Resynchronization Programs for Dairy Cows

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Take Home Messages

- First insemination in dairy cows may occur after observed estrus or after a timed AI but less than 50% of first insemination cows become pregnant. Most dairy cows, therefore, must be inseminated at least twice.
- Inseminating second-service cows based on an observed return to estrus is the most economically efficient method for second insemination. Many non-pregnant cows, however, fail to display estrus before pregnancy examination.
- Employing a resynchronization system will generally increase efficiency by reducing the interval between inseminations for cows that are not pregnant.
- If a resynchronization system is used then the most important considerations are:
  - the interval between first insemination and the resynchronized insemination;
  - the conception rate to the resynchronized insemination; and
  - the speed and accuracy of the pregnancy diagnosis method (chemical test, ultrasound test, or palpation) that is needed for the system.
- The shortest interval from first insemination to second resynchronized insemination that has been demonstrated thus far is 21 days. The 21 d program uses a d 18 pregnancy test combined with a Rapid Resynchronization.

Introduction

The past 10 years of research has led to the development of improved methods for the management of first insemination in dairy cows. These improved methods include the development and optimization of new activity monitoring systems for the detection of estrus (Galon, 2010) and also the development of better (more pregnancies per AI) systems for timed AI (Presynch Ovsynch, G6G, Double Ovsynch, etc.). Most herds use a combination of estrus detection and timed AI for first insemination. The combination of the two will reduce the interval to first service and also reduce the amount of synchronization drugs used before first insemination.

Given the tremendous success of first insemination programs, the focus has now shifted to second insemination for cows that are not pregnant after first insemination. These cows present added complexity to reproductive management because they may or may not be pregnant when the resynchronization program is started.

In theory, the non-pregnant cows will return to estrus approximately 21 d after first insemination. In practice the return to estrus interval is 18 to 28 d (Chebel et al., 2006). The variable return to estrus interval 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 within the group). Many inseminated cows that are not pregnant will not express estrus before pregnancy examination. The combination of a poorly synchronized natural return to estrus and also poor estrus expression in non-pregnant cows makes second insemination a difficult task.

Challenges associated with second insemination have led to programs that are specifically designed for second insemination or “resynchronization” (resynch programs). Although the author will use the terms second insemination and resynchronization, the reader is reminded that the “second insemination” may actually be the third or greater insemination if the cow has been inseminated multiple times. The systems used for resynchronizing second or greater inseminations are essentially the same.

■ Key Considerations for Second Insemination

The first key consideration is that all of the general rules for efficient postpartum reproduction apply to second insemination. Clean, healthy cows that are in good body condition and are cycling will achieve the greatest second insemination success. There is no postpartum reproductive program that can fully overcome metabolic problems during the transition period, uterine disease (endometritis/metritis), low postpartum body condition score or anovulation during the breeding period. Pre-partum and postpartum nutrition and transition cow management are essential elements of a successful reproductive program.

The second key consideration is that a cow’s reproductive cycle is “synchronized” at first insemination. Most programs for second insemination (estrus detection or otherwise), therefore, make the assumption that the cow is at a given stage of cycle at a specific number of days after first insemination. Because of this assumption, the first step for optimizing a second insemination system (achieving the greatest success) is to optimize the first insemination system. Optimizing first insemination means that it is necessary to minimize the number of cows that are inseminated but are not truly in estrus (estrous detection systems) and (or) minimize the number of cows that are submitted for timed AI but are not properly synchronized for ovulation. In both cases (estrus detection systems or timed AI systems) the key element is the quality of the reproductive program that is in place [efficient and accurate estrus detection and (or) compliance to timed AI programs]. Herds that have excellent first insemination success will generally have excellent second insemination success because the elements of good reproduction are the same regardless of the insemination number.

The third thing to recognize is that conception rate for cows at second insemination matters. It is a key consideration for any program (estrous detection systems or timed AI systems). Second insemination conception rates are generally 5 to 10 percentage points lower than first insemination conception rates. The best second insemination programs will approach a 5 percentage point difference or even achieve a lesser difference for first and second insemination. The decrease in conception rate for second insemination can be explained by the fact that cows with greatest fertility become pregnant after first insemination leaving a population of lesser fertility cows for second insemination. If a second insemination program is used and conception rates are greater than 10 percentage points less than first insemination then alternative programs for second insemination should be explored. The overall efficiency of reproductive programs is reduced greatly when second (or later) insemination cows have a large reduction in fertility relative to first insemination. This large reduction in fertility may be overcome by alternative programs.

Finally, the most efficient systems for resynchronization (shortest interval between first and second insemination) will stress the capacity for accurate pregnancy diagnosis. Most resynchronization systems employ a prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injection. Pregnant cows will abort if treated with $PGF_{2\alpha}$. It is necessary, therefore, to diagnose pregnancy within the resynchronization program. Traditional methods for diagnosing pregnancy such as rectal palpation can be used but the time from first insemination to pregnancy diagnosis leads to an extended interval between breeding for cows that are not pregnant at pregnancy diagnosis. Transrectal ultrasound can be employed to shorten the breeding interval but pregnancy exams before d 30 take too much time for most practitioners. Blood pregnancy tests [pregnancy associated glycoproteins (PAG), for example; Green et al., 2009] can be used after d 25 but these tests present additional challenges including the required time for sample collection, submission and turn-around times, and the fact that a blood test does not give an instantaneous cow-side result. The newest systems for pregnancy diagnosis employ tests that are accurate at 18 d after insemination but these tests cannot be completed easily.

▪ The Original System for Resynchronization – One injection of $PGF_{2\alpha}$

The original system for resynchronization in dairy cows was the single injection of $PGF_{2\alpha}$ system. Cows that were diagnosed not pregnant with a corpus luteum (CL) were given $PGF_{2\alpha}$ to induce estrus. This system is simple and does work but has been supplanted by alternative systems. The primary limitation to the system is that the age of the CL is not known and therefore the CL may not regress in response to the $PGF_{2\alpha}$ injection. Dairy cows are also poor in terms of estrous expression. Cows that respond to the $PGF_{2\alpha}$ may not show overt signs of estrus and will not be inseminated. For this reason, many farms do not use a single injection of $PGF_{2\alpha}$ as a method of resynchronization in cows.

▪ Using Progesterone to Block Estrus and Ovulation and Synchronize Second Insemination

Treating cows with progesterone from 14 to 21 d after insemination blocks estrus and ovulation in non-pregnant cows that regress their CL. The treatment causes resynchronization because cows that would otherwise come back into estrus from d 14 to 21 are delayed and come back into estrus after d 21. The improved synchrony in non-pregnant cows reduces the time devoted to estrous detection and may stimulate estrous expression by increasing the number of cows in estrus on a given day. The use of progesterone for resynchronization had its origins in New Zealand and Australia where controlled-internal drug-releasing devices (CIDR; Pfizer Animal Health, Kalamazoo, Michigan, USA) containing progesterone were used for first insemination and then were re-used for resynchronization (McDougall and Loeffler, 2004).

In the United States, large-scale field trials were completed where CIDR devices were administered to dairy cows from 13 to 21 d after first insemination (Chenault et al., 2003). These trials were conducted as part of the regulatory approval process for CIDR devices in lactating cows. The CIDR-treated cows had improved synchrony of returns to estrus compared with control (three day window for return to estrus). The pregnancy rate to the initial AI was reduced, however, for CIDR-treated cows [32.7% (CIDR) versus 36.7% (Control)]. Despite greater synchrony of return to estrus, the pregnancy rate during the 9 d 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 d 13 to 20 after first insemination. The total percentage of non-pregnant cows that showed estrus 20 to 26 d after first insemination (approximately 30%), however, was similar for CIDR and control. The poor 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. It may be possible to combine CIDR-based resynchronization systems such as these with automated estrous detection systems (activity monitors) to increase the percentage of cows that are detected in estrus and inseminated after CIDR removal.

▪ Synchronizing Second Insemination with Ovsynch

One of the limitations for resynchronization with progesterone is the requirement for estrous detection following treatment. An alternative approach would be a system for timed AI of second insemination. Pursley et al. (1997) demonstrated in their classic paper that Ovsynch (timed AI) could be applied repeatedly to dairy cows after pregnancy diagnosis. In their study, cows were either inseminated after observed estrus (Control group) or were not observed for estrus but instead were treated with Ovsynch when they were diagnosed non-pregnant. Ovsynch-treated cows became pregnant at a faster rate than controls. Timed AI, therefore, could be successfully applied as the only method for reinsemination in non-pregnant dairy cows.

The original work of Pursley et al. (1997) led to a large number of studies in which Ovsynch programs were tested for second insemination. These studies addressed the best interval after first insemination to start Ovsynch and also different methods of presynchronization before Ovsynch. The overall goal of these studies was to improve second insemination timed AI pregnancy rate. Few studies have attempted to optimize the percentage of cows observed in estrus and inseminated before the Ovsynch is applied. It is the author's opinion that studies conducted in the next 10 years will focus on breeding cows in estrus before Ovsynch is used. At this time, however, this topic will not be reviewed because there is very little data on the topic and success rates are largely dependent on estrous detection efficiency within the herd.

When to Start Ovsynch Relative to Pregnancy Diagnosis

The first injection of GnRH within the Ovsynch program does not damage the pregnancy if the cow is pregnant. The injection can be given to all cows, therefore, approximately 1 wk before pregnancy diagnosis. If the GnRH is given in this manner, all cows are "set up" for the timed AI regardless of whether they are eventually diagnosed pregnant or not pregnant. Cows that are diagnosed non-pregnant can be injected with $PGF_{2\alpha}$ and 48 to 72 h later injected with GnRH before timed AI (Figure 1). Chebel et al. (2003)

demonstrated that cows started on Ovsynch before pregnancy diagnosis (GnRH injection on d 21, ultrasound diagnosis on d 28, $PGF_{2\alpha}$ injection for non-pregnant cows on d 28 and GnRH injection and timed AI on d 30) had similar first insemination and second insemination pregnancy rates when compared with cows started on Ovsynch after pregnancy diagnosis. The disadvantage to starting cows before pregnancy diagnosis is that all cows are initially treated with GnRH. In the pregnant cows, the GnRH treatment serves no purpose. The advantage of the system is that the interval between first insemination and second insemination is shortened by 7 d.

Fricke et al. (2003) further examined the appropriate time for resynchronization with Ovsynch. In their study, cows were started on the resynchronization protocol on d 19, 26, or 33 after first insemination. The interval to the start of Ovsynch (19, 26, or 33 d) did not affect first insemination conception rate (31% across all groups). The Fricke et al. (2003) study, therefore, 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 greater for cows started on Ovsynch on d 26 (34%) or d 33 (38%) compared with d 19 (23%).

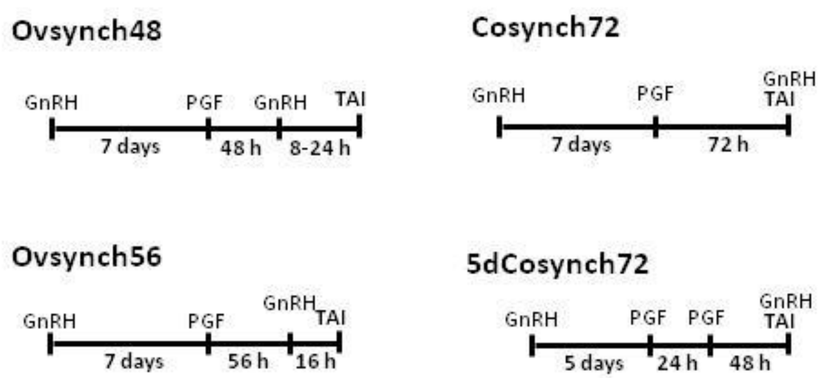

Figure 1. Timelines for timed AI (TAI) protocols that can be used for resynchronization of non-pregnant dairy cows. The programs can be started before pregnancy diagnosis and completed for cows that are later found not pregnant (the $PGF_{2\alpha}$ should not be given to pregnant cows). An alternative is to start the program after pregnancy diagnosis.

The conclusions from Fricke et al. (2003) led to the common recommendation that Ovsynch should be started after pregnancy diagnosis on d 32 (d32Ovsynch program). There are at least five reasons for this recommendation. First, cows have at least one month to come back into estrus before the resynchronization is started. When Ovsynch is started earlier (before pregnancy diagnosis, for example on d 19 or 21), cows will ovulate a follicle and will not show estrus because they form a CL and have circulating progesterone (blocks estrus and ovulation). The effect is to short-circuit the normal return to estrus and force more cows into the timed AI program. The second reason that the d32Ovsynch program is recommended is that ultrasound diagnosis of pregnancy on d 32 is fast because the horn is fluid filled and the fetus is large (Fricke, 2002). Third, if ultrasound diagnosis is done on d 32 then cows can be started back on Ovsynch immediately so that their treatments are given on the same days of the week as those used for first insemination cows. This reduces confusion (i.e., all cows get $PGF_{2\alpha}$ on Monday and are AI on Thursday, etc.; regardless of whether they are first or second insemination cows). Fourth, using Ovsynch after pregnancy diagnosis ensures that only non-pregnant cows are treated. Finally the d32Ovsynch program yields excellent fertility to timed AI.

Bisinotto et al. (2010) showed that a 5 d CIDR_Ovsynch program was also effective when started on d 34 (2 d after pregnancy diagnosis). In practice there are many variations to the general theme of starting cows on a timed AI program after non-pregnancy diagnosis.

Employing a Presynchronization Treatment before Ovsynch

Cows with a CL at the start of Ovsynch and that ovulate in response to the first GnRH generally perform better at timed AI (improved pregnancy rates). Attempts have been made, therefore, to increase the number of cows with a CL and ovulating to first GnRH by pre-treating with $PGF_{2\alpha}$, GnRH, or hCG within resynchronization systems (Figure 2).

Silva et al. (2007) employed a $PGF_{2\alpha}$-based method of presynchronization before a resynchronization Ovsynch. In their study, control cows were started on a traditional d32Ovsynch (Control) or were given $PGF_{2\alpha}$ on d 34 and started on Ovsynch on d 46. The $PGF_{2\alpha}$ regresses the CL in theory and places cows at d 5 to 10 of the estrous cycle (optimal time) when the Ovsynch is started. The $PGF_{2\alpha}$-treated cows had a 10 percentage point improvement in pregnancy rate (25.6% versus 35.2% pregnant when examined on d 66). The limitation of the program was that treated cows were inseminated 14 d later than control (control inseminations were d 42 after first insemination whereas $PGF_{2\alpha}$ -treated cows were on d 56). The authors speculated about the importance of the increase in conception rate relative to the delay in second insemination but left this as an open question for future study.

Dewey et al. (2010) compared a traditional Ovsynch resynchronization program (Cosynch72 started after pregnancy diagnosis on d 39) with a program that employed a pre-treatment injection of GnRH 7 d before the start of Cosynch72 in non-pregnant cows. They found that the pre-treatment with GnRH increased the conception rate to the Cosynch72 by approximately 9 percentage points. The increase was associated with an improved response to the first GnRH injection within the Cosynch72 program. Adding a CIDR to the Cosynch72 program (i.e., CIDR_Cosynch72) also increased the conception rate relative to the control program (approximately 7 percentage point improvement relative to Cosynch72). Regardless of the program, all cows were reinseminated at a 49 d interval from first insemination.

The Silva et al. (2007) and Dewey et al. (2010) studies demonstrated an improvement in timed AI conception rate for resynchronization. Both studies, however, tested programs with extremely long intervals between inseminations (56 or 49 d). The benefit of resynchronization diminishes as the interval between insemination increases; particularly when estrous detection rates are low (Cabrera et al., 2011). A program called “Double Ovsynch” can also be used for resynchronization (Giordano et al., 2009) and also has a 9 percentage point improvement relative to d32Ovsynch. The Double Ovsynch program, however, has an interval between inseminations of 49 d. More promising methods for presynchronization would enable shorter intervals between the first insemination and the reinsemination. One such method (termed “HGPG Resynch”) employed an injection of human chorionic gonadotropin (hCG; used to ovulate a follicle) on d 18 after insemination followed by an Ovsynch56 program started on d 25 (Giordano et al., 2010). The HGPG program gave a 9 percentage point improvement over the d 25 program alone. The very high timed AI conception rate was achieved with a 35 d interval between first and second insemination.

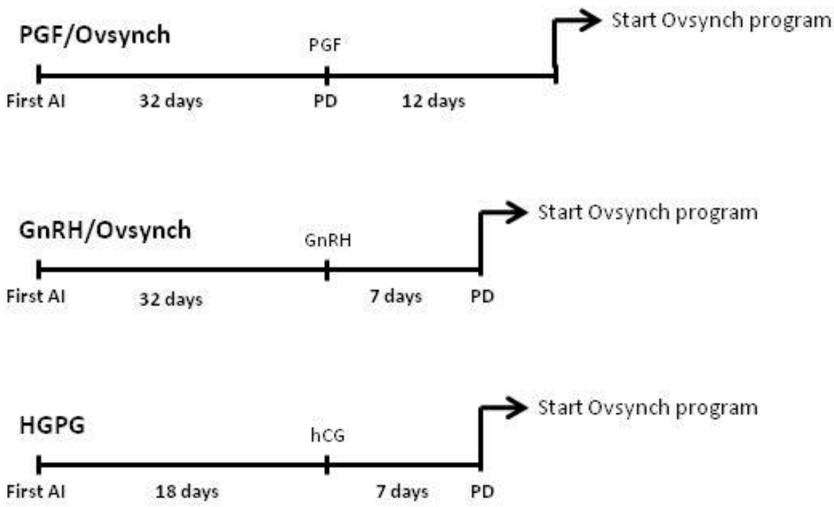

Figure 2. Presynchronization treatments that can be used to improve Ovsynch for resynchronization [PGF/Ovsynch (Silva et al., 2007), GnRH/Ovsynch (Dewey et al., 2010), and HGPG (Giordano et al., 2010)]. See text for details. PD = pregnancy diagnosis.

Combining a Blood Pregnancy Test (PAG) with an Ovsynch Resynchronization Program

Chemical pregnancy tests offer the opportunity to shorten the interval between inseminations. Two studies have examined chemical pregnancy testing within a resynchronization system. Silva et al. (2009) started an Ovsynch54 program for resynchronization on either d 25 (resulting in second insemination on d 35) or on d 32 (resulting in second insemination 7 d later; d 42). Cows started on the d 25 program were tested for pregnancy on d 27 by using a PAG ELISA. Cows on the d 32 program were tested for pregnancy by using ultrasound on d 39. The authors found that the pregnancy rates for the two treatments were similar (approximately 27% overall at the final pregnancy diagnosis). The conclusion was that a PAG ELISA could be used for pregnancy diagnosis as early as d 27 and that the program described could achieve a 35 d interval between first and second insemination with acceptable results.

Green et al. (2011) shortened the interval between first and second insemination further by starting a CIDR_Ovsynch program on d 18 after insemination and PAG testing cows at CIDR removal on d 25. Cows that were diagnosed not pregnant were injected with $PGF_{2\alpha}$ and reinseminated by d 28 (4 wk interval between first insemination and resynchronized second insemination for cows that were diagnosed not pregnant at d 25). The PAG-based program was compared to a CIDR_Ovsynch program that was begun on d 25 (non-pregnant cows inseminated on d 35). The authors found that both programs yielded similar second insemination conception rates (approximately 32%).

The data from the Green et al. (2011) demonstrated that it is possible to achieve an acceptable second insemination conception rate for cows that are programmed to receive their second insemination 28 d after first insemination. The 28 d interval is only 7 d longer that the traditional 21 d estrous cycle return interval. The program described by Green et al. (2011) requires a chemical pregnancy test that can detect pregnancies at 25 d after first insemination. A feature of the program is that cows are unlikely to express estrus before the pregnancy diagnosis and timed AI because a CIDR (containing progesterone) is inserted from d 18 to 25. The progesterone blocks estrus and ovulation. Hence, the program has a disadvantage in that cows will not naturally return to estrus. The advantage is that all non-pregnant cows will have a 28 d interval between first and second inseminations. The program described by Green et al. (2011) was tested in a small herd and thus the data are preliminary. Larger studies that compare other promising programs (for example HGPG) with this PAG-based CIDR_Ovsynch program will need to be completed.

▪ Methods that Employ an Early Pregnancy Diagnosis Followed by Rapid Resynchronization

Most non-pregnant cows cycle back 18 to 24 d after first insemination but their estrus (if expressed) is not detected. If ultrasound pregnancy diagnosis is done at approximately d 30 then non-pregnant cows will be between d 6 and 12 of the subsequent estrous cycle. There is a $PGF_{2\alpha}$-responsive CL and a dominant follicle on the ovary at this time. A simple two injection system ($PGF_{2\alpha}$ given to regress the CL and GnRH given 48 to 72 h later to cause ovulation; Rapid Resynchronization) is possible. Cows can be inseminated 0 to 24 h after the GnRH injection. The advantage of the system is that it is fast (2 to 3 d from non-pregnant diagnosis to reinsemination). The disadvantage is that some cows will not respond or respond poorly to the $PGF_{2\alpha}$ and (or) the GnRH because they are at the incorrect stage of the cycle. Stevenson et al. (2003) demonstrated that dairy cows could be reinseminated by using Rapid Resynchronization. Pregnancy was diagnosed on d 27 to 29 after first insemination. After pregnancy diagnosis, non-pregnant cows were either left untreated (Control), were injected with $PGF_{2\alpha}$ and inseminated at estrus (or timed AI at 72 to 80 h for those not seen in estrus), or were injected with $PGF_{2\alpha}$, GnRH, and timed AI (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 d less in treated cows ($PGF_{2\alpha}$-alone or Rapid Resynchronization) compared with controls.

We compared Rapid Resynchronization to resynchronization with Ovsynch (Meyer et al., 2007). Dairy cows were assigned to receive either Ovsynch treatment started on d 22 and completed on d 31 (timed AI) or Rapid Resynchronization treatment started on d 29 and completed on d 31 (timed AI). The cows treated with Ovsynch for resynchronization had pregnancy rates that were similar to cows treated with Rapid Resynchronization. The shorter Rapid Resynchronization program that was begun on d 29 and completed 2 d later was as effective as Ovsynch in this study.

Can Pregnancy Diagnosis and Second Insemination Be Done Before the Expected Return to Estrus in Non-pregnant Cows?

There are two other time periods after first insemination that are suitable for Rapid Resynchronization. The first period is during the first follicular wave after insemination (d 6 to 12) and the second period is during the second follicular wave after insemination (d 16 to 21). We treated postpartum dairy cows with Rapid Resynchronization and timed AI beginning on d 17 of the estrous cycle and achieved a 53% pregnancy rate (Lucy, Scheer, and Spain; unpublished). The problem with these two time periods (d 6 to 12 or d 16 to 21) is that the bovine embryo cannot be detected by ultrasound during at this time. A method for chemical pregnancy diagnosis, therefore, is required. A variety of uterine genes (Isg15, Oas1, and Mx2) are up-regulated during maternal recognition of pregnancy in cattle (d 16 to 20; Green et al., 2010b). We completed an experiment in which dairy heifers were inseminated and then pregnancy diagnosis was done on d 18 by using an Oas1 and progesterone test (Green et al., 2010a). We then began Rapid Resynchronization on day 19 so that non-pregnant heifers were inseminated on d 21 (Figure 3). The experiment demonstrated that dairy heifers could be pregnancy tested and reinseminated at a 3 wk interval between timed AI. The challenge is that the d 18 test is difficult to do in the laboratory (requires at least 8 h). If a simple d 18 test could be developed then testing cows on d 18 and performing a rapid resynchronization will be a viable method for resynchronization of non-pregnant cows. The 21 d reinsemination interval would be shorter (on average) and more compact than the normal interval for return to estrus (18 to 28 d after first insemination).

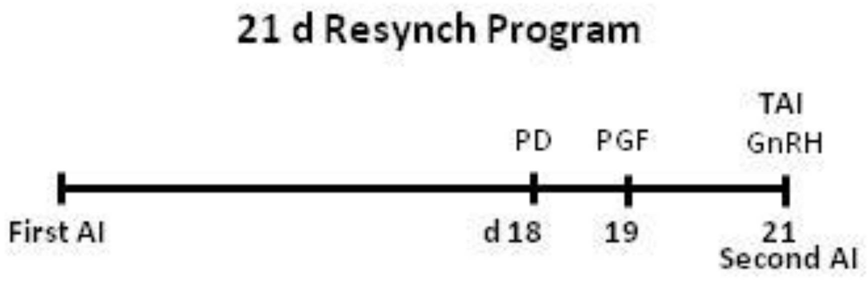

Figure 3. A program that achieves a 21 d interval between first insemination and second insemination in heifers that are not pregnant to first insemination (Green et al., 2010a). The program uses a d 18 pregnancy test combined with a Rapid Resynchronization. See text for details. PD = pregnancy diagnosis.

▪ What Factors Determine the Best System?

The most efficient way to reinseminate a non-pregnant cow is to observe the return to estrus and inseminate her. Aside from the 21 d program (previous paragraph, Figure 3), none of the current systems are superior to breeding a cow that is observed at the return to estrus. Breeding cows in estrus also saves drug expense and generally yields high fertility. The problem is that most cows either do not express estrus when they cycle back or have very poor estrous expression. If resynchronization is used, and if all other aspects are equal, then the program with the shortest interval from first insemination to second insemination is always best economically (Cabrera et al., 2011). The question is rarely this simple, however, because some of the shorter systems require more difficult methods of pregnancy diagnosis (PAG test or early ultrasound exams) or may not yield equivalent pregnancy rates compared with the longer programs. The economic analysis of resynchronization programs is an active area of investigation.

▪ Summary

Most cows are not pregnant after first insemination. Performing second inseminations after a spontaneous estrus is best but many cows fail to express estrus during the expected return interval. Treating cows with progesterone 14 to 21 d after first insemination will concentrate returns to estrus but low rates of estrous expression decrease the efficiency of progesterone-alone systems. A timed AI system that uses Ovsynch may be the best approach because all cows are inseminated. Ovsynch can be started before pregnancy diagnosis or after pregnancy diagnosis. Presynchronization programs for Ovsynch may improve pregnancy rate at second insemination

but presynchronization typically adds 1 to 2 wk to the insemination interval. A blood PAG test can be used to create a 28 d interval from first insemination to reinsemination. Reinseminating non-pregnant cows at a 3 wk interval is also possible with a d 18 blood pregnancy test and a rapid resynchronization. The d 18 test is difficult to do and not commercially available. If a viable on-farm test could be developed then a resynchronization interval of 21 d is possible by using a d 18 pregnancy test.

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