Progesterone Supplementation during Timed Artificial Insemination Programs in Dairy Cows

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TAKE HOME MESSAGES

- Incorporation of a single intravaginal insert containing progesterone to timed AI programs increases P/AI by 10% on day 60 after insemination, which was observed primarily in cows without a CL at the first GnRH injection.
- The benefit from supplemental progesterone is greater when synchrony of ovulation is expected to be low, such as in cows enrolled in the timed AI program during late diestrus and those without a CL at the $PGF_{2\alpha}$ injection.
- Progesterone supplementation improves P/AI primarily when estrus detection is not performed during the timed AI program. Herds with accurate detection of estrus in which cows have the opportunity to be inseminated before timed AI do not benefit from supplemental progesterone.
- Cows supplemented with progesterone during the timed AI program are 16% less likely to lose their pregnancies between the first and second month of gestation.
- A single ultrasound examination performed at the first GnRH injection is sufficient to identify a low-fertility cohort based on the absence of CL and increasing the dose of supplemental progesterone by using two intravaginal inserts restores P/AI in cows without CL at the first GnRH similar to that of cows in diestrus.

INTRODUCTION

Protocols for synchronization of ovulation allow for systematic control of reproduction and improve pregnancy rate in dairy herds, particularly when detection of estrus is inadequate (Tenhagen et al., 2004). Widespread use of timed artificial insemination (**AI**) in the U.S. has led to a reduction in average time to first postpartum AI and calving interval during the last decade (Norman et al., 2009). Such improvement was achieved, however, mostly by increasing insemination risk because pregnancy per AI (**P/AI**) remained stagnant during the same period. Because synchronization protocols provide a unique opportunity to manipulate the physiological processes that govern reproduction, recent research has focused on optimizing fertility in groups of individual lactating dairy cows subjected to timed AI programs.

Maintenance of adequate concentrations of progesterone throughout the synchronization protocol is critical for the success of timed AI programs. For instance, from 10 to 25% of cows enrolled in the Ovsynch protocol lack a corpus luteum (**CL**) at the injection of $PGF_{2\alpha}$ (Stevenson et al., 2008; Chebel et al., 2013; Bilby et al., 2013), which impairs synchrony of ovulation in response to the final GnRH and reduces P/AI in cows subjected to timed AI (Vasconcelos et al., 1999; Stevenson et al., 2008).

Moreover, low concentrations of progesterone before ovulation have been linked with changes in the diameter of the ovulatory follicle and follicular fluid composition (Cerri et al., 2011a; Cerri et al., 2011b), poor embryo quality on day 7 after AI (Cunha et al., 2008; Rivera et al., 2011), and reduced P/AI in dairy cows (Bisinotto et al., 2010a; Denicol et al., 2012). Extensive steroid catabolism by splanchnic tissues associated with elevated feed intake results in smaller circulating concentrations of progesterone in high-producing dairy cows compared with non-lactating heifers, which is

considered one of the causes of reduced fertility in lactating animals (Sangsritavong et al., 2002; Sartori et al., 2002; Sartori et al., 2004). Furthermore, 30% of lactating dairy cows do not have a CL at the initiation of the synchronization protocol and are exposed to suboptimal progesterone concentrations during growth of the ovulatory follicle (Stevenson et al., 2008; Bisinotto et al., 2010a).

These results support the incorporation of exogenous progesterone into GnRH+$PGF_{2\alpha}$-based timed AI protocols to increase synchrony of ovulation and enhance follicular maturation. Nevertheless, the benefits from supplemental progesterone are influenced by the cow's physiological state in addition to characteristics of the reproductive program implemented in the herd. From a decision making standpoint, understanding the factors that interfere with progesterone supplementation is critical to improve fertility in lactating dairy cows subjected to timed AI.

SUPPLEMENTING PROGESTERONE DURING TIMED AI PROGRAMS

Effect of Supplemental Progesterone on the Synchrony of Ovulation

Success of synchronization protocols relies on induction of ovulation within a narrow time frame so that cows can be inseminated at a prescheduled time. Cows that ovulate at the initiation of the timed AI program are likely to bear a CL 5 to 7 days later when $PGF_{2\alpha}$ is administered and to have a synchronous ovulation in response to the final GnRH injection (Vasconcelos et al., 1999). Ovulatory response to the first GnRH injection, however, ranges from only 60 to 70% after presynchronization protocols (Chebel et al., 2006; Galvão et al., 2007; Souza et al., 2008) and between 40 and 50% in cows subjected to resynchronization programs (Bilby et al., 2013; Bisinotto et al., 2015a). Cows that are enrolled in the timed AI protocol during mid to late diestrus have reduced ovulatory responses to the first GnRH and are at a greater risk of their CL regressing CL before the injection of $PGF_{2\alpha}$, which impairs the synchrony of ovulation around the time of insemination (Vasconcelos et al., 1999).

Use of commercially available intravaginal inserts for controlled release of progesterone increases circulating concentrations from 0.8 to 1.3 ng/mL in lactating dairy cows (Cerri et al., 2009; Bisinotto et al., 2015c). Albeit the amount of progesterone released by a single insert is arguably insufficient to affect the development of the ovulatory follicle (Dewey et al., 2010; Bisinotto et al., 2010b; Colazo et al., 2013), it inhibits estrus and prevents spontaneous LH surges during the treatment period (Rathbone et al., 2001; Bisinotto et al., 2015c). Therefore, supplementing progesterone with an intravaginal insert from the first GnRH to the $PGF_{2\alpha}$ injection of the timed AI program reduces the proportion of cows that ovulate prematurely and improves synchrony of ovulation in response to the final GnRH injection (Bisinotto et al., 2010b; Colazo et al., 2013).

Because of the aforementioned benefits, supplementation with progesterone during timed AI programs has been shown to improve P/AI when synchrony of ovulation is expected to be reduced. In a study by Stevenson et al. (2006), incorporation of an intravaginal insert containing progesterone to the Ovsynch protocol increased P/AI primarily in cows lacking a CL at the $PGF_{2\alpha}$ injection (i.e. anovular cows that did not ovulate in response to the first GnRH and estrus-cycling cows that had spontaneous luteolysis), whereas no effect was observed in cows bearing a CL. Furthermore, Bilby et al. (2013) evaluated the effect of supplementing progesterone during a resynchronization program initiated either 32 or 39 days after the previous AI when the majority of cows are anticipated to be approximately days 11 through 18 of the estrous cycle, respectively. Initiating the timed AI protocol during late diestrus increased the proportion of cows without a CL at the $PGF_{2\alpha}$ injection, which was greater for cows resynchronized on day 39 than day 32 after AI (20.6 vs. 12.1%). As expected, a treatment by day interaction was observed because supplemental progesterone increased P/AI when resynchronization was initiated on day 39 but not on day 32 after AI.

Factors that Affect the Benefits of Progesterone Supplementation during Timed AI Programs

Although previous studies indicate that progesterone concentrations in the week preceding estrus were greater in cows that conceived compared with herd mates that failed to become pregnant after AI (Folman et al., 1973; Meisterling and Dailey, 1987), progesterone supplementation has not improved P/AI in lactating dairy cows subjected to timed AI in a consistent manner. In fact, overall increases in P/AI in response to supplemental progesterone have been reported by some (El-Zarkouny et al., 2004; Stevenson et al., 2006; Bisinotto et al., 2010b; Colazo et al., 2013), but not all authors (Chebel et al., 2013; Bilby et al., 2013). The heterogeneity among studies reflects the differences in physiological and managerial conditions in which progesterone supplementation was applied in each study. Therefore, identifying the cows that benefit the most from supplemental progesterone as well as management factors that interfere with the response to supplementation is critical to optimize reproductive performance in dairy herds.

A systematic review of the literature revealed that progesterone supplementation during the timed AI protocol increased P/AI in lactating dairy cows by 10% (adjusted risk ratio = 1.10; Control = 31.5 vs. Treated = 35.3%) on day 60 after AI (Bisinotto et al., 2015b). The benefit from supplemental progesterone was observed mostly in cows without a CL at the initiation of the timed AI program (adjusted risk ratio = 1.16; Control = 27.3 vs. Treated = 33.3%; $P < 0.01$) compared with those that had a CL at the first GnRH injection (adjusted risk ratio = 1.05; Control = 35.3 vs. Treated = 37.5%; $P = 0.09$). Nevertheless, the positive effect from progesterone supplementation was observed exclusively in studies in which cows were not observed for signs of estrus during the timed AI program (Table 1). If estrus was not detected during the synchronization protocol and cows could only be inseminated at scheduled timed AI, supplementing progesterone increased P/AI by 20% on day 60 after AI. Conversely, insemination of cows in estrus before timed AI reduced the benefit of progesterone supplementation to only 4% and no difference in P/AI was observed between supplemented and control cows. The interaction between detection of estrus (AI before scheduled timed AI) and progesterone supplementation likely involves the timing of ovulation relative to timed AI. From 5 to 10% of cows are expected to display signs of estrus during the synchronization protocol (Chebel et al., 2013; Bisinotto et al., 2015a). In such cases, progesterone supplementation is expected to improve the synchrony of ovulation and increase P/AI. On the other hand, insemination of cows in estrus during the timed AI program results in satisfactory P/AI (Chebel et al., 2013) and lessens the detrimental effect of premature ovulation; thus, reducing the potential benefit of supplemental progesterone.

Table 1. Effect of progesterone supplementation during timed AI programs on P/AI in lactating dairy cows according to detection of estrus concurrently with the synchronization protocol[1]

	Without estrous detection			With estrous detection		
Item	Control	Treated	RR (95%CI)[2]	Control	Treated	RR (95%CI)
	--- % (n) ---			--- % (n) ---		
P/AI at day 32 after AI	33.9[a]	41.4[b]	1.16	36.2	37.1	1.02
	(3,192)	(3,096)	(1.09-1.24)	(5,206)	(5,189)	(0.84-1.20)
P/AI at day 60 after AI	30.6[a]	38.5[b]	1.20	32.0	33.4	1.04
	(3,190)	(3,095)	(1.10-1.29)	(5,172)	(5,153)	(0.92-1.16)

[a,b]Proportions within estrus-detection category with different superscripts differ ($P < 0.05$).
[1]The experimental design did not allow for the assessment of the main effect of estrus detection (no estrus detection vs. estrus detection).
[2]Risk ratio of the effect of progesterone supplementation compared with untreated controls (reference group) and its respective 95% confidence interval (i.e., RR >1 indicate greater P/AI in cows supplemented with progesterone during the timed AI program compared with controls).

Although presynchronization of the estrous cycle improves ovulatory response to the initial GnRH (Bisinotto and Santos, 2012) and synchrony of ovulation at insemination (El-Zarkouny et al., 2004), the benefit of supplementing progesterone during the timed AI program was not affected by the use of presynchronization protocols (Bisinotto et al., 2015b). The lack of effect of presynchronization on the benefit of supplemental progesterone might have been masked by the presence of anovular cows, which represent approximately 25% of dairy cows at the end of the voluntary waiting period (Stevenson et al., 2008; Bisinotto et al., 2010a), and which do not respond to the $PGF_{2\alpha}$-based protocols used in the studies included in the meta-analysis. Further investigation is needed to determine the effect of estrus-cycle status during presynchronization on the outcomes from progesterone supplementation, as well as the effects of progesterone supplementation following GnRH-based presynchronization protocols that are known to increase the proportion of cows with a functional CL at the initiation of the timed AI program (Souza et al., 2008).

Progesterone supplementation during timed AI programs also had a marginal effect on the risk of pregnancy loss between the first and second month of gestation, which was 16% smaller for supplemented cows compared with untreated controls (adjusted risk ratio = 0.84; Control = 11.6 vs. Treated = 9.1%; P = 0.09; Bisinotto et al., 2015b). As stated earlier, the amount of progesterone released by a single intravaginal insert increases serum concentrations only by 0.8 to 1.3 ng/mL in lactating dairy cows (Cerri et al., 2009; Bisinotto et al., 2015c) and is unlikely to exert major impacts on follicle maturation. Nevertheless, progesterone supplementation before ovulation altered uterine gland morphology and vascularization in cows (Shaham-Albalancy et al., 1997), which might affect subsequent placentation and maintenance of gestation.

Progesterone Supplementation for Cows without CL

Extensive research has demonstrated that the absence of a mature CL at initiation of the timed AI program reduces fertility in lactating dairy cows. Cows without a CL at the first GnRH injection have smaller concentrations of progesterone during growth of the ovulatory follicle and reduced P/AI compared with herdmates in diestrus when the synchronization protocol was initiated (Bisinotto et al., 2010a; Denicol et al., 2012). Importantly, estrus-cycling cows that do not bear a CL at the first injection of GnRH have similar P/AI compared with anovular counterparts indicating that reduced progesterone concentrations during follicular development is a major component

of low fertility in anestrous cows (Bisinotto et al., 2010a). The underlying mechanism by which suboptimal progesterone concentrations impair fertility responses is multifactorial and involve changes in LH pulse frequency (Endo et al., 2012), increased growth rate and altered composition of follicular fluid in the ovulatory follicle (Cerri et al., 2011a), reduced embryo quality in the first week after AI (Cunha et al., 2008; Rivera et al., 2011), altered morphology of uterine glands and increased oxytocin-induced $PGF_{2\alpha}$ synthesis in the subsequent diestrus (Shaham-Albalancy et al., 1997; Cerri et al., 2011a).

A low fertility cohort of cows with insufficient concentrations of progesterone during growth of the ovulatory follicle can be identified based on the absence of CL at the initiation of the timed AI program. Cows without CL in a single ultrasound examination performed at the first GnRH injection of Ovsynch are 15 to 40% less likely to become pregnant compared with cows in diestrus depending on the reproductive management used (Bisinotto et al., 2013; Bisinotto et al., 2015a). This decrease in fertility is critical considering that 30% of lactating dairy cows lack a CL at the initiation of the timed AI protocol (Stevenson et al., 2008; Bisinotto et al., 2010a; Bisinotto et al., 2013)–a cohort that encompasses anovular and estrus-cycling cows during the follicular phase of the estrous cycle. Nevertheless, results from a meta-analysis showed that the incorporation of a single intravaginal insert containing progesterone to the synchronization protocol did not improve P/AI when cows in estrus were inseminated before timed AI, which was observed for both cows with and without CL at the first GnRH injection (Bisinotto et al., 2015b). Notably, progesterone supplementation did not restore P/AI in cows without CL similar to that observed in cows bearing a CL.

Because the amount of progesterone released by commercially available inserts is insufficient to mimic the presence of a mature CL in lactating dairy cows (Cerri et al., 2009), use of multiple inserts during timed AI programs has been evaluated as an alternative to improve P/AI in cows without CL (Bisinotto et al., 2013; Bisinotto et al., 2015a). Progesterone supplementation using two controlled internal drug release (**CIDR**) inserts from the initial GnRH to the injection of $PGF_{2\alpha}$ elevated circulating concentrations of progesterone and restored P/AI in lactating dairy cows without CL at the initiation of the 5-day timed AI and the Ovsynch-56 protocols (Table 2). In contrast to the meta-analysis results, progesterone supplementation increased P/AI in dairy cows that were observed for signs of estrus and had the opportunity to be inseminated before scheduled timed AI. Interestingly, when supplemented cows were stratified based on plasma progesterone during the treatment period, P/AI in cows with concentrations <2.0 ng/mL was similar to that of non-supplemented controls (Bisinotto et al., 2015a). A linear effect of progesterone concentrations during the timed AI program on the probability of pregnancy was observed and no detrimental impact of excessively elevated concentrations on fertility was depicted within the range described by the authors (0.7 to 7.0 ng/mL). Therefore, concentrations of progesterone in plasma ≥2.0 ng/mL seem to be necessary to improve P/AI in cows without a CL at the initiation of the timed AI program.

Table 2. Effect of the presence of a CL and progesterone supplementation using two controlled internal drug release (CIDR) inserts for cows without CL at the initiation of the timed AI program

	Treatment[1]			
Item	Control	2CIDR	Diestrus	*P*-value
5-day timed AI[2]				
Progesterone, ng/mL [3]	0.51 ± 0.27[b]	2.65 ± 0.26[a]	3.40 ± 0.25[a]	< 0.01
Pregnant day 34, % (no.)	30.8 (234)[b]	46.8 (218)[a]	49.9 (946)[a]	< 0.01
Pregnant day 62, % (no.)	28.6 (234)[b]	43.7 (215)[a]	47.3 (641)[a]	< 0.01
Pregnancy loss, % (no.)	6.6 (72)	5.1 (99)	4.7 (4.67)	0.72
Ovsynch-56[4]				
Progesterone, ng/mL [3]	0.92 ± 0.27[c]	2.77 ± 0.25[b]	4.93 ± 0.26[a]	< 0.01
Pregnant day 32, % (no.)	31.3 (649)[b]	42.2 (633)[a]	38.4 (640)[a]	< 0.01
Pregnant day 60, % (no.)	28.9 (642)[b,B]	37.2 (630)[a]	33.9 (633)[A]	0.01
Pregnancy loss, % (no.)	8.5 (208)	11.4 (260)	8.8 (231)	0.48

[a,b,c]Values within row with different superscripts differ ($P < 0.05$).
[A,B]Values within row with different superscripts tended ($0.05 < P \leq 0.10$) to differ.
[1]Control = cows without a CL at the first GnRH; 2CIDR = cows without a CL at the first GnRH and treated with two CIDR inserts; Diestrus = cows with a CL at the first GnRH.
[2]5-day timed AI: day −8 GnRH; day −3 and −2 $PGF_{2\alpha}$; day 0 GnRH and timed AI (Bisinotto et al., 2013).
[3]Average concentrations from 1 day after the initial GnRH to the day of first $PGF_{2\alpha}$.treatment.
[4]Ovsynch-56: day −10 GnRH; day −3 $PGF_{2\alpha}$; GnRH at 56 hours after $PGF_{2\alpha}$; day 0 timed AI (Bisinotto et al., 2015a).

Treatment had no remarkable effect on P/AI in cows observed in estrus on the day of insemination (i.e., the combination of cows inseminated in estrus before the final GnRH injection and those in estrus on the day of timed AI) in either study (Figure 1). On the other hand, P/AI was greater for treated with two CIDR inserts (2CIDR) and for cows in diestrus (Diestrus) at the onset of progesterone supplementation than for Controls when no signs of estrus were observed at timed AI. Approximately 35% of cows without CL are detected in estrus when subjected to synchronization protocols (Bisinotto et al., 2013; Bisinotto et al., 2015a) and identifying this cohort before the first GnRH would allow for further refinement of supplementation strategies. A recent study in grazing dairy cows indicated that concentrations of anti-Müllerian hormone (**AMH**) might be related with the interaction between progesterone concentration during follicular development and expression of estrus during timed AI programs (Ribeiro et al., 2014). The proportion of cows detected in estrus at timed AI was greater in cows with low AMH concentration (low = 49.3% vs. intermediate = 39.4% vs. high = 34.5%). For cows with intermediate and high AMH concentrations, P/AI was smaller for those with progesterone concentrations in plasma <1 ng/mL at the initial GnRH injection compared with counterparts with a functional CL. Nevertheless, concentration of progesterone at the initiation of the timed AI program did not affect P/AI in cows with low circulating AMH. Although it is reasonable to speculate that cows with low AMH do not benefit from progesterone supplementation during the timed AI program, this hypothesis is yet to be tested.

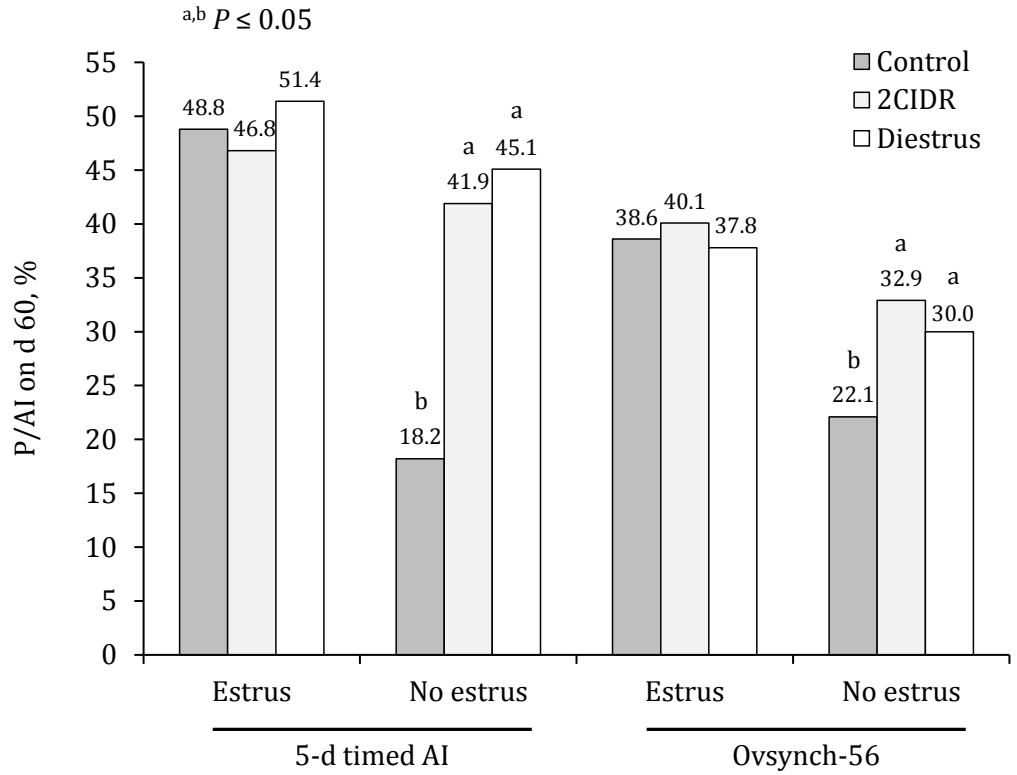

Figure 1. Interaction between presence of CL at the initiation of the synchronization protocol and the expression of estrus at the time of AI.

CONCLUSIONS

A continuous trend for consolidation of the dairy industry has been observed worldwide and large herds will rely progressively more on population-driven approaches to optimize reproduction, including strategies for identification of low-fertility cohorts that respond to therapy and adequate interventions to enhance individual fertility. Studies support the concept that supplementation with progesterone during timed AI programs improves synchrony of ovulation and P/AI in lactating dairy cows. Nonetheless, such benefits are dependent upon the physiological state of the cow and aspects of the reproductive management implemented in the herd. Incorporation of a single intravaginal insert containing progesterone to timed AI programs reduced pregnancy losses and increased P/AI by 20% in herds in which estrus is not performed, whereas no benefit was observed when cows in estrus were inseminated before scheduled timed AI. A single ultrasound examination is sufficient to define a low-fertility cohort based on the absence of CL at the initial GnRH injection. Finally, increasing the dose of supplemental progesterone reestablished P/AI in cows without CL similar to those in diestrus. Collectively, these results outline physiological and managerial conditions in which progesterone supplementation enhances fertility in lactating dairy cows subjected to timed AI protocols.

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