New Research in Controlled Breeding Programs for Dairy Cattle

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Take Home Messages

- The 7-day Ovsynch protocol improves insemination rate but does not increase conception rate because approximately one third of the animals are not properly synchronized.
- Synchronization and conception rate could be increased by adopting a presynchronization protocol or utilizing a progesterone-releasing intravaginal insert in Ovsynch-treated cows.
- Pregnancy per AI could be increased by replacing the second GnRH of Ovsynch by porcine LH or delaying the second injection of GnRH to 56 hours.
- New timed AI protocols that reduce the period of follicular dominance and extend the period of proestrus would appear to increase pregnancy per AI in dairy heifers and lactating dairy cows.

Introduction

Poor and inadequate estrus detection are the major causes of a low insemination rate and subsequent reduced reproductive efficiency in dairy cattle in Canada, so the use of controlled breeding programs has become an integral part of reproductive management in dairy herds. Protocols that synchronize follicular dynamics, corpus luteum (CL) regression and ovulation, and allow for timed artificial insemination (TAI) result in improved reproductive performance because all animals are inseminated whether they show estrus or not. In this regard, the 7-day Ovsynch program has been adopted in many dairy herds, but it has its limitations because of the effects of stage of follicle development on response to gonadotropin-releasing hormone (GnRH). Hence, new strategies have been developed to maximize pregnancy per AI (P/AI) in lactating dairy cows. In dairy heifers, while GnRH-based protocols (i.e. Ovsynch and Cosynch) have resulted in poor P/AI, the addition of a progesterone-releasing device into these protocols has increased P/AI. Recently, refinement of these protocols has resulted in further improvements in P/AI in heifers. In this manuscript, new research on the development of TAI protocols will be discussed.

▪ The Ovsynch Protocol

The 7-day Ovsynch protocol was developed to allow TAI without the necessity to detect estrus (Pursley et al., 1995). The first injection of GnRH given to synchronize follicular development (if ovulation of an existing dominant follicle occurs) is followed by an injection of prostaglandin F2α (PGF) 7 days later and a second injection of GnRH 48 hours later (for synchronizing ovulation). The TAI is recommended at 16-20 hours after the second GnRH injection. Cosynch is a modified 7-day Ovsynch protocol in which the second GnRH treatment is given concurrently with TAI, thereby reducing one handling event and making this protocol more acceptable to producers.

Lactating dairy cows subjected to Ovsynch have pregnancy rates (defined as the number of pregnant cows over the number of those treated or eligible) similar to those obtained after AI with estrus detection (Pursley et al., 1997; de La Sota et al., 1998). However, conception rate (defined as the number of pregnant cows over the number of those inseminated) is usually lower in Ovsynch-treated cows because ovulation is not adequately synchronized in approximately one third of the animals. We reported that 11% of cows ovulated before TAI, 15% did not respond to treatment with PGF and another 9% did not ovulate after the second treatment with GnRH (Colazo et al., 2009); indicating that synchronization rate [defined as cows that had a regressed CL and ovulated within 24 hours after TAI] was only 68% after Ovsynch. The variability in synchronization with the Ovsynch protocol is mainly due to the stage of the estrous cycle at the time of the first GnRH treatment (Vasconcelos et al., 1999; Moreira et al., 2000). Initiation of the Ovsynch during metestrus (days 1 to 4 of the estrous cycle), when the dominant follicle is not sufficiently developed to ovulate in response to first GnRH, will result in two possible scenarios. Firstly, the dominant follicle regresses and a new follicular wave emerges around the day of PGF administration. Under this scenario, the new dominant follicle will not be sufficiently developed and fails to ovulate after the second injection of GnRH. These cows usually show heat within 2 weeks after the second injection of GnRH. In the second scenario, the dominant follicle does not ovulate after the first GnRH but persists and ovulates after the second GnRH; fertility will be compromised following TAI due to prolonged follicle dominance. Initiation of the Ovsynch during late diestrus (days 13 to 17), when the CL is beginning to regress, will result in ovulation before TAI. Finally, if the Ovsynch protocol begins during proestrus (days 18 to 21), the first injection of GnRH will induce ovulation but the new CL might not respond to the injection of PGF 7 days later. All these observations indicate that cows between days 5 and 12 of the estrous cycle would have a higher probability of responding to the Ovsynch protocol and become pregnant.

▪ Improving the Response to the Ovsynch Protocol

Presynchronization

A program called "Presynch-Ovsynch" was developed by the University of Florida (Table 1). This program involves the application of two PGF injections (14 days apart) and the initiation of Ovsynch 12 days after the second PGF treatment. The goal is to have most of the animals between days 5 and 12 of the estrous cycle when starting the Ovsynch. In two separate studies, the P/AI following TAI was higher in cows treated with the "Presynch-Ovsynch" than in those treated with Ovsynch (49 vs. 37%, Moreira et al., 2001; 47 vs. 37%, El-Zarkouny et al., 2004).

Table 1. Daily injection schedule of a presynchronization program with 2 PGF injections (14 days apart) and initiation of Ovsynch 12 days after the second PGF (Adapted from Moreira et al., 2001).

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Satday
			PGF-PM			
			PGF-PM			
	GnRH-AM					
	PGF-AM		GnRH-PM	AI-AM		

The effect of a different interval between the second PGF of Presynch and initiation of Ovsynch on P/AI in lactating dairy cows has been examined in recent studies. While the first two studies showed that the interval of 12 days between the second PGF and the start of Ovsynch improves P/AI by 10 to 12 percentile points, the dairy industry adopted an interval of 14 days so that treatments are done on the same days of the week. However, new studies have shown that the 12 days interval results in higher percentages of P/AI (Galvão et al., 2007; Stevenson, 2011; Colazo et al., 2013).

Researchers at the University of California, Davis, investigated whether a decrease from 14 to 11 days between the last PGF of Presynch and first GnRH of Heatsynch would improve P/AI to TAI (Galvão et al., 2007). The Davis group used the Heatsynch protocol (a modified Ovsynch that includes 1 mg of estradiol cypionate in lieu of a second GnRH treatment). A third group, which included treatment with GnRH 4 days following the last PGF of the 11-day Presynch was also investigated in this study. A reduction from 14 to 11 days interval between Presynch and initiation of Heatsynch increased the percentage of animals ovulating to the first GnRH (61 versus 45%) and consequently P/AI (41 versus 34%). The increase in the proportion of animals ovulating to the first GnRH of Heatsynch was only observed in those animals that were cycling. The inclusion of a GnRH treatment after the second PGF of the 11-day Presynch increased the percentage of animals with a CL at the time of the first GnRH of the Heatsynch protocol (88 versus 74%) but did not increase the ovulatory response to the first GnRH (62 versus 61%) or P/AI (40 versus 41%).

In another study (Stevenson, 2011), with a variable percentage of acyclic cows (69 to 84%) among groups, a 12-day interval from last PGF of Presynch to initial GnRH resulted in numerically higher P/AI (37%) at 32 days after TAI than 14 (32%) or 10 days (35%), or Control (no presynchronization; 34%). In addition, presynchronization with a CIDR insert for 7 days plus PGF administration at CIDR removal which occurred either 3 or 10 days before initial GnRH did not improve P/AI. Cows in this study were subjected to a Cosynch with TAI 72 hours after PGF.

We recently compared ovarian response (i.e. ovulatory response to first GnRH and overall synchronization rate) and P/AI in 241 cycling lactating dairy cows subjected to the Ovsynch protocol initiated either 9 or 12 days after the second PGF of the Presynch protocol (Colazo et al., 2012). Ovarian responses were determined by plasma progesterone concentration and transrectal ultrasonography. Cows that had a regressed CL (< 1 ng/mL progesterone at TAI) and ovulated within 24 hours after last GnRH treatment were considered as “synchronized”. Percentage of cows that ovulated after first GnRH (62 versus 62%) and overall synchronization rate (75 versus 80%) did not differ between cows subjected to Ovsynch protocol initiated either 9 or 12 days after the second PGF of the Presynch protocol. However, reducing the interval from 12 to 9 days reduced the P/AI at 32 (34 versus 44%) and 60 days (32 versus 43%) after TAI.

Undoubtedly, the increased P/AI in dairy cows that are presynchronized is because the Ovsynch protocol is initiated during a more appropriate stage of the estrous cycle. This results in a reduced proportion of animals with premature ovulations, a greater proportion responding to the first GnRH treatment and perhaps an increased concentration of circulating progesterone before TAI. But we should not rule out the possible beneficial effects that the PGF treatments of Presynch might exert on the uterus, simply by increasing the number of estrous cycles before first service, as repeated progesterone priming of the uterus is known to improve the chances for conception.

Based on studies described here, an interval of 11 or 12 days is more appropriate than 9, 10 or 14 days from second PGF of Presynch to initiation of Ovsynch. While presynchronization with PGF is effective in cyclic cows, it would appear to have no benefits in acyclic cows as they do not have a CL. Other presynchronization protocols that include the use of GnRH or a progesterone-releasing device (CIDR or PRID) for 7 d have been proposed in dairy herds with a high incidence of acyclic cows.

A novel presynchronization protocol has been reported by researchers from the University of Michigan (Bello et al., 2006). The aim of this new protocol is to increase the percentage of animals that respond to the first GnRH injection of the Ovsynch protocol. In this study 137 lactating cows were divided into four groups, one group received no presynchronization treatment before Ovsynch and the other three groups were treated with PGF followed 2 days later with GnRH administered at 4, 5 or 6 days before the Ovsynch. The percentage of animals ovulating following the first GnRH of the Ovsynch protocol was 54, 56, 67 and 85%, respectively. Pregnancy per AI tended to be higher in those animals in which the Ovsynch was initiated at 6 days after presynchronization with PGF and GnRH (G6G; Table 2) than in those which received Ovsynch without presynchronization (50 versus 27%). Recently, this G6G presynchronization protocol has been evaluated in a larger number of animals, resulting in similar P/AI (Ribeiro et al., 2011).

Table 2. Daily injection schedule of a presynchronization program with PGF and GnRH and initiation of Ovsynch 6 days after the GnRH of Presynch (G6G; adapted from Bello et al., 2006).

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	PGF-AM		GnRH-AM			
		GnRH-AM				
		PGF-AM		GnRH-PM	AI-AM	

Most recently, another new presynchronization system has been described (Double Ovsynch; Table 3) resulting in improved P/AI over the Presynch-Ovsynch protocol (50 versus 42%; Souza et al., 2008). Basically, a double Ovsynch protocol involves two Ovsynch protocols following one after the other with the third GnRH treatment being administered 7 days after the second. The improved P/AI are probably the result of two factors; the availability of a dominant follicle that will ovulate following the third GnRH, and the elevated levels of progesterone prior to the administration of PGF (Wiltbank et al., 2012).

Table 3. Daily injection schedule of the Double Ovsynch protocol (Adapted from Souza et al., 2008).

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
					GnRH-AM	
					PGF-AM	
	GnRH-AM					
	GnRH-AM					
	PGF-AM		GnRH-PM	AI-AM		

Management of Acyclic Cows

It is estimated that approximately 10 to 30% of lactating cows are not cycling at day 60 postpartum (Wiltbank et al., 2002). Therefore, recent work in our laboratory examined whether the Ovsynch treatment would be effective in acyclic animals. While most acyclic animals ovulate after the first injection of GnRH, some authors have shown that P/AI is lower than in cows that are cycling at initiation of Ovsynch (22 versus 42%; Thatcher et al., 2002). Our research data from four studies revealed that 64% of 228 acyclic cows ovulated to the first GnRH injection, and the P/AI was significantly greater in those that ovulated compared to cows that did not ovulate (41 versus 11%). Conversely, only 33% of 864 cyclic cows ovulated to the first GnRH but the difference in P/AI between those that ovulated compared to cows that did not ovulate (41 versus 35%) was not as significant. It seems that ovulation in response to first GnRH of Ovsynch is not required for conception in cyclic cows whereas there is a substantial reduction in P/AI in acyclic cows that do not ovulate to initial GnRH treatment. Thus, determining the ovulatory response to first GnRH treatment in acyclic cows by transrectal ultrasonography would be of significant value in predicting the probability of pregnancy.

Although the overall P/AI was lower in cows that were not cycling at the beginning of the GnRH-based program (30 versus 37%), our data further support the notion that GnRH-based protocols facilitate the insemination of acyclic cows regulating their ovarian function and resulting in acceptable P/AI after TAI. Impaired establishment and maintenance of pregnancy has been reported to be responsible for the reduced fertility in acyclic cows (Santos et al., 2009), which may experience as much as 33% embryonic mortality (Colazo et al., 2013). An alternative to further enhance P/AI and decrease pregnancy loss in acyclic animals treated with the Ovsynch protocol could be the use of a progesterone-releasing intravaginal device between first GnRH and PGF administration and/or after TAI.

Supplementing Progesterone before AI

One of the causes of poor fertility in high producing dairy cows is inadequate progesterone, a key hormone of pregnancy. The effects of progesterone secretion on embryonic development and fertility in cattle have been recently reviewed (Inskeep, 2004). A simple and practical approach to supplement progesterone is via an intravaginal insert.

The following two early studies showed that the use of a progesterone-releasing intravaginal device before the second injection of PGF in dairy cows treated with two doses given 14 days apart increases fertility. Folman et al. (1990) reported a quadratic relationship between plasma progesterone 3 days before insemination and conception rate. In this study, more dairy cows became pregnant to AI when they received treatment with a PRID (1.55 g progesterone) for 8 days before the second injection of PGF (66%) than those inseminated after a single treatment with PGF (39%). In another study in New Zealand (Xu et al., 1997), 593 cows were treated with two doses of PGF separated by 14 days followed by estrus detection and AI; a second group of 608 dairy cows were treated with a CIDR device (1.9 g progesterone) 5 days prior to second PGF treatment. Progesterone supplementation before AI increased the expression of estrus (90 versus 83%) and the P/AI (65 versus 60%).

Considering the above studies and knowing that modern high producing dairy cows have lower circulating progesterone than heifers, one could speculate that supplementation with exogenous progesterone during the Ovsynch program would increase P/AI in lactating cows (Figure 1).

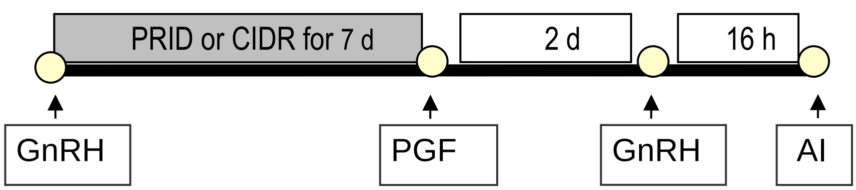

Figure 1. Treatment diagram of a 7-day Ovsynch protocol that includes a progesterone-releasing intravaginal device (PRID or CIDR) between first GnRH and PGF treatment.

In this regard, El-Zarkouny et al. (2004) compared the P/AI in 182 lactating cows treated with the Ovsynch protocol with or without the addition of a CIDR.

Based on serum progesterone concentration, only 44% of the cows were cycling at initiation of treatment. Pregnancy per AI at 29 (59 versus 36%) and 57 days (45 versus 20%) after TAI were greater in cows treated with Ovsynch + CIDR than in those treated with Ovsynch alone. Fertility was particularly enhanced in acyclic animals; P/AI in cows treated with Ovsynch + CIDR was 64% compared with 27% in cows treated with Ovsynch without CIDR. In a second experiment by the same authors, in a dairy herd with 80% of cows cycling at the beginning of Ovsynch, adding a CIDR did not improve P/AI (43 versus 32% in Ovsynch and Ovsynch + CIDR respectively).

The benefit of supplementing progesterone in lactating dairy cows subjected to an Ovsynch protocol was also demonstrated in a large study in Mexico (Melendez et al., 2006). Cows received two treatments of PGF 14 days apart and were inseminated if they showed heat after the second PGF. Those not detected in estrus (n = 1175) were divided into two groups: one group was subjected to the Ovsynch + CIDR protocol and the other group to Ovsynch without CIDR. Although the proportion of acyclic cows was not determined, it could be assumed that a vast majority of the cows used in this study were non-cycling because they were not detected in estrus after second PGF treatment. A comparable percentage of animals (89 and 95%) with plasma progesterone > 1 ng / mL 14 days after TAI suggests that both TAI protocols resulted in similar response and induction of cyclicity. However, P/AI were greater in cows subjected to Ovsynch + CIDR protocol (31 versus 23%).

In yet another study (Stevenson et al., 2006), the use of a CIDR in cows treated with Ovsynch led to a 10-percentile increase in P/AI (50 versus 40%). Neither cyclicity status at initiation of Ovsynch nor its interaction with the administration of a CIDR insert affected the P/AI. However, CIDR treatment increased P/AI in those animals with low progesterone levels at the time of administration of PGF in the Ovsynch protocol (17 to 33% and 19 to 38% in acyclic and cyclic cows, respectively). Results from this study suggest that the addition of a CIDR increases the fertility of cows that may not respond to the first GnRH of Ovsynch (acyclic) or those during late diestrus (cyclic) at initiation of Ovsynch.

Most recently, we (Colazo et al., 2013) determined the efficacy of an intravaginal insert containing 1.55 g of progesterone (PRID, Vetoquinol Canada) given before TAI on ovarian response, plasma progesterone concentrations, P/AI and pregnancy loss in 608 lactating dairy cows. PRID is a progesterone-releasing insert available in the veterinary market and licensed for use in dairy heifers and lactating cows. Cows were subjected to a 7-day Ovsynch with or without presynchronization. Cows given a PRID during the Ovsynch protocol had a higher synchronization rate (78 versus 71%) because fewer cows had premature ovulation (6 versus 11%). Administration of a PRID insert pre-TAI tended to increase plasma progesterone concentrations at PGF treatment (4.4 ± 0.2 and 3.9 ± 0.2 ng/mL for PRID- and no PRID-treated cows) and increased P/AI in cows subjected to Ovsynch without presynchronization (41 versus 25% for PRID- and no PRID-treated cows). However, adding a PRID during the Ovsynch protocol did not significantly increase P/AI in presynchronized cows (42 versus 40% for PRID- and no PRID-treated cows). Although administration of a PRID in cows subjected to Ovsynch without presynchronization improved the overall synchronization rate and subsequent P/AI, the increase in P/AI was also observed when only synchronized Ovsynch cows were considered (52 versus 38% for PRID- and no PRID-treated cows).

Regarding acyclic cows, the inclusion of a PRID increased P/AI from 22 to 35%. However, as discussed above, pregnancy success was highly dependent on ovulatory response to the first GnRH. In this regard, P/AI in cows that did not ovulate to first GnRH was extremely low and did not differ significantly whether (9%) or not (4%) they received a PRID during Ovsynch. Thus, the common recommendation to providing acyclic cows a progesterone supplement via a single intravaginal device before breeding is not fully supported by our findings.

It is important to note that the majority of cows in the Ovsynch group had been previously inseminated and diagnosed as non-pregnant 32 days after AI (Resynchronization). Hence, our finding has a practical implication that supplementing with exogenous progesterone via PRID during resynchronization starting on day 32 after the previous AI will enhance subsequent P/AI. Similarly, the incorporation of a CIDR insert to dairy cows subjected to a 5-day TAI protocol during resynchronization starting on day 34 after the previous AI improved P/AI (51 versus 43%; Bissinotto et al., 2010).

On the contrary, cyclic cows that were presynchronized with PGF would have adequate levels of plasma progesterone at initiation of Ovsynch. Presynchronization also increases the probability of ovulation after the first injection of GnRH (46 versus 28% in our study), which would result in an additional CL and even more circulating progesterone. Hence, we infer that progesterone supplementation before TAI would not benefit cows subjected to a Presynch-Ovsynch protocol because their ovarian synchrony is already optimized, leaving little room for further improvement.

In agreement with our findings, other authors have reported no benefit in supplementing progesterone pre-TAI in presynchronized lactating dairy cows. In a second experiment by Zarkouny et al. (2004), in a dairy herd that utilizes presynchronization with PGF and with approximately 80% cyclic cows at the beginning of Ovsynch, adding a CIDR did not improve P/AI at 29 days after TAI (45 versus 48% in Ovsynch and Ovsynch + CIDR respectively). Similar results were reported by Galvão et al. (2004); inclusion of a CIDR did not improve P/AI to TAI (36 versus 39%) in lactating dairy cows (> 80% cycling) presynchronized with PGF and treated with the Heatsynch protocol.

Improving the Fertility of TAI Protocols

Supplementing Progesterone after AI

Studies in which progesterone has been supplemented during the post-AI period have yielded inconsistent results depending on timing of initiation of treatment. A review of several studies revealed an overall increase in P/AI of 10% when progesterone supplementation commenced during early luteal phase (Mann and Lamming, 1999). Most of these studies utilized lactating dairy cows that may be considered low or medium producing cows nowadays. With that in mind, administration of a PRID for 7 days, starting 4.5 days post-TAI has been evaluated by our group as a strategy to improve P/AI and reduce pregnancy losses in high producing dairy cows (Colazo et al., 2013). We observed a positive linear association between the plasma concentration of progesterone at PRID removal post-TAI and predicted probability of pregnancy at 32 days after TAI. In other words, cows with greater plasma progesterone concentrations at 11.5 days after TAI were more likely to become pregnant. Although supplementing progesterone post-TAI via a PRID increased plasma progesterone concentrations by an average of 0.9 ng/mL, this increase was insufficient to enhance P/AI in our study.

Our finding is in agreement with more recent reports. Arndt et al. (2009) did not observe an improvement in P/AI in cows treated from days 4 to18 post-AI with a CIDR containing 1.38 g of progesterone. Similarly, Scott et al. (2009) found that the probability of pregnancy to AI was not significantly different in cows receiving a PRID (36%) between 5 and 12 days post-AI compared to those given a placebo intravaginal insert (38%). In contrast, Larson et al. (2007) reported that P/AI tended to increase in cows given a once-used CIDR insert, which originally contained 1.9 g of progesterone, from day 3.5 to day 10 post-AI. It seems that progesterone supplementation post-AI via commercially available intravaginal inserts would have either no effect or only a modest effect on pregnancy outcome in high producing dairy cows.

Pregnancy losses were reduced (6 versus 11%) between 32 and 60 days of gestation in cows given a PRID for 7 days, starting 4.5 days post-AI. The reduction in pregnancy loss was particularly substantial in acyclic cows (6 versus 33%). Based on our findings, we infer that an increase in circulating progesterone during early embryonic development (3.5 to 10.5 days post-ovulation) plays an important role in maintenance of pregnancy in acyclic cows subjected to timed-AI protocols. However, this finding should not be over-interpreted because only a limited number of acyclic cows (n=107) were evaluated in our study.

Reducing the Length of Follicular Dominance and Extending the Period of Proestrus

Researchers at the Ohio State University proposed that reducing the interval from initial GnRH to PGF from 7 to 5 days of Cosynch + CIDR protocol and extending the interval from PGF treatment to second GnRH treatment (proestrus) will result in improved P/AI (Figure 2). Results with this modification were first reported in beef cows. Bridges et al. (2008) showed that a 5-day Cosynch + CIDR protocol with TAI at 72 hours after CIDR removal resulted in greater P/AI compared to a 7-day Cosynch + CIDR with TAI at 60 hours after CIDR removal. It is important to note that two applications of PGF 6 to 24 hours apart were needed for adequate CL regression in a 5-day Cosynch + CIDR protocol. Findings from this and other studies with beef cattle encouraged researchers to examine the 5-day TAI protocol in lactating dairy cows.

With the objective to determine the effect of reducing the period of follicle dominance on P/AI, Santos et al. (2010) compared a 7- versus a 5-day Cosynch with TAI at 72 hours in 933 presynchronized dairy cows. As previously reported, two treatments of PGF were needed to induce luteolysis in cows subjected to a 5-day Cosynch protocol. Pregnancy per AI was greater for 5-day Cosynch (38%) than for 7-day Cosynch (31%).

More recently, we compared response to PGF, synchrony of ovulation, and P/AI in a 5- versus a 7-day Ovsynch + PRID protocol with TAI at 72 hours in 500 lactating dairy cows (Colazo et al. unpublished). We also examined whether the initial GnRH was necessary to achieve acceptable P/AI . The percentage of cows ovulating before TAI was greater for 7-day Ovsynch + PRID (17%) than for 5-day Ovsynch + PRID (9%). Conversely, the percentage of cows that did not respond to PGF was greater for 5-d Ovsynch + PRID plus 2 PGF (10%) than for 7-day Ovsynch + PRID (1%). The overall P/AI at 32 days did not differ between groups but a two-way interaction of treatment by number of inseminations on P/TAI was detected. At first insemination, cows subjected to a 7-day protocol had greater P/AI (42%) compared to cows subjected to a 5-day protocol (28%). However, at subsequent inseminations (Resynchronization), the 5-day Ovsynch + PRID protocol resulted in greater P/AI (45%) than the 7-day Ovsynch + PRID protocol (30%). Overall, P/AI did not differ whether or not cows received initial GnRH at PRID insertion (36 versus 38%), but cows that did not receive initial GnRH experienced more pregnancy losses between 32 and 60 days after TAI (15 versus 8%).

It seems that the 5-day TAI protocol plus a progesterone-releasing insert, which reduces the duration of follicular dominance and increases the length of the proestrus period, can enhance fertility in lactating dairy cows. The high

P/AI achieved with this protocol, in particular during resynchronization, is very encouraging.

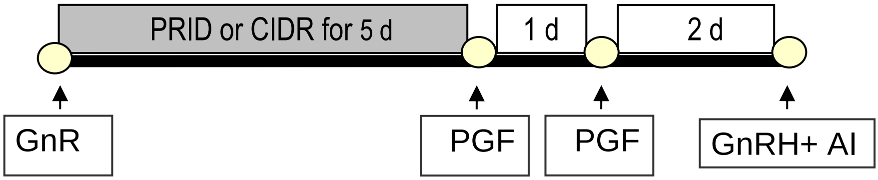

Figure 2. Treatment diagram of the new 5-day Cosynch protocol for lactating dairy cows.

Improving the Periovulatory Hormonal Environment

Although ovulation occurs in approximately 90% of treated animals following the second GnRH, those ovulations could result in the formation of a less functional CL compared to spontaneously occurring ovulations. Several authors have reported a high proportion of re-inseminations within two weeks after TAI in animals that had been treated with Ovsynch. Administration of 100 µg of GnRH (recommended dose) induces a release of LH with a duration of 4-5 hours which is far shorter than the duration of endogenous LH during natural estrus (Chenault et al., 1975; Colazo et al., 2008). Therefore, researchers have studied different alternatives for replacing the second GnRH treatment in an Ovsynch protocol.

One alternative to synchronize ovulation in a TAI program is the ability of estradiol to induce the release of GnRH by the hypothalamus when circulating progesterone is low. In addition, treatment with estradiol increases the expression of the GnRH receptors in the pituitary and thereby contributes to an increased release of LH. Indeed, estradiol-induced LH release lasts about 10 hours (Bó et al., 1994), which is comparable to the duration of the release of endogenous LH (Chenault et al., 1975). In particular, 1 mg of estradiol cypionate (ECP) has been used in lieu of the second GnRH treatment. The protocol is called "Heatsynch", because a higher percentage of animals show heat with this protocol than with Ovsynch. However, ECP has been withdrawn from the veterinary market in North America.

The low plasma estradiol concentrations before AI (i.e. low expression of estrus) in high producing dairy cows, especially in those treated with Ovsynch, is of concern among investigators. Although the expression of estrus with the Heatsynch protocol is increased, the P/AI has not been improved in all cases. Therefore, other authors have investigated the incorporation of 1 mg of estradiol-17β 8 hours before the second injection of

GnRH in cows subjected to Ovsynch (Souza et al., 2007). There was an increase in the expression of estrus among cows treated with estradiol (80 versus 44%) but P/AI was only improved in cows with BCS < 2.5 (40 versus 28% for treated and non-treated with estradiol).

With the idea of evaluating treatments that increase the concentration of plasma LH before ovulation, our group recently investigated the use of porcine LH (pLH; Lutropin-V) to replace the first and/or second injection of GnRH in dairy cows subjected to an Ovsynch (Colazo et al., 2009). A total of 605 cows at three different locations were enrolled in a 2 X 2 experimental design. Cows received 100 µg of GnRH or 25 mg of pLH at the beginning or end of a standard 7-day Ovsynch. A greater percentage of cows ovulated after the first treatment with GnRH versus pLH (61 versus 44%), but this had no effect on P/AI. Those cows that received GnRH at intiation of Ovsynch and pLH instead of a second GnRH injection had greater P/AI than cows treated with a standard Ovsynch utilizing two injections of GnRH (42 versus 28%). Contrary to our expectations, pLH treated cows had similar plasma progesterone post-TAI than cows treated with GnRH, indicating that a mechanism other than post-ovulation luteal function was responsible for the increased fertility in this study.

Another alternative to improve follicle maturity and periovulatory concentrations of estradiol is the use of equine chorionic gonadotropin (eCG). We have examined the effects of eCG at the time of PGF injection on preovulatory follicle size and fertility in lactating beef cows subjected to a Cosynch protocol (Small et al., 2009). Treatment with 400 IU of eCG increased P/AI in primiparous cows that were not presynchronized, confirming that eCG may be useful in Ovsynch or Cosynch protocols in cows that were early postpartum or under nutritional stress. Additional research is needed to evaluate the supplementation with eCG in lactating dairy cows subjected to GnRH-based protocols in Canada.

Optimizing the Interval from PGF to Second Gnrh and Timing of Insemination Following Gnrh Treatment

A common question among dairy producers is the optimal interval between PGF and the second GnRH treatment, and the optimal time to AI cows that have been synchronized with the Ovsynch protocol.

Pursley et al. (1998) designed a study with 733 lactating dairy cows to investigate the optimal time of AI when using the Ovsynch protocol. Cows were inseminated at 0, 8, 16, 24 or 32 hours after the second GnRH treatment, which was given 48 hours after PGF. All cows ovulated between 24 and 32 hours after second GnRH. Pregnancy per AI at 32 days increased from 0 to 16 hours (37 versus 45%) with a subsequent decrease at 24 hours (41%) and a further decrease at 32 hours (32%). This study suggests that the optimal time to inseminate cows subjected to the Ovsynch protocol is 16 hours after the second injection of GnRH administered 48 hours after PGF. However, from a practical point of view, a 16 hours interval is difficult for dairy herds milked twice daily. Therefore, many dairy producers inseminate cows at either 0 or 24 hours after administration of the second GnRH.

In a study at Kansas State University (Portaluppi and Stevenson, 2005), 665 dairy cows subjected to the Presynch-Ovsynch protocol were divided into three groups: one group was inseminated at the time of the second GnRH (48 hours after PGF; Cosynch 48), a second group was inseminated 24 hours after the second GnRH (72 hours after the PGF; Ovsynch) and a third group was inseminated concurrent with second GnRH given 72 hours after PGF (Cosynch 72). Pregnancy diagnosis was performed by rectal palpation 40-41 days after TAI; P/AI were 23, 24 and 31% for groups one, two and three, respectively. These results suggest that in cows treated with Presynch-Ovsynch protocol inseminations at 0 or 24 hours after second GnRH would result in fewer pregnancies than inseminations concurrent with second GnRH at 72 h after PGF. In the latter group, approximately two-third of the animals showed estrus at 72 hours after injection of PGF, indicating that in presynchronized cows delaying the time of second GnRH may optimize preovulatory follicle maturity.

In a preliminary study, Peters et al. (1999) examined the effect of delaying the second GnRH on synchrony of ovulation in dairy cows subjected to a 7-day Ovsynch. In the first study, from a total of 11 cows treated with the second GnRH between 56 and 60 hours after administration of PGF, 10 ovulated within 24 hours (between 72 and 96 hours after PGF). This observation was confirmed later in a subsequent study. Although this early study showed that cows subjected to Ovsynch will have a more synchronous ovulation if the administration of second GnRH is between 56 to 60 hours after PGF, the effect of this approach on P/AI in lactating dairy cows was not investigated until recently (Brusveen et al., 2008). A total of 927 dairy cows (1507 services) were assigned to receive the second GnRH injection in the Ovsynch protocol at 48, 56 or 72 hours after PGF. Cows were TAI at the time of administration of GnRH (48- and 72-hour groups) or 16 hours later (56-hour group). All cows were initially treated with two injections of PGF and the Ovsynch protocol began at 11 days after the second PGF. Pregnancy diagnosis was performed by transrectal ultrasonography between 31 and 33 days post-TAI. Open cows were synchronized again (resynchronization) at that time with one of the three protocols without presynchronization. Overall, P/AI was lower in cows receiving GnRH at 48 (29) or 72 hours (25%) after PGF and inseminated concurrently than in those given GnRH at 56 hours and inseminated 16 hours later (39%). In the latter group, P/AI was 45% in cows that had been presynchronized with PGF.

As discussed above, timing of second GnRH and interval to insemination seems to affect P/AI in lactating dairy cows subjected to a 7-day Ovsynch. However, timing of insemination following second GnRH might not affect P/AI of cows subjected to a 5-day TAI program. In this regard, GnRH treatment concurrent with TAI (46%) did not affect P/AI of lactating dairy cows subjected to a 5-day TAI protocol compared with the administration of GnRH 16 hours before AI (56 hours after first PGF treatment; 46%; Bissinotto et al., 2010).

▪ Controlled Breeding Program for Dairy Heifers.

The Ovsynch and Cosynch protocols have resulted in poor P/AI in dairy (Schmitt et al., 1996; Pursley et al., 1997) and beef (Colazo et al., 2004) heifers, mainly because approximately 20% of heifers show premature estrus (Colazo et al., 2004). The addition of an intravaginal progesterone insert (CIDR), into the 7-day Cosynch protocol (between first GnRH and PGF treatment) prevented early ovulations and improved P/AI (39 versus 68%) in beef heifers (Martinez et al., 2002).

Reduced ovulatory follicle size, lower plasma estradiol concentrations and decreased luteal function have also been speculated as the reason of reduced fertility in GnRH-treated heifers. In an attempt to improve the quality of ovulatory follicle and further increase P/AI, modifications of the GnRH-based protocols have been done. In dairy heifers subjected to a 8-day Ovsynch + CIDR protocol, administration of PGF 24 hours before CIDR removal resulted in increased P/AI compared to PGF given at CIDR removal (62 versus 54%) or a conventional 7-day Ovsynch + CIDR protocol (56%; Ambrose et al., 2008). However, the former protocol required that heifers be handled 5 times, which would be less acceptable to producers.

Reducing the period of follicular dominance and extending the period of proestrus has also been shown to improve P/AI in dairy heifers. Rabaglino and coworkers (2010) tested the 5-day Cosynch + CIDR protocol with a single or two PGF treatments in dairy heifers and attained 53 to 59% P/AI after TAI in two separate experiments. It was also concluded that a single PGF treatment is enough to induce luteolysis in dairy heifers subjected to a 5-day Cosynch + CIDR protocol.

Recently, we compared a 5- versus a 7-day Cosynch + PRID protocol in dairy heifers given a single dose of PGF at PRID removal (Colazo and Ambrose, 2011). Pregnancy per AI did not differ between 5- (59%) and 7-day Cosynch (58%) + PRID. Hence, our study does not suggest any benefit of one controlled breeding protocol over the other in dairy heifers. Interestingly, ovulation response to first GnRH treatment was only 25% in heifers subjected to the 5-day Cosynch + PRID protocol, and a larger proportion of heifers that did not ovulate became pregnant (65 versus 45%). Apparently, the 5-day protocol avoids aged follicles.

Given that GnRH induces ovulation in a very small percentage of heifers, we have questioned whether GnRH administration is required at the beginning of the 5-day Cosynch + PRID protocol. Therefore, in another experiment we determined whether the initial GnRH was necessary to achieve acceptable P/AI in dairy heifers subjected to a 5-day Cosynch + PRID protocol (Colazo and Ambrose, 2011). Pregnancy per AI did not differ whether or not heifers received GnRH at PRID insertion (68 versus 71%). Based on this finding, we concluded that initial injection of GnRH at PRID insertion in a 5-day Cosynch + PRID protocol was not essential to achieve acceptable P/AI in dairy heifers.

In an ongoing study, we are investigating whether the modified 5-day Cosynch + PRID protocol (without the initial GnRH; Fig. 3) is suitable for the use of sexed semen in dairy heifers. In a two by two experimental design, cycling heifers are divided to receive either 2 PGF treatments 14 days apart with insemination approximately 12 hours after estrus detection or the modified 5-day Cosynch + PRID protocol with TAI at 72 hours after PRID removal. Heifers are inseminated with either conventional or sexed semen from two sires per replicate. Preliminary results show that overall P/AI is 10 percentile higher in heifers inseminated after estrus detection than in those subjected to TAI after the modified 5-day Cosynch + PRID protocol. Nevertheless, TAI heifers had acceptable P/AI of 59 and 69% following insemination with either sexed or conventional semen, respectively.

Evidently, the modified 5-day Cosynch + PRID protocol with TAI at 72 hours after PRID removal can potentially improve P/AI in dairy heifers with reduced (3 versus 4 times) animal handling.

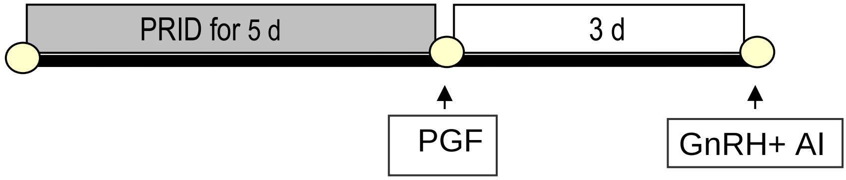

Figure 3. Treatment diagram of the modified 5-day Cosynch protocol for dairy heifers.

▪ Conclusions

The 7-day Ovsynch protocol is an important tool that has facilitated the use of TAI in dairy herds in North America, but it has its limitations. Presynchronization with PGF increases P/AI by 10 to 12%; intervals of 11 or 12 days between second PGF of Presynch and initiation of Ovsynch are more appropriate than intervals of 9, 10 or 14 days. While presynchronization with

PGF is effective in cyclic cows, other approaches which include GnRH (e.g. G6G and Double Ovsynch) could potentially increase P/AI in acyclic cows. Progesterone supplementation via an intravaginal device between the first GnRH and PGF administration increased fertility especially in resynchronized cows. Progesterone supplementation post-TAI reduced pregnancy loss, mainly in acyclic cows. In cows subjected to a Presynch-Ovsynch, administration of second GnRH at 56 hours after PGF and TAI 16 hours later has resulted in more P/AI. A 5-day TAI protocol plus a progesterone-releasing device can potentially enhance fertility in cows and facilitate the use of sexed semen in heifers.

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