at a random stage of the cycle (Gümen et al., 2003; Stevenson et al., 2008). Thus, cows without activity that received an Ovsynch protocol had a P/AI similar to that of cycling cows starting an Ovsynch protocol at a random stage of the cycle. Thus, aggressive submission of cows to an Ovsynch protocol after failing to be detected with increased activity is an effective management strategy to establish pregnancy in this subgroup of cows.

Table 1. *Effect of treatment on percentage of lactating Holstein cows with activity based on an activity monitoring system, and pregnancies per AI (P/AI) for cows with or without activity and inseminated to activity (AI) or inseminated after synchronization of ovulation (TAI). Adapted from Fricke et al., 2014.*

	Treatment	
Item	1	2
Cows with increased activity, % (n/n)	69 (230/335)	70 (232/331)
P/AI 35 d after AI, % (n/n)		
Cows with activity receiving AI	30[a] (68/230)	-
Cows with activity receiving TAI	-	41[b] (96/232)
Cows with no activity receiving TAI	36 (37/104)	35 (35/99)
Overall P/AI 35 d after AI, % (n/n)	32[c] (105/333)	40[d] (131/331)
P/AI 67 d after AI, % (n/n)		
Cows with activity receiving AI	27 (62/230)	-
Cows with activity receiving TAI	-	40 (92/232)
Cows with no activity receiving TAI	33 (34/104)	35 (35/99)
Overall P/AI 67 d after AI, % (n/n)	29[c] (96/333)	38[d] (127/331)

[a,b]Within a treatment by activity subgroup, statistical contrast differed ($P = 0.004$).

[c,d]Within a row, percentages with different superscripts differed ($P = 0.0454$).

[1]Treatments were: 1) cows inseminated based on an activity monitoring system after the second $PGF_{2\alpha}$ injection of a Presynch-Ovsynch protocol with cows not detected with activity receiving timed AI after completing the Presynch-Ovsynch protocol; 2) cows receiving timed AI after a Presynch-Ovsynch protocol.

Overall, 31% of cows in treatment 1, and 100% of cows in treatment 2 were submitted to the Ovsynch portion of the synchronization protocol, and blood samples were collected from a subgroup (~85%) of cows in each treatment at the first GnRH injection of the Ovsynch protocol to determine progesterone concentration at the onset of the protocol (Fricke et al., 2014). Surprisingly, over 50% of these cows had progesterone concentrations $\geq$ 1 ng/mL at the first GnRH injection of the protocol, and similar results were observed for cows in treatment 2 that were not detected with activity after presynchronization. Thus, many cows without activity after presynchronization likely ovulated in the absence of detectable activity resulting in high progesterone at G1 of the Ovsynch protocol. These results agree with the 10% of cows that ovulated but failed to be detected with activity by the activity monitoring system (Valenza et al., 2012). Results from Fricke et al. (2014) support a management strategy in which the 30% of cows not detected with activity are aggressively submitted to an Ovsynch protocol rather than continuing to detect activity using an activity monitoring system.

Based on two experiments assessing an activity monitoring system on a large commercial dairy farm in the U.S. (Valenza et al., 2012; Fricke et al., 2014), only about 70% of cows were inseminated based on the activity monitoring system. These data underscore the importance of implementing a comprehensive reproductive management program for identification and treatment of cows that would otherwise not be inseminated. Although use of an activity monitoring system to inseminate cows based on activity reduced days to first AI, cows receiving 100% timed AI after completing a Presynch-Ovsynch protocol had more P/AI (Fricke et al., 2014). The trade-off between AI service rate and P/AI was reflected by an economic analysis in which varying levels of estrous detection and three levels of P/AI were compared (Giordano et al., 2012b). When P/AI to detected estrus in a herd is poor, allowing cows to complete the Presynch-Ovsynch protocol and receive a timed AI would be a preferred strategy; conversely, whenever P/AI to AI after a detected estrus is 30% or 35% there was always an economic advantage to inseminating cows detected in estrus across all levels of estrous detection. We conclude that although a variety of strategies can be used to submit cows for first and subsequent AI, synchronization of ovulation and timed AI is a beneficial strategy to inseminate cows not detected by the activity monitoring system.

Fertility Programs. Even though the Presynch-Ovsynch protocol was originally developed to increase the fertility of cows submitted to TAI, many farms inseminate cows detected in estrus after the second $PGF_{2\alpha}$ treatment of a Presynch-Ovsynch protocol followed by submission of cows not detected in estrus to an Ovsynch-56 protocol. A recent meta-analysis, however, supports the idea that incorporation of AI to estrus into a Presynch-Ovsynch protocol decreases P/AI for cows not detected in estrus and submitted to timed AI than when all cows were allowed to complete the protocol and receive timed AI (Borchardt et al., 2016). This decrease in P/AI to timed AI occurs because cycling cows that would have been presynchronized so that G1 occurred at an optimal time of the estrous cycle are removed from the protocol after AI to estrus thereby negating the presynchronization effect. Further, anovular cows submitted to a Presynch-Ovsynch protocol have decreased P/AI than their cycling herd mates. Because anovular cows lack a CL and therefore do not respond to the two initial $PGF_{2\alpha}$ treatments of a Presynch-Ovsynch protocol, G1 occurs in a low progesterone environment resulting in fewer P/AI to timed AI. Because anovular cows represent nearly 1 in 4 cows submitted for first timed AI, presynchronization strategies using $PGF_{2\alpha}$ alone with or without inclusion of detection of estrus do not yield high P/AI to Timed AI.

Two limitations of presynchronization strategies that use $PGF_{2\alpha}$ alone (e.g., Presynch-Ovsynch) are that 1) $PGF_{2\alpha}$ does not affect anovular cows or resolve the anovular condition before G1 of the Ovsynch protocol, and 2) follicular growth is not tightly synchronized after two sequential $PGF_{2\alpha}$ treatments administered 14 d apart. By contrast, presynchronization strategies that combine GnRH and $PGF_{2\alpha}$ overcome both of these limitations thereby increasing fertility to TAI. For example, when an Ovsynch protocol was used to presynchronize cows before G1 of an Ovsynch protocol (i.e., a **Double-Ovsynch** protocol), cows submitted to the Double-Ovsynch protocol had more P/AI than cows submitted to a Presynch-Ovsynch protocol (Souza et al., 2008; Herlihy et al., 2012). Presynchronization strategies tested to date have used a combination of GnRH and $PGF_{2\alpha}$ 6 to 7 d before G1 (i.e., **G6G**; Bello et al., 2006).

Several experiments have assessed the effect of an additional $PGF_{2\alpha}$ treatment within a Double-Ovsynch protocol to decrease progesterone at G2 (Brusveen et al., 2009; Wiltbank et al., 2015). Adding a second $PGF_{2\alpha}$ treatment 24 h after the first $PGF_{2\alpha}$ treatment during the second Ovsynch portion of a Double-Ovsynch protocol for first TAI dramatically increased the proportion of cows undergoing complete luteal regression thereby increasing the proportion of pregnant primiparous cows by 4.6% and the proportion of pregnant multiparous cows by 23% (Table 2; Wiltbank et al., 2015). Thus, the treatment by parity effect on P/AI to timed AI after a Double-Ovsynch protocol discussed previously can at least partially be explained by a lack of luteal regression in multiparous cows that can be overcome by adding a second $PGF_{2\alpha}$ before G2 of the protocol.

Table 2. *Effect of 1 vs. 2 prostaglandin $F_{2\alpha}$ (PGF) treatments on pregnancies per AI (P/AI) in lactating dairy cows (adapted from Wiltbank et al., 2015).*

	P/AI, % (n/n)			
Item	1 PGF	2 PGF	Difference (%)	P-value
Primiparous	46 (41/89)	48 (40/83)	5	0.45
Multiparous	37 (37/101)	45 (45/100)	23	0.14
P-value	0.24	0.77		
Overall	41 (78/90)	46 (85/183)	13	0.17

Taken together, these data support that ~50% of cows become pregnant to first TAI after a modified Double-Ovsynch protocol. The advantages of using 100% TAI after a modified Double-Ovsynch protocol for first timed AI include precise control of the interval from calving to first AI such that all cows in the herd receive timed AI within 7 d of the end of the VWP (in herds in which timed AI is conducted weekly) and yielding exceptionally high fertility to first timed AI. When optimized, these two factors dramatically increase the 21-d pregnancy rate. Presynchronization strategies that combine GnRH and $PGF_{2\alpha}$ to resolve anovular conditions and optimize timing of initiation of G1 and addition of a second $PGF_{2\alpha}$ treatment 24 h after the first $PGF_{2\alpha}$ treatment within an Ovsynch protocol currently represents the most aggressive method for submitting cows for first AI while also yielding high P/AI to timed AI.

Technologies for Nonpregnancy Diagnosis

DO identify nonpregnant cows early after AI
DON'T identify nonpregnant cows TOO early after AI

Transrectal Ultrasonography. As a pregnancy diagnosis method, transrectal ultrasonography is accurate and rapid, and the outcome of the test is known immediately at the time the test is conducted. Transrectal ultrasonography has begun to displace transrectal palpation as the direct method of choice by veterinarians for pregnancy diagnosis (Caraviello et al., 2006). Because many experienced bovine practitioners can accurately diagnose pregnancy as early as 35 days after AI using transrectal palpation, pregnancy examination using transrectal ultrasonography 28 to 34 days after AI only reduces the interval from insemination to pregnancy diagnosis by a few days. Although ultrasound conducted at ≥ 45 days post breeding did not increase accuracy of pregnancy diagnosis for an experienced palpator, it may improve diagnostic accuracy of a less experienced one (Galland et al., 1994). The rate of pregnancy loss and the efficacy of strategies to reinseminate cows at various stages post breeding also play a role in determining the advantages and disadvantages on the timing of pregnancy diagnosis and resynchronization (Fricke et al., 2003).

To determine the accuracy of early pregnancy diagnosis using transrectal ultrasonography, we conducted a field trial on a commercial dairy farm milking ~2,000 cows (Giordano and Fricke, 2012a). Pregnancy status was determined 29 days after timed AI using transrectal ultrasonography (Easi-scan, BCF Technology Ltd.) based on the following criteria: presence or absence of a CL, presence, absence, volume and appearance of uterine fluid typical for a 29-day conceptus, presence or absence of an embryo with a heartbeat. Cows were classified as 1) not-pregnant: presence or absence of a CL, absence of uterine fluid, or insufficient uterine fluid, and absence of an embryo; 2) pregnant: CL present, normal uterine fluid, and no embryo; 3) pregnant embryo: CL present, normal uterine fluid, and at least one embryo visualized; and 4) questionable pregnant: CL present, and one or more of the following: uterine fluid, insufficient uterine fluid, and either no embryo or a non-viable embryo. At 39 and 74 days after timed AI, pregnancy status was determined using transrectal palpation, and pregnancy loss occurring between each pregnancy examination was calculated.

Results from this experiment are shown in Table 3. Overall, 802 cows were classified not-pregnant 29 days after timed AI, whereas 799 cows were classified not-pregnant 39 days after timed AI resulting in a not-pregnant misdiagnosis rate of 0.5% (4/802) for transrectal ultrasonography 29 days after timed AI. At 29 days after timed AI, 1,116 cows were classified as either pregnant with an embryo visualized (68%), pregnant based on uterine fluid alone (29%), or questionable pregnant (3%). Among questionable pregnant cows, 69% were classified as not pregnant 39 days after timed AI, and an additional 46% were classified as not pregnant 74 days after timed AI. For cows classified pregnant 29 days after timed AI, more ($P < 0.01$) cows diagnosed based on uterine fluid only than fluid and the presence of an embryo were classified as not pregnant using transrectal palpation 39 days after timed AI. Similarly, more cows diagnosed pregnant based on uterine fluid alone than cows diagnosed pregnant based on visualization of an embryo with a heartbeat were classified as not pregnant using transrectal palpation 74 days after timed AI. From the initial pregnancy examination at 29 days to the last examination 74 days after timed AI, more cows diagnosed pregnant based on uterine fluid alone than cows diagnosed pregnant based on visualization of an embryo with a heartbeat were classified as not pregnant using transrectal palpation 74 days after timed AI. Cows classified pregnant based on uterine fluid alone 29 days after timed AI were 3.8 (95% CI = 2.7 to 5.4) times more likely to be classified not-pregnant 74 days after timed AI than cows diagnosed pregnant based on visualization of an embryo with a heartbeat.

Table 3. *Pregnancy loss by pregnancy classification for lactating Holstein cows diagnosed pregnant using ultrasonography 29 d after timed AI (adapted from Fricke et al., 2016).*

	Pregnancy classification[1]		
Item	Pregnant	Uterine fluid	Questionable
	---------- %, (n/n) ----------		
29 d after timed AI	68 (758/1,116)	29 (322/1,116)	3 (36/1,116)
Pregnancy loss			
29 to 39 d	4^a (30/758)	18^b (57/322)	69^c (25/36)
39 to 74 d	5^a (39/728)	12^b (32/265)	46^c (5/11)
Total loss	9^a (69/758)	28^b (89/322)	83^c (30/36)

[a,b,c]Within a row, proportions with different superscripts differ ($P<0.001$).

[1]Lactating Holstein cows diagnosed pregnant were classified based on the following criteria using transrectal ultrasonography: Pregnant: visualization of a CL ipsilateral to the gravid uterine horn, visualization of an amount of non-echogenic uterine fluid in accordance to stage of pregnancy, and visualization of an embryo with a heartbeat; Uterine fluid: visualization of a CL ipsilateral to the gravid uterine horn, visualization of an amount of non-echogenic uterine fluid in accordance to stage of pregnancy but without visualization of the embryo; Questionable: visualization of a CL ipsilateral to the gravid uterine horn with insufficient uterine fluid for the stage of pregnancy.

Based on these data, we concluded that the accuracy of pregnancy outcomes using transrectal ultrasonography increase dramatically when an embryo with a heartbeat is visualized compared to outcomes based only on the presence of a CL and the volume of uterine fluid in the absence of a visualized embryo with a heartbeat. The presence of a large proportion of cows with a CL and fluid was visualized in the absence of an embryo with a heartbeat is likely due to a high degree of early pregnancy loss in dairy cows. In one experiment, 44% of dairy cows diagnosed not pregnant 32 days after timed AI had extended luteal phases (Ricci et al., 2014). Based on our results, early pregnancy diagnosis should not be conducted earlier than an embryo with a heartbeat can be rapidly and reliably detected in pregnant cows under on-farm conditions using transrectal ultrasonography (~30 days after AI) to reduce the negative impact of false positive results.

Pregnancy-Associated Glycoproteins. Pregnancy-associated glycoproteins belong to a large family of inactive aspartic proteinases expressed by the placenta of domestic ruminants including cows, ewes, and goats (Haugejorden et al., 2006). In cattle, the PAG gene family comprises at least 22 transcribed genes as well as some variants (Prakash et al., 2009). Mean PAG concentrations in cattle increase from 15 to 35 d in gestation; however, variation in plasma PAG levels among cows precludes PAG testing as a reliable indicator of pregnancy until about 26 to 30 d after AI (Zoli et al., 1992; Humblot, 2001). Assessment of pregnancy status through detection of placental PAG or PSPB levels in maternal blood (Sasser et al.,1986; Zoli et al 1992; Green et al 2005) is now used to evaluate pregnancy status within the context of a reproductive management scheme on commercial dairies (Silva et al., 2007, 2009; Sinedino et al., 2014). A commercial test for detecting PAG levels in milk (The IDEXX Milk Pregnancy Test, IDEXX Laboratories, Westbrook, ME) has been developed and marketed to the dairy industry.

Few studies have compared factors associated with PAG levels in blood and milk of dairy cows early in gestation and the impact these factors may have on the accuracy of pregnancy diagnosis. We conducted an experiment to determine factors affecting PAG levels in blood and milk of dairy cows during early gestation (Ricci et al., 2015). Lactating Holstein cows were synchronized to receive their first AI at a fixed time. Blood and milk samples were collected 25 and 32 d after TAI, and pregnancy status was determined 32 d after TAI using transrectal ultrasonography. Cows diagnosed pregnant with singletons continued the experiment in which blood and milk samples were collected and pregnancy status was assessed weekly using transrectal ultrasonography from 39 to 102 d after TAI. Plasma and milk samples were assayed for PAG concentrations using commercial ELISA kits.

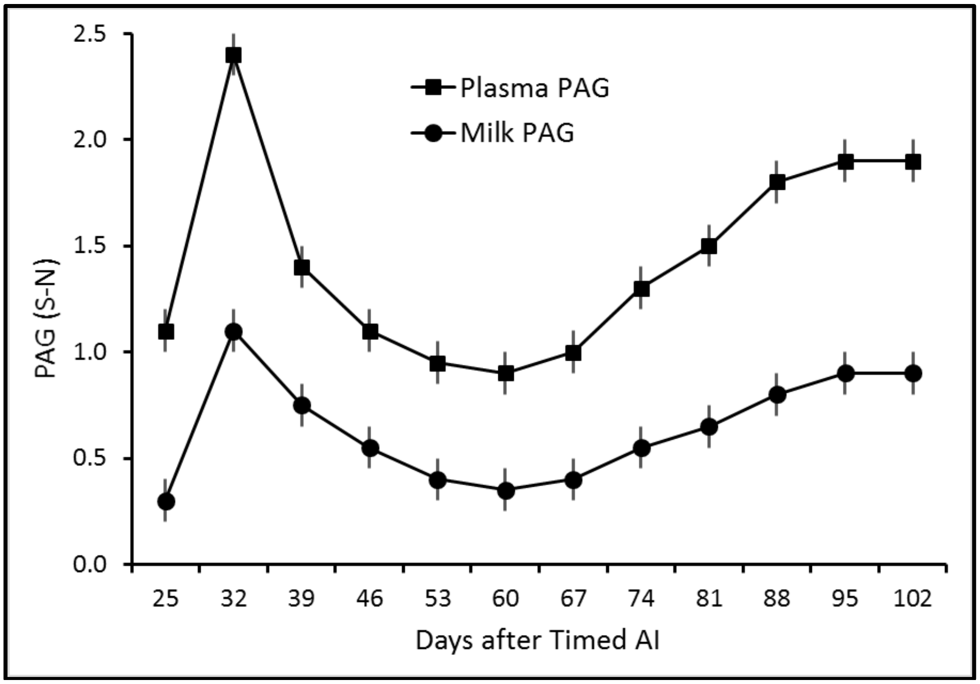

Figure 1*. Plasma and milk pregnancy-associated glycoprotein (PAG) profiles for Holstein cows (n = 48) that maintained pregnancy from 25 to 102 d after AI. ELISA outcomes were calculated from the optical density (OD) of the sample (corrected by subtraction of the reference wavelength OD of the sample (S) minus the OD of the negative control (N) at 450 nm with both values corrected by subtraction of the reference wavelength OD of the negative control), which resulted in an S-N value. Plasma and milk PAG levels were affected by week after AI (P < 0.01). Adapted from Ricci et al., 2015.*

The incidence of pregnancy loss from 32 to 102 d after AI among cows bearing a singleton was 13% (7/55), which is similar to the 13% pregnancy loss between 29 ± 2 and 44 ± 6 d after AI reported in a summary of 14 studies (Santos et al., 2004). For the plasma PAG ELISA, all but one cow that underwent pregnancy loss tested positive, whereas all cows undergoing pregnancy loss tested positive at one or more time points for the milk PAG test. Similarly, 5 of 7 cows that had inconclusive results based on the plasma PAG test before the loss occurred compared with 3 of 7 cows based on the milk PAG test. Thus, PAG levels detected by these ELISA tests in the present study have a half-life in maternal circulation resulting in a 7 to 14 d delay in identification of cows undergoing pregnancy loss based on plasma or milk PAG levels compared with transrectal ultrasonography.

Profiles of PAG in plasma and milk of cows that maintained pregnancy from 25 to 102 days in gestation are shown in Figure 1. Factors associated with PAG levels in dairy cows included stage of gestation, parity, pregnancy loss, and milk production. Based on plasma and milk PAG profiles, the optimal time to conduct a first pregnancy diagnosis is around 32 days after AI coinciding with an early peak in PAG levels. We concluded that because of the occurrence of pregnancy loss, all pregnant cows should be retested 74 days after AI or later when plasma and milk PAG levels in pregnant cows have rebounded from their nadir.

Technologies for Second and Greater Insemination

DO couple a nonpregnancy diagnosis with a strategy to rapidly re-inseminate cows
DON'T submit nonpregnant cows without a CL to a Resynch protocol

Return to Estrus after AI. Accurate identification of cows returning to estrus from 18 to 24 days after AI is the easiest and least costly method for determining nonpregnancy early after insemination. This assumption, however, is being challenged by new research and long-recognized reproductive problems. First, estrous detection efficiency is estimated to be less than 50% on most dairy farms in the United States (Senger, 1994). Only, 51.5% of the eligible cows were detected in estrus and inseminated in a recent study in which detection of estrus was performed through continuous monitoring with activity tags after a previous insemination until pregnancy diagnosis 32 ± 3 d after AI, (Giordano et al., 2015). Second, estrous cycle duration varies widely with a high degree of variability among individual cows (Remnant et al., 2015). Finally, the high rate of pregnancy loss in dairy cows can increase the interval from insemination to return to estrus for cows that establish pregnancy early then undergo pregnancy loss later during gestation (Ricci et al., 2014).

Resynch. Whereas presynchronization strategies have yielded significant increases in fertility to first TAI, many herds struggle with poor fertility to an Ovsynch protocol used for TAI at second and greater services (i.e., **Resynch**). Based on progesterone profiles during the Ovsynch protocol, the best indicator of poor fertility to timed AI is low progesterone (i.e., cows lacking a CL) at the $PGF_{2\alpha}$ treatment. One of the first strategies to optimize fertility to Resynch TAI attempted to determine the optimal interval after an initial TAI to initiate G1 based on the physiology of the estrous cycle (Fricke et al., 2003). Assuming an estrous cycle duration of 21 to 23 d, initiating a Resynch protocol 32 d after AI corresponds to starting the Resynch protocol around day 6 to 14 of the estrous cycle, a stage of the estrous cycle when a dominant follicle capable of ovulating and a CL with mid-level progesterone should be present. Cows identified not pregnant 32 days after AI with a CL at G1 have greater fertility to TAI than cows without a CL (Giordano et al., 2012c; Lopes et al., 2013). In several studies however, 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; Giordano et al., 2015). When cows were synchronized for first timed AI and progesterone 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, G1 occurs in a low-progesterone environment in up to one-third of nonpregnant cows submitted to a Resynch protocol which leads to a lack of complete luteal regression after treatment with $PGF_{2\alpha}$ 7 d later and low fertility to TAI.

***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)[1]. Adapted from Carvalho et al., 2015.*

	Treatment	
Item	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 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)

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

We conducted an experiment to determine the effect of adding a second $PGF_{2\alpha}$ treatment 24 hours after the first within an Ovsynch protocol would increase P/AI to TAI after a Resynch protocol (Carvalho et al., 2015). A greater proportion of cows receiving 1 $PGF_{2\alpha}$ treatment had incomplete luteal regression (≥ 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/AI than cows receiving 1 $PGF_{2\alpha}$ treatment, whereas for cows with P4 concentrations ≥ 1.0 ng/mL at G1, P/AI did not differ ($P = 0.46$) between cows receiving 1 vs. 2 $PGF_{2\alpha}$ treatments (Table 4).

A benefit of transrectal ultrasonography over transrectal palpation for nonpregnancy diagnosis is the opportunity to more accurately determine the ovarian status of cows at a nonpregnancy diagnosis facilitating the assignment of cows to different treatment alternatives. For example, use of an Ovsynch protocol for resynchronization of cows identified not pregnant 32 days after AI resulted in greater conception rates when cows were identified with a CL compared to cows without a CL at the first GnRH treatment of the protocol (Giordano et al., 2012c; Lopes et al., 2013). Treatment of cows without a CL at the first GnRH treatment of an Ovsynch protocol with exogenous progesterone (i.e., a CIDR insert) increased fertility at first as well as resynch timed AI in lactating dairy cows (Chebel et al., 2010; Bilby et al., 2013). Treatment of cows with a CL ≥20 mm at nonpregnancy diagnosis with a PGF injection increased the overall proportion of cows inseminated after a detected estrus for second and subsequent AI services (Giordano et al., 2015). Based on these data, many veterinarians now use the presence or absence of a CL at a nonpregnancy diagnosis to improve outcomes to timed AI protocols used to resynchronize nonpregnant cows or to increase the proportion of cows inseminated in estrus after a previous insemination.

Conclusions

Reproductive management programs that successfully integrate new technologies for submission of cows for first AI, for early nonpregnancy diagnosis, and for resubmission of nonpregnant cows for second and greater AI can now yield reproductive performance that is unprecedented in herds of high-producing Holstein dairy cows. Although correct integration of these technologies 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., 2014), and poor uterine health (Lima et al., 2013).

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