Controlled Breeding Programs for Heifers

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Introduction

Improving the reproductive efficiency of replacement heifers is important, as heifers represent a large cost to the farm before they become productive, i.e. calve. Fixed time artificial insemination (FTAI) protocols have been shown to increase the insemination risk and reduce the interval between introduction to the breeding pen or beginning of the breeding season and first AI compared to AI after detected estrus on an estrus synchronization protocol (Silva et al., 2015). However, the gonadotropin releasing hormone (GnRH)-based FTAI protocols (i.e. Ovsynch and Cosynch) used on lactating cows have resulted in poor pregnancy per AI (P/AI) in heifers, mainly due to premature estrus (Colazo et al., 2004). The addition of an intravaginal device containing progesterone (IDP4; e.g. CIDR, Cue-Mate® or PRID) between first GnRH and prostaglandin $F_{2\alpha}$ (PGF) treatment of the 7-d Cosynch protocol prevented early ovulations and improved P/AI (68 vs. 39%) in beef heifers (Martínez et al., 2002). Studies have also looked at reducing the protocol to 5 days (Table 1), which brings both pros and cons compared to the 7-d Cosynch. The 5-d protocol reduces the period of follicular dominance and extends the proestrus period, which has been shown to increase P/AI in beef cows (Bridges et al., 2008).

Modifying the 5-d Cosynch Protocol

In the traditional 5-d Cosynch protocol the initial GnRH is given to induce ovulation and the start of a new follicular wave, resulting in a new corpus luteum (CL) by the time the IDP4 is removed 5 days later. The new CL formed after the initial GnRH may not respond to a single PGF treatment given 5 days later and thus require a 2nd PGF to ensure luteolysis compared to the 7-d protocol. However, the initial GnRH is only really effective at inducing ovulation when given in the presence of a dominant follicle ≥10 mm in diameter.

Therefore, the first modification to be addressed in the 5-d Cosynch protocol is the need for a 2nd PGF treatment. A study from our group compared the 5 and 7-d Cosynch protocols in dairy heifers, using only 1 PGF in both, and we found no difference in P/AI (59 vs. 58%; Colazo and Ambrose, 2011). Despite using only 1 injection of PGF in the 5-d protocol luteal regression occurred in 96.4% of heifers. This indicates that the 2nd PGF may not be necessary and removing it would reduce the amount of handling required and cost of the synchronization protocol. In a study comparing the use of 1 or 2 PGF injections in both dairy and beef heifers, the authors found no difference in P/AI, supporting the theory that the 2nd PGF is not needed in heifers (Kasimanickam et al., 2014). However, Lima et al. (2013) reported that the optimal 5-d protocol requires 2 PGF injections, as the luteolysis of newly formed CLs increases with 2nd PGF, although it has no effect on older CLs. The authors observed that 34% of cows ovulated to the initial GnRH injection, resulting in a new CL at the time of IDP4 removal, indicating that the 2nd PGF is not necessary in 66% of animals. Providing 2 injections of PGF does not appear to be necessary for improved fertility in most heifers and also leads into another modification of the 5-d Cosynch protocol.

In our study, Colazo and Ambrose (2011), we found that only 25% of heifers ovulated to the initial GnRH and that a larger proportion of heifers that did not ovulate to the first GnRH became pregnant (65 vs. 45%). These results indicate that the initial GnRH may not be necessary and removing this step would negate the need for the 2nd PGF and also reduce cost of the protocol (Table 1). Furthermore, our 2011 study compared the 5-d protocol with (+GnRH) or without (-GnRH) the initial GnRH and we observed no difference in P/AI (68 vs. 71%). Our group also conducted a comparison study on beef heifers (Lopez-Helguera et al., 2015) and again we found no difference between the +GnRH and –GnRH protocols on P/AI (53 vs. 48%). Similar results were also observed on beef heifers by Cruppe et al. (2014) comparing the + and –GnRH treatments (51 vs. 55%). However, previous studies in this area have found conflicting results. In the study conducted by Lima et al. (2013) the +GnRH protocol using 2 PGF increased fertility in dairy heifers compared to the –GnRH with 1 PGF with a P/AI of 62 vs. 53%. While Kasimanickam et al. (2014) found no effect of the GnRH on the fertility of dairy heifers (51 vs. 54%), the authors observed an increase in P/AI when beef heifers were on the +GnRH with 1 PGF protocol compared to –GnRH (60 vs. 50%). This study reported that the initial GnRH increased P/AI when the follicle was ≥ 8 mm compared to –GnRH (70 vs. 47%), which suggests that a dominant follicle not eliminated at IDP4 insertion, will result in an aged oocyte and reduced fertility in beef heifers. While the results on fertility for each 5-d Cosynch protocol is variable, veterinarians and producers should also consider the labor and cost required to select an optimal method.

Table 1. Daily injection schedule of the 7-d Cosynch, traditional 5-d Cosynch (+GnRH & 2 PGF) and modified 5-d Cosynch (-GnRH & 1PGF) protocols.

Protocol	Tues	Wed	Thur	Fri	Sat	Sun	Mon	Tues	Wed	Thurs
7-d Cosynch	(AM) GnRH +IDP4							(AM) PGF -IDP4		(PM) GnRH FTAI
5-d +GnRH **2 PGF**		(AM) GnRH +IDP4					(AM) PGF -IDP4	(AM) PGF		(AM) GnRH FTAI
5-d **-GnRH**		(AM) +IDP4					(AM) PGF -IDP4			(AM) GnRH FTAI

+IDP4: insertion of the intravaginal device containing progesterone.
-IDP4: removal of the intravaginal device containing progesterone.
GnRH: gonadotropin releasing hormone.
PGF: prostaglandin $F_{2\alpha}$.
FTAI: fixed time artificial insemination.

Managing Acyclic Heifers

Non-cyclicity is more often seen in beef heifers and pasture based dairy heifers. Heifers that are acyclic at the beginning of the breeding season have lower fertility and are more likely to be open at the end of the breeding season. Results from several studies with more than 4,000 FTAI in beef heifers subjected to a 7-d estradiol plus progesterone protocol shown that fertility to initial AI is 10 to 15% lower in acyclic compared to cyclic animals (Colazo, unpublished).

In a more recent study we found that 21% (226/1062) of beef heifers were acyclic, which resulted in a lower P/AI than cyclic heifers (41 vs. 59%; Lopez-Helguera et al., 2015). The proportion of acyclic heifers varied from 8 to 27% among the three herds. In this study, we compared the +GnRH and – GnRH with 1 PGF protocols and although there was no effect on the fertility of cyclic heifers, the +GnRH protocol increased P/AI in acyclic heifers compared to the –GnRH (50 vs. 35%). We also found that 1/3 of the acyclic heifers remained acyclic after the synchronization and AI, and they were more likely to lose their pregnancy (6 vs. 3%). Therefore, at the end of the breeding season (after 2 rounds of AI and natural service for 45 more days) more acyclic heifers remained open (7 vs. 3%). These results identify a couple of approaches that veterinarians and producers could use to increase fertility in acyclic heifers. First, veterinarians could target acyclic heifers by scanning the ovaries prior to the FTAI protocol and assign non-cycling heifers to the +GnRH protocol. Second, producers could assign all heifers to the +GnRH protocol, which would positively affect acyclic heifers and have no negative effect on cyclic heifers. The first option will increase veterinary costs but will decrease the amount of GnRH used and can also help identify other problem heifers, which further improves the overall fertility of the protocol. Our results also question whether acyclic heifers should be synchronized and FTAI. Although the 5-d protocol will induce some acyclic heifers to cycle and improve their chances of getting pregnant, the lower fertility and the higher risk of losing the pregnancy would negatively affect the cost-effectiveness of implementing the synchronization program.

Using Sex-Selected Semen

The use of sex-selected semen on heifers helps to reduce the risk of dystocia in first-time calving as well as increase the rate of genetic improvement in the herd. However, conception rates using sex-selected semen are lower than conventional semen due to lower sperm concentration, damage to the sperm during the selection process, and reduced duration of optimal fertility in the female reproductive tract (Seidel, 2014). As the duration of optimal fertility is reduced with the use of sex-selected semen it is essential that heifers are ovulating close to the time of FTAI. Although our 2011 study did not look at different types of semen, we did observe that 75% of heifers in the –GnRH protocol ovulated within 24 hours of FTAI, which was greater than in the +GnRH treatment and could benefit sex-selected semen. Macmillan et al. (2017) compared the + and –GnRH protocols on both conventional and sex-selected semen and although the results were not statistically significant there was a numerical increase in P/AI in sex-selected semen when the –GnRH protocol was used compared to the +GnRH (60 vs. 50%). These results suggest that –GnRH 5-d Cosynch protocol is more beneficial in heifers when using sex-selected semen as the timing of AI coincides more closely with ovulation. There is also evidence suggesting estrus detection may have a role to play in increasing heifer fertility to sex-selected semen.

Estrus Detection

Although estrus detection (ED) is not required in FTAI protocols, including a short period of estrus detection between IDP4 removal and AI may increase reproductive efficiency. We have previously reported that ovulations within 24 h after AI results in higher P/AI (Colazo and Ambrose, 2011) and that the interval from AI to ovulation was shorter and less variable when heifers were inseminated on ED compared with FTAI (16 ± 0.7 vs. 21 ± 1.3 h; Colazo and Mapletoft, 2017). In our recent study, Macmillan et al. (2017) compared the + and –GnRH protocols but also observed heifers for estrus on d 1.5 (PM) and 2 (AM) after IDP4 removal (Day 0 – AM); if heifers were in estrus they were bred on d 2 PM after IDP4 removal as opposed to d 3 AM. We found that heifers bred on ED tended to have an increased P/AI (68 vs. 58%) compared to FTAI and that more heifers were bred based on ED in the –GnRH protocol (22 vs. 14%) compared to the +GnRH protocol. This could explain why the P/AI tended to be higher in the –GnRH in comparison to the +GnRH protocol (66 vs. 57%) and why the P/AI was numerically higher for sex-selected semen in the –GnRH protocol, as mentioned earlier.

In a study conducted on beef heifers (Colazo et al., 2016), we used Estrotect™ ED patches to detect estrus between IDP4 removal and FTAI, although all cows were bred by FTAI on d 3 after IDP4 removal. Patches were scored as 0 no color change, 1 ≤50 % color change, 2 >50 % color change and 3 missing. Overall, heifers with a score of 2 had increased P/AI (65%) compared to heifers with a score of 0 (43%) or 1 (47%) and heifers scored 3 had an intermediate P/AI (59%), which indicates patches may have been missing due to estrus behaviour. The effect of the ED patch score on fertility was also dependent on type of semen used. With conventional semen, P/AI was higher with heifers scored 2 compared to those scored 1 (70 vs. 49%). With sex-selected semen, P/AI was higher with patches scored 2 (60%) and 3 (58%) compared to both 0 (36%) and 1 (37%). A practical use for these patches when using different semen types would be to use the sex-selected semen only on heifers with patches scored 2 or 3 and using conventional semen on the rest of the heifers to increase overall pregnancy rate and effectiveness of sex-selected semen. These results also suggest that the patches could be used to decide the timing of AI. In this study 19% (170/890) of heifers had patches scored 0, which suggests they may benefit from a delayed AI to allow them to come into estrus and ovulate closer to insemination. We are currently investigating the use of Estrotect patches to identify heifers for an early (56 h after IDP4 removal) or late (80 h after IDP4 removal) AI based on ED at 56 h after IDP4 removal. Delaying estrus may increase the P/AI of heifers that would have scored 0 or 1 at 72 h, thus increasing the overall herd pregnancy rate. Breeding heifers based on ED, whether before or after the scheduled FTAI, removes the use of GnRH concurrent with AI, thus reducing overall cost of the protocol as well. Despite an increase in labor and/or vet costs, selecting FTAI protocols that increase the occurrence of naturally occurring estrus may improve pregnancy rates, particularly when using sex-selected semen.

Resynchronization using FTAI

Decreasing the interval between AI and resynchronization is projected to reduce the time to pregnancy, and thus increase overall pregnancy rate. However, when Sinedino et al. (2014) compared an early (commenced 28-d after first AI) and late (commenced 46-d after first AI) resynchronization more cows in the late group were inseminated on detected estrus before the assigned resynch protocol, which increased P/AI and resulted in a similar rate of pregnancy in both groups. In our study with dairy heifers (Macmillan et al., 2017) we compared 2 resynch protocols with interbreeding intervals of either 35- or 42-d with heifers on either the + or – GnRH 5-d Cosynch protocols for each breeding. We achieved a cumulative pregnancy rate of 81% after 2 breedings and 97% after 4. There was no difference in P/AI between the 35- and 42-d resynch groups (54 vs. 60%), however less heifers in the 35-d group were bred based on detected estrus compared to the 42-d group (7.8 vs. 23%). The 42-d resynch likely increased the number of heifers bred on ED as the protocol ended when heifers were expected to show natural estrus, 2 estrous cycles after the initial AI.

An inter-breeding interval of even 35 days would be considered a long interval in systems with a shortened breeding season. However, if the first AI is performed the first day of the breeding season, and second AI 35 days later, then it is possible to achieve an overall pregnancy rate of 80% after two rounds of AI in a 35-d breeding period. Furthermore, if heifers are exposed to cleanup bulls for an extra estrous cycle a cumulative overall 90% pregnancy rate after a 63-d breeding season is practically achievable.

The use of the 5-d Cosynch plus protocol allows to achieve acceptable pregnancy rates in both beef and dairy heifers. This protocol can be further modified to suit the needs of individual producers. The +GnRH with 2 injections of PGF protocol has been shown to increase pregnancy per AI, especially in acyclic heifers, however the –GnRH protocol with 1 PGF has had comparable results in cyclic heifers and removes the labor and cost of the extra PGF injection from the protocol. The –GnRH protocol has also had beneficial results when used with sex-selected semen, particularly when a limited amount of estrus detection is added to the protocol. Finally, a longer resynch interval that coincides more closely with the natural estrous cycle increases the number of heifers bred on detected estrus. Choosing an optimal breeding protocol depends on the goals of the producer in addition to labor and costs of veterinary services as well as the cost of each protocol.

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Table 2. Comparison of pregnancy per AI (P/AI) among different modifications of the 5-d Cosynch protocols in beef and dairy heifers.

Study	n	-GnRH/1PGF	+GnRH/1PGF	+GnRH/2PGF	P-Value*
Macmillan et al. 2017	370	66%	57%	--	0.08
Colazo et al. 2016 Overall	1,034	48%	53%	--	>0.10
Colazo et al. 2016 Cyclic	890	62%	57%	--	>0.10
Colazo et al. 2016 Acyclic	140	35%	50%	--	<0.05
Lopez-H et al. 2015 Cyclic	836	64%	--	--	--
Lopez-H et al. 2015 Acyclic	226	--	40%	--	--
Kasimanickam et al. 2014 Dairy	1,137	51%	54%	55%	>0.10
Kasimanickam et al. 2014 Beef	1,018	50%	60%	58%	<0.05
Cruppe et al. 2014	823	55%	51%	--	>0.10
Lima et al. 2013 Exp. 1	1,106	54%	--	59%	<0.05
Lima et al. 2013 Exp. 2	2,144	53%	--	62%	<0.05
Colazo and Ambrose, 2011	56	68%	71%	--	>0.10

GnRH: gonadotropin releasing hormone.
PGF: prostaglandin $F_{2\alpha}$.
*P-values indicate a significant difference (<0.05), a tendency for difference (0.05 < P < 0.10) or no difference (> 0.10) between P/AI values.

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