Evaluation of Progesterone Supplementation in a Prostaglandin $F_{2\alpha}$ -Based Presynchronization Protocol Before Timed Insemination

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ABSTRACT

The objective of the study was to determine the effects of treatment with a controlled internal drug-release (CIDR) insert containing progesterone in a $PGF_{2\alpha}$ -based presynchronization protocol on pregnancy rates at first service in lactating Holstein cows. A total of 1,318 (656 treatment and 662 control) cows from 5 farms were used in the analysis. Cows received a CIDR insert as part of the presynchronization protocol of 2 $PGF_{2\alpha}$ injections given 14 d apart. The CIDR insert was applied during 7 d before the second $PGF_{2\alpha}$ injection, whereas control cows received no CIDR insert. Serum progesterone concentrations were measured in samples collected at 37 ± 3 d in milk (DIM; 7 d after the first $PGF_{2\alpha}$ injection) and at 58 ± 3 DIM, just before initiation of the Ovsynch protocol. According to serum concentrations of progesterone, cows were classified as having either high (≥ 1 ng/mL) or low (< 1 ng/mL) progesterone. The proportion of cows with low progesterone at 37 ± 3 DIM was similar for cows treated later with the CIDR insert (60.7%; $n = 654$) and for control cows (59.2%; $n = 657$). In contrast, use of the CIDR insert resulted in fewer low-progesterone cows (17.4%; $n = 402$) compared with control cows (30.6%; $n = 399$) at 58 ± 3 DIM. No significant effect of the CIDR insert was detected on overall pregnancy rates. Pregnancy rates, as measured by the percentage of cows pregnant at 37 ± 3 d post timed artificial insemination, for control cows having high or low progesterone at 58 ± 3 DIM were 46.6 and 22.1%, respectively. For the CIDR group, pregnancy rates were 40.4 and 11.4%, respectively, for high- and low-progesterone cows at 58 ± 3 DIM. Overall pregnancy rates were 36.4 and 34.5% for control cows and cows receiving the CIDR insert, respectively. A significant decreasing trend was observed in the proportion of cows having low progesterone as the body condition score increased, at 37 ± 3 and 58 ± 3 DIM. A significant increasing trend in the pregnancy rate was observed as body condition score increased. In conclusion, incor-

poration of CIDR inserts into a presynchronization protocol reduced the proportion of cows having low progesterone; however, the pregnancy rate did not differ between control cows and those receiving the CIDR insert. Earlier expression of estrus after the second $PGF_{2\alpha}$ injection, and consequently improper timing of initiation of the Ovsynch protocol, could have negatively affected fertility in the CIDR-treated cows.

Key words: controlled internal drug-release insert, presynchronization, dairy cow

INTRODUCTION

Reproductive failure is an important cause of economic loss for dairy farmers (Plaizier et al., 1996). Inefficient detection of estrus, anestrus, and poor expression of estrus are major reasons for reproductive failure. To reduce dependency on the detection of visible signs of estrus, ovulation-synchronization protocols were developed to allow cows to be inseminated at a fixed time.

The Ovsynch protocol [injection of GnRH 7 d before and 48 h after a single injection of $PGF_{2\alpha}$, with timed AI (TAI) at 16 h after the second GnRH injection; Pursley et al., 1995] is the most commonly used TAI protocol. However, response to the Ovsynch protocol can vary according to the stage of the estrous cycle at the time of the first GnRH injection. Initiation of the TAI protocol when the cow is in the late luteal phase might lead to premature regression of the corpus luteum (CL) and premature estrus before the second GnRH injection (DeJarnette et al., 2001). Initiation of the TAI protocol when the cow is in proestrus might result in incomplete luteal regression following injection of $PGF_{2\alpha}$ shortly after estrus, leading to conception failure (Burke et al., 1996). Initiation of the TAI protocol when the cow is in metestrus might result in the failure of synchronization of the new follicular wave (Moreira et al., 2000). Therefore, for best results, the Ovsynch protocol should be initiated between d 5 and 12 of the cycle (Vasconcelos et al., 1999).

An increased probability that cows are in the early luteal phase at initiation of the Ovsynch protocol can be achieved by presynchronizing the estrous cycles in cows before initiating Ovsynch. Presynchronization using 2 injections of $PGF_{2\alpha}$ given 14 d apart has been

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shown to synchronize 90 to 95% of cyclic cows (Ferguson and Galligan, 1993; Moreira et al., 2001). Anestrus is common early postpartum, with as many as 49% of cows still anestrus by 50 DIM (Opsomer et al., 2000) and 23% of cows still anestrus at first service 53 ± 3 DIM (Moreira et al., 2001). Anestrus or anovulatory cows do not respond well to GnRH- and PGF_{2 α} -based synchronization protocols and have reduced pregnancy rates compared with cows that are cycling at the time Ovsynch is initiated. Pregnancy rates in anestrus cows after Ovsynch are reported to be as low as 0% of 11 cows (Murugavel et al., 2003) and 9% of 33 cows (Gümen et al., 2003). Furthermore, given the prevalence of anestrus and its detrimental effect on reproductive performance, establishing cyclicity in anestrus cows presents an opportunity to improve the overall reproductive performance of the herd.

Hormonal treatment protocols that include a period of progesterone supplementation have been shown to induce estrus successfully in previously anestrus cows. Subsequent estrous cycles of anestrus cows previously supplemented with progesterone were of a normal duration, and pregnancy rates were similar to those of control cows (Rhodes et al., 2003). A presynchronization protocol that includes a period of progesterone preceding the initiation of a TAI protocol might reduce the number of anestrus cows, and therefore improve reproductive performance. The objective of our study was to determine the effects of progesterone, administered via a controlled internal drug-release (CIDR) insert during a presynchronization protocol, on the proportion of cows having elevated serum progesterone (≥ 1 ng/mL) before initiating the Ovsynch and on the resulting fertility after a TAI protocol at first service in lactating Holstein cows.

MATERIALS AND METHODS

Study Farms

The experiment was conducted on 5 commercial Holstein dairy farms located in Cayuga County, New York, between November 2004 and May 2005. Farms A, B, and C each consisted of approximately 1,000 milking cows. Farm D consisted of 2,500 milking cows, and farm E consisted of 800 milking cows. Lactating dairy cows were housed in free-stall facilities and milked 3 times daily. Cows in the experiment were fed a TMR consisting of about 55% forage (corn silage, haylage, alfalfa silage, alfalfa hay, and wheat straw) and 45% concentrate (cornmeal, soybean meal, canola, cottonseed, and citrus pulp). The diet was formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows weighing 650 kg and producing 45 kg of 3.5% FCM.

Study Design and Treatment Description

Cows were assigned randomly to receive a CIDR insert or to serve as controls within farm and study week (Figure 1). A randomization spreadsheet was created using the random number function of Excel (Microsoft, Redmond, WA). Each week, every cow that was between 27 and 33 DIM was enrolled in the study. Identification numbers were recorded on preprinted allocation lists as cows exited the milking parlor or as the veterinarians walked through the pens. A new randomization sheet was used each week and each farm enrolled cows separately from the other farms.

All cows received (i.m.) 25 mg of PGF_{2 α} (Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) at 30 ± 3 DIM. At 37 ± 3 DIM, cows from farms D and E were scored for body condition on a scale of 1 (thin) to 5 (obese) using a quarter-point system (Edmonson et al., 1989). Before statistical analysis of the BCS data, scores were rounded to 0.5 increments. Also, 18 cows with a BCS < 2 were collapsed into the BCS = 2.0 category, and 14 cows with a BCS of > 3.5 were included in the 3.5 category. Cows in the CIDR treatment received a CIDR insert (EAZI-BREED CIDR insert; Pharmacia Animal Health) at 37 ± 3 DIM, whereas control cows received no treatment. At 44 ± 3 d DIM, the CIDR insert was removed and all cows in both groups received a second injection of PGF_{2 α} . Every cow in the study was then started on the Ovsynch program at 58 ± 3 DIM with a 100- μ g (i.m.) injection of GnRH (Cystorelin, Merial Ltd., Athens, GA). Cows were injected 7 d later with PGF_{2 α} and with a second dose of GnRH 48 h after the PGF_{2 α} injection. All cows received their first insemination (TAI) 16 h after the second GnRH injection. Semen of proven sires from different commercial studs was used.

Pregnancy was diagnosed by palpation per rectum at 37 ± 3 d following the TAI in all 5 farms by herd veterinarians; cows that returned to estrus before pregnancy diagnosis was performed were considered not pregnant. Therefore, as used in this study, pregnancy rate is the proportion of cows pregnant at 37 ± 3 d after TAI.

Progesterone Concentrations

At 37 ± 3 DIM, a blood sample was collected from all the cows enrolled in the study. For cows in the CIDR treatment, blood was collected immediately before application of the CIDR insert. On farms D and E, a second blood sample was collected at 58 ± 3 DIM before the first injection of GnRH of the Ovsynch protocol. Blood samples were collected from a coccygeal vessel by venipuncture into blood collection tubes (BD Vacutainer; Preanalytical Solutions, Franklin Lake, NJ) using a 2.5-

Figure 1. Presynchronization and ovulation synchronization protocols for cows treated with a controlled internal drug-release (CIDR) insert (Eazi-Breed; Pharmacia Animal Health, Kalamazoo, MI) and control cows. Cows in the CIDR treatment received a new CIDR insert vaginally on d 37 ± 3 DIM for 7 d and were given an injection of PGF_{2α} after CIDR insert removal. Cows were inseminated at a fixed time (timed AI, TAI) as part of the Ovsynch protocol (16 h after the second GnRH injection).

cm, 20-g blood collection needle. Blood samples were kept on ice and transported to the laboratory within 12 h from the time of collection. At the laboratory, the samples were centrifuged for 20 min at $1,500 \times g$ at room temperature, and serum was harvested into sample mailing tubes (VWR International, West Chester, PA), frozen, and stored at -20°C . At the end of the study, the samples were sent to the New York State Animal Health Diagnostic Center at Cornell University to measure concentrations of progesterone by RIA (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA). Samples were assayed in duplicate and averaged. Interassay and intraassay coefficients were 7.6 and 5.9%, respectively. Cows having a concentration of serum progesterone ≥ 1 ng/mL were assumed to have an active CL at the time of sampling (Murugavel et al., 2003; Galvão et al., 2004).

Statistical Analyses

Two dichotomous variables were created to classify the results of the progesterone assays. Cows were categorized at 37 ± 3 and 58 ± 3 DIM as having high progesterone when serum progesterone was ≥ 1 ng/mL and as having low progesterone when serum progesterone was < 1 ng/mL. The first variable (P1) represented the progesterone concentration of the first sample taken at 37 ± 3 DIM, which was collected just before insertion of the CIDR insert or 7 d after the first PGF_{2α} injection. This variable was calculated for all cows enrolled in each of the 5 dairies. The second variable (P2) represented the second progesterone sample collected at 58 ± 3 DIM, which was the day of the first GnRH injection of the Ovsynch protocol. Samples to calculate P2 were available only from farms D and E.

The effects of the CIDR treatment and other factors on pregnancy at 37 ± 3 d post TAI were analyzed using 2 separate logistic regression models. For both models, pregnancy (yes or no) was included as a dichotomous response variable. The first model used data from all 5 herds and included explanatory variables coding for farm, lactation number (1, 2, and 3+), P1 (high vs. low), and CIDR (treatment vs. control) and for all 2-way interactions among the main effects. The second model analyzed data from herds D and E. This allowed the inclusion of 2 additional explanatory variables, P2 (high vs. low) and BCS, which were measured only in these 2 herds. The BCS was included as a categorical variable with 4 levels (2.0, 2.5, 3.0, and 3.5). Backward stepwise variable selection was used to select significant explanatory factors for the models. Variables were retained in the model if either the main effects or their interactions were significant ($P < 0.05$). Following variable selection, individual models also were used to evaluate the effects of specific variables of interest that did not meet the criterion for retention in the models. Potential confounding effects were assessed by comparing the parameter estimates of statistically significant factors with and without other main effects forced into the model.

The effects of CIDR treatment and other factors at P2 also were analyzed by using logistic regression with data from herds D and E. The response variable for this model was the proportion of cows having high progesterone at P2 and the explanatory variables were farm, lactation group, P1, and CIDR treatment. Variable selection among the main effects and their 2-way interactions was done using backward stepwise selection as described for the preceding models. The LOGIS-

TIC procedure of SAS (SAS Inst. Inc., Cary NC) was used for all logistic regression models.

The association of BCS with the proportions of cows having low progesterone at P1 and P2 and pregnancy status at 37 ± 3 d post TAI was analyzed using the 2-sided Cochran-Armitage trend test in the FREQ procedure of SAS. Chi-squared tests were used to test the statistical significance of crude associations between categorical variables.

RESULTS

Descriptive Statistics

A total of 1,466 cows (733 CIDR and 733 control) were enrolled in our study. Seventy-one control cows and 77 cows in the CIDR treatment were excluded from the analysis because they were culled before the end of the study or had the wrong breeding date (failure to comply with the research protocol). Therefore, 1,318 cows were available for analysis, with 191, 118, 198, 591, and 220 cows from farms A, B, C, D, and E, respectively. Overall, the random assignment of cows to treatments was effective, as shown by the lack of association of lactation group and progesterone concentration with CIDR treatment. There was a tendency for treatment cows to be somewhat unequally ($P = 0.07$) distributed in the highest BCS category, which had the fewest cows. Farms had similar percentages of cows pregnant after TAI but differed ($P < 0.05$) with respect to the proportion of cows having high progesterone at 37 ± 3 DIM, distribution of lactation numbers, and BCS.

Pregnancy Rate

Based on data from all cows (excluding 7 with missing progesterone concentrations at P1), farm, lactation group, and CIDR treatment did not affect the pregnancy rate and were dropped from the logistic regression models. The proportion of cows having high progesterone at P1 was associated with greater pregnancy rates (odds ratio = 1.5; 95% confidence interval 1.2, 1.9) after TAI. The odds ratio at P1 was virtually unchanged when farm, lactation group, and CIDR treatment were forced into the model. The pregnancy rate was 41.7% of 525 cows having high progesterone at P1 and 31.5% of 786 cows having low progesterone. For cows subsequently treated with a CIDR insert or control cows, the percentages pregnant were 34.5 and 36.4%, respectively.

Both the proportion of cows having high progesterone at P2 and the proportion in the CIDR treatment had effects on the pregnancy rate in the logistic regression analysis of data from farms D and E (excluding 11 observations with missing data). No interaction between these 2 variables was detected. Farm, BCS, lacta-

Table 1. Pregnancy rates of lactating dairy cows at 2 of 5 farms as categorized by concentrations of progesterone at 58 ± 3 DIM¹

Concentrations of progesterone, ng/mL	Control	CIDR	Average
	[% (no.)]		
≥ 1	46.6 ^a (277)	40.4 ^a (332)	43.2 ^a (609)
< 1	22.1 ^b (122)	11.4 ^b (70)	18.2 ^b (192)
Total	38.7 (399)	35.5 (402)	

^{a,b}Percentages within columns having different superscript letters differ ($P < 0.05$).

¹All cows received 2 injections of PGF_{2 α} 14 d apart, beginning at 37 ± 3 DIM, to presynchronize the estrous cycles. Cows were treated with a controlled internal drug-release (CIDR) insert (Eazi-Breed; Pharmacia Animal Health, Kalamazoo, MI) during the 7 d before the second PGF_{2 α} injection or were not treated (control).

tion group, and the proportion of cows having high progesterone at P1 were dropped from the model. When controlling for CIDR treatment, the odds ratio for the effect of high progesterone at P2 and being diagnosed as pregnant was 3.6 (95% confidence interval 2.4, 5.4). When adjusted for the proportion of cows with high progesterone at P2, CIDR treatment had a detrimental effect on the proportion pregnant (odds ratio = 0.7; 95% confidence interval 0.5, 0.98). This effect reflects the fact that the proportion pregnant tended to be less for the CIDR-treated cows than for control cows (Table 1). There were no important changes in the proportions of cows having high progesterone at P2 and CIDR treatment estimates when the other main effects were forced into the model. Although not significant in the multivariable logistic regression models when accounting for CIDR treatment and progesterone at P2, the proportion of pregnant cows tended ($P = 0.10$) to be greater among first- and second-lactation cows than among those in third or greater lactation, and pregnancy rates also increased ($P = 0.10$) with greater BCS. The proportion of cows having high progesterone at P2 may in part represent an intermediate step in the effect of other factors on pregnancy rate, such as progesterone at P1, lactation, or BCS. This may account for the lack of significance of these variables when progesterone at P2 was included in the model.

Proportion of Cows Having High Progesterone

In the logistic regression analysis of factors influencing the proportion of cows having high progesterone at P2, lactation group was not significant and was dropped from the model, whereas farm, BCS, proportion of cows having high progesterone at P1, and CIDR treatment were all retained. None of the 2-way interactions were significant. Cows treated with the CIDR insert were more likely than control cows to have high progesterone

Figure 2. Proportion of cows having low serum progesterone (<1 ng/mL) at 37 ± 3 DIM (P1) vs. BCS at 2 farms. A decreasing trend was observed in the proportion of cows having low progesterone as the BCS increased ($P < 0.001$; using the Cochran-Armitage trend test). Numbers above the bars represent the total number of cows per BCS group.

at P2 (odds ratio = 2.5; 95% confidence interval 1.7, 3.5). High progesterone at enrollment (P1) also had a strong association with that at P2 (odds ratio = 7.2; 95% confidence interval 4.6, 11.3). Increasing the BCS had a significant positive ($P = 0.02$) association with high progesterone at P2 in the logistic regression model. The parameter estimates for farm, BCS, P1, and CIDR treatment were similar with and without lactation group in the model.

BCS

Body condition score had a significant relationship with the proportion of cows having low progesterone at P1 (Figure 2), with a decreasing trend in the proportion of cows having low progesterone as the BCS increased (Cochran-Armitage trend test; $P < 0.001$). A similar effect was found for the relationship between BCS and the proportion of cows having low progesterone at P2 (Figure 3; Cochran-Armitage trend test; $P < 0.001$). Although not significant in the multivariable logistic regression analysis described above, the crude association of BCS with pregnancy rate was significant (Cochran-Armitage trend test; $P < 0.02$). Having a high BCS at enrollment among cows at farms D and E was related to an increase in the proportion of cows pregnant after TAI (Figure 4).

DISCUSSION

The frequency of low-progesterone cows at 65 DIM has been reported to be between 20% (Moreira et al., 2001) and 40% (Cerri et al., 2004). Our progesterone samples were obtained earlier (58 ± 3 DIM) compared

with the literature, but the 23.9% (of 801) incidence of low-progesterone cows in the present study was similar to that in previous reports. The present study was not designed to characterize cyclicity precisely. Although the second blood sample was collected 14 d after the second injection of PGF_{2α}, the time between the first and second blood samples was 21 d, instead of the commonly used 14 d between samples (Chebel et al., 2006).

The proportion of low-progesterone cows enrolled in the study at 37 ± 3 DIM was 60.0% of 1,311 cows. Progesterone samples collected at 58 ± 3 DIM (P2) were used to evaluate the effects of treatment on the proportion of high-progesterone cows before initiation of the TAI protocol. The proportion of cows with low progesterone at P2 was 30.6% of 399 control cows and 17.4% of 402 cows in the CIDR treatment. Rhodes et al. (2003) and Chebel et al. (2006) have also found similar benefits of the CIDR insert in reducing the incidence of low-progesterone cows.

Cows in the BCS = 2 group had greater proportions of low progesterone at 37 ± 3 and 58 ± 3 DIM compared with those in the BCS = 3.5 group. First-parity cows did not have a greater proportion of low progesterone compared with second-parity cows or those in their third or greater parity. Primiparous cows are described as having a greater incidence of low progesterone when compared with multiparous cows (Lucy et al., 1992; Moreira et al., 2001), presumably because of their greater nutritional requirement for growth and lactation. It is unclear why parity did not affect the proportions of cows with low progesterone in this study. Primiparous cows also have been reported to have greater fertility after TAI when compared with multiparous

Figure 3. Proportion of cows having low serum progesterone (<1 ng/mL) at 58 ± 3 DIM (P2) vs. BCS at 2 farms. A decreasing trend was observed in the proportion of cows having low progesterone as the BCS increased ($P = 0.001$; using the Cochran-Armitage trend test). Numbers above the bars represent the total number of cows per BCS group.

cows (Stevenson et al., 2003; Chebel et al., 2004; Sterry et al., 2006).

Moreira et al. (2001) reported that the pregnancy rate for cows having low progesterone at the initiation of Ovsynch was lower (18.6% of 242 cows) than that for cows having high progesterone (43.1% of 873 cows), regardless of treatment. In our study, cows having low progesterone at 58 ± 3 DIM had a pregnancy rate of 18.2% of 192 cows compared with 43.2% of 609 cows having high progesterone. There was no significant effect of CIDR treatment on the pregnancy rate, despite reducing the proportion of low-progesterone cows. Furthermore, logistic regression analysis of data from farms D and E, performed to detect the effect of progesterone on the pregnancy rate, indicated a significant

detrimental effect of treatment on the pregnancy rate. It is interesting that the overall effect of treatment was not significant, but when the logistic regression was adjusted for the effect of the proportion of cows having high progesterone at 58 ± 3 DIM, the CIDR treatment had a detrimental effect on the pregnancy rate. That is because among all cows, regardless of progesterone status, there were fewer pregnant cows in the CIDR treatment than in the control treatment.

Vasconcelos et al. (1999) showed that 96% of cows in the fifth to ninth day of the estrous cycle (early luteal phase) at the time of Ovsynch initiation ovulated in response to the first GnRH injection, but for cows in d 10 to 16 of estrus (late luteal phase), this incidence decreased to 54%. Cows in the late luteal phase that

Figure 4. Pregnancy rate by BCS for cows at 2 farms. The pregnancy rate increased ($P = 0.015$) with increasing BCS (using the Cochran-Armitage trend test). Numbers above the bars represent the total number of cows per BCS group.

did not ovulate after the first injection of GnRH might have early CL regression before the PGF_{2α} injection. They also subsequently have a lower incidence of ovulation after the second GnRH injection and a decreased pregnancy rate compared with cows in the early luteal phase (Moreira et al., 2000). Chebel et al. (2006) observed that estrus was better synchronized and occurred earlier following the combination of Presynch (2 injections of PGF_{2α}) and a CIDR insert—more than 55% by the fourth day after the second PGF_{2α}, compared with only about 46.3% when using PGF_{2α} alone. This would result in more cows being in the late luteal phase instead of the intended early luteal phase at the time of Ovsynch initiation. A shorter interval between the last PGF_{2α} injection of the CIDR presynchronization protocol and the initiation of Ovsynch should improve the pregnancy rate by increasing the proportion of cows in the early luteal phase at the initiation of Ovsynch. Chebel et al. (2006) also observed that cows that were presynchronized with 2 injections of PGF_{2α} tended to have a greater incidence of ovulation after the first GnRH injection of the Ovsynch protocol when compared with cows exposed to the combination of Presynch and the CIDR insert. These findings support the hypothesis that cows receiving a combined PGF_{2α}-CIDR presynchronization can benefit from a shorter interval between the second PGF_{2α} injection of the presynchronization protocol and initiation of the Ovsynch protocol.

Cows treated with the CIDR insert and having low progesterone at 58 ± 3 DIM also tended to have a decreased pregnancy rate compared with control cows (farms D and E). Depending on the cause of anestrus, some anestrus cows may be more readily responsive to progesterone from the CIDR insert. Studies (Gümen et al., 2003; Murugavel et al., 2003) have shown that some anestrus cows do respond to the Ovsynch protocol and become pregnant. Therefore, some control cows having low progesterone at 58 DIM might have responded to the Ovsynch protocol. In contrast, these same responsive cows treated with the CIDR insert may have been induced to cycle earlier in response to progesterone and were classified as having high progesterone at 58 DIM, leaving only anestrus cows that would not respond to progesterone.

CONCLUSIONS

Presynchronization of estrous cycles by using the CIDR insert significantly decreased the incidence of cows having low progesterone at the initiation of Ovsynch. Treatment with progesterone via the CIDR insert did not increase pregnancy rates at first service. Earlier expression of estrus after the second PGF_{2α} injection, and consequently improper timing of initiation

of the Ovsynch protocol, could have negatively influenced the pregnancy rate in cows exposed to the CIDR insert.

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REFERENCES

- Burke, J. M., R. L. De La Sota, C. A. Risco, C. R. Staples, E. J. P. Schmitt, and W. W. Thatcher. 1996. Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. *J. Dairy Sci.* 79:1385–1393.
- Cerri, R. L. A., J. E. P. Santos, S. O. Juchem, K. N. Galvão, and R. C. Chebel. 2004. Timed artificial insemination with estradiol cypionate or insemination at estrus in high producing dairy cows. *J. Dairy Sci.* 87:3704–3715.
- Chebel, R. C., J. E. P. Santos, R. L. A. Cerri, H. M. Rutigliano, and R. G. S. Bruno. 2006. Reproduction on dairy cows following progesterone insert presynchronization and resynchronization protocols. *J. Dairy Sci.* 89:4205–4219.
- Chebel, R. C., J. E. P. Santos, J. P. Reynolds, R. L. A. Cerri, S. O. Juchem, and M. Overton. 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. *Anim. Reprod. Sci.* 84:239–255.
- DeJarnette, J. M., R. R. Salverson, and C. E. Marshall. 2001. Incidence of premature estrus in lactating dairy cows and conception rates to standing estrus or fixed-time inseminations after synchronization using GnRH and PGF_{2α}. *Anim. Reprod. Sci.* 67:27–35.
- Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart of Holstein dairy cows. *J. Dairy Sci.* 72:68–78.
- Ferguson, J. D., and D. T. Galligan. 1993. Prostaglandin synchronization programs in dairy herds—Part 1. *Comp. Contin. Educ. Pract. Vet.* 15:646–655.
- Galvão, K. N., J. E. P. Santos, S. O. Juchem, R. L. A. Cerri, A. C. Coscioni, and M. Villasenor. 2004. Effect of addition of a progesterone intravaginal insert to a timed insemination protocol using estradiol cypionate on ovulation rate, pregnancy rate, and late embryonic loss in lactating dairy cows. *J. Anim. Sci.* 82:3508–3517.
- Gümen, A., J. N. Guenther, and M. C. Wiltbank. 2003. Follicular size and response to ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. *J. Dairy Sci.* 86:3184–3194.
- Lucy, M. C., C. R. Staples, W. W. Thatcher, P. S. Erickson, R. M. Cleale, J. L. Firkins, J. H. Clark, M. R. Murphy, and B. O. Brodie. 1992. Influence of diet composition, dry-matter intake, milk production and energy balance on time of postpartum ovulation and fertility in dairy cows. *Anim. Prod.* 54:323–331.
- Moreira, F., R. L. de la Sota, T. Diaz, and W. W. Thatcher. 2000. Effect of day of the estrus cycle at initiation of a timed artificial insemination protocol on reproduction responses in dairy heifers. *J. Anim. Sci.* 78:1568–1576.
- 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.

- Murugavel, K., J. L. Yáñez, P. Santolaria, M. López-Béjar, and F. López-Gatius. 2003. Luteal activity at the onset of a timed insemination protocol affects reproductive outcome in early postpartum dairy cows. *Theriogenology* 60:583–593.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Opsomer, G., Y. T. Gröhn, J. Hertl, M. Coryn, H. Deluyker, and A. de Kruif. 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: A field study. *Theriogenology* 53:841–857.
- Plaizier, J. C. B., G. J. King, J. C. M. Dekkers, and K. Lissemore. 1996. Estimation of economic value of indices for reproductive performance in dairy herds using computer simulation. *J. Dairy Sci.* 80:2775–2783.
- Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF_{2α} and GnRH. *J. Dairy Sci.* 44:915–923.
- Rhodes, F. M., S. McDougall, C. R. Burke, G. A. Verkerk, and K. L. MacMillan. 2003. Invited review: Treatment of cows with an extended postpartum anestrous interval. *J. Dairy Sci.* 86:1876–1894.
- Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Effect of interval from timed AI to initiation of resynchronization of ovulation on fertility of lactating dairy cows. *J. Dairy Sci.* 89:2099–2109.
- Stevenson, J. S., J. A. Cartmill, B. A. Hensley, and S. Z. El-Zarkouny. 2003. Conception rates of dairy cows following early non-pregnant diagnosis by ultrasonography and subsequent treatments with shortened Ovsynch protocol. *Theriogenology* 60:475–483.
- 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.