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Updates on Reproductive Management of Dairy Cows and Heifers

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Reproductive efficiency continues to be a challenge for the dairy industry. Generally, less than 45% of cows are pregnant after the first postpartum AI and significantly smaller percentage of cows (< 30%) are pregnant after resynchronized AI. Another significant impediment to adequate reproductive performance is the reduced estrus detection rate, also known as AI submission rate. Consequently, a large percentage of dairies (74.8%) reported to use estrus/ovulation synchronization protocols for first postpartum AI (Caraviello et al., 2008). Although the aim of such reproductive programs is to increase AI submission rate and ultimately percentage of cows that become pregnant, often the use of timed AI protocols results in smaller pregnancy per AI (P/AI) compared with insemination in estrus.

Cow factors (i.e. anovular condition, postparturient diseases, mastitis, lameness, etc.) have a profound impact on reproductive performance of dairy cows but, when GnRH-based ovulation synchronization protocols (i.e. Ovsynch) are implemented, the following also have a significant impact on P/AI: ovulation to the first GnRH, length of dominance of the ovulatory follicle, synchrony of luteolysis, and interval from ovulation to AI.

The timing of initiation of ovulation synchronization protocols is fundamental because it will determine the likelihood of ovulation to the first GnRH injection, timing of luteolysis during the protocol, length of dominance of the ovulatory follicle, and ultimately synchrony of the estrous cycle. Cows that start synchronization protocols during metestrus (1 to 4 d of the estrous cycle) are less likely to ovulate (< 25% ovulate) in response to the first GnRH (Vasconcelos et al., 1999), which will result in prolonged dominance of the ovulatory follicle and reduced embryo quality (Cerri et al., 2009) and P/AI (Chebel et al., 2006). If ovulation synchronization protocols are initiated during early diestrus (5 to 9 d of the estrous cycle), percentage of cows that ovulate to the first GnRH (d 0) is high (> 90%), a new follicular wave is recruited within 40-48 h after the GnRH, a CL is present at the time of the prostaglandin (PG) $F_{2\alpha}$ injection (d 7 = 12 to 16 d of the estrous cycle) resulting in synchronized luteolysis, and synchronized ovulation is induced with the second GnRH given on d 9.5 (Vasconcelos et al., 1999). The start of synchronization protocols during late diestrus (10 to 16 d of the estrous cycle) poses problems because a small percentage of cows ovulate to the first GnRH (< 55%) and a significant percentage of them may undergo spontaneous luteolysis before the $PGF_{2\alpha}$ injection (given on d 17 to '23' of the estrous cycle), which are likely to result in prolonged dominance of ovulatory follicles and ovulation of aged follicles and lack of synchrony at the end of the protocol, respectively (Vasconcelos et al., 1999). Finally, the start of ovulation synchronization protocols during the proestrus (17 and 22 d of the estrous cycle) will result in reasonable ovulation in response to the first GnRH (~ 75%); however, because spontaneous luteolysis occurs at approximately d 17 of the estrous cycle, luteolysis would not be synchronized by the $PGF_{2\alpha}$ injection, resulting in lack of synchrony of the estrous cycle. Therefore, when implementing ovulation synchronization protocols the estrous cycle has to be presynchronized to assure that a large percentage of cows start such protocols between d 5 and 9 of the estrous cycle.

In the following pages we will discuss methods to presynchronize the estrous cycle before submission to ovulation synchronization protocols for first postpartum AI and resynchronized AI. Further, we will discuss recent research that has demonstrated that reducing the length of the dominance period of the ovulatory follicle by reducing the interval between the GnRH and the $PGF_{2\alpha}$ injections could improve P/AI.

First Postpartum AI

The most commonly known and implemented presynchronization protocol is the Presynch, which is composed of two injections of $PGF_{2\alpha}$ given 14 d apart with the second $PGF_{2\alpha}$ given 12 d before the start of the Ovsynch. This results in good synchrony of the estrous cycle after the second $PGF_{2\alpha}$ injection of the Presynch, with approximately 85% of cycling cows displaying estrus within two to seven days. Consequently, by starting the Ovsynch approximately 12 d after the second $PGF_{2\alpha}$ of the Presynch the majority of cyclic cows would be between d 5 and 10 of the estrous cycle (early diestrus). The positive effects of Presynch on P/AI of cows submitted to the Ovsynch protocol was demonstrated in a study conducted in Florida in which cows submitted to the Presynch-Ovsynch had P/AI approximately 10 percentage units greater than those submitted to the Ovsynch (Moreira et al., 2001). For convenience, researchers altered the Presynch-Ovsynch protocol so that most injections could be given in two days of the week ($PGF_{2\alpha}$-MON, 14 d later $PGF_{2\alpha}$-MON, 14 d later GnRH-MON, 7 d later $PGF_{2\alpha}$-MON, 2.5 d later GnRH-WED); however, the interval between the second $PGF_{2\alpha}$ of the Presynch and the first GnRH of the Ovsynch was extended from 12 to 14 d. Although initially it was suggested that extending the interval between the second $PGF_{2\alpha}$ of the Presynch and the start of the Ovsynch would not impact P/AI, a recent study demonstrated that cows submitted to a Presynch-Ovsynch with a 11 d interval between the second $PGF_{2\alpha}$ of the Presynch and the start of the Ovsynch had greater P/AI than cows with a 14 d interval (36.4 vs. 30.2%; Galvão et al., 2007). This can be explained by the greater proportion of cows ovulating to the first GnRH of the Ovsynch when the interval was 11 d instead of 14 d (61.4 vs. 44.7%; Galvão et al., 2007). Therefore, when the Presynch-Ovsynch is implemented the interval between the second $PGF_{2\alpha}$ of the Presynch and the start of the Ovsynch should be 10 to 12 d.

Much has been discussed about the Double-Ovsynch protocol lately. In this protocol cows are submitted to a ‘presynchronizing Ovsynch’ followed seven d later by the ‘breeding Ovsynch’. The protocol would be: GnRH d -17, $PGF_{2\alpha}$ d -10, GnRH d -7, GnRH d 0, $PGF_{2\alpha}$ d 7, GnRH d 9.5, and TAI d 10. As such, it is expected that approximately 40-50% of cows would ovulate to the first GnRH given on d -17 and a significantly larger proportion would ovulate to the GnRH given on d -7. This would cause the recruitment of a new follicular wave approximately 40-48 h later, which would result in a large percentage of cows starting the ‘breeding Ovsynch’ in early diestrus and a large percentage of cows ovulating in response to the GnRH given on d 0. In the study published in which this protocol was compared to the Presynch-Ovsynch protocol (Souza et al., 2008), cows submitted to the Double-Ovsynch had greater P/AI than cows submitted to the Presynch-Ovsynch (54.9 vs. 38.5%). This can be explained by the fact that cows in the Double-Ovsynch treatment were less likely to have P4 < 1 ng/mL (9.4 vs.

33.3%) at the beginning of the 'breeding-Ovsynch' and were more likely to ovulate to the first GnRH injection of the 'breeding-Ovsynch' (71.8 vs. 66.7%). This results in growth of follicles under high P4 environment and better quality oocytes available for fertilization. One of the very interesting findings of this study, however, is that P/AI was only greater for first lactation cows submitted to the Double-Ovsynch (65.2 vs. 37.5%), whereas in mature cows there were no differences (45.2 vs. 39.3%). Although it is not clear why Double-Ovsynch only increased P/AI of first lactation cows, it is possible that the additional two injections of GnRH given during this protocol stimulated resumption of cyclicity of more first lactation cows, which are known to have extended anovular periods postpartum. Therefore, in herds that have a large percentage of first lactation cows and are facing extended anovular period postpartum the Double-Ovsynch protocol may be very useful.

Five day synchronization

As mentioned above, the length of dominance of the ovulatory follicle plays a very important role on embryo quality and P/AI. In a study in which period of dominance of the ovulatory follicle was extended by starting the Ovsynch protocol during metestrus instead of diestrus (d 3 vs. d 6 of the estrous cycle) cows that had period of dominance of 5.8 d produced better quality embryos than those that had period of dominance of 8.1 d (Cerri et al., 2009).

Therefore, it was hypothesized that by reducing the interval between the first GnRH and the $PGF_{2\alpha}$ injections of the timed AI protocol in 48 h it would be possible to improve P/AI. To do so it was necessary to determine whether one or two injections of $PGF_{2\alpha}$ would be needed to induce luteolysis of the newly formed corpus luteum (CL). In one experiment, the following treatments were evaluated to determine whether one or two injections of $PGF_{2\alpha}$ would be necessary to induce luteolysis of newly formed CL: 7dCos – d 0 GnRH, d 7 $PGF_{2\alpha}$, d 10 GnRH+TAI; 5dCos1PG – d 0 GnRH, d 5 $PGF_{2\alpha}$, d 8 GnRH+TAI; and 5dCos2PG – d 0 GnRH, d 5 and 6 $PGF_{2\alpha}$, d 8 GnRH+TAI (Chebel et al., 2008). As observed in table 1, cows in the 5dCos1PG were less likely to have luteolysis, whereas 5dCos2PG cows had the greatest rate of luteolysis. As expected, because of the shorter interval between the first and second injections of GnRH, the diameter of the ovulatory follicle was smaller for 5dCos1PG and 5dCos2PG cows compared with 7dCos cows.

Table 1. Effect of reducing the period of follicle dominance on ovarian responses and luteal regression of dairy cows during the timed AI protocols

	Treatment			
	7dCos	5dCos1PG	5dCos2PG	*P*
Progesterone, ng/mL				
At AI	1.14 ± 0.16^{b}	1.80 ± 0.18^{a}	0.77 ± 0.20^{b}	<0.01
CL regression at AI,[1] %				
All cows	79.0 (45/57)^{b}	59.1 (26/44)^{c}	95.7^{a} (45/47)	<0.01
Cows ovulating to 1st GnRH	71.4^{b} (25/35)	51.7^{c} (15/29)	96.0^{a} (24/25)	0.01
Diameter of ovulatory follicle, mm				
At $PGF_{2\alpha}$	$14.5^{a} \pm 0.6$	$13.0^{b} \pm 0.6$	$12.9^{b} \pm 0.6$	0.05
At AI	$18.3^{a} \pm 0.6$	$17.5^{ab} \pm 0.6$	$16.5^{b} \pm 0.6$	0.02

2nd GnRH of timed AI				
Ovulation,[2] %	88.7 (55/62)	78.9 (41/52)	84.3 (43/51)	0.36
Synchronization,[3] %	69.4[a] (43/62)	42.3[b] (22/52)	78.4[a] (40/51)	<0.01

[a,b,c] Superscripts in the same row differ ($P < 0.05$).
[d,e] Superscript in the same row differ ($P = 0.06$).
[1] Cows with progesterone ≥ 1 ng/mL at $PGF_{2\alpha}$ and < 1 ng/mL at AI.
[2] Ovulation within the first 48 h of the final GnRH of the timed AI protocols.
[3] Cows that regressed their CL (progesterone ≥ 1 ng/mL at $PGF_{2\alpha}$ and < 1 ng/mL at AI) and ovulated within 48 h of timed AI.

From this initial experiment we concluded that two injections of $PGF_{2\alpha}$ would be necessary to induce luteolysis and assure appropriate synchronization of the estrous cycle when the interval between the GnRH and $PGF_{2\alpha}$ injections was reduced in 48 h. In a follow-up experiment, we submitted cows to either the 7dCos or the 5dCos2PG. As observed in table 2, when only cows that had luteolysis were evaluated, P/AI of cows in the 5dCos2PG was increased in 4.2 percentage points compared with 7dCos (Chebel et al., 2008).

Table 2. Effect of reducing the period of follicle dominance on fertility responses of dairy cows that had luteolysis

	Treatment		
	7dCos	5dCoS2PG	*P*
	% (n/n)		
Pregnant			
Day 38	33.9 (144/425)	39.3 (175/445)	0.02
Day 66	32.5 (138/425)	36.7 (163/444)	0.02
Pregnancy loss	4.2 (6/144)	6.3 (11/174)	0.52

Therefore, we conclude that it is possible to increase the P/AI when the period of dominance of the ovulatory follicle is reduced by reducing the interval between the GnRH and the $PGF_{2\alpha}$ interval, but only if two injections of $PGF_{2\alpha}$ are given.

Use of intravaginal progesterone inserts (CIDR) for first AI

Since 2003, intra-vaginal progesterone devices (CIDR) are available in the US. This device, when used between the GnRH and the $PGF_{2\alpha}$ injections of the timed AI protocol, has the potential to improve synchrony of the estrous cycle by impeding cows that undergo spontaneous luteolysis during the timed AI protocol ovulate before its conclusion. Although effects of using the CIDR device during the timed AI protocol on P/AI have been inconsistent, in a recent study conducted in seven dairy herds across the US with a large number of cows (1,665 cows) we determined that cows treated with CIDR device during the timed AI protocol had P/AI 5.1 percentage points greater than cows not treated with CIDR device (35.1 vs. 30.0%; Chebel et al., 2010a). In other studies, it was determined that cows treated with CIDR device were less likely to have asynchronous estrous cycle after the timed AI (3.8 vs. 14.3%; Lima et al., 2009) and were less likely to have short luteal phase after the timed AI (13.0 vs. 21.6%; Cerri et al., 2009) than those not treated with CIDR. These physiological responses to the treatment with CIDR devices help explain the expected improvements in P/AI.

Resynchronization

Resynchronization of non-pregnant cows continues to be a challenge for reproductive performance of dairy cows. According to published studies and on-farm data, P/AI of cows diagnosed not pregnant and resynchronized with the Ovsynch protocol is usually < 30%. As described above, it is critical that cows start the timed AI protocol between d 5 and 9 of the estrous cycle. Considering that the length of the estrous cycle of lactating dairy cows is approximately 22 d, one could suggest that resynchronization protocols should be started between 27 and 31 d after AI. Several studies have evaluated P/AI of cows that started the resynchronization protocol at different intervals after AI. Although strong evidence exists to suggest that starting the resynchronization protocol 19-21 d after AI results in reduced P/AI, other studies have failed to prove that starting the resynchronization protocol at 25-28 d after AI results in different P/AI compared with starting the resynchronization 32-33 d after AI. This can be explained by the fact that only approximately 50% of previously inseminated cows return to estrus between 20-24 d after AI. This is caused by the following factors: 15% of cows are not properly synchronized by timed AI protocols and 10% of cows inseminated in 'estrus' are not actually in estrus; 15% of cows have short luteal phase after first postpartum AI; and, although 55% of cows have a viable embryo at 6 d after AI, 30% of them have embryonic loss between 6 and 28 d after AI. These cows are cows that have altered pattern of return to estrus (< 20 or > 24 d) and would not be in early diestrus if resynchronization protocols were started between 27 and 31 d after AI.

Therefore, it becomes evident that methods to presynchronize the resynchronization protocol have to be developed and implemented to increase P/AI of cows diagnosed not pregnant. Below we discuss a few new methods of presynchronizing the resynchronization protocol.

Silva et al. (2007) evaluated a $PGF_{2\alpha}$-based presynchronization protocol before the start of the resynchronization. Therefore, cows were examined for pregnancy at 32 d after AI. Half of the cows diagnosed not pregnant were submitted to the Ovsynch protocol starting at 33 d after AI and were re-inseminated at 43 d after AI, whereas the other half of the cows were presynchronized with an injection of $PGF_{2\alpha}$ at 34 d after AI, were submitted to the Ovsynch at 46 d after AI and were re-inseminated at 56 d after AI. The hypothesis was that by giving $PGF_{2\alpha}$ at 34 d after AI a large percentage of cows would start a new estrus cycle between 36 and 41 d after AI and consequently start the Ovsynch between d 5 and 10 of the estrous cycle. Pregnancy per AI was increased by presynchronizing cows with $PGF_{2\alpha}$ (35.2 vs. 25.6%). Interestingly, the percentage of cows that ovulated to the first GnRH injection of the Ovsynch was not different (53.9 vs. 49.3%) and, therefore, it was suggested that increases in P/AI may have been the result of improved uterine health because of the additional $PGF_{2\alpha}$ injection and the extra time between inseminations.

Giordano et al. (2009) compared the reproductive performance of cows resynchronized with the Ovsynch or Double-Ovsynch. Cows resynchronized with the Ovsynch received the first GnRH at 32 d after AI and, if diagnosed not pregnant at 39 d after AI, they received the $PGF_{2\alpha}$ at 39 d after AI, the second GnRH at 41 d and TAI at

42 d. Cows resynchronized with the Double-Ovsynch received a GnRH at 22 d after AI, and if diagnosed not pregnant at 29 d after AI they received a $PGF_{2\alpha}$, at d 32 a GnRH, at d 39 a GnRH, at d 46 a $PGF_{2\alpha}$, at d 48 a GnRH, and at d 49 the TAI. Cows resynchronized with the Double-Ovsynch were more likely to ovulate to the first GnRH (85.4 vs. 68.9%) and had greater P/AI (38.5 vs. 30.0%).

A critical issue with both of these protocols is that the interval between AI was increased by 7 d in both protocols, despite the fact that cows in the Double-Ovsynch treatment were examined for pregnancy by ultrasound 10 d earlier. One possible alternative to reduce the interval between AI when presynchronizing with a $PGF_{2\alpha}$ injection is to inseminate cows that display estrus during the 12 d before the start of the Ovsynch protocol.

A good alternative to presynchronize the estrous cycle of cows of unknown pregnancy status is to treat them with GnRH injection. Considering that cows with follicles > 10 mm in diameter are likely to ovulate to a GnRH injection, it is expected that GnRH given at random would result in ovulation in approximately > 40% of lactating dairy cows. Another alternative is to treat cows with a CIDR device during the resynchronization protocol because, as mentioned above, treatment with a CIDR device during a timed AI protocol results in improved synchronization of the estrous cycle. These alternatives were evaluated in a recent study conducted in AZ and CA (Dewey et al., 2009).

In this study, cows in the GGPG treatment were presynchronized with an injection of GnRH given at 32 d after AI and if diagnosed not pregnant on d 39 after AI they were submitted to the Cosynch72 (d 0 GnRH, d 7 $PGF_{2\alpha}$, and d 10 GnRH+TAI). Cows in the CIDR treatment diagnosed not pregnant on d 39 after AI received the Cosynch72 with the addition of a CIDR device between the GnRH and the $PGF_{2\alpha}$, given on d 0 and 7, respectively. The control treatment was the Cosynch72 protocol that started on d 39 after AI if cows were diagnosed not pregnant. As observed in table 3, cows in the GGPG protocol had more CL and were more likely to have P4 > 1 ng/mL on d 0 (day of the first GnRH injection of the Cosynch72), which is likely resultant from ovulations occurring in response to the GnRH injection given 7 d before the start of the Cosynch72. More importantly, greater percentage of GGPG cows ovulated in response to the first GnRH of the Cosynch72 given on d 0, which consequently resulted in greater number of CL on d 7 for GGPG cows. These physiological responses resulted in greater P/AI for GGPG cows (31.2%) than control cows (22.1%). Further, CIDR cows (29.5%) had P/AI similar to that of GGPG cows and greater than control cows.

Table 3. Ovarian responses to the different resynchronization protocols

Items	Treatments			*P* – value
	Control	GGPG	CIDR	
Number of CL on d 0 (±SEM)	1.05 ± 0.11^{a}	1.30 ± 0.11^{b}	1.05 ± 0.11^{a}	< 0.01
Cows with $P_4 \geq 1$ ng/mL on d 0, %	72.0^{a}	$84.7^{b,A}$	66.1^{B}	0.09
Cows that ovulated to the GnRH given on d 0, %	36.6^{a}	48.4^{b}	29.6^{a}	< 0.01
Number of CL on d 7 (±SEM)	1.03 ± 0.14^{a}	1.41 ± 0.14^{b}	0.97 ± 0.13^{a}	< 0.01
Cows with luteolysis between d 0 and 7, %	26.8	28.4	25.3	0.58

[a,b] Values within a row with different superscripts differ ($P < 0.05$).
[A, B] Values within a row with different superscripts differ ($P < 0.10$).

Presynchronizing cows with a GnRH 7 d before the start of the resynchronization and treating cows with a CIDR device during the resynchronization protocol increased P/AI in nearly 8 percentage points without extending the interval between AI.

An interesting finding of this study is the small percentage (~ 25%) of cows that had luteolysis between the first GnRH injection (d 0) and the $PGF_{2\alpha}$ injection (d 7) of the Cosynch. The days when these injections were given would correspond, in cows with estrous cycle length of 22 d, to the beginning of the proestrus of the estrous cycle immediately after AI and early metestrus of the second estrous cycle after AI, respectively. This demonstrates that a large percentage of cows do not have the expected 22 d interval to return to estrus after a previous AI.

Insemination based on signs of estrus continues to be key

Although constantly new timed AI protocols are developed and evaluated, the importance of inseminating cows based on signs of estrus continues to be significant. Only in situations in which facilities or personnel available do not allow for daily observation of estrus and insemination should 100% timed AI protocols be recommended. In herds with adequate estrus-detection accuracy it is expected that P/AI of cows inseminated in estrus to be similar or greater than that of cows inseminated at fixed time. For example, in the study by Chebel et al. (2010a) cows inseminated in estrus after the second injection of $PGF_{2\alpha}$ of the Presynch protocol had P/AI that ranged from 25.3 to 41.2% and in only 2 of the 7 herds P/AI of cows inseminated in estrus was smaller than that of cows inseminated at fixed time (range – 22.4 to 46.9%). In another study (Chebel et al., 2010b), we evaluated the reproductive and economic performances of cows submitted to the Presynch-Ovsynch with or without 'cherry-picking' (insemination of cows that displayed estrus between the second $PGF_{2\alpha}$ of the Presynch and the beginning of the Ovsynch). In this study, P/AI after first AI was not different between cows in the 'cherry-picking' and '100% timed AI' protocols (Table 4).

Table 4. Effect of inseminating cows in estrus following a presynchronization protocol on reproductive and economic performance of lactating Holstein cows.

Items	Treatments		P – value
	Cherry-picking	100% timed AI	
Number	321	318	
Percentage inseminated on estrus at first AI, %	58.9	0.0	< 0.01
Days in milk at first AI (± SEM)	64.7 ± 0.4	74.2 ± 0.5	< 0.01
P/AI at 32 d after first AI, %	33.0	39.6	0.14
P/AI at 60 d after first AI, %	25.3	31.1	0.20
Pregnancy loss from 32 to 60 d after first AI, %	22.9	21.4	0.97
Cost of synchronization for first AI	19.1 ± 0.02	21.0 0.02	< 0.01
Balance[1] after 305 DIM	447.6 ± 28.7	400.0 ± 28.7	0.21

[1]Balance was calculated based on cost of synchronization for first AI, reproductive status at the end of 305 DIM, cost of replacement and salvage value, and income over feed cost.

When we evaluated the rate at which cows became pregnant (Figure 1), it was evident that inseminating 100% of cows at fixed time did not improve reproductive efficiency significantly to pay for the additional cost of first AI synchronization protocols.

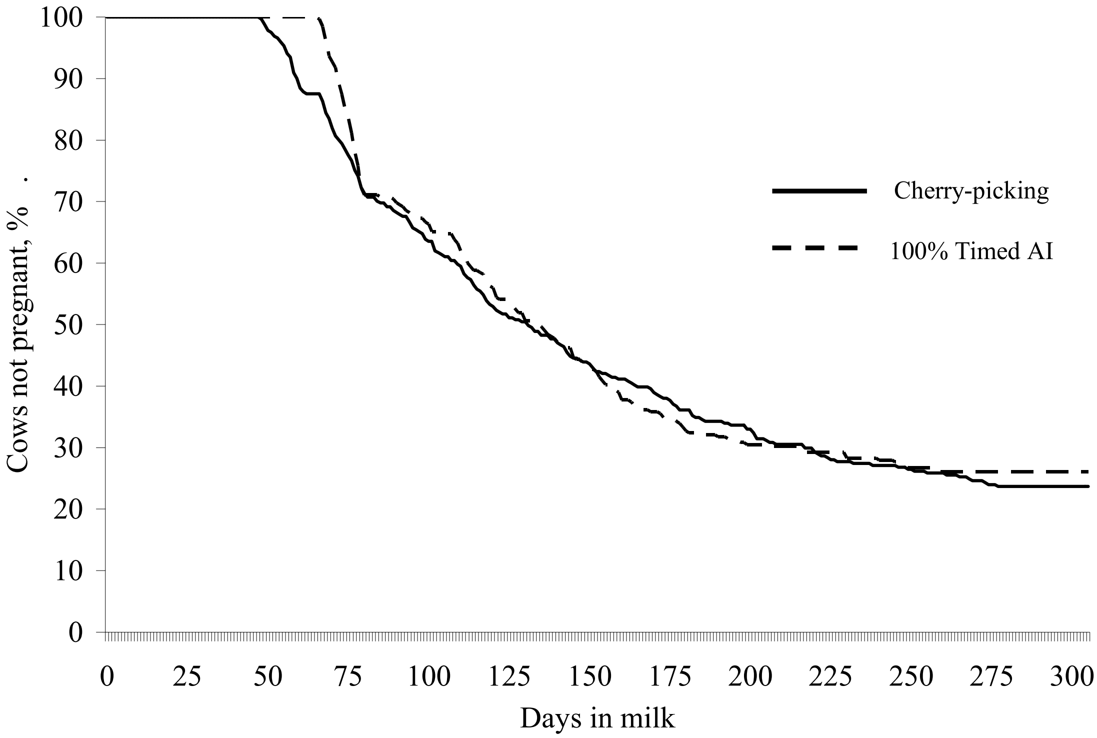

Figure 1. Effect of 'cherry-picking' after the Presynch on interval from parturition to pregnancy (*P* = 0.22). Mean (±SEM) and median interval from parturition to establishment of a new pregnancy: 'Cherry-picking' = 154.0 ± 4.7 and 125 and '100% Timed AI' = 153.4 ± 4.2 and 134.5.

The importance of inseminating cows in estrus becomes even more important when dealing with re-insemination of cows previously inseminated. In the study by Dewey et al. (2008), cows that displayed estrus from 32 to 46 d after previous AI and were re-inseminated had P/AI = 37.1%, whereas cows re-inseminated at fixed time (on d 49 after previous AI) had P/AI = 28.4%. The findings of the study by Dewey et al. (2008) are reinforced by on-farm data. Below (Table 5) are the P/AI of cows re-inseminated based on signs of estrus or at fixed time in four herds across the US (data extracted from Dairy Comp 305).

Table 5. Pregnancy per AI (P/AI) of cows re-inseminated based on signs of estrus or at fixed time

Herd	Lactating dairy cows, no.	3.5% Fat corrected milk (lb/d)	P/AI, %

			Estrus	Timed AI
A	537	80	37	27
B	1,016	90	29	22
C	3,379	82	20	12
D	5,778	70	38	28

Not only is insemination based on signs of estrus expected to improve P/AI in herds with good estrus-detection accuracy, it is also expected to reduce costs associated with timed AI protocols. For example, in a herd with 5,000 lactating dairy cows the cost of first postpartum AI would be approximately $ 50,000/year if the Presynch-Ovsynch is implemented and 100% of cows are inseminated at fixed time (5,000 cows x 5 injections x $ 2/injection). However, if daily estrous detection is implemented and 50% of cows are inseminated in estrus after the second injection of the Presynch and before the start of the Ovsynch expenses with the timed AI protocol for first AI would be $ 35,000/year (2,500 cows x 2 injections x $ 2/injection + 2,500 cows x 5 injections x $ 2/injection) and the additional cost for estrous detection in the first 22 d after the end of the voluntary waiting period (interval from the end Presynch to the end of the Ovsynch protocol) would be approximately $ 4,500 (5,000 cows x 22 d x 15 seconds/cow/d x $ 10/hour). Therefore, according to the scenarios described above, the expected savings by inseminating cows based on signs of estrus would be approximately $ 10,000, which corresponds to the findings described above (Chebel et al., 2010b).

Reproductive management of dairy heifers

Assuring that heifers calve at approximately 24 mo of age is likely to result in greater profitability because they would become part of the lactating herd sooner, as long as they reach the adequate height and weight postpartum for their specific breed (i.e. Holstein ≥ 1,250 lb of BW and 55 inches wither height).

In general, once heifers achieve puberty and weight approximately 60% of their mature body weight, reproductive efficiency should be good. Data from a heifer-grower outfit in Idaho was evaluated (Chebel et al., 2007). In this herd, heifers selected to enter the breeding pen received an injection of $PGF_{2\alpha}$ and were observed for estrus daily and when in estrus were inseminated. Those heifers not inseminated within 11 d after entering the breeding pen were treated for a second time with $PGF_{2\alpha}$. The median interval from entering the breeding pen and first insemination was 6 d and 98.7% of heifers were inseminated within 22 d after entering the breeding pen. Further, the P/AI after first AI was 67.8%, pregnancy loss from 35 to 90 d after AI was 3.4%, and the median interval from entering the breeding pen and establishment of pregnancy was 14 d (Chebel et al., 2007). Heifers are more fertile than lactating dairy cows because they should have healthier reproductive tract (particularly uterus), they have greater circulating concentrations of estradiol and progesterone (better signs of estrus and embryo growth, respectively, among other things), and the length of dominance of the ovulatory follicle is usually smaller (consequence of more follicular waves and greater estradiol).

Therefore, simple estrus-synchronization protocols based on $PGF_{2\alpha}$ are likely to result in good reproductive performance and tend to be cheapest. In a study conducted at a dairy farm in Idaho (Stevenson et al., 2007), we evaluated 3 reproductive programs for dairy heifers: NoSynch – heifers received no treatment and were inseminated at spontaneous estrus; PGFsynch – heifers received an injection of $PGF_{2\alpha}$ at enrollment and were inseminated in estrus, but if not observed in estrus within 14 d heifers received a second injection of $PGF_{2\alpha}$ and continued to be observed for estrus; CIDRSynch – heifers received a CIDR device at enrollment, 7 d later CIDR was removed and heifers received a $PGF_{2\alpha}$ injection, heifers were observed for estrus and were inseminated for 72 h after CIDR removal, and those not inseminated by 72 h after CIDR removal received timed AI+GnRH. At 28 d after enrollment heifers were moved to a bull-pen.

Table 6. Reproductive performance of Holstein heifers submitted to different reproductive protocols.

Items	Treatment			*P*-value
	NoSynch	PGFSynch	CIDRSynch	
Interval from enrollment to first AI, d (± SEM)	12.1 ± 0.6^{a}	8.7 ± 0.5^{b}	9.7 ± 0.5^{b}	< 0.01
Pregnant 32 ± 3 d after AI, % (no.)	63.7 (135)^{a}	67.7 (86/127)^{a}	54.3 (76/140)^{b}	< 0.01
Pregnancy rates after 28 d, % (no.)	58.9 (146)	62.8 (137)	54.3 (140)	> 0.10
Female calves, % (no.)	37.5 (48)	48.0 (50)	45.7 (35)	0.68

^{a,b} Means having different superscript letters within row differ $P < 0.05$.

As observed in Table 6, P/AI was greater for heifers inseminated after spontaneous or $PGF_{2\alpha}$-induced estrus (Stevenson et al., 2007). When we evaluated the costs of the reproductive programs and the economic return (cost per pregnancy generated) from each of them we observed that the cheapest program was the PGFSynch and that the PGFSynch resulted in the smallest cost per pregnancy generated (Table 7).

Table 7. Economic outcomes of different reproductive protocols according to 35 different scenarios[1].

Reproductive Protocols	Breeding program cost		Cost per pregnancy generated	
	Mean (±SEM), $	Range, $	Mean (±SEM), $	Range, $
NoSynch	$29.77 \pm 0.26^{a,A}$	14.38, 40.81	42.01 ± 0.42^{a}	20.29, 57.59
PGFSynch	$29.16 \pm 0.26^{a,B}$	15.90, 38.43	40.02 ± 0.42^{b}	21.81, 52.73

CIDRSynch	34.20 ± 0.26^{b}	21.86, 42.85	49.84 ± 0.42^{c}	31.86, 62.44

[a,b,c,d] Means having different superscript letters within column differ $P < 0.05$.

[A,B] Means having different superscript letters within column tended to differ $P \leq 0.10$.

[1] Evaluation of different scenarios – price assumptions regarding cost of drugs, labor, and rearing heifers were made. As the price of one item varied the price of the other items remained the same.

Other groups have evaluated the use of timed AI protocols in dairy heifers. In general, P/AI of heifers submitted to Ovsynch or a 6d-Cosynch protocol has ranged from 29.5 to 45.5%. These results are somewhat discouraging because of the labor and cost involved with implementing such programs and because in general dairy heifers display good estrus and are very fertile and should have P/AI > 55% when inseminated based on signs of estrus.

In situations in which no labor or facilities exist to allow for daily observation of estrus for at least 30 d, a new timed AI protocol may result in acceptable P/AI (Rabaglino et al., 2010). Heifers submitted to a 5d-CIDRSynch protocol had greater P/AI (> 55%) than the other timed AI protocols tested in heifers thus far. In this protocol heifers received a GnRH injection and a CIDR device, 5 d later the CIDR device was removed and an injection of $PGF_{2\alpha}$ was given, and 3 d later heifers received timed AI concurrent with GnRH. It is interesting to note that one injection of $PGF_{2\alpha}$ given 5 d after the initial GnRH was sufficient to induce luteolysis and acceptable P/AI in heifers, whereas in lactating dairy cows one injection of $PGF_{2\alpha}$ given 5 d after the GnRH was not sufficient (Chebel et al., 2008). Corpora lutea younger than 5 d are not responsive to $PGF_{2\alpha}$; therefore, those cows and heifers that ovulate to the first GnRH injection and formed a new CL are the ones at greater risk of not responding to the $PGF_{2\alpha}$ given 5 d later. The key difference between lactating dairy cows and heifers, however, was the significantly greater percentage of cows (> 63%; Chebel et al., 2008) that ovulated to the first GnRH of the protocol compared with the smaller expected percentage of heifers that would ovulate to the GnRH given at random stages of the estrous cycle (15 to 35%; Stevenson et al., 2007). Because of the expected small percentage of heifers that would ovulate to the first GnRH of the protocol, it is expected that not treating heifers with this first GnRH injection would not impact P/AI significantly in the 5d-CIDRSynch protocol.

Conclusions

Although many alternatives for synchronization of estrus and/or ovulation of lactating dairy cows and even heifers exist, management aspects (i.e. quality of the transition period, health, cow comfort, nutrition, stocking density, maturity, etc.) have to be taken into consideration and carefully evaluated when facing poorer than expected reproductive performance. If one decides to adopt synchronization protocols, appropriate facilities and labor-force must be available, farm personnel should be trained and understand the consequences of poor compliance, compliance-monitoring systems must be designed, and constant monitoring of results have to be part of the program.

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