Timed AI in Context: Fitting Reproductive Programs to Cows' Physiological Needs

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Take Home Messages

- Protocols for synchronization of ovulation and timed artificial insemination (AI) allow for systematic control of reproduction and provide a unique platform to assess cows' endocrine milieu around the time of insemination.
- Optimal reproductive performance based on pregnancy rate and profitability is observed in herds that use a combination of estrus detection and timed AI protocols.
- Estrus detection efficiency and accuracy as well as compliance to synchronization protocols are vital for reproductive performance and economic success of dairy herds.
- Strategies to identify females that fail to respond to synchronization protocols based on ultrasonography and milk progesterone profiling implemented cow side can be used to diverge low-fertility cohorts to alternate fertility programs.
- Progesterone concentrations in plasma above 2 ng/ml during growth of the ovulatory follicle increase pregnancy per AI. Targeted progesterone supplementation improves pregnancy outcomes in cows without a corpus luteum (CL) at the initiation of the timed AI protocol.

Introduction

Reproductive efficiency is a key component of a dairy herd's sustainability because it controls average milk yield, culling decisions, and availability of replacement heifers. In fact, simulation models consistently describe pregnancy rate as being positively associated with the economic success of dairy farms (Giordano et al., 2012; Galvão et al., 2013). Programs for synchronization of ovulation and timed artificial insemination (AI) allow for systematic control of reproduction, which has disseminated its use worldwide.

The use of timed AI is particularly attractive in situations where estrus detection is inadequate (Tenhagen et al., 2004). Assuming compliance with hormonal treatments and proper estrus detection practices, a combination of timed AI and estrus detection results in the greatest reproductive efficiency and ensuing profitability (Giordano et al., 2012, Galvão et al., 2013).

Besides increasing insemination rate by controlling time to first AI postpartum and the interval between subsequent services, synchronization programs provide a unique platform for evaluation of cows' physiological status around the time of insemination. Use of ultrasonography and assessment of hormonal profiles at key time points allow for identification of cows that fail to respond to the synchronization protocol and those that benefit from alternative fertility programs, such as targeted progesterone supplementation. In particular, for a dairy industry under constant public scrutiny over the use of hormonal therapies and blanket treatments, the future of reproductive management is likely to benefit from the population approach of timed AI associated with an individual approach based on objective measures of a cow's' physiological state.

▪ Role and Benefits of Timed AI

The development of novel technology for detection of estrus and the current state involving the use of hormonal therapy in dairy cattle have fostered discussions about future strategies to manage reproduction in the absence of timed AI. The main objective of this section is not to expand on this debate, but rather to provide some insights on potential consequences of such change.

Based on previously published work and on-farm data, Galvão et al. (2013) estimated productive and financial outputs of various strategies to manage reproduction in lactating dairy cows (Table 1). As expected, reproductive performance and profitability in herds that only inseminated cows in estrus rely heavily on efficiency (i.e., proportion of estrus events detected) and accuracy of detection (i.e., proportion of estrus correctly detected). Likewise, advantages from management systems that use only estrus detection compared with programs that use timed AI only also rely on estrus detection rate and accuracy. The results from Galvão et al. (2013) agree with those from Giordano et al. (2012) in that poorly executed estrus detection used concurrently with timed AI resulted in financial losses ranging between US$7.9 and 17.4/cow/year. Both studies also agree that the greatest reproductive performance and profitability are obtained when well-executed estrus detection and timed AI are used in unison, which streams from increased income over feed costs and reduced replacement costs. Peer-reviewed randomized trials comparing automated heat detection devices with commonly used estrus detection techniques (e.g., daily tail chalking or painting) remain sparse and additional data will be key to refine the expectations of how technology will help reshape reproductive management of high-producing dairy cows.

Table 1. Financial outputs of a stochastic dynamic Monte-Carlo simulation model comparing reproductive programs for lactating dairy cows based on estrus detection and timed AI (adapted from Galvão et al., 2013).

Item	ED_{40-85}[1]	ED_{60-95}[2]	TAI_{95}[3]	TAI_{95} ED_{60-95}[4]
21-day pregnancy rate, %	10.9[d]	16.1[b]	15.5[c]	26.8[a]
Culling, %	40.3[c]	33.0[b]	32.8[b]	27.5[a]
Milk sales, US$/cow/yr.	3,450.5[a]	3,433.3[b]	3,391.2[d]	3,405.2[c]
Replacement costs, US$/cow/yr.	645.2[c]	528.4[b]	525.3[b]	439.2[a]
Breeding costs, US$/cow/yr.	91.1[a]	94.1[c]	91.6[b]	123.0[d]
Feeding costs, US$/cow/yr.	1,801.9[d]	1,782.0[c]	1,773.1[b]	1,766.3[a]
Profit, US$/cow/year	254.6[d]	348.3[b]	315.5[c]	375.2[a]

Simulations considered milk prices at US$0.33/kg (US$15.00/CWT).
[1] Use of estrus detection only with 40% efficiency and 85% accuracy.
[2] Use of estrus detection only with 60% efficiency and 95% accuracy.
[3] Use of timed AI only with 95% compliance with synchronization protocols.
[4] Concurrent use of estrus detection (60% efficiency and 95% accuracy) and timed AI (95% compliance).
[a-d] Values with different superscripts differ (P < 0.05). Superscripts ordered in alphabetical sequence from best to worst.

The impact of eliminating hormonal therapy from reproductive management was assessed in two randomized clinical trials (M. Liebenstein, personal communication). In the first study, cows were inseminated in estrus based on removal of tail paint either with no intervention (No Synch; n = 351) or following treatment with $PGF_{2\alpha}$ every 14 days for cows not yet inseminated and at non-pregnancy diagnosis (PGF + ED; n = 349). Voluntary waiting period was 30 days in milk (DIM) and only AI following spontaneous estrus was performed in both groups until 48 DIM. Restricting the use of $PGF_{2\alpha}$ delayed first AI postpartum (62 vs. 56 DIM), increased the proportion of cows not inseminated by 91 DIM (15.6 vs. 3.3%), and decreased 21-day insemination rate during the first five cycles after the voluntary waiting period (64.6 vs. 75.4%). However, such decrease in insemination rate was insufficient to significantly reduce 21-day pregnancy rate, which was 23.8% for No Synch and 26.6% for PGF + ED. Figure 1A illustrates the hazard of pregnancy over time for the two experimental groups. In the second study, cows were inseminated in estrus based on removal of tail paint without the use of exogenous hormones (No Synch; n = 510) or with a combination of estrus detection and timed AI (TAI + ED; n = 505). Voluntary waiting period was 30 DIM and only AI following spontaneous estrus was performed in both groups until 48 DIM. Restricting the use of timed AI delayed first AI postpartum (65 vs. 56 DIM) and increased the proportion of cows not inseminated by 91 DIM (17.3 vs. 2.5%). Moreover, cows not eligible to receive timed AI had a lower 21-day insemination rate (67.5 vs. 76.0%) and 21-day pregnancy rate (23.7 vs. 29.6%) during the first five cycles after the voluntary waiting period. Hazard of pregnancy between 48 and 250 DIM was 28.2% lower for cows not eligible to receive hormonal therapy (Figure 1B), which resulted in smaller proportions of cows pregnant at 150 (77.9 vs. 83.7%) and at 250 DIM (85.3 vs 90.3%) and increased the risk of culling by 250 DIM (13.9 vs. 9.3%). Financial simulations yielded that yearly operating income per cow in No Sync systems was reduced by US$53 compared with PGF + ED and by US$75 compared with TAI + ED.

▪ Managing Cows That do Not Respond to Synchronization

Fertility responses following timed AI depend on adequate response to synchronization protocols. For synchronization programs commonly used in North America, pregnancy per AI (P/AI) is greater in cows that ovulate in response to the first GnRH injection, bear a functional corpus luteum (CL) at $PGF_{2α}$ injection five to sevem days later, undergo complete luteolysis following treatment with $PGF_{2α}$, and ovulate eight to 16 hours after insemination (Pursley et al., 1998; Stevenson et al., 2008; Bisinotto and Santos, 2012). It is important to highlight that adequate response begins with compliance to the schedule of injections. Galvão et al. (2013) estimated that reducing the proportion of correct treatments from 95 to 85% reduces P/AI by 30 to 32% and 21-day pregnancy rate by 36%. Such reduction in reproductive performance led to a decrease in yearly net profit per cow between US$76 and 78 depending on milk prices.

Despite major advances in timed AI programs and development of presynchronization strategies (Moreira et al., 2001, Souza et al., 2008), work from multiple research groups has defined low-fertility cohorts of cows that fail to respond to key hormonal treatments. For instance, from 10 to 20% of cows subjected to GnRH/$PGF_{2α}$-based protocols do not have a CL at the injection of $PGF_{2α}$ (Bisinotto et al., 2013; Giordano et al., 2016). This cohort encompasses cows without a CL that do not ovulate in response to the first GnRH injection and cows that undergo spontaneous luteolysis before the injection of $PGF_{2α}$ (Stevenson et al., 2008, Bisinotto et al., 2013). This is particularly important because cows that are at a greater risk of premature luteolysis are also more likely to ovulate before scheduled timed AI, which generates an asynchrony between sperm and oocyte availability (Vasconcelos et al., 1999).

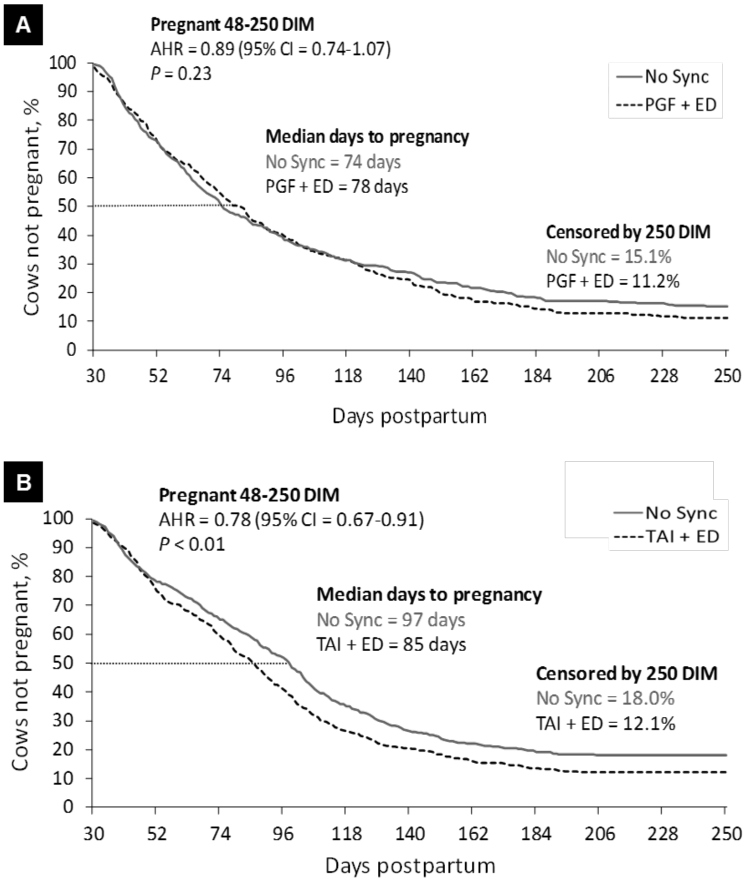

Figure 1. Time to pregnancy for cows inseminated following estrus detection and not treated with exogenous reproductive hormones (No Sync) compared with cows exposed to estrus detection and eligible for treatment with $PGF_{2\alpha}$ (PGF + ED; panel A) and cows exposed to estrus detection and eligible to receive a GnRH/$PGF_{2\alpha}$-based timed AI protocol (TAI + ED; panel B). AHR = adjusted hazard ratio, which measures the change in rate at which No Synch cows became pregnant compared with PGF + ED (hazard of pregnancy was 12.4% smaller for No Synch, *P* = 0.23) or TAI + ED (hazard of pregnancy was 28.2% smaller for No Synch, *P* < 0.01); CI = confidence interval.

Cows without a CL at the injection of $PGF_{2\alpha}$ can be identified using ultrasonography with reasonable sensitivity, specificity, and reliability among different technicians. In agreement with the studies presented above, absence of a CL at $PGF_{2\alpha}$ is a useful predictor of reduced fertility following timed AI. Pregnancy risk in cows lacking a CL and those with a CL smaller than 10 mm in diameter at $PGF_{2\alpha}$ was 3.1-fold smaller (10.3 vs. 33.2%) compared with pregnancy risk in herdmates with a CL larger than this threshold (Giordano et al., 2016). Cows without a CL at $PGF_{2\alpha}$ represented 18.9% of the total enrolled in a resynchronization protocol and identification of this low-fertility cohort allows for the implementation of targeted fertility programs. In the same study, cows received the initial GnRH injection seven days before non-pregnancy diagnosis and those without a CL at $PGF_{2\alpha}$ were randomized into one of two treatments. Cows without a CL were either re-enrolled in the Ovsynch protocol (i.e., GnRH, seven days later $PGF_{2\alpha}$, 56 hours later GnRH, 16 hours later timed AI) and treated with an intravaginal insert for controlled release of progesterone from the first GnRH to $PGF_{2\alpha}$ or received an injection of GnRH followed by the Ovsynch protocol starting seven days later. Both strategies improved P/AI, which was similar to that observed in cows bearing a CL at $PGF_{2\alpha}$. In fact, using information on ovarian structures for allocation of cows to alternate resynchronization strategies reduced overall time to pregnancy by 12 days (Wijma et al., 2018).

As described above, identification of cows without a CL at the $PGF_{2\alpha}$ injection of resynchronization and implementation of alternative protocols can be executed somewhat easily in herds that use ultrasonography for non-pregnancy diagnosis (Giordano et al., 2016; Wijma et al., 2018). However, synchronization protocols for first insemination postpartum are not routinely associated with ultrasonographic assessment of the reproductive tract and application of a similar approach would result in additional animal handling. Likewise, herds wherein non-pregnancy diagnosis is based on transrectal palpation or laboratory assays (i.e., pregnancy-associated glycoproteins and pregnancy-specific protein B), reliable identification of cows without a CL at $PGF_{2\alpha}$ requires additional animal handling and equipment, and trained technicians. In such cases, progesterone profiling becomes an interesting alternative to identify cows that fail to respond to the synchronization protocol (Wilsdorf et al., 2016). Cows with progesterone concentrations in plasma ≤ 0.51-0.54 ng/ml at the injection of $PGF_{2\alpha}$ had fewer P/AI compared with herdmates with concentrations above this threshold (12.8 vs. 49.5%). Similar to the data from Giordano et al. (2016), 20.7% of cows were classified as not having a functional CL at $PGF_{2\alpha}$. Progesterone profiling was also evaluated on the day of timed AI and seven days after insemination (Wilsdorf et al., 2016). Pregnancy per AI was lower in cows with elevated concentrations of progesterone at AI (≥ 0.43-0.54 ng/ml; 11.1 vs. 49.5%) and those with small concentrations seven days later (≤ 1.43-1.84 ng/ml; 13.2 vs. 50.2%). Of particular importance, low-fertility cohorts detected at $PGF_{2\alpha}$ or insemination could be resynchronized before timed AI, thus reducing costs associated with semen and labour.

Although the potential benefits of applying plasma progesterone profiling to manage timed AI has been demonstrated (Wilsdorf et al., 2016), cow-side tests for evaluation of progesterone concentrations in blood are not currently available. On the other hand, the use of lateral flow immunochromatographic tests and parlour in-line progesterone analysis systems for assessment of progesterone concentrations in milk have the potential to generate timely information that can be incorporated into routine decision-making (Waldmann and Raud, 2016; Bruinje et al., 2018). Despite limited sensitivity (0.58), the use of lateral flow immunochromatographic tests for identification of cows without a functional CL yielded elevated specificity (0.93) and positive predictive value (0.91; B.O. Omontese, personal communication). In the same study, 26.4% of cows subjected to a 5-day Cosynch protocol were classified as not having a functional CL at the injection of $PGF_{2\alpha}$ and had fewer P/AI compared with herdmates with elevated milk progesterone concentrations (17.4 vs. 37.5%). In addition, 11.6% of cows did not undergo complete luteolysis prior to timed AI based on milk progesterone profiling and had fewer P/AI compared with cows without a functional CL at insemination (13.4 vs. 34.8%).

Financial benefits from identifying cows that fail to respond to the synchronization program were estimated based on individual cow value at 240 DIM (Cabrera, 2012). When simulating a scenario wherein timed AI is used together with estrus detection, re-enrolling cows without a CL at $PGF_{2\alpha}$ in the Cosynch program increased yearly profit per cow by US\$7.16 (1st lactation), 13.28 (2nd lactation), and 11.65 (3rd lactation) compared with no testing (B.O. Omontese, personal communication). Expected benefits were larger for herds that use timed AI exclusively (1st lactation = US\$10.24; 2nd lactation = US\$22.70; 3rd lactation = US\$21.37). Testing cows on the day of AI resulted in smaller financial gains both in system with (1st lactation = US\$ 2.82; 2nd lactation = US\$3.20; 3rd lactation = US\$1.98) and without estrus detection (1st lactation = US\$0.36; 2nd lactation = US\$3.67; 3rd lactation = US\$2.11).

▪ Strategies to Supplement Progesterone to Dairy Cows

Early studies in lactating cows already recognized the importance of elevated concentrations of progesterone in blood preceding estrus and AI (Fonseca et al., 1983; Meisterling and Dailey, 1987). The exact mechanism that underlies reduced fertility in cows without a functional CL during development of the ovulatory follicle has not been entirely defined (Santos et al., 2016). However, insufficient progesterone concentrations before ovulation have been associated with reduced early embryonic survival and upregulation of luteolytic machinery early during the subsequent estrous cycle (Cerri et al., 2011a,b; Rivera et al., 2011). Although the importance of progesterone during growth of the ovulatory follicle is supported by a large body of literature (Wiltbank et al., 2014), effectiveness of supplementing progesterone to lactating dairy cows varies according to herd- and individual-related factors.

The primary cohort of cows expected to benefit from supplemental progesterone are those that do not bear a functional CL during the development of the ovulatory follicle, which includes anovular and cyclic females that ovulate the dominant follicle from the first wave (Bisinotto et al., 2010). In the context of synchronization programs, this low-fertility cohort can be identified via a single ultrasound examination of the ovaries at the first GnRH injection (Bisinotto et al., 2013; Bisinotto et al., 2015a). Approximately one third of cows enrolled in timed AI programs lack a CL when the synchronization protocol is initiated and proceed to have fewer P/AI compared with counterparts with a CL (Bisinotto et al., 2013, Bisinotto et al., 2015a). Commercially available intravaginal inserts increase progesterone concentrations in plasma of lactating dairy cows by approximately 0.8–1.0 ng/ml (Cerri et al., 2009; Lima et al., 2009), which is less than typically observed in cows with a mature CL during diestrus. Increasing the dose of supplemental progesterone in lactating dairy cows without a CL by using two intravaginal inserts concurrently reestablished P/AI similar to that observed in cows with a CL when the synchronization protocol was initiated. In fact, cows without a CL treated with supplemental progesterone via two intravaginal inserts in which concentrations in plasma failed to reach 2 ng/ml had P/AI similar to untreated controls (Bisinotto et al., 2015a). Only when circulating concentrations of progesterone were elevated above this threshold, P/AI became comparable to that observed in cows with a CL at the first GnRH.

Five to 7% of cows are observed in estrus between the first GnRH and $PGF_{2\alpha}$ injections of the synchronization protocol (Chebel et al., 2013; Bisinotto et al., 2015c). If inseminated upon detected estrus, these cows have similar P/AI compared with herdmates that receive timed AI (Chebel et al., 2013; Bisinotto et al., 2015c). Nevertheless, in management systems that rely exclusively on timed AI, cows that express signs of estrus during the synchronization protocol are still inseminated at scheduled timed AI and therefore are expected to have poor P/AI due to an asynchrony between ovulation and the timing of insemination. Although commercially available intravaginal inserts do not sustain sufficient release of progesterone to mimic the presence of a mature CL in lactating dairy cows, such level of supplementation reduces the incidence of premature estrus and improves synchrony of ovulation (Chebel et al., 2010; Colazo et al., 2013; Bisinotto et al., 2015c). For this reason, supplementing progesterone to all cows enrolled in synchronization programs using a single intravaginal insert increased P/AI by 19% in herds that use only timed AI for management of insemination, but did not yield any benefits when applied in herds that use a combination of timed AI and estrus detection (Bisinotto et al., 2015b).

Conclusions

Protocols for synchronization of ovulation and timed AI allow dairymen to manage reproduction on a proactive and systematic manner. Reproductive programs that combine efficient and accurate estrus detection with timed AI often result in increased pregnancy rates and profitability, which has popularized its implementation worldwide. Besides being an attractive tool for management of AI, synchronization protocols provide a unique platform to assess cows' endocrine milieu around the time of insemination. Assessment of ovarian structures using ultrasonography and on-farm assays for milk progesterone profiling allow for the identification of cows that fail to respond to the synchronization protocols or have insufficient progesterone concentrations to optimize P/AI. Such low-fertility cohorts can then be diverged to receive alternate fertility programs based on individual needs. These strategies benefit both from a population approach intrinsic to timed AI and an individual approach based on objective measures of a cow's physiological state.

References

Bisinotto, R.S., R.C. Chebel, and J.E.P. Santos. 2010. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. J. Dairy Sci. 93:3578-3587.

Bisinotto, R.S., and J.E.P. Santos. 2012. The use of endocrine treatments to improve pregnancy rates in cattle. Reprod. Fertil. Dev. 24:258-266.

Bisinotto, R.S., E.S. Ribeiro, F.S. Lima, N. Martinez, L.F. Greco, L.F. Barbosa, P.P. Bueno, L.F. Scagion, W.W. Thatcher, and J.E.P. Santos. 2013. Targeted progesterone supplementation improves fertility in lactating dairy cows without a corpus luteum at the initiation of the timed artificial insemination protocol. J. Dairy Sci. 96:2214-2225.

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

Bisinotto, R.S., I.J. Lean, W.W. Thatcher, and J.E.P. Santos. 2015b. Meta-analysis of progesterone supplementation during timed artificial insemination programs in dairy cows. J. Dairy Sci. 98:2472-2487.

Bisinotto, R.S., M.B. Pansani, L.O. Castro, C.D. Narciso, L.D. Sinedino, N. Martinez, P.E. Carneiro, W.W. Thatcher, and J.E.P. Santos. 2015c. Effect of progesterone supplementation on fertility responses of lactating dairy cows with corpus luteum at the initiation of the Ovsynch protocol. Theriogenology 83:257-265.

Bruinje, T.C., M.G. Colazo, E.S. Ribeiro, M. Gobikrushanth, and D.J. Ambrose. 2018. Using in-line milk progesterone data to characterize

parameters of luteal activity and their association with fertility in Holstein cows. J. Dairy Sci. (in press).
Cabrera, V.E. 2012. A simple formulation and solution to the replacement problem: a practical tool to assess the economic cow value, the value of a new pregnancy, and the cost of a pregnancy loss. J. Dairy Sci. 95:4683-4698.
Cerri, R.L.A., H.M. Rutigliano, R.G. Bruno, and J.E.P. Santos. 2009. Progesterone concentration, follicular development and induction of cyclicity in dairy cows receiving intravaginal progesterone inserts. Anim. Reprod. Sci. 110(:56-70.
Cerri, R.L.A., R.C. Chebel, F. Rivera, C.D. Narciso, R.A. Oliveira, M. Amstalden, G.M. Baez-Sandoval, L.J. Oliveira, W.W. Thatcher, and J.E.P. Santos. 2011a. Concentration of progesterone during the development of the ovulatory follicle: II. Ovarian and uterine responses. J. Dairy Sci. 94:3352-3365.
Cerri, R.L.A., R.C. Chebel, F. Rivera, C.D. Narciso, R.A. Oliveira, W.W. Thatcher, and J.E.P. Santos. 2011b. Concentration of progesterone during the development of the ovulatory follicle: I. Ovarian and embryonic responses. J. Dairy Sci. 94:3342-3351.
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 controlled internal drug release inserts during ovulation synchronization protocols in lactating dairy cows. J. Dairy Sci. 93:922-931.
Chebel, R.C., A.A. Scanavez, P.R. Silva, J.G. Moraes, L.G. Mendonça, and G. Lopes Jr. 2013. Evaluation of presynchronized resynchronization protocols for lactating dairy cows. J. Dairy Sci. 96:1009-1020.
Colazo, M.G., A. Dourey, R. Rajamahendran, and D.J. Ambrose. 2013. Progesterone supplementation before timed AI increased ovulation synchrony and pregnancy per AI, and supplementation after timed AI reduced pregnancy losses in lactating dairy cows. Theriogenology 79:833-841.
Fonseca, F.A., J.H. Britt, B.T. McDaniel, J.C. Wilk, and A.H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66:1128-1147.
Galvão, K.N., P. Federico, A. De Vries, and G.M. Schuenemann. 2013. Economic comparison of reproductive programs for dairy herds using estrus detection, timed artificial insemination, or a combination. J. Dairy Sci. 96:2681-2693.
Giordano, J.O., A.S. Kalantari, P.M. Fricke, M.C. Wiltbank, and V.E. Cabrera. 2012. A daily herd Markov-chain model to study the reproductive and economic impact of reproductive programs combining timed artificial insemination and estrus detection. J. Dairy Sci. 95:5442-5460.

Giordano, J.O., M.J. Thomas, G. Catucuamba, M.D. Curler, M. Masello, M.L. Stangaferro, and R. Wijma. 2016. Reproductive management strategies to improve the fertility of cows with a suboptimal response to resynchronization of ovulation. J. Dairy Sci. 99:2967-2978.

Lima, J.R., F.A. Rivera, C.D. Narciso, R. Oliveira, R.C. Chebel, and J.E.P. Santos. 2009. Effect of increasing amounts of supplemental progesterone in a timed artificial insemination protocol on fertility of lactating dairy cows. J. Dairy Sci. 92:5436-5446.

Meisterling, E.M., and R.A. Dailey. 1987. Use of concentrations of progesterone and estradiol-17 beta in milk in monitoring postpartum ovarian function in dairy cows. J. Dairy Sci. 70:2154-2161.

Moreira, F., C. Orlandi, C.A. Risco, R. Mattos, F. Lopes, and W.W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84:1646-1659.

Pursley, J.R., R.W. Silcox, and M.C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J. Dairy Sci. 81:2139-2144.

Rivera, F.A., L.G. Mendonça, G. Lopes, Jr., J.E.P. Santos, R.V. Perez, M. Amstalden, A. Correa-Calderon, and R.C. Chebel. 2011. Reduced progesterone concentration during growth of the first follicular wave affects embryo quality but has no effect on embryo survival post transfer in lactating dairy cows. Reproduction 141:333-342.

Santos, J.E.P., R.S. Bisinotto, and E.S. Ribeiro. 2016. Mechanisms underlying reduced fertility in anovular dairy cows. Theriogenology 86:254-262.

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.

Stevenson, J.S., D.E. Tenhouse, R.L. Krisher, G.C. Lamb, J.E. Larson, C.R. Dahlen, J.R. Pursley, N.M. Bello, P.M. Fricke, M.C. Wiltbank, D.J. Brusveen, M. Burkhart, R.S. Youngquist, and H.A. Garverick. 2008. Detection of anovulation by heatmount detectors and transrectal ultrasonography before treatment with progesterone in a timed insemination protocol. J. Dairy Sci. 91:2901-2915.

Tenhagen, B.A., M. Drillich, R. Surholt, and W. Heuwieser. 2004. Comparison of timed AI after synchronized ovulation to AI at estrus: reproductive and economic considerations. J. Dairy Sci. 87:85-94.

Vasconcelos, J.L., R.W. Silcox, G.J. 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.

Waldmann, A., and A. Raud. 2016. Comparison of a lateral flow milk progesterone test with enzyme immunoassay as an aid for reproductive status determination in cows. Vet. Rec. 178:260.

Wijma, R., M.M. Perez, 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. 101:1697-1707.

Wilsdorf, L.J., D.H. Keisler, S.E. Poock, W.R. Lamberson, R.C. Escalante, and M.C. Lucy. 2016. Short communication: Test for nonpregnancy in dairy cows based on plasma progesterone concentrations before and after timed artificial insemination. J. Dairy Sci. 99:5858-5865.

Wiltbank, M.C., A.H. Souza, P.D. Carvalho, A.P. Cunha, J.O. Giordano, P.M. Fricke, G.M. Baez, and M.G. Diskin. 2014. Physiological and practical effects of progesterone on reproduction in dairy cattle. Animal 8 Suppl. 1:70-81.