Second Insemination Breeding Strategies for Dairy Cows

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Take Home Message

- There are a variety of methods for synchronizing first insemination in dairy cows but less than 50% of first insemination cows become pregnant; thus most dairy cows must be reinseminated.
- Inseminating second-service cows based on observed return to estrus is inefficient because many non-pregnant cows fail to display estrus or have a delayed return to estrus after first insemination.
- Some second insemination systems concentrate estrus return intervals but these systems generally do not increase the total number of reinseminated cows because of low rates of estrous expression.
- Employing timed artificial insemination (timed AI) within a second insemination system will improve the overall response.

Introduction

Partial synchronization of second insemination is achieved when first insemination is synchronized. This is because synchronization of first insemination groups cows at the same stage of the estrous cycle. Non-pregnant cows, therefore, return to estrus at approximately the same time. In practice, the return to estrus is variable, however, with most cows returning to estrus 20 to 24 days after first insemination (Chenault et al., 2003). The variable return to estrus can be explained by the normal variation in estrous cycle length, early embryonic death (causing partial extension of the estrous cycle) and the fact that some cows do not respond to the first synchronization (i.e., they were never actually synchronous with the group).

Less than half of the cows that fail to conceive after first insemination (non-pregnant) are detected in estrus at the expected time following first synchronised insemination (Chenault et al., 2003). Non-pregnant cows that do

not return to estrus have been termed “phantom cows”; owing to their illusionary nature (appearing to be pregnant but in reality are not; Cavalieri et al., 2003). The presence of phantom cows creates a serious reproductive challenge. Under traditional reproductive management, a phantom cow is not detected until pregnancy exam; 40 to 60 days after initial insemination. A variety of causes for phantom cows have been found. The causes include: 1) estrus without ovulation following initial treatment; 2) estrus and ovulation following initial treatment but subsequent return to anestrus; 3) ovulation between days 14 to 28 but failure to express estrus before ovulation; 4) abnormally long inter-estrous intervals (i.e., greater than 24 days); or 5) early pregnancy loss. The risk factors for phantom cows are similar to those for anestrous cows (e.g., low body condition, early postpartum, first parity, etc; Nation et al., 2001; Cavalieri et al., 2003).

■ Methods for Synchronizing Second Insemination

Programming second insemination is difficult because there is a mixture of pregnant and non-pregnant cows after first insemination. Cows must be pregnancy diagnosed before prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injection because pregnant cows will abort if treated with $PGF_{2\alpha}$. Methods for diagnosing early pregnancy such as transrectal ultrasound (Fricke, 2002) can be employed so that $PGF_{2\alpha}$ can be given in resynchronization systems (see below). If pregnancy diagnosis is not done then progesterone is typically supplemented late in the estrous cycle after first insemination. Cows that are not pregnant to insemination have a variable return to estrus (discussed above). Progesterone supplementation blocks the return to estrus of non-pregnant cows and increases the synchrony of estrus in non-pregnant cows when progesterone is withdrawn.

Using a Progesterone-Releasing Device to Synchronize Second Insemination

The use of progesterone for resynchronization had its origins in New Zealand and Australia where controlled-internal drug-releasing (CIDR; Pfizer Animal Health, Kalamazoo, Michigan, USA) devices containing progesterone were used for first insemination and then were re-used for resynchronization. In practice, the CIDR device is inserted for a period of 6 to 8 days beginning on days 14 to 16 after insemination. An estradiol benzoate injection (0.5 to 1 mg) may be given at device insertion for the purpose of follicular wave synchronization. Estradiol benzoate (0.5 to 1 mg) is injected at device removal to increase estrous behaviour and improve the overall response (Eagles et al., 2001; McDougall, 2001; McDougall 2003; McDougall and Loeffler, 2004). Some systems also include a second period of resynchronisation by intravaginal progesterone application and estradiol injection (Cavalieri et al., 2004).

McDougall and Loeffler (2004) tested the efficacy of an estradiol benzoate-CIDR resynchronization system. They also determined if an injection of gonadotropin-releasing hormone (GnRH) could be substituted for the first estradiol benzoate injection (alternative method to synchronize the follicular wave). Their study employed New Zealand dairy cows previously treated for anestrus and inseminated. The resynchronization treatments resulted in more cows inseminated between 14 and 28 days after first insemination (Table 1). The estradiol benzoate-CIDR-treated cows had a higher pregnancy rate at the end of the breeding period.

Table 1. Estrous response after resynchronization, conception rate at second insemination, and final pregnancy rates for New Zealand dairy cows that were either control or treated with one of two different resynchronization systems (McDougall and Loeffler, 2004).

	Resynchronization system[1]		
	Control	**EB-CIDR-EB**	**GnRH-CIDR-EB**
Total cows enrolled, n	491	244	236
Estrous response[2], % (n)	55.1 (134)	79.1 (106)	69.8 (97)
Conception rate[3], % (n)	42.8 (104)	61.2 (82)	49.6 (69)
Final pregnancy rate[4], % (n)	88.3 (370)	95.0 (191)	88.6 (178)

[1]Control cows were untreated after first insemination; EB-CIDR-EB cows were treated approximately 14 days after first estrus with estradiol benzoate (0.5 mg at CIDR insertion), a CIDR (progesterone-containing) device for 6 days, and estradiol benzoate (0.5 mg) one day after CIDR removal; GnRH-CIDR-EB were treated with GnRH at CIDR insertion and otherwise treated as EB-CIDR-EB.

[2]Percentage of non-pregnant cows returning to estrus (days 14 to 28).

[3]Coneption rate for second insemination.

[4]Final pregnancy rate (all cows and all inseminations).

Large-scale field trials were recently completed in the United States where CIDR devices were administered to dairy cows from 13 to 21 days after first insemination (Chenault et al., 2003). The CIDR-treated cows had improved synchrony of returns to estrus compared to control (three day window for return to estrus; Table 2). The pregnancy rate to the initial AI was slightly reduced for CIDR-treated cows [32.7% (CIDR) versus 36.7% (Control)]. Despite greater synchrony of return to estrus, the pregnancy rate during the nine day resynchrony period was similar for treated and control cows. El-Zarkouny and Stevenson (2004) detected an increase in synchrony of return to estrus in cows treated with CIDR from day 13 to 20 after first insemination. However, the total percentage of non-pregnant cows that showed estrus 20 to 26 days after first insemination (approximately 30%) was similar for CIDR and control. The poor resynchronization response for Chenault et al. (2003) and El-Zarkouny and Stevenson (2004) may relate to the dependence that these specific CIDR programs place on estrous detection. Improved efficiency may be achieved by using an estradiol benzoate injection at the end of progesterone treatment (and

thus increasing the intensity of estrous behaviour; McDougall and Loeffler, 2004) or implementing a timed AI that does not depend on the expression of estrus.

Table 2. Estrous response after resynchronization, conception rate at second insemination, and final pregnancy rates for United States dairy cows that were either control or treated with a CIDR device for 14 to 21 days after first insemination (Chenault et al., 2003).

	Resynchronization system[1]	
	Control	CIDR
Estrous response[2], % (n)	19.3 (544)	34.1 (589)
Conception rate[3], % (n)	30.9 (194)	26.7 (266)
Final pregnancy rate[4], % (n)	44.0 (857)	41.2 (871)

[1]Control cows were untreated after first insemination; CIDR cows were treated with a CIDR (progesterone-containing) device on days 14 to 21 after first insemination.

[2]Percentage of non-pregnant cows returning to estrus within the 3-day resynchronization window.

[3]Coneption rate for second insemination.

[4]Final pregnancy rate (first and second inseminations).

Synchronizing Second Insemination for Timed AI using Ovsynch

One of the limitations for resynchronization with progesterone is the requirement for estrous detection following treatment. A better approach would be a system for timed AI of second insemination. Pursley et al. (1997) demonstrated that timed AI (Ovsynch) could be applied repeatedly to dairy cows after pregnancy diagnosis. In their study, cows were placed back on Ovsynch when they were diagnosed non-pregnant by ultrasound (32 to 38 days after insemination). Ovsynch-treated cows became pregnant at a faster rate than control (inseminated at observed estrus). Thus, timed AI can be successfully applied for first and subsequent inseminations after pregnancy diagnosis is done.

The first injection of GnRH within the Ovsynch program does not damage the pregnancy in pregnant cows (Chebel et al., 2003). Thus, the first GnRH injection can be given to all cows approximately one week before pregnancy diagnosis. Cows that are subsequently diagnosed non-pregnant can be injected with $PGF_{2\alpha}$ and 48 hours later injected with GnRH before timed AI. If pregnancy diagnosis by ultrasound occurs on day 28 then re-insemination of non-pregnant cows by timed AI occurs on day 30 or 31 (Chebel et al., 2003).

Chebel et al. (2003) demonstrated that cows treated for resynchronization in this manner (GnRH injection on day 21, ultrasound diagnosis on day 28, $PGF_{2\alpha}$ injection for non-pregnant cows on day 28 and GnRH injection and timed AI on day 30) had similar first insemination and second insemination pregnancy rates

when compared to cows started on resynchronization after day 28 pregnancy diagnosis.

Fricke et al. (2003) examined further the appropriate time for resynchronization using Ovsynch. In their study, cows were started on the resynchronization protocol on day 19, 26, or 33 after first insemination. The interval to resynchronization (19, 26, or 33 days) did not affect first insemination conception rate (31% across all groups). Thus, the Fricke et al. (2003) study agreed with the Chebel et al. (2003) study that showed no deleterious effect of GnRH on the first insemination pregnancies. Fricke et al. (2003), however, showed that second insemination pregnancy rates were higher for cows started on Ovsynch on day 26 (34%) or day 33 (38%) compared to day 19 (23%).

Synchronizing Second Insemination for Timed AI Using Rapid Resynchronization

It may be possible to use the inherent synchrony caused by the first synchronization to develop better methods for resynchronization. Most non-pregnant cows are in estrus 20 to 24 days after first insemination. If ultrasound pregnancy diagnosis is done at approximately day 29 then non-pregnant cows will be between days 5 and 9 of the subsequent estrous cycle. This early phase of the estrous cycle represents a period when a PGF2α-responsive corpus luteum and a dominant follicle are present on the ovary. Thus, a simple two injection system (PGF2α given to regress the corpus luteum and GnRH given 48 hours later to cause ovulation; Rapid Resynchronization) is possible. Cows can be inseminated 0 to 24 hours after the GnRH injection. Stevenson et al. (2003) demonstrated that dairy cows could be reinseminated and timed AI by using Rapid Resynchronization. Pregnancy was diagnosed on days 27 to 29 after first insemination. After pregnancy diagnosis, non-pregnant cows were either left untreated (Control), were injected with PGF2α and inseminated at estrus (or timed AI at 72 to 80 hours for those not seen in estrus), or were injected with PGF2α, GnRH, and timed AI (Rapid Resynchronization). Compared to control, the average interval from first to second insemination was decreased by 24 days for PGF2α-alone and Rapid Resynchronization (Table 3). Conception rates were similar regardless of the system (control, PGF2α-alone or Rapid Resynchronization). The combination of a shorter interval from first to second insemination with equivalent conception rates led to calving to conception intervals that were 22 to 23 days less in treated cows (PGF2α-alone or Rapid Resynchronization) compared to controls.

Table 3. Days from first to second insemination, conception rate at second insemination, and days from calving to conception for control dairy cows and dairy cows resynchronized with $PGF_{2\alpha}$-alone or resynchronized with Rapid Resynchronization (Stevenson et al., 2003).

	Resynchronization system[1]		
	Control	$PGF_{2\alpha}$-alone	Rapid Resynchronization
First to second insemination, days (n)	55 ± 1 (189)	31 ± 2 (108)	31 ± 1 (160)
Second insemination conception rate, % (n)	22.8 (189)	22.2 (108)	23.3 (160)
Calving to conception, days (n)	179 ± 8 (154)	157 ± 10 (82)	156 ± 7 (137)

[1]Control cows were untreated after first insemination; $PGF_{2\alpha}$-alone cows were injected with $PGF_{2\alpha}$ and inseminated at estrus (or timed AI at 72 to 80 hours for those not seen in estrus); Rapid Resynchronization cows were injected with $PGF_{2\alpha}$, injected with GnRH (48 hours after $PGF_{2\alpha}$) and timed AI 16 to 20 hours after GnRH.

We compared Rapid Resynchronization to resynchronization with Ovsynch (Meyer et al., 2004). Dairy cows were randomly assigned to receive either GnRH (Ovsynch treatment) or no injection (Rapid Resynchronization treatment) on d 22 after first insemination. All cows diagnosed non-pregnant by ultrasound were treated with $PGF_{2\alpha}$ on d 29, GnRH on d 31 and timed AI (4 to 8 hours after GnRH). Cows treated with GnRH on day 22 (Ovsynch resynchronization) had pregnancy rates that were similar to cows that had not been treated with GnRH on day 22 (Rapid Resynchronization) (Table 4). Thus, pre-treatment with GnRH may not be necessary if Rapid Resynchronization is done 29 days after first insemination.

Table 4. Pregnancy rates for dairy cows that were resynchronized with either Ovsynch or Rapid Resynchronization (Meyer et al., 2004).

	Resynchronization system[1]	
	Ovsynch	Rapid Resynchronization
Number of cows, n	154	163
Pregnancy rate, %	24.6	23.9

[1]Dairy cows were randomly assigned to receive either GnRH (Ovsynch) or no injection (Rapid Resynchronization) on d 22 after first insemination. All cows diagnosed non-pregnant by ultrasound were treated with $PGF_{2\alpha}$ on d 29, GnRH on d 31 and timed AI (4 to 8 hours after GnRH).

Can Second Insemination Be Done Before the Expected Return to Estrus?

Rapid Resynchronization can be used after ultrasonography exam. There are two other time periods after first insemination for Rapid Resynchronization (when a corpus luteum and dominant follicle are on the ovary). The first period is during the first follicular wave after insemination and the second period is during the second follicular wave after insemination. We treated non-pregnant lactating postpartum dairy cows with Rapid Resynchronization and timed AI beginning on day 17 of the estrous cycle and achieved a 53% pregnancy rate (n = 53 cows; Lucy, Scheer, and Spain; unpublished). Thus, the second period for Rapid Resynchronization (second follicular wave after first insemination) can be exploited. The problem with these first two time periods is that the bovine embryo cannot be ultrasonographically imaged during this time. A method for biochemical pregnancy diagnosis, therefore, is required. A variety of uterine genes are up-regulated during maternal recognition of pregnancy in cattle (days 15 to 20; Austin et al., 2004; Hicks et al., 2003). If early pregnancy tests can be developed for these proteins then the second window for Rapid Resynchronization (second follicular wave after first insemination) is opened. Pregnancy testing cows on day 18 and applying Rapid Resynchronization would enable the reinsemination of non-pregnant cows 21 days after first insemination. Thus, the reinsemination interval would be shorter than the normal interval for return to estrus (20 to 24 days after first insemination).

■ Summary

Most cows are not pregnant after first insemination. Performing second inseminations after a spontaneous second estrus is inefficient because many cows fail to express estrus during the expected return interval (20 to 24 days after first insemination). Treating cows with progesterone 14 to 21 days after first insemination will concentrate returns to estrus but low rates of estrous expression decrease the efficiency of progesterone-alone systems. Adding estradiol benzoate to a progesterone-alone system will increase estrous behaviour and improve overall responses. A timed AI system that uses Ovsynch may be the best approach because all cows are inseminated. Ovsynch can be started before pregnancy diagnosis so that second insemination cows are time inseminated about 30 days after first insemination. The first GnRH injection of Ovsynch may not be necessary if pregnancy diagnosis is done 28 to 29 days after first insemination (Rapid Resynchronization). Reinseminating non-pregnant cows before their spontaneous return to estrus (i.e., before 21 days after first insemination) may be possible if a biochemical test for early pregnancy is developed.

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