New Strategies to Maximize Pregnancy Outcomes

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▪ Take-Home Messages

- Periparturient clinical disease reduces fertilization rates, embryo quality, embryo growth and pregnancy outcome.
- Presynchronization of estrous cycles before first postpartum AI improves pregnancy per AI (P/AI) compared with applying only the Ovsynch timed AI program.
- Using Presynch ($PGF_{2\alpha}$)-Ovsynch to setup first postpartum AI increased P/AI by exposing cows to the entire program compared with inseminating cows detected earlier in estrus after Presynch $PGF_{2\alpha}$ treatments.
- Incorporating GnRH into Presynch programs (G-6-G, PG-3-G, or Double Ovsynch) before Ovsynch increased pregnancy outcomes at first AI services compared with standard Presynch 'PGF_{2a}' programs, especially in herds with larger percentages of anovular cows.
- Double Ovsynch increases P/AI at first service in primiparous cows compared with Presynch ($PGF_{2\alpha}$)-Ovsynch, whereas no increase in fertility is observed in multiparous cows.
- Resynchronization programs that include administering GnRH 7 days before starting the Resynch-Ovsynch program seem to improve pregnancy outcomes.
- Incorporating PGF_{2a} as part of a presynchronization program before Resynch-Ovsynch facilitates estrus expression and reduces the proportion of cows requiring timed AI, whereas GnRH as part of a presynchronization Resynch program inhibits estrus expression and increases the proportion of cows requiring timed AI.
- Incorporating progesterone via intravaginal inserts increased pregnancy outcome at day 60 after timed AI and reduced pregnancy loss in cows receiving their first postpartum AI.
- Administering post-AI treatments of human chorionic gonadotropin or GnRH may induce ancillary luteal tissue, increase progesterone concentrations, and improve pregnancy outcomes by 3.0 to 3.5 percentage units.
- Applying a pregnancy-associated glycoprotein pregnancy test is a viable alternative to a weekly transrectal ultrasound or palpation pregnancy diagnosis to allow earlier detection of nonpregnant cows.

▪ Introduction

Although overall fertility of lactating dairy cows in North America and elsewhere followed a downward trend since the 1950s (Butler and Smith, 1989), annual U.S. milk yield per cow increased 4.4 times from 2,409 kg in the 1950s to 10,500 kg in 2018. Based on a sample of comparatively less-productive dairy cows in the United Kingdom, fertility also decreased from 1975 to 1982 and from 1995 to 1998 (Royal et al., 2000). During that period, conception rates after first services decreased from 56 to 40% despite similar intervals to first service, whereas calving intervals increased from 370 to 390 days.

Since 2000, however, an increasing upward trend in daughter pregnancy rate (DPR) has occurred (Wiltbank and Pursley, 2014). For every unit increase or decrease in DPR (a value derived from days open), the 21-day pregnancy rate of a sire's daughters increased or decreased by 1%. During the past decade, while milk yield continued to increase, a dramatic increase of approximately 5% in DPR has occurred, which should translate into a reduction of 20 days open for their daughters when they become cows. Although the association between milk production and reproductive performance of dairy cows based on some metrics (i.e., days open, calving interval) is generally believed to be antagonistic, the antagonism between milk production and reproductive performance is not universal. In fact, recent work (Bello et al., 2012) supports a highly heterogeneous association between milk production and reproductive performance, whereby heterogeneity is partitioned across several scales and driven by many contributing factors, both physiological and managerial. It is clear in many herds that sound management practices overcome this potential negative relationship to achieve acceptable rates of reproductive efficiency.

Reproductive inefficiency of dairy cattle causes great frustration and potential lost income for dairy producers (Call and Stevenson, 1985). Even under optimal conditions, the reproductive process is less than perfect because of multiple factors involved in producing a live calf. To manage the complexities of the estrous cycle, understanding of many interrelated physiological functions is critical. Further, reproductive efficiency involves successful management of not only cows, but also the people who milk, feed, house, inseminate, and care for them.

Although benefits of improving reproduction are apparent, specific causes of poor reproductive performance are difficult to identify and not resolved easily. To improve reproductive efficiency, the limiting factors or bottlenecks must be addressed (Senger, 2001). Some potential bottlenecks over which dairy management teams can exert major control include: 1) estrus-detection efficiency, 2) reducing estrus-detection errors, 3) improving inseminator skills, 4) reducing errors in semen thawing and handling to prevent ‘cold shock’ of semen, 5) selecting for AI bull fertility and calving ease, 6) strategic use of sexed semen, 7) reducing heat stress, 8) addressing health and poor footing conditions, 9) improving health of transition cows, and 10) optimizing frequency of ‘open’ checks of potentially pregnant cows When management focuses on these bottlenecks, improvement in reproductive efficiency is possible.

▪ Voluntary Waiting Period

Several physiological changes including uterine involution and recurrence of ovarian follicular waves and normal estrous cycles must occur early postpartum to facilitate good fertility at first AI. Many factors affect these outcomes including, but not limited to, body condition, energy balance (milk yield and dry matter intake), parity, season, and disease (Crowe et al., 2014).

Results from Germany (Tenhagen et al., 2003) demonstrated that lengthening the voluntary waiting period (VWP) by 3 weeks from 53 ± 3 to 77 ± 3 days in milk (DIM) in low milk-producing cows or from 77 ± 3 to 98 ± 3 DIM in high milk-producing cows increased fertility at first AI when applying a timed AI program. Extending duration of VWP from 60 to 88 DIM increased P/AI to first service (VWP60 = 41%; VWP88 = 47%; Stangaferro et al., 2018). The greatest benefit of extending VWP on first-service P/AI was detected in primiparous cows (VWP60 = 46%; VWP88 = 55%) because P/AI did not differ in multiparous cows (VWP60 = 36%; VWP88 = 40%). Physiological status more conducive to pregnancy—characterized by improved uterine health, greater body condition score, reduced systemic inflammation, and to a lesser extent, more time to resume ovarian cyclicity—explained the increment in P/AI to first service. Despite having greater P/AI to first service, cows with the longer VWP had delayed time to pregnancy during lactation and greater risk of leaving the herd, particularly for multiparous cows. This shift in pregnancy timing led to an overall extension of the lactation length (+ 13 days), which resulted in greater total milk yield per lactation (+ 491 kg) but not greater milk yield per day of lactation.

▪ Postpartum Disease

Approximately 50% of the dairy cows in the U.S. suffer from at least one disease event during the first 60 DIM. Transition from pregnancy (no lactation) to lactation (not pregnant) presents the greatest risk of culling and death for a dairy cow. During this transition period, a number of metabolic and endocrine adaptations must occur to keep cows healthy. Most cows face a negative energy balance during early lactation because the cow cannot consume adequate energy to meet the demands of milk yield until later in lactation when daily dry matter intake peaks, often after peak in milk in older cows.

Calving-related disorders and diseases that affect the reproductive tract are major contributors to poor fertility. Dystocia (calving difficulty), metritis, and clinical endometritis were observed in 14.6, 16.1, and 20.8% of postpartum dairy cows in large U.S. confinement herds, respectively (Ribeiro et al., 2013). Cows that have one of the aforementioned disorders were 50 to 63% less likely to resume estrous cycles by the end of the VWP, and were 25 to 38% less likely to become pregnant following the first AI-breeding compared with healthy cows.

Direct effects of clinical disease on reproductive traits have been reported (Ribeiro et al., 2016). Embryos collected on day 5 to 6 after AI were evaluated for fertilization and grade quality. It was clear that cows suffering from at least one case of clinical disease had reduced fertilization rates and compromised embryo quality as early as 5 to 6 days after insemination. When conceptuses (embryos and their developing placenta) were collected on day 15 after AI, fewer cows with clinical diseases were pregnant. In fact, when day 15 conceptuses were evaluated, 83.9% of those collected from cows without a diagnosis of a clinical disease were elongated as expected for a day 15 bovine conceptus. In contrast, only 28.6% of them were elongated when they originated from cows with a diagnosis of clinical disease event during the first 60 DIM. When one clinical disease was diagnosed during the first 60 days, percentage of cows pregnant on day 15 after AI decreased from 49.4 to 29.8%. Uterine disease reduced the percentage of cows pregnant by more than half (49.4 to 20%). This carryover effect of disease on reproductive responses, embryo survival and maintenance of pregnancy in lactating dairy cows was independent of the category of postpartum health disorder (Mohtashamipour et al., 2019).

▪ Timed AI Programs for First Services

Ovsynch

The most common timed AI system is Ovsynch (injections of GnRH 7 days before (G-1) and 56 hours after $PGF_{2\alpha}$ (G-2), with AI administered at 72 hours after $PGF_{2\alpha}$ or 16 hours after G-2 treatment (Figure 1). Some variations have included a 5-day program with G-1 administered on day 0 and doses of $PGF_{2\alpha}$ given on day 5 and 6 with timed AI on day 8 (at the time of G-2). In the peer-reviewed literature (Wiltbank and Pursley, 2014), hundreds of articles have cited the original Ovsynch article and numerous articles use the term Ovsynch in the title (n = 6,870). Obviously, the 'Ovsynch' program has become an integral part of dairy reproductive programs during the 20 years since the original publication. A recent review of ovulation synchronization for management of reproduction in dairy cows provides insights into current methods and limitations (Bisinotto et al., 2014).

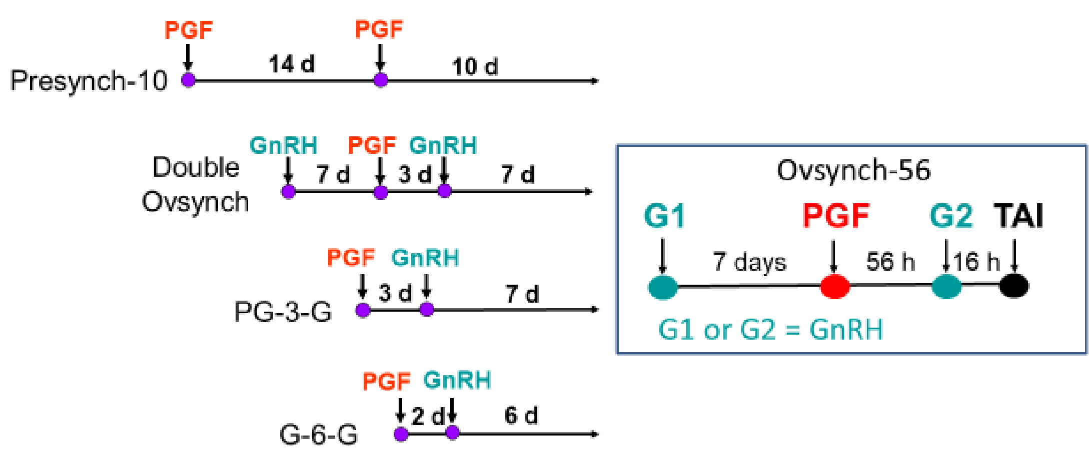

Figure 1. Standard presynchronization '$PGF_{2\alpha}$' – Ovsynch or Presynch-Ovsynch (e.g., Presynch-10 shown here or the interval from the last PGF injection to the start of Ovsynch can be 14, 12, or 11 days) and presynchronization 'GnRH' options (e.g., Double Ovsynch, PG-3-G, or G-6-G).

Presynch ‘$PGF_{2α}$’ Programs

Early studies indicated that pregnancy outcomes at first AI service after calving were improved when cows were at specific stages of the estrous cycle before initiating a timed AI program. Cows beginning the timed AI program on days 5 through 12 of the estrous cycle had greater ovulatory responses to G-1 and greater fertility than cows at other stages of the estrous cycle (Vasconcelos et al., 1999). The first Presynch ‘$PGF_{2α}$’ program tested was the Presynch-12 program. Permutations of the standard Presynch have been applied (e.g., Presynch-14, Presynch-11, and Presynch-10) where the two injections of $PGF_{2α}$ were consistently administered 14 days apart, but the interval from the last injection to the onset of the timed AI program was either 14, 11, or 10 days, respectively (Figure 1).

Presynch ‘GnRH’ Programs

The major limitation to Presynch ‘$PGF_{2α}$’ programs is their inability to improve fertility in anovular cows (i.e., those not having initiated estrous cycles since calving), which may represent up to 41% of dairy cows at the end of the VWP (Bisinotto et al., 2014). Including GnRH with $PGF_{2α}$ in a Presynch program increased the odds for pregnancy by 1.65 times (Bisinotto et al., 2013) and resulted in new presynchronization programs that add GnRH to $PGF_{2α}$ (e.g., Double Ovsynch, PG-3-G, and G-6-G; Figure 1).

Presynch 5-Day Programs

Ovulation to G-1 of Ovsynch improves synchronization of the estrous cycle and reduces the period of follicular dominance; both factors are associated with greater pregnancy outcomes (Vasconcelos et al., 1999; Santos et al., 2010). As a result, studies comparing 5-day vs. 7-day Ovsynch programs tested in dairy (Santos et al., 2010) and beef cattle (Bridges et al., 2008) sometimes produced greater pregnancy outcomes in the 5-day programs because proestrus is prolonged and follicular dominance is reduced in more cows. One limitation to the 5-day program is the inability of a single dose of $PGF_{2α}$ to induce complete corpus luteum (CL) regression (particularly the ‘new’ CL that resulted from GnRH-induced ovulation). Therefore, two doses of $PGF_{2α}$ are required (one on day 5 and another on day 6) to optimize luteal regression by either $PGF_{2α}$ or by one of its analogues (Riberio et al., 2012a). Even larger single doses administered on day 5 were less effective in the 5-day program when the one 50-mg dose (dinoprost or Lutalyse) was applied, but both $PGF_{2α}$ dose-frequencies (one 50-mg dose or two 25 mg doses [24 hours apart]) effectively induced complete luteolysis in the 7-day program (Stevenson et al., 2018).

▪ Comparative Fertility of First-AI Programs

Ovsynch

A meta-analysis examined 71 treatment and control comparisons extracted from 53 research papers (Rabiee et al., 2005). Programs evaluated included Ovsynch, natural breeding, single, double, or triple prostaglandin injections, Select Synch, Heat Synch, and modified Ovsynch. Pregnancy rates for Ovsynch programs did not differ from those any of those breeding programs. What these studies did not evaluate was that all cows submitted to a timed-AI program, which is independent of expressed estrus as a prerequisite for AI, were inseminated at one time, and reduced the variation and postpartum interval to semen exposure at first AI. The findings demonstrated that the Ovsynch program could benefit dairy operations because it allows for timed AI of lactating cows without detection of estrus. There was, however, little or no significant improvement in pregnancy rates using Ovsynch over other programs and the costs of labor and hormone administration should be considered when selecting this form of reproductive technology for routine use.

Presynch ‘$PGF_{2\alpha}$’ (Presynch-Ovsynch) Programs

Studies demonstrated that presynchronization of estrous cycles to days 5 to 12 after estrus before applying Ovsynch could improve fertility at the timed AI. Two early studies (Moreira et al., 2001; El-Zarkouny et al., 2004) tested whether estrous cycles could be staged in cows to meet this ideal by applying two injections of $PGF_{2\alpha}$ (named Presynch-12) administered 14 days apart and then initiating the timed AI program 12 days after the second $PGF_{2\alpha}$ injection. In the preceding two studies and in another study employing the Presynch-14 program (Navanukraw et al., 2004), the Presynch programs were superior to Ovsynch alone for timed AI pregnancy outcome.

In nearly all published studies, these Presynch ‘$PGF_{2\alpha}$’ systems have produced greater pregnancy outcomes in cows than in those submitted to the timed AI program at random stages of the estrous cycle without Presynch. Furthermore, greater pregnancy outcomes were reported in cows treated with Presynch-11 than with Presynch-14 before a timed AI program (Galvão et al., 2007), probably because more cows were at the ideal stage of the cycle after Presynch-11 treatment (Figure 1). Further shortening the interval between Presynch and Ovsynch to less than 10 days may reduce pregnancy outcomes (Colazo et al., 2013). Overall, lactating dairy cows exposed to Presynch ‘$PGF_{2\alpha}$’ programs for presynchronization have 42% greater odds of pregnancy compared with cows receiving only the timed AI program (Bisinotto et al., 2014).

These Presynch ‘$PGF_{2\alpha}$’ programs also have the flexibility of choosing to inseminate cows detected in estrus after $PGF_{2\alpha}$ (Melendez et al., 2006; Chebel and Santos, 2010; Chebel et al., 2010) but it may come at a cost. A meta-analysis examined the question whether to inseminate cows that were detected in estrus after $PGF_{2\alpha}$ and before cows completed the entire Presynch ‘$PGF_{2\alpha}$’ program (Borchardt et al., 2016). Cows from another 20 experimental groups including 8,124 cows submitted to first AI using a Presynch-Ovsynch protocol were used. The overall proportion of P/AI for cows inseminated early (EDAI + timed AI) was less than that of cows that completed the program and received the timed AI (Figure 2). Information regarding pregnancy loss at 60 days after AI was available for 5,200 cows and did not differ between groups(Figure 2).

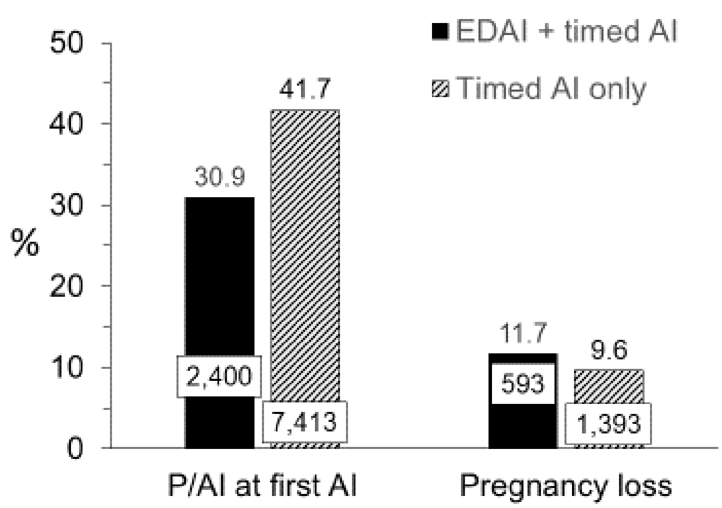

Figure 2. Pregnancy outcomes (assessed at days 28 to 42 after AI) in cows exposed to standard Presynch-Ovsynch program before first postpartum AI for cows inseminated after the second $PGF_{2\alpha}$ treatment upon estrus detection or that received the timed AI (ED + timed AI) compared with cows only receiving the timed AI (timed AI only). Pregnancy losses were assessed at days 42 to 74 after AI.

Presynch 'GnRH' Programs

Comparisons of various Presynch programs showed that G-6-G produced greater pregnancy outcomes than Presynch-11 when Cosynch-72 (combining timed AI and G-2 at 72 hours after $PGF_{2\alpha}$) was used (Ribeiro et al., 2011). When Presynch-12 was applied before timed AI, pregnancy outcome was similar in Ovsynch-56 vs. Cosynch-72 (Bisinotto et al., 2010). Comparison of two Presynch programs and two times for administering G-2 and timed AI (56 vs. 72 hours; Ribeiro et al., 2012b) produced no significant difference in pregnancy outcomes at day 30 after timed AI. At day 65 after timed AI, however, fewer cows treated with Presynch-10 and injected with GnRH and inseminated at 58 hours were pregnant compared with those injected with GnRH and inseminated at 72 hours. In contrast, no such differences were detected when cows were exposed to Double Ovsynch before the timed AI program.

In some cases, Double Ovsynch (Souza et al., 2008; Herlihy et al., 2012) and PG-3-G (Stevenson and Pulley, 2012) may produce greater pregnancy outcomes than the two standard Presynch '$PGF_{2\alpha}$' variants (Presynch-12 or Presynch-10). Another recent meta-analysis including a total of 25 articles with 27 experimental groups from 63 herds including 21,046 cows submitted to first timed AI using either a Presynch-Ovsynch or a Double-Ovsynch protocol were reviewed (Borchardt et al., 2017). Information was available for P/AI for 7,400 primiparous cows and 10,999 multiparous cows. Primiparous cows treated with Double Ovsynch had greater P/AI than cows exposed to Presynch '$PGF_{2\alpha}$' programs, whereas no differences were detected in multiparous cows (Figure 3). The overall proportion of pregnancy loss was 11.3% and 11.7% on day 60 after AI for Presynch-Ovsynch and Double-Ovsynch, respectively.

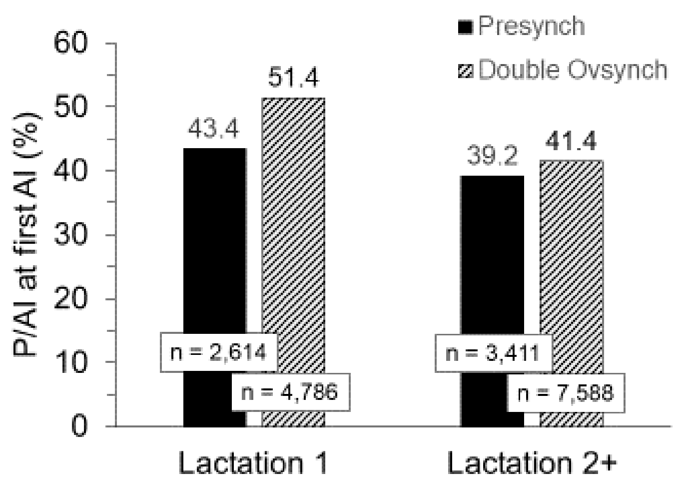

Figure 3. Pregnancy per AI (assessed at days 28 to 42 after AI) in primiparous (lactation 1) and multiparous (lactation 2+) cows exposed to Presynch-Ovsynch or Double Ovsynch before first postpartum AI. Pregnancy losses were assessed at days 42 to 74 after AI.

The PG-3-G program produced greater timed AI pregnancy outcomes in four herds during summer than did the Presynch 10 program and tended to improve P/AI compared with that in cows inseminated early before completing the entire program (Figure 4; Stevenson and Pulley, 2012). During cooler and cold seasons of the year, both presynchronization programs produced greater P/AI than that in cows inseminated early.

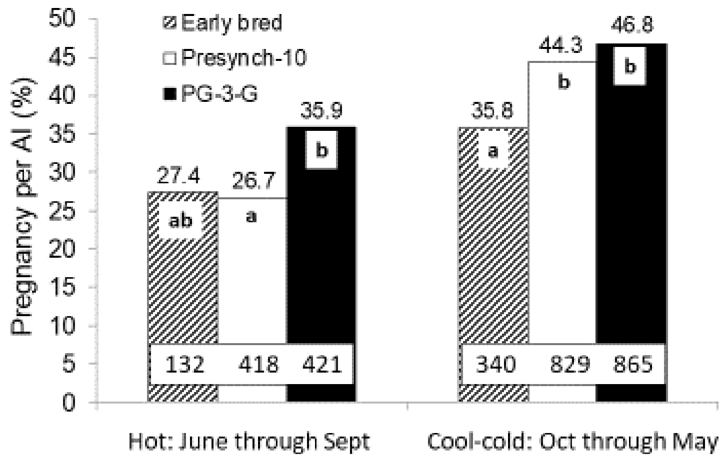

Figure 4. Pregnancy per AI (assessed at days 32 to 38 after AI) in cows exposed to Presynch-Ovsynch (Presynch-10) or PG-3-G before first postpartum AI during summer and cooler seasons. Early bred (EB) cows were inseminated upon detected estrus before the scheduled timed AI. Pregnancy loss at days 60 to 66 did not differ for EB, Presynch-10, and PG-3-G treatments (4.0, 6.8, and 9.3%, respectively).

Programs Including Progesterone

A review of the literature (meta-analysis) indicates that use of a single CIDR (controlled internal drug release) containing progesterone administered during the period between G-1 and the $PGF_{2\alpha}$ of the timed AI protocol increased pregnancy outcome at day 60 after AI by 18% compared with untreated controls (34.2% vs. 29.6%; Bisinotto et al., 2015b). The benefit from progesterone supplementation was similar for cows with and without a CL at G-1. Nevertheless, P/AI for cows without CL treated with a single insert was 10.5% less than that of untreated cows that had a CL at the initiation of the timed AI program (29.0% vs. 32.0%). Incorporating a single progesterone insert as part of a timed AI program increased fertility in cows that lacked a CL at the first GnRH injection, but did not restore fertility to the same level as those cows starting the timed AI program in diestrus (i.e., having a CL), likely related to the amount of progesterone released.

Several recent studies have attempted to target potential problem cows with progesterone as part of timed AI programs. Ovaries were examined for the presence of a CL by ultrasound at the beginning of Ovsynch after earlier treatment with two injections of $PGF_{2\alpha}$ 14 days apart (Presynch, Stevenson et al., 2008). Cows without a CL were treated with Ovsynch with or without progesterone via a CIDR insert. Cows with a CL served as a control. Cows without a CL had greater P/AI at 33 days after timed AI when treated with progesterone (CIDR) compared with control cows with no CL or cows having a CL at the onset of Ovsynch (Figure 5; left panel). In two other reports (Bisinotto et al., 2013, 2015a), cows without a CL at the onset of a 5-day or 7-day Ovsynch program were treated with two CIDR inserts and P/AI was compared with contemporary no-CL cows and cows in diestrus (bearing a CL). Pregnancy per AI at 32 days after timed AI did not differ between cows with a CL and those treated with two CIDR inserts but was greater than that in untreated, no-CL cows (Figure 5; middle and right panel).

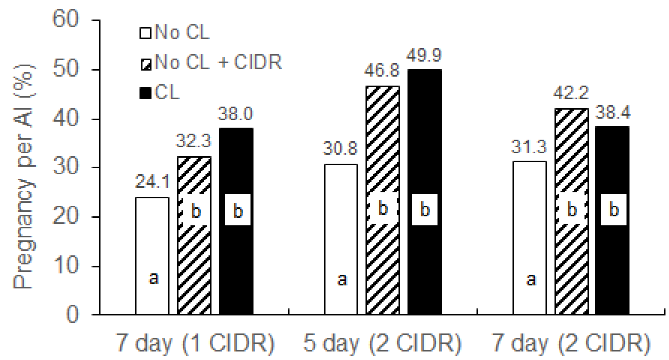

Figure 5. Pregnancy per AI for cows at first postpartum AI after treatment with either a 5-day or 7-day Ovsynch timed AI program. Cows with no corpus luteum (CL) were either controls or received an intravaginal progesterone-releasing insert (CIDR) and were compared with cows starting the Ovsynch treatment with a CL.

Progesterone supplementation during a timed AI program tended to reduce the risk of pregnancy loss between day 32 and 60 of gestation, which corresponded to 2.5 percentage units based on data of a meta-analysis, from 11.6 to 9.1% (Bisinotto et al., 2015b). The benefit of progesterone supplementation in reducing pregnancy loss was not affected by service number, use of presynchronization, or detection of estrus during the timed AI program. Reduced risk of pregnancy loss in response to progesterone supplementation was not affected by the presence of a CL at the initiation of the synchronization protocol.

▪ Post Insemination Treatments

A recent meta-analysis examined the effects of either GnRH or human chorionic gonadotropin (hCG) administered during the luteal phase after AI (Besbaci et al., 2019). No difference in P/AI was detected between cows treated with hCG or GnRH. Compared with no treatment, treatment with GnRH vs. control (45.9 vs. 43.8%) or hCG vs. control (42.0 vs. 39.1%), respectively, improved the chances of P/AI in cows with very poor (< 30%) and poor (30.1 to 45%) fertility, whereas treatment did not benefit cows with very good fertility (> 60%). Moreover, treatment with GnRH and hCG improved the chances of P/AI in primiparous cows, especially those with very poor fertility. Treatments with GnRH on or before day 10 post-AI did not differ from controls (41.1 vs. 40.4%), but GnRH administered after day 10 increased P/AI (51.2 vs. 47.7%) compared with controls, respectively. Treatment with hCG at doses greater than 2,500 IU was associated with increased chances of P/AI compared with smaller doses.

▪ Repeat Service (Resynch) Programs

Early Not-Pregnant Diagnosis

Increased efficiency of reproduction occurs when the not-pregnant diagnosis (NPD) in previously inseminated cows is determined as soon as possible (Fricke, 2002). Means to detect not-pregnant status relatively soon after insemination include:

- Monitoring of blood concentrations of progesterone or commercially available kits to measure blood or milk pregnancy-associated glycoproteins (PAG)
- Presence of estrus determined by visual heat detection or heat-detection aids, or by employing other electronic methods (pressure-sensitive, rump-mounted transmitters or physical activity monitors)
- Absence of uterine fluid, CL, and embryo via transrectal ultrasonography

Early detection of NPD facilitates earlier re-insemination of open cows. Although detection of pregnancy status earlier is associated with improved overall herd fertility measures, more embryo loss is observed than when pregnancy status is detected later. Methods to facilitate re-insemination of open cows are described hereafter.

Presynchronization before Resynch (No or Limited Estrus Detection)

In some herds, cows diagnosed not pregnant are started on a timed AI program on the day of NPD. In other herds, in order to re-inseminate cows sooner after a NPD, the first GnRH injection (G-1) of the timed AI is administered to all cows eligible for the next pregnancy diagnosis (unknown pregnancy status) either 5 or 7 days before NPD depending on which timed AI program is used (i.e., 5-day vs. 7-day Ovsynch).

Applying a presynchronization treatment such as GnRH or hCG to ovulate a follicle and initiate a new follicular wave in cows with unknown pregnancy status has been tested for its profertility effects. Simple Resynch-Ovsynch programs initiated at day 32 or 39 after previous AI were compared with treatments that included a presynchronization GnRH or hCG injection administered 7 days before Ovsynch. At both initiation times, the pre-GnRH or pre-hCG injection increased pregnancy risk by 4 to 5 percentage units (Table 1).

Table 1. Pregnancy per AI (P/AI) of nonpregnant dairy cows exposed to a 7-day Resynch-Ovsynch timed AI program initiated at a not-pregnant diagnosis at either 32 or 39 days after previous AI with or without presynchronization injections of either GnRH or hCG.

Program	P/AI 32 to 39 days after AI, %	No. of studies
Control Resynch[1]	29.0 (3,657)[3]	11
Pre-GnRH[2] or pre-hCG[2] + Resynch	33.5 (2,996)	8

[1]Ovsynch was started at the non-pregnant diagnosis (NPD).
[2]GnRH or hCG injection administered 7 days before NPD and starting Ovsynch-56.
[3]No. of cows.

Presynchronization before Resynch (Estrus Detection)

In the previously cited summary (Table 1), detection of estrus was not applied and all cows received only the timed AI. In herds in which cows are housed in dry lots or free stall barns with or without turnout dry lots detection of estrus is often applied to cows in addition to using timed AI programs for cows not detected in estrus. In subsequent studies, a pre-GnRH injection was applied as a presynchronization treatment and cows were inseminated when detected in estrus. The timed AI program was discontinued when cows were re-inseminated at estrus, or in the absence of estrus, the complete timed AI program was carried out.

In two such studies (Table 2), fewer cows were detected in estrus and inseminated before the timed AI program was initiated when the pre-GnRH injection was applied compared with the control Resynch (37.4 vs. 50.3%; calculated from Table 2 data), even though resulting P/AI was not different. In the face of similar P/AI after insemination, cows detected in estrus and inseminated became pregnant earlier than timed AI cows.

Table 2. Pregnancy per AI (P/AI) risk of nonpregnant cows exposed to a 7-day Resynch-Ovsynch timed AI program initiated at not-pregnant diagnosis at either 32 or 39 days after previous AI: Insemination at estrus or at appointment (timed AI).

Program	P/AI 32 to 39 days after AI, %	No. of studies
Control Resynch	32.3 (1,501)[2]	
Estrus	39.1 (756)	2
Timed AI	25.4 (745)	2
Pre-GnRH + Resynch[1]	33.9 (1,398)	
Estrus	41.2 (523)	2
Timed AI	29.6 (875)	2

[1] GnRH injection administered 7 days before Ovsynch-56.
[2] No. of cows.

Subsequent studies endeavoured to presynchronize estrous cycles with $PGF_{2\alpha}$ to facilitate estrus expression and earlier re-insemination after a NPD. In most cases fertility was not improved (Chebel et al., 2013), but more cows detected in estrus were inseminated earlier than cows assigned to a timed AI program. In general, when applying Resynch programs to dairy cows at NPD, employing presynchronization $PGF_{2\alpha}$ facilitates estrus expression, whereas using a pre-GnRH or Pre hCG injection suppresses estrus expression. Another study demonstrated that when $PGF_{2\alpha}$ was administered at NPD or 3 days after NPD compared with a pre-GnRH-Resynch, less than 24% of the cows received the timed AI scheduled 7 or 14 days after NPD because cows already were inseminated upon detected estrus compared with 78% of GnRH-Resynch cows receiving the timed AI (Bruno et al., 2013).

Use of Progesterone in Resynch Programs

Addition of progesterone in the form of a CIDR to nonpregnant cows in designed studies in 7-day Resynch-Ovsynch programs initiated at the NPD failed to increase pregnancy risk of cows, whereas when tested in a 5-day Ovsynch program initiated at day 32, progesterone increased pregnancy outcome (Bisinotto et al., 2010). As previously noted, the greatest pregnancy advantage accrues from applying progesterone to cows without a CL rather than those with a CL.

We conducted a recent study in three herds using a shortened version of Ovsynch program that excluded GnRH-1 to resynchronize ovulation in cows bearing a CL after a NPD. In addition, we included progesterone supplementation with the Ovsynch program for cows without a CL to determine if shorter inter-insemination intervals and P/AI in either treatment would differ from that of cows treated with the OVS treatment (Sauls-Hiesterman et al., 2019). Cows (n = 1,584) were enrolled in the study and assigned to one of three treatments at NPD (32 ± 3 days after AI [day 0]). Cows with a detected CL were assigned randomly to: 1) a modified Ovsynch (OVS: G-1 — 7 days — $PGF_{2\alpha}$-1 — 24 h — $PGF_{2\alpha}$-2 — 32 h — G-2 — 16 h — AI) or 2) Short Synch (SS: $PGF_{2\alpha}$-1 — 24 h — $PGF_{2\alpha}$-2 — 32 h — G-2 — 16 h — AI). Cows with no CL were assigned to OVS plus a progesterone insert (CIDR) administered between G-1 and $PGF_{2\alpha}$-1. Mean and median inter-insemination intervals were less in SS cows (mean = 34.3 ± 0.05 days [median = 35 days] than in OVS cows (40.2 ± 0.05 days [42 days]), but that in OVS cows did not differ from OVS + CIDR cows (41.4 ± 0.05 days [42 days]). Herd technicians were more accurate in visually detecting a functional CL than a non-functional CL (81.2 vs. 61.1%). Pregnancy per AI at 32 days after AI was less for SS (16.5% [n = 115]) than OVS (29.3% [n = 133] when a functional CL was inaccurately detected, but did not differ when a functional CL was detected accurately (27.6% [n = 561] vs. 30.3% [n = 508]). Pregnancy per AI did not differ between OVS and OVS + CIDR cows regardless of CL status. Short synch is an alternative to the entire modified Ovsynch program to produce similar P/AI when the CL status was detected accurately, and regardless of functional CL status, SS reduced inter-insemination intervals by 7 days.

▪ Timing of GnRH before Timed AI

Success of timed AI programs depends on adequate duration of proestrus and proper timing of insemination relative to ovulation. For the standard Ovsynch program, with 7 days between the initial GnRH and $PGF_{2\alpha}$ injections, administering the final GnRH 56 hours after $PGF_{2\alpha}$ and performing AI 16 hours later seems to optimize P/AI in dairy cows (Pursley et al., 1998). Conversely, extending the proestrus longer than 56 hours and inseminating cows concurrently with the final GnRH injection may reduce fertility in dairy cows (Sterry et al., 2006; Brusveen et al., 2008). Allowing 56 h of proestrus provides additional growth of the ovulatory follicle and increased exposure to estradiol (Peters and Pursley, 2003), which is thought to be needed to avoid short estrus cycles after induced ovulation. For 7-day Ovsynch programs, optimal timing of the second (or breeding) injection of GnRH (G-2) is approximately 56 hours after $PGF_{2\alpha}$ (Ovsynch-56) with AI occurring approximately 16 hours later (or 72 hours after $PGF_{2\alpha}$ compared with timed AI at either 48 or 72 hours, concurrent with the second GnRH injection (Table 3).

For 7-day Ovsynch programs, which were preceded by Presynch-11, optimal timing of the second or breeding injection of GnRH is approximately 56 h after PGF_{2a} (Ovsynch-56) with AI occurring approximately 16 hours later or 72 hours after PGF_{2a} (Brusveen et al., 2008). When GnRH + AI at 72 hours after PGF_{2a} occurred (Cosynch-72), pregnancy outcomes at 31 days after timed AI were less than when GnRH was administered at 56 h (27.5 vs. 45.2%). In contrast, when GnRH + AI occurred at 48 hours after PGF_{2a} (Cosynch-48), pregnancy outcome at 31 days post-timed AI did not differ from Ovysnch-56 (38.2 vs. 45.2%), respectively.

Table 3. Pregnancy per AI (P/AI) of dairy cows exposed to a 7-day Ovsynch timed AI program and various times of GnRH injection relative to $PGF_{2\alpha}$.

Program	P/AI 28 to 40 days after AI, %	No. of studies
Cosynch-48[1]	28.4 (1,640)[4]	6
Cosynch-72[2]	29.0 (1,582)	7
Ovsynch-56[3]	33.7 (1,729)	5

[1]Cosynch 48 = GnRH (G-2) and AI at 48 hours after $PGF_{2\alpha}$.
[2]Cosynch72 = GnRH (G-2) and AI at 72 hours after $PGF_{2\alpha}$.
[3]Ovsynch-56 = GnRH (G-2) at 56 hours after $PGF_{2\alpha}$ and AI 16 hours later.
[4]No. of cows.

▪ Frequency of $PGF_{2\alpha}$ Treatments in Ovsynch

A few studies have tested the addition of a second dose of $PGF_{2\alpha}$ administered 24 hours after the standard $PGF_{2\alpha}$ treatment in Ovsynch (Figure 1) in cows inseminated at first postpartum AI. Luteolytic risk (progesterone < 0.5 ng/mL at 72 hours after the first or only $PGF_{2\alpha}$ treatment) increased from approximately 83% with one standard dose of $PGF_{2\alpha}$ to 88% with one double dose of prostaglandin $F_{2\alpha}$ to 97% with two standard doses administered 24 hours apart (Wiltbank et al., 2015; Stevenson et al., 2018). In the former study consisting of 11 herds, the increase in P/AI was only 3 percentage points for cows receiving two vs. one $PGF_{2\alpha}$ treatment.

▪ Using PAG Testing in Reproductive Management

Using blood or milk testing of PAG in lieu of transrectal ultrasound or palpation to detect pregnancy is commonplace. It allows any size herd to conduct a weekly pregnancy diagnosis when the herd veterinarian is unavailable and serves the purpose of finding nonpregnant cows sooner than later. Most DHI labs offer this service for samples beginning at 28 days after AI. Based on PAG profiles in plasma and milk samples collected weekly, the optimal time to conduct a first pregnancy diagnosis is approximately 32 days after AI when plasma and milk PAG concentrations are at an early peak (Ricci et al., 2015). Because of the occurrence of pregnancy loss, all pregnant cows should be submitted for a confirming pregnancy diagnosis 74 days or more after AI when relative PAG concentrations in plasma and milk of pregnant cows have rebounded from their nadir.

▪ Conclusions

A magic program does not exist that will result in pregnancy success without the hard work of animal care that includes cow comfort, good health, balanced diets, and excellent insemination skills. One must exercise caution when considering changes to current programs and operating procedures that are not broken. A critical point to remember when considering the value of reproductive changes is that profit gains follow the law of diminishing returns. In other words, gains are greater when improvements are made from a very low starting point, but the exact value will vary depending on economic conditions. For example, using an example with U.S. dairy milk prices and 21-day pregnancy risk (21-day PR), the economic benefit from increasing 21-day PR from 16 to 18% (approximately $40/cow) is much greater than attempting to move it from 26 to 28% (< $10/cow). Whatever the milk price, increasing the 21-day PR results in diminishing economic returns. This example is for a herd producing 12,250 kg (305-day mature equivalent basis) with $20 per cwt. milk, a 60-day VWP, a market cow value of $0.55 per cwt, lactating feed cost of $0.05 per kg of dry matter, dry cow feed costs of $2.75 per day, newborn calf values of $200 for heifers and $50 for bulls, a replacement heifer cost of $1,800, labor cost of $15 per hour, a cost per A.I. service of $21, and an annual nonfeed transition management cost of $300, including the predicted cost of fresh cow disease.

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