Reproductive Programs for Florida Dairy Herds

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Introduction

Reproductive efficiency is a major component of economic success in dairy herds. Recently, it was estimated that the average value of a pregnancy was US $278 in high-producing herds in the US, whereas the cost of a pregnancy loss was substantially greater (De Vries, 2006).

In the past, most dairy herds used reproductive programs that relied upon observation of estrus up to a certain number of days in milk (DIM), and subsequent intervention was only implemented in cows with advanced DIM and no insemination. Typically, interventions were based on palpation per rectum of the reproductive tract and a decision was made based upon detection of ovarian structures. More recently, reproductive programs have taken a slightly different approach and the goal is to be more proactive and work with groups of cows. In most cases, the focus is to increase the rate at which eligible cows become pregnant and, for that, use of systematic breeding protocols have become an integral portion of reproductive management in dairy herds (Caraviello et al., 2006). The development of controlled breeding programs have allowed producers to minimize the variation in the interval from calving to first AI, increase the rate at which eligible cows become pregnant and, consequently, reduce the interval from calving to pregnancy in a more consistent manner. For these programs to succeed, cows need to be managed as groups in a consistent manner and programs should follow physiological basis to optimize fertility, but they also need to not be extremely complicated that producers would find them difficult to implement.

Implementing Reproductive Programs for First AI

It is clear that high-producing lactating dairy cows have compromised duration and intensity of estrous expression (Wiltbank et al., 2006; Yaniz et al., 2006). Therefore, implementation of reproductive programs based on synchronization of estrus, ovulation, or both is needed to optimize reproductive efficiency in dairy herds.

Managing Anovular Cows

In high-producing dairy herds, 6 to 59% of the postpartum Holstein cows do not resume cyclicity by 60 d postpartum or before the first postpartum AI (Santos et al., 2009; Stevenson et al., 2006). These cows experience reduced pregnancy per AI (P/AI) and increased pregnancy loss following the first insemination (Chebel et al., 2006; Santos et al., 2004; Santos et al., 2009).

A method to induce cyclicity in anovular cows is to administer exogenous progesterone by using controlled internal drug release (CIDR) impregnated with progesterone (Gumen and Wiltbank, 2005). When anovular cows were treated with a new or a 7-d used autoclaved CIDR originally containing 1.38 g of progesterone (Table 1), induction of cyclicity was increased and short-cycling was reduced in cows receiving supplemental progesterone, but these effects were not sufficient to improve proportion of cows pregnant and pregnancy loss (Cerri et al., 2009).

Table 1. Effect of a new or 7-d used autoclaved controlled internal drug-releasing (CIDR) containing progesterone on reproductive responses of anovular cows[1]

	Treatment			P [2]	
Item	Control	New CIDR	Used CIDR	CIDR	Type
	% (number of cows)				
Cyclic	34.1 (120)	50.3 (199)	46.4 (196)	0.02	0.55
Short-cycling[3]	21.6 (74)	11.8 (110)	14.2 (120)	0.09	0.67
Pregnant					
d 38	36.8 (117)	43.6 (195)	37.8 (193)	0.86	0.36
d 66	32.5 (117)	39.4 (76/193)	35.4 (192)	0.61	0.57
Pregnancy loss	11.6 (43)	8.4 (83)	5.6 (72)	0.40	0.48

[1] Adapted from Cerri et al. (2009).
[2] CIDR = effect of CIDR (control vs. new + used CIDR); Type = effect of type of CIDR.
[3] Reinsemination of cows between 6 and 17 d after the initial AI.

The use of supplemental progesterone to reestablish ovulatory cycles in high-producing anovular cows does not seem to be warranted. When used prior to first postpartum insemination to induce cyclicity in anovular cows, the resulting P/AI are usually not altered (Cerri et al., 2009; Chebel et al., 2006). When incorporated as part of a timed AI program, the efficacy of progesterone inserts in improving fertility of anovular cows is also questionable (Lima et al, 2009b; Stevenson et al., 2006). When compared with timed AI protocols, treatment of anovular cows (cystic) using intravaginal inserts containing progesterone were less economical, a difference of approximately US $11.4 (De Vries et al., 2006). Therefore, other methods than just progesterone inserts are recommended to induce cyclicity and increase the risk or a cow to become pregnant to an insemination.

Timed AI Protocols

Manipulation of the estrous cycle to improve service rate and fertility usually impacts positively on pregnancy rate. Timed AI protocols rely on control of the estrous cycle by synchronizing follicular development, CL regression and, ultimately, ovulation to allow for insemination at fixed time with adequate P/AI (Thatcher et al., 2001). Such programs have become an integral part of reproductive management in herds (Caraviello et al., 2006), and adoption has been widespread because of the recognized problems with expression and detection of estrus in dairy cows.

The most accepted timed AI protocol in dairy herds in the US is the Ovsynch and CoSynch protocols, which consist of an injection of GnRH given at random stages of the estrous cycle, followed 7 d later by a luteolytic dose of $PGF_{2\alpha}$. For the Ovsynch, a final GnRH injection is given at 48 to 56 h after $PGF_{2\alpha}$ and fixed-time AI is performed 12 to 16 h later. When the CoSynch is utilized, cows are fixed-time inseminated 48 or 72 h after the $PGF_{2\alpha}$, and GnRH is given concomitantly with timed AI. These protocols have been implemented very successfully in many commercial dairy farms as a strategy for AI during the first postpartum service, as well as for re-insemination of nonpregnant cows. Although timed AI protocols allow for insemination without the need for estrous detection, approximately 10 to 15% of the cows will display signs of estrus during the protocol and they should be inseminated promptly if maximum pregnancy rate is to be achieved.

Pursley et al. (1997a) evaluated P/AI in lactating dairy cows (n = 310) and heifers (n = 155) when AI was performed following the Ovsynch protocol or a synchronization program utilizing only $PGF_{2\alpha}$ injections. Cow in the $PGF_{2\alpha}$ treatment received as many as 3 injections 14 d apart if signs of estrus had not been observed. All control cows not detected in estrus after the third injection of $PGF_{2\alpha}$ were timed AI 72 to 80 h after that injection. Pregnancies per AI for the two programs were similar and it averaged 38%. For the lactating cows, estrous detection rate during the first 2 injections of $PGF_{2\alpha}$ averaged 54.0% following each injection, with an overall 81.8% for the 28-d period. Because of the low estrous detection rate in the $PGF_{2\alpha}$ group, cows enrolled in the Ovsynch timed AI protocol experienced greater pregnancy rate. In a subsequent study by the same group (Pursley et al., 1997b), lactating dairy cows from 3 commercial herds (n = 333) were randomly assigned to either the Ovsynch protocol or AI based on estrous detection with periodic use of $PGF_{2\alpha}$. Nonpregnant cows were re-inseminated using the original treatment. Median days postpartum to first AI (54 vs 83; $P < 0.001$) and days open (99 vs. 118: $P < 0.001$) were reduced in cows receiving the Ovsynch compared with cows inseminated following detection of estrus.

It is important to note that the positive effects of timed AI compared with more traditional reproductive programs based on detection of estrus on reproductive efficiency of a herd are only observed when P/AI are not reduced with timed AI, and detection of estrus is deficient (Tenhagen et al., 2004). When timed AI was implemented in 2 herds with distinct reproductive performance, the benefits from a systematic breeding program were more clearly demonstrated in the herd with poor estrous detection rate (Tenhagen et al., 2004).

Improving Response to Timed AI

Response to the Ovsynch protocol is optimized when cows ovulate to the first GnRH injection of the program, and when a responsive CL is present at the moment of the $PGF_{2\alpha}$ treatment (Chebel et al., 2006). Vasconcelos et al. (1999) initiated the Ovsynch protocol at different stages of the estrous cycle and observed that synchronization rate to the second GnRH injection was higher when cows received the first GnRH injection prior to day 12 of the estrous cycle. Also, initiation of the Ovsynch protocol between days 5 and 9 of the cycle resulted in the greatest ovulation rate. Ovulation to the first GnRH injection and initiation of a new follicular wave should improve pregnancy rate because an ovulatory follicle with reduced period of dominance is induced to ovulate (Austin et al., 1999). Furthermore, initiating the Ovsynch protocol prior to day 12 of the estrous cycle should minimize the number of cows that come into estrus and ovulate prior to the completion of the program.

The importance of inducing follicle turnover is demonstrated vividly by evaluating fertilization rates and embryo quality after timed AI following the induction of follicle turnover or not (Cerri et al. 2009b). They demonstrated that cows that did not ovulate to the first GnRH of the Ovsynch protocol, and those that had extended period of follicle dominance had reduced embryo quality.

Moreira et al. (2001) designed a presynchronization protocol to optimize response to the Ovsynch program by given 2 injections of $PGF_{2\alpha}$ 14 days apart, with the second injection given 12 days prior to the first GnRH of the timed AI protocol. This presynchronization program increased pregnancy rates at 32 and 74 days after timed AI in cyclic cows. Because of the convenience of giving injections on the same day of the week, many producers have opted for administering the $PGF_{2\alpha}$ injections of the presynchronization protocol on the same day of the injection of the Ovsynch protocol, which results in an interval between presynchronization and initiation of the Ovsynch of 14 days. Although presynchronizing cows 14 days before initiating the Ovsynch also improved P/AI compared with no presynchronization (Navanukraw et al., 2004), the interval is not optimal and results in poor ovulation rate to the initial GnRH of the Ovsynch (Chebel et al., 2006; Galvão et al., 2007).

We have recently demonstrated that reducing the interval between presynchronization and initiation of the timed AI from 14 to 11 days increased ovulation rate to the initial GnRH of the timed AI protocol and increased P/AI (Galvão et al., 2007).

Table 2. Effect of presynchronization treatment on ovulatory responses to the first GnRH of the timed AI, pregnancy per AI and pregnancy loss in dairy cows[1]

	Treatment[2]			*P* [3]	
	Control	PShort	PShortG	Interval	GnRH
Ovulation to 1st GnRH[4]	------------------- % (no.) -----------------				
Overall	44.7 (340)	61.4 (337)	62.2 (323)	<0.001	0.28
Cows with CL	37.2 (274)	54.4 (250)	59.7 (285)	<0.001	0.29
Cows without CL	75.8 (66)	81.6 (87)	81.6 (38)	0.34	0.99
Pregnant					
day 38	33.5 (412)	40.5 (410)	39.8 (392)	0.02	0.60
day 66	30.2 (410)	36.4 (409)	36.2 (392)	0.04	0.70
Pregnancy loss					
day 38 to 66	8.8 (136)	9.7 (165)	9.0 (156)	0.88	0.85

[1] Adapted from Galvão et al. (2007).
[2] Control = two injections of $PGF_{2\alpha}$ at 37 and 51 DIM, then enrolled in the timed AI 14 d later; PShort = two injections of $PGF_{2\alpha}$ at 40 and 54 DIM, then enrolled in the timed AI 11 d later; PShortG = same as PShort, but with an injection of GnRH 7 d before the first GnRH of the timed AI.
[3] Interval = contrast for the effect of 14 vs. 11 d interval (Control vs. PShort + PShortG); GnRH = contrast for the effect of GnRH 7 d before initiation of timed AI (PShort vs. PShortG).
[4] Ovulation to GnRH was evaluated in cows with or without a CL on the day of treatment.

Insemination or Not During Presynchronization

A common program adopted in many farms in California is to administer 2 $PGF_{2\alpha}$ injections at 14 d interval, with the second injection given at approximately 50 to 55 days postpartum. Cows are then inseminated following the second injection, and those not inseminated in the following 11 days are enrolled in the timed AI protocol. Because 45 to 55% of the cows display estrus and are inseminated following the second $PGF_{2\alpha}$ of the presynchronization, these cows ended up receiving their first AI early in the postpartum period. Studies have demonstrated that response to timed AI programs improves as the lactation progresses up to 70 to 90 days postpartum (Pursley et al., 1997; Tenhagen, 2005), and we have demonstrated that cows inseminated at estrus following the presynchronization have smaller P/AI than those inseminated after the completion of the entire program (presynchronized timed AI), 3 weeks later (Bruno et al., Chebel et al., 2006). However, insemination of cows at estrus during the presynchronization reduces the interval to first AI and costs associated with hormones and labor.

In an attempt to evaluate whether cows should be inseminated following presynchronization or subjected to timed AI, Chebel et al. (2005; 2006) assigned 1,019 Holstein cows to a presynchronization with PGF_{2a} (CON) or PGF_{2a} and CIDR (CTAI and CED). All cows received 2 injections of PGF_{2a} on days 35 ± 7 and 49 ± 7 after calving. Cows in CTAI and CED received a CIDR on d 42 ± 7. After the second PGF_{2a} and CIDR removal on day 49 ± 7, cows were observed for estrus, but only CON and CED were inseminated. On day 62 ± 7 CON and CED cows not inseminated in estrus and all CTAI began the Ovsynch and were timed AI on day 72 ± 7. Cows in CON and CED had smaller P/AI that CTAI on d 31 after the first AI, however, because they were inseminated on average 2 weeks earlier, median days open for the first 300 d postpartum were similar between CED and CTAI (Figure 1).

These results suggest that insemination of cows after the second PGF_{2a} of the presynchronization results in smaller P/AI, but because cows are inseminated earlier, days open are not affected. This gives flexibility to producers that might decide to inseminate cows that display estrus after the second $PGF_{2\alpha}$ of the presynchronization, or inseminate all cows at timed AI. The first will reduce costs with treatments, but the latter will optimize first service P/AI, with both resulting in similar time to pregnancy.

It is important to emphasize that in order for systematic breeding programs to work, there must be high compliance at every step of the program. Each individual farm has to develop a system to assure that cows receive the correct hormonal treatment on the correct day. Failure in complying with the programs can result in reduced insemination rate and P/AI. Because some programs require handling of cows multiple times to administer hormonal treatments, it is important that they tailored to the needs of the farm as long as critical steps are not ignored.

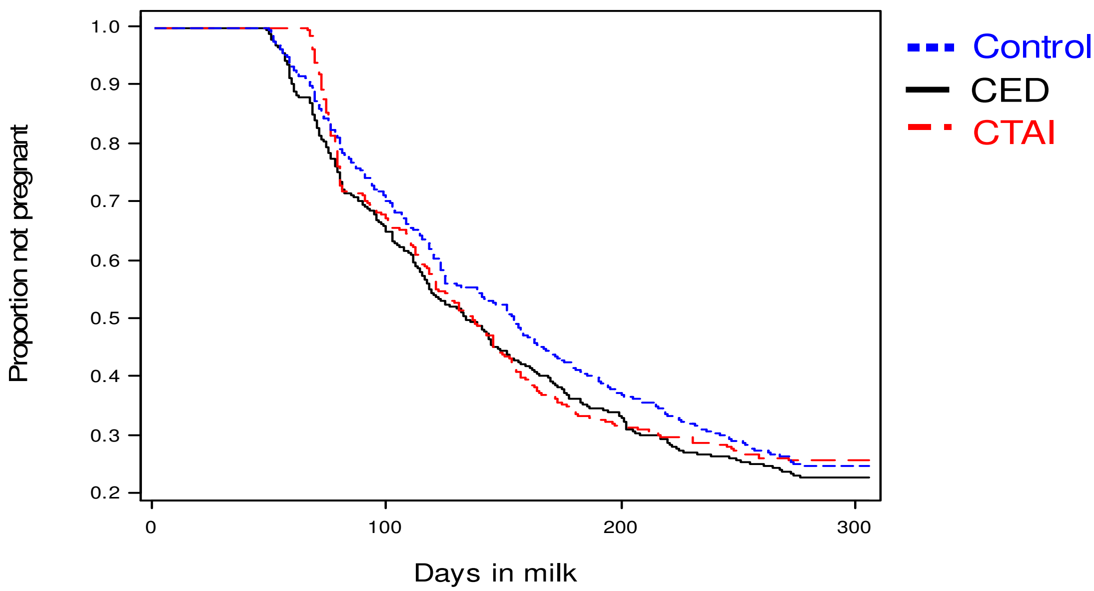

Figure 1. Survival curves for time to pregnancy in cows subjected to different insemination protocols for first AI. Median days open were 154, 133, and 136 for control, CED, and CTAI, respectively.

Use of Bulls for Breeding Programs

A common belief by dairy producers is that the use of natural service can overcome inefficiencies of the reproductive program when AI is used, particularly problems with estrous detection. A considerable proportion of dairy farms use natural service as the sole or as a component of their breeding program. In many cases, natural service is used after cows have been subjected to several unsuccessful attempts of AI. Despite the common use of natural service, very few controlled studies have compared the two systems for their effect of the reproductive performance of dairy cows. De Vries et al. (2005) compared herds that used natural service and others that used AI in the southeast region of the US and observed that bull breeding did not seem to improve measures of reproductive performance. Another observational study in California indicated that cows exposed to AI had increased rate of pregnancy than those exposed to natural service (Overton and Sischo, 2005). A problem with these studies is that none used randomly assigned cows under the same conditions within the same farm. In many cases, only cows that had received several AI were part of the natural service group.

Recently (Lima et al., 2009; Lima et al., 2011) compared natural service with AI by randomly assigning cows to one of the two treatments. In both groups, no detection of estrus was performed and inseminated cows were subjected to timed AI only. In Lima et al. (2009), 1,055 Holstein cows were blocked by parity and randomly assigned to timed AI (n = 543) or natural service (n = 512). Both groups received 2 doses of $PGF_{2\alpha}$, and those in natural service were exposed to Holstein bulls 14 d later, at 70 d postpartum. Cows remained with bulls for 223 d postpartum. Cows in timed AI were enrolled in the Ovsynch protocol 14 d after the second $PGF_{2\alpha}$, at 70 d postpartum, and received the first AI at 80 d postpartum. For timed AI, nonpregnant cows were re-inseminated every 35 d during the first 223 d postpartum. The pregnancy rate was greater for natural service than timed AI, which resulted in a small reduction in median days open (Figure 2, 111 vs. 116 d). The proportion of pregnant cows at 223 d postpartum was greater in the NS than TAI group (84.2 vs. 74.8%, respectively). Most of this benefit was attributed to the increased opportunities for insemination in the natural service than the timed AI group (Lima et al., 2009).

More recently, Lima et al. (2011) completed an experiment with 1,050 lactating Holstein cows subjected to either 1 (1TAI, n = 533) or 3 timed AI (3TAI, n = 517) following the double Ovsynch timed AI program (d -27 GnRH, d -20 PGF2a, d -17 GnRH, d -10 GnRH, d -3 PGF2a, d -1 GnRH, and d 0 AI) for first AI. Following the first AI, cows in 1TAI were subjected to natural service 1 week after insemination, whereas cows in nonpregnant cows 3TAI were re-inseminated every 42 d. After the third AI, cows in 3TAI were subjected to natural service. As expected, pregnancy at the first timed AI did not differ between 1TAI and 3TAI on d 60 after insemination (30.9 vs. 33.4%). Cows receiving 3TAI had greater (P = 0.04) rate of pregnancy than those in 1TAI (AHR=1.15; 95% CI=1.01-1.31; Figure 2). This resulted in median d open of 142 (95% CI=130-150) and 123 (95% CI=121-144) for 1TAI and 3TAI, respectively. Therefore, in spite of the long re-insemination interval, cows receiving 3TAI had improved reproductive performance than those receiving 1TAI (figure 2).

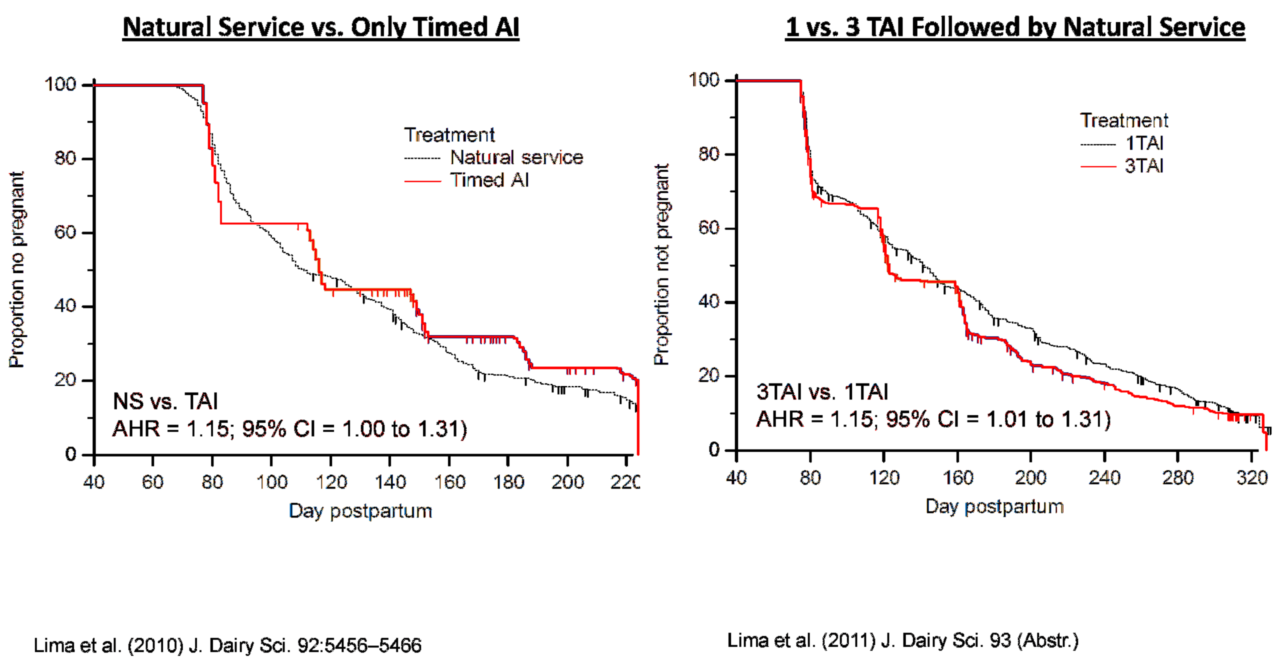

Figure 2. Survival curves for interval from calving to pregnancy in cows receiving natural service or timed AI only (Lima et al., 2009), or 1 vs. 3 timed AI (Lima et al., 2011).

Results from these two controlled randomized experiments give little support to the use of natural service to improve reproductive performance of lactating dairy cows. In fact, economic analyses of the two programs indicated that, despite the long inter-AI interval, timed AI only was more profitable than natural service (Lima et al., 2010).

References

Austin, E. J., M. Mihm, M. P. Ryan, D. H. Williams, and J. F. Roche. 1999. Effect of duration of dominance of the ovulatory follicle on onset of estrus and fertility in heifers. J. Anim. Sci. 77: 2219-2226.

Britt, J. H. 1975. Early postpartum breeding in dairy cows. A review. J. Dairy Sci.58:266-271.

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: 4723-4735.

Cerri, R. L. A., H. M. Rutigliano, R. G. S. Bruno, R. C. Chebel, and J. E. P. Santos. 2005. Effect of artificial insemination (AI) protocol on fertilization and embryo quality in high-producing dairy cows. J. Dairy Sci. 88 (Suppl. 1): 86 (Abstr.).

Cerri, R.L.A., H.M. Rutigliano, R.G.S. 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.

Chebel, R. C., H. M. Rutigliano, R. L. A. Cerri, R. G. S. Bruno, and J. E. P. Santos. 2005. Economic evaluation of pre-synchronization and resynchronization protocols in lactating dairy cows. J. Dairy Sci. 88(Suppl. 1): 46 (Abstr.).

Chebel, R. C., J. E. P. Santos, R. L. A. Cerri, H. M. Rutigliano, and R. G. S. Bruno. 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. J. Dairy Sci. 89: 4205-4219.

De Vries, A. 2006. Economic value of pregnancy in dairy cattle. J. Dairy Sci. 89: 3876-3885.

De Vries, A., M. B. Crane, J. A. Bartolome, P. Melendez, C. A. Risco, and L. F. Archbald. 2006. Economic comparison of timed artificial insemination and exogenous progesterone as treatments for ovarian cysts. J. Dairy Sci. 89: 3028-3037.

Ferguson, J. D., and D. T. Galligan. 1993. Reproductive programs in dairy herds. *In* Proc. Centr. Vet. Conf. pp. 161-178.

Galvão, K. N. M. F. Sá Filho, and J. E. P. Santos. 2007. Reducing the interval from presynchronization to initiation of timed AI improves fertility in dairy cows. J. Dairy Sci. *In Press*.

Gumen, A., and M. C. Wiltbank. 2005. Length of progesterone exposure needed to resolve large follicle anovular condition in dairy cows. Theriogenology 63: 202–218.

Lima, F.S., A. De Vries, C.A. Risco, J.E.P. Santos, and W.W. Thatcher. 2010. Economic comparison of natural service and timed artificial insemination breeding programs in dairy cattle. J. Dairy Sci. 93:4404–4413.

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

Lima, F.S., C.A. Risco, M.J. Thatcher, M.E. Benzaquen, L.F. Archbald, J.E.P. Santos, and W.W. Thatcher. 2009. Comparison of reproductive performance in lactating dairy cows bred by

natural service or timed artificial insemination. J. Dairy Sci. 92:5456–5466.
Lucy, M. C. 2006. Estrus: basic biology and improving estrous detection. In Proc. Dairy Cattle Reproductive Council Conference, November 6 to 8, Denver, CO, pp. 29-37.
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.
Navanukraw C., D. A. Redmer, L. P. Reynolds, J. D. Kirsch, A. T. Grazul-Bilska, and P. M. Fricke. 2004. A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. J. Dairy Sci. 87:1551-1557.
Pursley, R. J., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997a. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci. 80: 295-300.
Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997b. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80:301-306.
Santos, J. E. P., M. Villaseňor, E. J. DePeters, P. H. Robinson, and C. H. Holmberg. 2003. Type of cottonseed and gossypol in diets of lactating dairy cows: Plasma gossypol, reproduction, and health. J. Dairy Sci. 86: 892-905.
Santos, J. E. P., W. W. Thatcher, R. C. Chebel, R. L. A. Cerri, and K. N. Galvão. 2004. The effect of embryonic death rates in cattle on the efficacy of estrous synchronization programs. Anim. Reprod. Sci. 82-83C: 513-535.
Santos, J.E.P., H.M. Rutigliano, and M.F. Sá Filho. 2009. Risk factors for resumption of postpartum cyclicity and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110:207-221.
Stevenson, J. S., J. R. Pursley, H. A. Garverick, P. M. Fricke. D. J. Kesler, J. S. Ottobre, and M. C. Wiltbank. 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. J. Dairy Sci. 89: 2567-2578.
Thatcher, W. W., F. Moreira, J. E. P. Santos, R. C. Mattos, F. L Lopes, and S. M. Pancarci. 2001. Effects of animal drugs on reproductive performance and embryo production. Theriogenology 55: 75-89.
Tenhagen, B. A., C. Vogel, M. Drillich, G. Thiele, and W. Heuwieser. 2003. Influence of stage of lactation and milk production on conception rates after timed artificial insemination following Ovsynch. Theriogenology 60: 1527-1537.
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.
Tenhagen, B. A. 2005. Factors influencing conception rate after synchronization of ovulation and timed artificial insemination - a review. Dtsch. Tieraerztl. Wochenschr. 112: 136-141.
Vasconcelos, J. L. M., 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.
Wiltbank, M., H. Lopez, R. Sartori, S. Sangsritavong, A. Gumen. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65: 17-29.

Yaniz, J. L., P. Santolaria, A. Giribet, and F. Lopez-Gatius. 2006. Factors affecting walking activity at estrus during postpartum period and subsequent fertility in dairy cows. Theriogenology 66: 1943-1950.

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