Resynchronization Strategies

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Introduction

Many dairy farms in the U.S. have adopted systematic synchronization protocols and timed AI for submitting cows for first AI (Caraviello et al., 2006). Although reliance on synchronization of ovulation and timed AI for improving service rate to first AI reduces the impact of poor estrous detection, the increased AI submission rate to first timed AI often is followed by a time lag exceeding 60 d before cows failing to conceive are detected and reinseminated. Methods for early detection of nonpregnancy coupled with hormonal resynchronization systems that program nonpregnant cows to receive subsequent timed AI services are now being developed and assessed so that systematic reproductive management programs can be implemented to aggressively manage reproduction. Although studies have been conducted to resynchronize behavioral estrus among groups of previously inseminated cows (Chenault et al., 2003), the objective of this paper is to overview strategies for resynchronization of ovulation that allow for timed AI of cows failing to conceive to a prior AI service.

Optimization of Resynch Protocols

Because fertility to Resynch is poor for cows lacking a CL at the first GnRH or $PGF_{2\alpha}$ treatments of a Resynch protocol compared with those cows with a CL at these times (Fricke et al., 2003), alternative treatments aimed at increasing fertility of cows based on their stage of the cycle at initiation of Resynch may further improve an overall resychronization strategy. One strategy to optimize fertility to Resynch and timed AI has been to determine the optimal interval after timed AI to initiate Resynch based on assumptions regarding the physiology of the estrous cycle (Fricke et al., 2003; Sterry et al., 2006). Assuming an estrous cycle duration of 21 to 23 d, initiation of Resynch 32 to 33 d after timed AI should ensure that the first GnRH injection of Resynch occurs between Day 5 to 12 of the estrous cycle, a stage of the cycle when a CL should be present and that results in greater fertility when Ovsynch is initiated (Vasconcelos et al., 1999). Despite this logic, 16% to 22% of cows lack a CL 33 d after timed AI (Fricke et al., 2003; Sterry et al., 2006) suggesting that there is significant variation among a group of cows at various times after synchronization for first timed AI.

Luteal Dynamics after Timed AI

Variation in return to estrus in groups of cows submitted to AI can be readily observed in herds that use activity-monitoring systems that detect increases in physical activity associated with estrous behavior in dairy cows. Many farms that use activity-monitoring systems for this purpose are surprised that some cows that do not return to estrus after AI are diagnosed not pregnant at a subsequent pregnancy check by their veterinarian. Estrous cycle duration varies widely among individual cows and can range from 18 to 32 days rather than the expected 18 to 24 days (Remnant et al., 2015). Why some nonpregnant cows fail to return to estrus after AI is an interesting question indeed.

We conducted a study to evaluate progesterone concentrations and growth of the corpus luteum (**CL**) from 4 to 32 days after a timed AI to answer this question (Ricci et al., 2017). A total of 141 lactating Holstein cows were submitted to a Double-Ovsynch protocol for first timed AI, but four of these cows failed to synchronize and were removed from the study. Blood samples were collected three times a week (MWF) from 4 to 32 days after timed AI for analysis of blood progesterone and, at each blood sample collection, the ovaries of each cow were evaluated using ultrasound to determine the size of the CL.

Overall, 57 cows were diagnosed pregnant 32 days after timed AI and, as expected, progesterone and of the size of their CL increased from 4 to 15 days and then remained constant until 32 days after TAI.

For the 80 cows diagnosed open 32 days after timed AI, cows were grouped based on the day after timed AI that progesterone decreased to very low levels (Figure 1). This resulted in the following 5 groups of cows (Figure 1):

1) CL regression 15 days after timed AI (only one cow)
2) CL regression 18 to 22 days after timed AI (55.0%)
3) CL regression 25 to 27 days after timed AI (17.5%)
4) CL regression 29 to 32 days after timed AI (5.0%)
5) CL maintained until the pregnancy check 32 days after timed AI (21.3%).

Thus, only about half (55%) of the nonpregnant cows underwent CL regression at the expected time of luteolysis, whereas the remaining cows either had an extended luteal phase (23.7%) or never underwent luteal regression (21.3%) by the time of the pregnancy check.

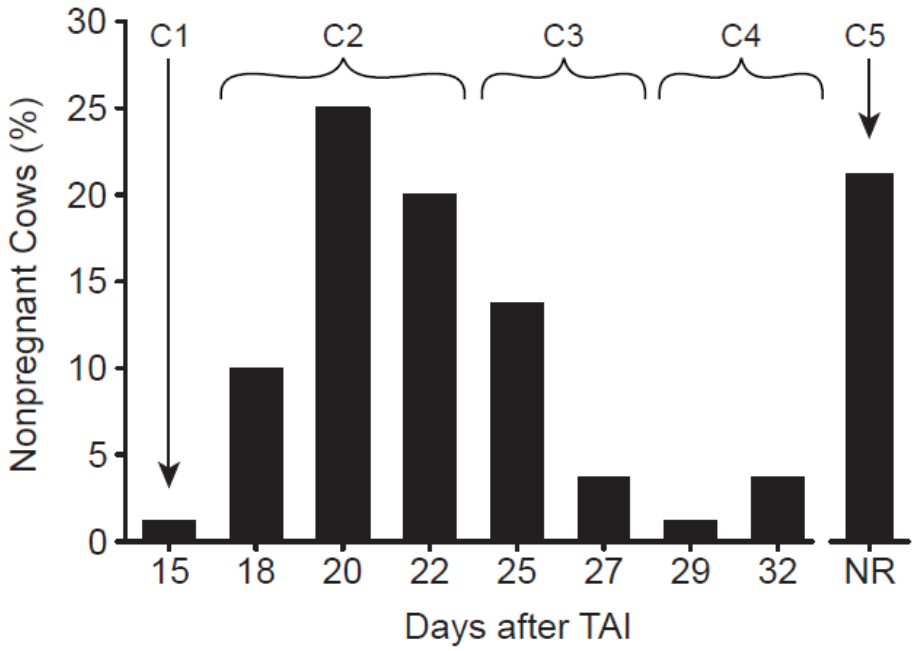

Figure 1. Day of CL regression for cows diagnosed nonpregnant 32 d after TAI. Cows were submitted to a Double-Ovsynch protocol for synchronization of ovulation for first timed artificial insemination. Day of CL regression was determined based on the day P4 first decreased to < 1.0 ng/mL. Cows were segregated into 5 Clusters (C1 – 5) based on a statistical Cluster analysis of the day of CL regression. NR = cows that did not regress their original CL by 32 d after TAI. Adapted from Ricci et al., 2017.

Results from Ricci et al. (2017) support that 21.3% of nonpregnant cows maintained their original CL until 32 days after timed AI. Thus, about 1 in 5 inseminated cows would not be expected to return to estrus within 32 d after insemination despite the most aggressive estrous detection. A phenomenon referred to as the “Phantom Cow Syndrome” occurs in seasonally calving herds in Australia and New Zealand in which cows inseminated on the mating start date fail to be detected in estrus within 24 d after AI and are subsequently diagnosed not pregnant 35 d after AI (Cavalieri et al., 2003).

To explain the extended luteal phases observed in our study, over half of the cows that failed to regress their CL by 32 days after timed AI had measurable pregnancy-associated glycoprotein (**PAG**) levels (a

blood pregnancy marker) indicating that they were initially pregnant but lost the pregnancy. Data from Ricci et al. (2017) in which P4 profiles and luteal dynamics were defined from 4 to 32 days after timed AI support that implementation of a Resynch protocol based on whether or not a nonpregnant cow has a CL is a better strategy for increasing fertility to timed AI than basing initiation of a Resynch protocol on a specific day after AI due to variability in CL regression among groups of cows.

Presence or Absence of a CL at G1 and Fertility to Resynch

Whereas presynchronization strategies have yielded significant increases in fertility to first timed AI, many herds struggle with poor fertility to an Ovsynch protocol used for timed AI at second and greater services (i.e., **Resynch**). Based on progesterone profiles during the Ovsynch protocol, the best indicator of poor fertility to timed AI is low progesterone (i.e., cows lacking a CL) at the $PGF_{2\alpha}$ treatment. In several studies, 16%, 22%, and 35% of cows diagnosed not pregnant 32 days after timed AI and that did not receive a GnRH treatment 7 days before pregnancy diagnosis lacked a CL at G1 (Fricke et al., 2003; Giordano et al., 2015). Thus, G1 occurs in a low-progesterone environment in up to one-third of nonpregnant cows submitted to a Resynch protocol which leads to a lack of complete luteal regression after treatment with $PGF_{2\alpha}$ 7 d later and low fertility to timed AI as described for first timed AI.

One strategy to treat nonpregnant cows without a CL at G1 is to supplement with exogenous progesterone during the Resynch protocol. Treatment of cows without a CL at G1 of an Ovsynch protocol with exogenous progesterone (i.e., a CIDR insert) for 7 days increased fertility at first as well as resynch timed AI (Chebel et al., 2010; Bilby et al., 2013; Bisinotto et al., 2015). Based on these data, many veterinarians now use the presence or absence of a CL at a nonpregnancy diagnosis to implement a strategy to attempt to increase fertility to Resynch timed AI or to increase the proportion of cows inseminated to a detected estrus after AI.

A Second $PGF_{2\alpha}$ Treatment before G2 Increases Fertility to Resynch

Many cows submitted to a Resynch protocol have poor fertility to timed AI because they initiate G1 in a low-progesterone environment resulting in lack of complete luteal regression at the end of the protocol. Based on an analysis of data from an experiment in which cows were resynchronized using a Double-Ovsynch protocol (Giordano et al., 2012), we classified cows based on the age and number of CL present at the $PGF_{2\alpha}$ treatment of an Ovsynch protocol and assessed the rate of complete luteal regression. Overall, 97% of cows bearing a single CL ~13 d of age underwent complete luteal regression, and 92% of cows bearing a CL ~13 d of age and a CL ~6 d of age underwent complete luteal regression. By contrast, only 64% of cows bearing a single CL ~6 d of age underwent complete luteal regression. Cows in which G1 occurs in a low progesterone environment (whether anovular or cycling and lacking a CL) have a high ovulatory response to G1 resulting in a single CL ~6 d of age at the $PGF_{2\alpha}$ treatment of the Ovsynch protocol. Approximately one-third of these cows fail to fully regress this young CL resulting in slightly increased progesterone levels at G2 which dramatically decreases P/AI after timed AI.

Similar to first timed AI, a greater proportion of cows receiving 1 $PGF_{2\alpha}$ treatment had incomplete luteal regression (≥ 0.4 ng/mL) than cows receiving 2 $PGF_{2\alpha}$ treatments during a Resynch protocol, regardless of progesterone concentrations at G1 (Carvalho et al., 2015). For cows with low (< 1.0 ng/mL) progesterone concentrations at G1, cows receiving 2 $PGF_{2\alpha}$ treatments had greater fertility to TAI than cows receiving 1 $PGF_{2\alpha}$ treatment, whereas for cows with high (≥ 1.0 ng/mL) progesterone concentrations at G1, fertility did not differ between cows receiving 1 vs. 2 $PGF_{2\alpha}$ treatments (Carvalho et al., 2015). Similar to protocols for first timed AI, addition of a second $PGF_{2\alpha}$ treatment 24 h after the first $PGF_{2\alpha}$ treatment in an Ovsynch protocol increases fertility to Resynch timed AI. The magnitude of the increase in P/AI, however, is greater for Resynch timed AI because more cows submitted to a Resynch protocol have low progesterone at G1. Taken together, these data support that presynchronization strategies before G1 of a Resynch protocol combined with differential treatment of

cows diagnosed not pregnant based on the presence or absence of a CL, and inclusion of a second $PGF_{2\alpha}$ treatment before G2 of a Resynch protocol can yield high fertility to timed AI.

Achieving a 40% 21-d Pregnancy Rate in a 30,000 lb. Dairy Herd

In 2014, we implemented an aggressive reproductive management system for first and Resynch timed AI based on the concepts presented in this review to manage the Allenstein Dairy Teaching Herd, which consists of approximately 550 Holstein cows located at the Emmons Blaine Dairy Cattle Research Center in Arlington, WI. This facility is one of three locations constituting the Integrated Dairy Facilities that serves the research, teaching, and outreach needs of the Department of Dairy Science and the School of Veterinary Medicine at the University of Wisconsin-Madison. Cows are milked twice daily and are fed a TMR that meets or exceeds NRC requirements for high-producing dairy cows. Only 23% of the cows at this location are primiparous because 100 primiparous cows are housed at the Marshfield Agricultural Research Station. Multiparous cows are treated with bST as per label recommendation, whereas primiparous cows do not receive bST. Average daily milk production is 98 lbs. and average ME305 for the cows at this location is 31,116 lbs.

First Timed AI. All cows are submitted for first TAI between 76 and 82 DIM after a Double-Ovsynch protocol as described by Souza et al. (2008). The Breeding-Ovsynch is conducted as an Ovsynch-56 protocol as described by Brusveen et al. (2009) with the addition of a second $PGF_{2\alpha}$ treatment 24 hours after the first $PGF_{2\alpha}$ treatment.

Resynch Timed AI. All cows are treated with GnRH 25 days after TAI. Pregnancy diagnosis is conducted using transrectal ultrasonography 32 days after TAI, and cows diagnosed not pregnant are classified as having or lacking a CL > 10 mm in diameter. Nonpregnant cows with a CL continue an Ovsynch-56 protocol by receiving a $PGF_{2\alpha}$ treatment 32 days after TAI with the addition a second $PGF_{2\alpha}$ treatment 24 hours after the first $PGF_{2\alpha}$ treatment. Nonpregnant cows lacking a CL restart an Ovsynch-56 protocol that includes a second $PGF_{2\alpha}$ treatment 24 hours after the first (i.e., GGPPG) as described by Carvalho et al. (2015). Intravaginal P4 inserts are included within the Ovsynch protocol for cows lacking a CL based on studies in which exogenous P4 increased P/AI for cows lacking a CL at initiation of an Ovsynch protocol to that of cows with a CL at initiation of an Ovsynch protocol (Bilby et al., 2013; Bisinotto et al., 2015).

Reproductive Performance. During the previous one-year period (September 2016 to September 2017), the adjusted 21-d pregnancy rate (based on a 76 day VWP) in the University of Wisconsin-Madison Arlington dairy herd averaged 41%. The 21-d service risk averaged 70%, and overall conception risk averaged 59% (n = 1,093). Conception risk to first timed AI averaged 57% (n = 546), conception risk to second timed AI averaged 64% (n = 268), and conception risk to third timed AI averaged 58% (n = 130). The first three timed AI occur from 76 to 170 DIM, and 92% of cows are pregnant after the first three timed AI.

Conclusion

This intensive reproductive management protocol based on the concepts presented in this review has resulted in reproductive performance that is unprecedented for a herd of high-producing Holstein dairy cows. This strategy is not the only way to resynchronize nonpregnant cows. Recent work supports a strategy in which rather than setting up all cows with GnRH before a nonpregnancy diagnosis, nonpregnant cows are simply segregated based on presence or absence of a CL (Wijma et al., 2017, 2018). Nonpregnant cows with a CL receive PGF and finish the remainder of an Ovsynch protocol, whereas nonpregnant cows lacking a CL restart an Ovsynch protocol with inclusion of a CIDR insert.

Although use of an ideal fertility program is important for achieving a high 21-day pregnancy rate, cows must be healthy to achieve high fertility. Many cow health factors have been reported to decrease P/AI to TAI including the incidence of mastitis between TAI and the first pregnancy diagnosis (Fuenzalida et

al., 2015), a decrease in body condition score during the first 21 days after calving (Carvalho et al., 2014), and poor uterine health (Lima et al., 2013).

References

Bilby, T. R., R. G. S. Bruno, K. J. Lager, R. C. Chebel, J. G. N. Moraes, P. M. Fricke, G. Lopes, Jr., J. O. Giordano, J. E. P. Santos, F. S. Lima, S. L. Pulley, and J. S. Stevenson. 2013. Supplemental progesterone and timing of resynchronization on pregnancy outcomes in lactating dairy cows. J. Dairy Sci. 96:7032-7042.

Bisinotto, R. S., L. O. Castro, M. B. Pansani, C. D. Narciso, N. Martinez, L. D. P. Sinedino, T. L. C. Pinto, N. S. Van de Burgwal , H. M. Bosman, R. S. Surjus, W. W. Thatcher, and J. E. P. Santos. 2015. Progesterone supplementation to lactating dairy cows without a corpus luteum at initiation of the Ovsynch protocol J. Dairy Sci. 98:2515-2528.

Brusveen, D. J., A. H. Souza, and M. C. Wiltbank. 2009. Effects of additional prostaglandin F2α and estradiol-17β during Ovsynch in lactating dairy cows. J. Dairy Sci. 92:1412-1422.

Caraviello, D. Z., K. A. Weigel, P. M. Fricke, M. C. Wiltbank, M. J. Florent, N. B. Cook, K. V. Nordlund, N. R. Zwald, and C. L. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. J Dairy Sci 89(12):4723-4735.

Carvalho, P. D., A. H. Souza, M. C. Amundson, K. S. Hackbart, M. J. Fuenzalida, M. M. Herlihy, H. Ayres, A. R. Dresch, L. M. Vieira, J. N. Guenther, P. M. Fricke, R. D. Shaver, and M. C. Wiltbank. 2014a. Relationships between fertility and postpartum changes in body condition and body weight in lactating dairy cows. J. Dairy Sci. 97:3666-3683.

Carvalho, P. D., M. J. Fuenzalida, A. Ricci, A. H. Souza, R. V. Barletta, M. C. Wiltbank, and P. M. Fricke. 2015. Modifications to Ovsynch improve fertility during resynchronization: Evaluation of presynchronization with GnRH 6 days before Ovsynch and addition of a second prostaglandin F2α treatment. J. Dairy Sci. 98:8741-8752.

Cavalieri, J., J. Morton, D. P. Nation, G. Hepworth, S. Pino, A. R. Rabiee, and K. L. Macmillan. 2003. Phantom cows: predisposing factors,causes and treatment strategies that have been attempted to reduce the prevalence within herds. Proc. Society of Dairy Cattle Veterinarians of the NZVA Annual Conference.

Chebel, R. C., M. J. Al-Hassan, P. M. Fricke, J. E. P. Santos, J. R. Lima, C. A. Martel, J. S. Stevenson, R. Garcia, and R. L. Ax. 2010. Supplementation of progesterone via internal drug release inserts during ovulation synchronization protocols in lactating dairy cows. J. Dairy Sci. 93:922-931.

Chenault, J. R., J. F. Boucher, K. J. Dame, J. A. Meyer, and S. L. Wood-Follis. 2003. Intravaginal progesterone insert to synchronize return to estrus of previously inseminated daory cows. J. Dairy Sci. 86:2039-2049.

Fricke, P. M., D. Z. Caraviello, K. A. Weigel, and M. L. Welle. 2003. Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. J. Dairy Sci. 86:3941-3950.

Fuenzalida, M. J., P. M. Fricke, and P. L. Ruegg. 2015. The association between occurrence and severity of subclinical and clinical mastitis on pregnancies per artificial insemination at first service of Holstein cows. J. Dairy Sci. 98:3791-3805.

Giordano, J. O., M. C. Wiltbank, J. N. Guenther, R. Pawlisch, S. Bas, A. P. Cunha, and P. M. Fricke. 2012. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch when compared to Ovsynch initiated 32 d after timed artificial insemination. J. Dairy Sci. 95:639-653.

Giordano, J. O., M. L. Stangaferro, R. Wijma, W. C. Chandler, and R. D. Watters. 2015. Reproductive performance of dairy cows managed with a program aimed at increasing insemination of cows in estrus based on increased physical activity and fertility of timed artificial inseminations. J. Dairy Sci. 98:2488-2501.

Lima, F. S., R. S. Bisinotto, E. S. Ribeiro, L. F. Greco, H. Ayres, M. G. Favoreto, M. R. Carvalho, K. N. Galvão, and J.E.P Santos. 2013. Effects of 1 or 2 treatments with prostaglandin F2α on subclinical endometritis and fertility in lactating dairy cows inseminated by timed artificial insemination. J. Dairy Sci. 96: 6480–6488.

Remnant, J. G., M. J. Green, J. N. Huxley, and C. D. Hudson. 2015. Variation in the interservice intervals of dairy cows in the United Kingdom. J. Dairy Sci. 98(2):889-897.

Ricci, A., P. D. Carvalho, M. C. Amundson, and P. M. Fricke. 2017. Characterization of luteal dynamics in lactating Holstein cows for 32 days after synchronization of ovulation and timed artificial insemination. J. Dairy Sci. 100:9851-9860.

Souza, A. H., H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 70:208-215.

Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Effect of interval from timed artificial insemination to initiation of resynchronization of ovulation on fertility of lactating dairy cows. J. Dairy Sci. 89:2099-2109.

Vasconcelos, J.L.M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067-1078.

Wijma, R., M. L. Stangaferro, M. Masello, G. E. Granados, and J. O. Giordano. 2017. Resynchronization of ovulation protocols for dairy cows including or not including gonadotropin-releasing hormone to induce a new follicular wave: Effects on re-insemination pattern, ovarian responses, and pregnancy outcomes. J. Dairy Sci. 100:7613-7625.

Wijma, R. M. M. Pérez, M. Masello, M. L. Stangaferro, and J. O. Giordano. 2018. A resynchronization of ovulation program based on ovarian structures present at nonpregnancy diagnosis reduced time to pregnancy in lactating dairy cows. J. Dairy Sci. (in press).