Effect of gonadotropin-releasing hormone or prostaglandin $F_{2\alpha}$ -based estrus synchronization programs for first or subsequent artificial insemination in lactating dairy cows

R. G. S. Bruno,*† A. M. Farias,* J. A. Hernández-Rivera,* A. E. Navarrette,* D. E. Hawkins,† and T. R. Bilby*¹

*Texas A&M AgriLife Research and Extension, Texas A&M System, Stephenville 76401

†Department of Agricultural Science, West Texas A&M University, Canyon 79015

ABSTRACT

The objective was to evaluate the effects of GnRH or PGF_{2 α} -based synchronization and resynchronization programs on fertility in lactating dairy cows. For experiment 1, cows ($n = 1,521$) were presynchronized with 2 injections of PGF given at 36 and 50 DIM and assigned to 1 of 3 protocols: Ovsynch [OVS; $n = 552$; GnRH injection, PGF_{2 α} injection 7 d later, GnRH injection 56 h later, and timed artificial insemination (TAI) 16 h later] beginning at 14 d after presynchronization (PS), GnRH-GnRH-PGF_{2 α} -GnRH (GGPG; $n = 402$) treatment with a GnRH injection given 7 d after PS and OVS 7 d later, or PGF_{2 α} -GnRH-PGF_{2 α} -GnRH (P7GPG; $n = 567$) treatment with a PGF_{2 α} injection given 7 d after PS and OVS beginning 7 d later. Experiment 2 cows ($n = 2,327$) were assigned to 1 of 3 resynchronization protocols 7 d before nonpregnancy diagnosis (NPD): GGPG cows ($n = 458$) received a GnRH injection at enrollment and OVS at NPD; P7GPG cows ($n = 940$) received a PGF_{2 α} injection at NPD and OVS 7 d later; and P11GPG cows ($n = 929$) received a PGF_{2 α} injection 3 d after NPD and OVS 11 d later. In both experiments, cows were artificially inseminated upon estrus detection (ED). In experiment 1, 52.3% of cows were artificially inseminated upon ED, with GGPG having reduced ED (GGPG = 46.8 vs. OVS = 50.7 and P7GPG = 57.7%). Treatments did not affect overall pregnancy per artificial insemination (P/AI) at 36 and 66 d after AI (OVS = 34.1 and 32.3, P7GPG = 34.6 and 31.9, and GGPG = 31.3 and 28.1%, respectively) or pregnancy loss but cows artificially inseminated upon ED had higher P/AI than cows undergoing TAI (ED cows = 37.9 vs. TAI cows = 28.8%). Treatment did not affect P/AI for cows artificially inseminated upon ED or TAI at 36 and 66 d after AI (OVS = 34.1 and 32.3, P7GPG = 34.6 and 31.9, and GGPG = 31.3 and 28.1%). Median days in milk

at first AI was affected by treatment (P7GPG = 59 vs. OVS = 68 and GGPG = 68 d). In experiment 2, GGPG reduced ED (GGPG = 23.3 vs. P7GPG = 74.9 and P11GPG = 79.6%). Treatment did not affect overall P/AI at 36 and 66 d after AI (GGPG = 29.2 and 25.8, P7GPG = 28.7 and 26.6, and P11GPG = 31.9 and 30.2%) or pregnancy loss. Cows artificially inseminated upon ED had greater P/AI than TAI cows (ED = 32.3 and TAI = 25.1%). However, treatment did not affect P/AI for cows artificially inseminated upon ED at 36 and 66 d after AI (GGPG = 29.6 and 27.3, P7GPG = 29.4 and 28.1, and P11GPG = 35.7 and 33.7%) or TAI (GGPG = 29.1 and 25.3, P7GPG = 25.0 and 22.1, and P11GPG = 16.9 and 16.9%). Median days between NPD and AI was affected by treatment (GGPG = 10 vs. P7GPG = 4 and P11GPG = 7 d). Prostaglandin-based programs increased ED and reduced interval to first AI and between AI. Gonadotropin-releasing hormone-based programs increased the proportion of TAI cows. Cows artificially inseminated upon ED had increased P/AI compared with TAI cows.

Key words: dairy cow, fertility, resynchronization, synchronization

INTRODUCTION

Within the last decade, hormonal manipulation of the estrous cycle has been observed more frequently in dairy herds worldwide. Estrus synchronization programs have been researched extensively since the late 1990s (Pursley et al. 1997; Moreira et al., 2001; Dewey et al., 2010). The main objective of estrus synchronization is to improve AI submission rate, decrease intervals between AI, reduce days open, and improve overall reproductive performance. Estrus synchronization and resynchronization programs have an advantage of allowing cows to be bred after completing the timed AI (TAI) protocol, even though signs of estrus are not observed. However, some disadvantages include increased cost for treatments and labor.

Most synchronization and resynchronization protocols are based on initiating with an injection of GnRH,

Received June 28, 2012.

Accepted November 9, 2012.

¹Corresponding author: trbilby@ag.tamu.edu

resulting in the release of LH and ovulation of a dominant follicle in 66 to 80% of dairy cows (Vasconcelos et al., 1999), decreasing up to 17 percentage units in the number of cows displaying signs of estrus (Bruno et al., 2011). Recently, data from our laboratory demonstrated that initiating estrus resynchronization with a GnRH injection 17 d after previous AI reduced the number of cows displaying estrus. In addition, cows TAI had lower risk for pregnancy than cows AI upon estrus detection (Bruno et al., 2011).

Several strategies have been developed to improve reproductive performance in cows submitted to TAI protocols such as presynchronization (**PS**; Moreira et al., 2001; Dewey et al., 2010). Presynchronization with 2 injections of PGF_{2α} given 12 (Moreira et al., 2001) or 14 d (Navanukraw et al., 2004) before the initiation of the TAI protocol showed improvements in pregnancy per AI (**P/AI**). In addition, shortening the interval between PS and the beginning of Ovsynch (**OVS**) from 14 to 11 d improved fertility (Galvão et al., 2007). However, some commercial dairies still use a 14-d interval due to the ability to manage injections on 1 d of the week. Dewey et al. (2010) compared 3 resynchronization protocols in dairy cows and reported an improvement in P/AI when a GnRH injection was given 7 d before initiation of the resynchronization TAI protocol.

Although these studies have indicated that herd fertility can be improved by implementing a synchronization or resynchronization protocol, most of the studies evaluated only fertility to TAI and did not use estrus detection or did not evaluate the fertility of cows artificially inseminated based on observed estrus. Evaluating only fertility to TAI can be misleading, as a large portion of dairies in the United States use estrus detection as part of their reproduction program (USDA, 2009) and some reproduction programs may influence the proportion of cows artificially inseminated upon detected estrus. A common practice on commercial dairies in the United States is to artificially inseminate upon detection of estrus within a synchronization program (USDA, 2009), thereby increasing the proportion of subfertile cows to receive TAI (Stevenson and Phatak, 2005). When this breeding strategy is implemented, better fertility is observed in cows artificially inseminated upon detection of estrus (Stevenson and Phatak, 2005). After a PS strategy is implemented, approximately half of the cows are artificially inseminated upon estrus detection and the other half complete the TAI protocol (Jordan et al., 2002; Bruno et al., 2009), which is dependent on estrus-detection level of the dairy farm.

Reproduction programs on dairies should be designed to evaluate not only fertility of TAI but overall reproductive efficiency, which would include both TAI and AI upon detection of estrus. Furthermore, reproduction

programs that maximize the number of cows artificially inseminated upon estrus detection might yield a higher risk for P/AI and improve overall herd fertility.

Two experiments were designed to evaluate the entire reproduction program (i.e., both cows artificially inseminated upon estrus detection and TAI) for first service (experiment 1) and subsequent AI (experiment 2). In both experiments, different synchronization protocols were used that would allow more cows to be artificially inseminated upon estrus detection and (or) improve fertility for the TAI protocol.

The objective of the first experiment was to evaluate the effect of adding a GnRH or PGF_{2α} injection in between the PS and OVS protocols for first AI on reproductive performance in lactating dairy cows. The objective of the second experiment was to compare reproductive performance of cows submitted to a GnRH-based resynchronization protocol with 2 other PGF_{2α}-based resynchronization protocols. We hypothesized that in both synchronization and resynchronization programs, the PGF_{2α}-based programs would allow more cows to be artificially inseminated upon estrus detection, whereas GnRH-based protocols would improve fertility of TAI, therefore increasing the overall risk for pregnancy.

MATERIALS AND METHODS

Animals, Housing, and Diets

Both experiments were conducted simultaneously on a single commercial dairy farm located in Hereford, Texas between March and November 2011. Both primiparous ($n = 1,681$) and multiparous ($n = 2,167$) lactating Holstein cows were housed in open corrals equipped with metal framework shade at the center of the pen, providing approximately 5 m² of shade per cow and clean water available ad libitum. All cows received the same diet twice daily as a TMR allowing ≤5% feed refusals daily. Diets were formulated to meet or exceed NRC (2001) recommendations for lactating mature cows producing >40 kg of 3.7% FCM/d.

All animals participating in this experiment were milked twice per day and monitored daily for signs of diseases. If any health issues occurred, animals were moved to hospital pens and appropriate treatment was performed by following dairy protocols until total recovery. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at Texas A&M University (College Station).

Experiment 1

Treatments. All cows had their estrous cycle pre-synchronized with 2 i.m. injections of 25 mg of PGF_{2α}

(Lutalyse; 5 mg/mL of dinoprost tromethamine sterile solution; Pfizer Animal Health, Madison, NJ) given 14 d apart, at 36 ± 3 and 50 ± 3 DIM. At the time of the second PGF_{2α} injection, cows (n = 1,521) were blocked by parity and DIM and randomly assigned to 1 of 3 reproductive programs (Figure 1): cows in the GnRH-GnRH-PGF_{2α}-GnRH (**GGPG**; n = 402) group received an injection of 86 µg of GnRH (Fertagyl; 43 µg/mL of gonadorelin sterile solution, equivalent to 50 µg/mL of gonadorelin diacetate tetrahydrate; Intervet/Merck Animal Health, Summit, NJ) 7 d after PS at 57 ± 3 DIM, followed by the OVS protocol (GnRH injection, PGF_{2α} injection 7 d later, GnRH injection 56 h later, and TAI 16 h later) 7 d later at 64 ± 3 DIM; cows in the PGF_{2α}-GnRH-PGF_{2α}-GnRH (**P7GPG**; n = 567) group received an injection of PGF_{2α} 7 d after

PS at 57 ± 3 DIM, followed by the OVS protocol 7 d later at 64 ± 3 DIM; cows in the OVS (n = 552) group were used as control and received only the OVS protocol 14 d after PS at 64 ± 3 DIM. All activities in experiment 1 are depicted in Figure 1.

Cows were allowed to be inseminated upon detected estrus at any time after PS. All cows had their tail heads painted with chalk (All-Weather Paintstik, livestock marker; LA-CO Industries Inc., Elk Grove Village, IL) daily after PS and were considered in estrus upon removal of chalk. Cows in estrus were artificially inseminated on the same morning as estrus was detected by 1 of 2 experienced farm technicians. All cows artificially inseminated upon estrus detection were removed from subsequent injections of the TAI protocol.

Figure 1. Diagram of experimental activities in experiment 1. GGPG = GnRH-based synchronization program; P7GPG = PGF_{2α}-based synchronization program; OVS = Presynch-Ovsynch synchronization program; P and PGF = 25 mg of PGF_{2α} (dinoprost tromethamine; Lutalyse; 5 mg/mL; Pfizer Animal Health, Madison, NJ); G and GnRH = 86 µg of GnRH (gonadorelin sterile solution; Fertagyl; 43 µg/mL; Intervet/Merck Animal Health, Summit, NJ); TAI = timed AI; PD = pregnancy diagnosis; ● = blood sample and ultrasound scan.

BCS, Ovarian Structures, and Blood Collection. At experiment enrollment, all cows were scored for body condition using a 5-point scale according to Ferguson et al. (1994). In addition, a subsample of 300 cows completing the TAI program (100 cows/treatment) had their ovaries examined on the day of first GnRH injection (64 ± 3 DIM) and again on the day of PGF_{2 α} injection (71 ± 3 DIM) of the OVS TAI protocol, using a real-time portable ultrasound scanner (Ibex Pro; E. I. Medical Imaging, Loveland, CO) equipped with 7.5-MHz rectal linear probe. Ovulation to first GnRH injection of the OVS TAI protocol was determined by presence of at least 1 follicle larger than 10 mm at the initiation of the protocol, which was replaced by a corpus luteum (CL) at the time of PGF_{2 α} injection.

Blood samples (10 mL) were also collected on the day of first GnRH injection and again on the day of PGF_{2 α} injection of the OVS TAI protocol from the same subsample of cows that had their ovaries scanned (100 cows/treatment) by puncture of the coccygeal vein or artery into evacuated tubes containing EDTA (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Immediately after collection, samples were placed on ice and processed at the laboratory within 4 h of collection. Blood tubes were centrifuged at $3,000 \times g$ for 15 min at 4°C for plasma separation. Plasma was frozen at -25°C and later analyzed for plasma progesterone (P₄) by a solid-phase RIA using a commercial kit (Coat-A-Count; Siemens Healthcare Diagnostics Inc., Los Angeles, CA). A single assay was performed with all samples. The assay sensitivity was 0.1 ng/mL and the intra- and interassay coefficients of variation were 5.2 and 8.3%, respectively. Plasma P₄ concentration was used to determine cyclicity and reproductive program response. Cows with P₄ concentrations <1 ng/mL were considered to be without a functional CL and cows with P₄ $\geq$ 1 ng/mL were considered to have a functional CL (Ribeiro et al., 2011).

Pregnancy Diagnosis. Pregnancy status was diagnosed at 36 ± 3 d after AI by transrectal ultrasonography of the reproductive tract. Observation of embryonic fluid, appearance of the embryo, and embryonic heartbeat were used as determinants of pregnancy. Pregnancies were reconfirmed at 66 ± 3 d after AI. Pregnancy per AI was calculated at 36 and 66 d after AI and pregnancy loss between these 2 evaluation points was also calculated. Pregnancy rate (PR) at d 66 was also calculated on a weekly basis from experiment enrollment until TAI for each treatment.

Experiment 2

Treatments. A week before nonpregnancy diagnosis (NPD) at 29 ± 3 d after previous AI, cows were

blocked by parity, times inseminated, projected 305-d milk yield and DIM, and randomly assigned to 1 of 3 resynchronization programs (Figure 2). Cows assigned to the GGPG protocol ($n = 458$) received an injection of 86 μ g of GnRH (Fertagyl; 43 μ g/mL of gonadorelin sterile solution; Intervet/Merck Animal Health) 7 d before the NPD and nonpregnant cows started the OVS TAI protocol at the time of NPD. Cows assigned to the P7GPG protocol ($n = 940$) received an injection of PGF_{2 α} at the time of NPD and started the OVS TAI protocol 7 d later. Cows assigned to the P11GPG protocol ($n = 929$) received an injection of PGF_{2 α} 3 d after the NPD and started the OVS TAI protocol 11 d later. All activities in experiment 2 are depicted in Figure 2.

Cows were allowed to be inseminated upon estrus detection at any time after experiment enrollment. Estrus detection was performed daily by 2 experienced technicians as described in experiment 1 and all cows artificially inseminated upon detected estrus were removed from subsequent injections of the TAI protocol.

Reenrollment of Previously Resynchronized Cows. Cows were allowed to be reenrolled in the experiment for further resynchronization at any time they fulfilled the enrollment criterion. At any time a cow was enrolled in this experiment, cows were randomly assigned to treatments 7 d before NPD.

BCS, Ovarian Structures, and Blood Collection. At NPD, all cows were scored for BCS using a 5-point scale as described on experiment 1. In addition, a subsample of 300 cows completing the TAI program (100 cows/treatment) had their ovaries examined on the day of first GnRH injection and again at PGF_{2 α} injection of the TAI protocol, as described on experiment 1.

Blood samples (10 mL) were collected on day of first GnRH injection and again at PGF_{2 α} injection of the OVS TAI protocol from the same sub-sample of cows that had their ovaries scanned (100 cows/treatment) as described on experiment 1. The assay sensitivity was 0.1 ng/mL and the intra- and interassay CV were 5.1 and 8.9%, respectively.

Pregnancy Diagnosis. Pregnancy status was diagnosed at 36 ± 3 and 66 ± 3 d after AI by transrectal ultrasonography of the reproductive tract as described for experiment 1. Pregnancy per AI, PR, and pregnancy loss were calculated similarly as for experiment 1.

Experimental Design and Statistical Analyses

The experimental design for both experiments was a randomized complete block design (Kuehl, 1994). In experiment 1, cows were blocked according to parity and DIM. Within each block, cows were randomly assigned on a weekly basis to 1 of 3 synchronization protocols.

Figure 2. Diagram of experimental activities in experiment 2. GGPG = GnRH-based resynchronization program; P7GPG = resynchronization program starting with PGF_{2α} at nonpregnancy diagnosis, followed by Ovsynch 7 d later; P11GPG = resynchronization program starting with PGF_{2α} 3 d after nonpregnancy diagnosis, followed by Ovsynch 11 d later; P = 25 mg of PGF_{2α} (dinoprost tromethamine; Lutalyse; 5 mg/mL; Pfizer Animal Health, Madison, NJ); G = 86 µg of GnRH (gonadorelin sterile solution; Fertagyl; 43 µg/mL; Intervet/Merck Animal Health, Summit, NJ); TAI = timed AI; PD = pregnancy diagnosis; ● = blood sample and ultrasound scan.

In experiment 2, cows were blocked by parity, number of times inseminated, projected 305-d milk yield, and DIM. Within each block, cows were randomly assigned to 1 of 3 resynchronization protocols.

A sample size calculation was performed (Minitab 15; Minitab Inc., 2000) to estimate the number of experimental units required to detect a 7-percentage unit difference in P/AI with 95% confidence ($\alpha = 0.05$) and 80% protection against a type II error ($\beta = 0.20$), given that P/AI ranges from 30 to 40% for first AI and 22 to 35% for subsequent AI. Furthermore, the number of experimental units provided enough pregnant cows at 66 ± 3 d after AI to detect statistical significance with a 8-percentage unit difference in pregnancy loss among treatments ($\alpha = 0.05$; $\beta = 0.20$).

Descriptive statistics were generated with PROC UNIVARIATE, MEANS, and FREQ of SAS (SAS 9.2; SAS Institute, 2008). Plasma P₄ concentration data (with normal distribution) were transformed into 2 categories (high or low concentration) following a binomial distribution. Concentrations higher than 1 ng/mL were defined as high and concentrations ≤ 1 ng/mL were defined as low plasma P₄ concentration. Binomially

distributed data such as occurrence of estrus, P/AI, plasma P₄ levels (high or low), and ovulation to first GnRH of the TAI protocol were analyzed by logistic regression using PROC LOGISTIC of SAS. A backward stepwise regression model was used (Allison, 1999), and the full model included the effects of treatment, parity, BCS, previous lactation milk yield, number of times inseminated at enrollment, and the interactions between treatment and the respective explanatory variables. Variables were continuously removed from the model by the Wald statistic criterion if the significance was >0.15 . Treatment was forced into all models and the Akaike information criterion was used to define the best model to be used for each dependent variable analysis. Treatment differences with $P \leq 0.05$ were considered significant, and $0.05 < P \leq 0.10$ were designated as a tendency to differ.

For both experiments, the effect of reproductive program on time from PS (experiment 1) or experiment enrollment (experiment 2) to AI were analyzed by a nonparametric Kruskal-Wallis analysis of ranks test. In both experiments, the data distribution was skewed to the right due to TAI protocol generating a distribution-

free data. The median-days interval between PS or experiment enrollment and first AI was also analyzed by survival analysis using the product limit method of the Kaplan-Meier model (Kaplan and Meier, 1958) by PROC LIFETEST using a Weibull distribution to illustrate the interval differences in survival curves among the 3 reproductive programs in each experiment.

RESULTS

Experiment 1

Milk Yield and BCS. A total of 915 multiparous and 606 primiparous lactating dairy cows ($n = 1,521$) were enrolled in experiment 1, of which 552 were assigned to the OVS protocol, 402 were assigned to the GGPG protocol, and 567 were assigned to the P7GPG protocol. The average milk yield at experiment enrollment was not different across reproductive programs (OVS = 37.7 ± 0.39 , GGPG = 38.5 ± 0.45 , and P7GPG = 38.4 ± 0.40 kg/d). The BCS at enrollment did not differ among treatments and within each treatment, the median BCS for multiparous and primiparous cows were 2.75 and 3.00, respectively.

Estrus Detection and Median Days to AI. Estrus was observed daily throughout the synchronization program and cows were allowed to be artificially inseminated on the same morning as estrus was detected. The proportion of cows displaying estrus within 7 d after PS did not differ across treatments. However, a large proportion of cows receiving the P7GPG program were identified in estrus within the second week after PS. Overall, the P7GPG program had the largest proportion of cows being artificially inseminated upon estrus detection, whereas the GGPG program had the largest proportion of cows completing the TAI protocol (Table 1). Estrus detection was affected ($P < 0.001$) by parity but no interaction between parity and type of reproductive program was detected [multiparous = 58.7 and primiparous = 42.6%, adjusted odds ratio (AOR) = 1.62; 95% CI = 1.19 to 2.21]. The nonparametric method Kruskal-Wallis rank test showed differences ($P < 0.001$) in survival curves across synchronization programs for the median-day interval between PS and first service, which was 11 d for cows assigned to the P7GPG program, whereas it was 34 and 22 d for the GGPG and OVS cows, respectively (Figure 3). The median days in lactation to first AI was 59 d for the P7GPG program, whereas for both GGPG and OVS, the median days to first AI was 68 d.

Fertility Responses. Overall, P/AI at 36 and 66 d after first service were not different between types of synchronization programs (Table 1). However, cows inseminated upon estrus detection had greater ($P < 0.01$)

P/AI at 36 d after AI than TAI cows (37.9 vs. 28.8%; AOR = 1.43; 95% CI = 1.10 to 1.86) and also at 66 d after AI (35.2 vs. 26.5%; AOR = 1.58; 95% CI = 1.26 to 1.98). Parity also affected P/AI at 36 and 66 d after AI in which primiparous cows had greater P/AI ($P < 0.01$) than multiparous cows at both 36 (36.9 vs. 31.3%; AOR = 1.39; 95% CI = 1.12 to 1.74) and at 66 d after AI (34.2 vs. 28.9%; AOR = 1.37; 95% CI = 1.10 to 1.72). As expected, the PR on d 66 from the first week after PS and before initiating the treatments did not differ ($P = 0.24$). However, in the second week (from 8 to 14 d after experiment enrollment), cows receiving the P7GPG protocol had an increased ($P < 0.01$) PR compared with the other 2 protocols (Table 1).

Ovarian Responses and Circulating Progesterone. Ovulation to first GnRH of the OVS TAI protocol did not differ across synchronization protocols and averaged 54.5% (Table 2). Plasma P_4 concentration on day of TAI protocol initiation was affected by synchronization protocol. A higher proportion of cows assigned to the GGPG protocol had a P_4 concentration >1 ng/mL compared with OVS and P7GPG protocols at first GnRH injection of the TAI protocol (Table 2). Nevertheless, the proportion of cows with P_4 concentration >1 ng/mL at the time of PGF_{2α} injection of the OVS TAI protocol did not differ across synchronization protocols (Table 2).

Experiment 2

Milk Yield and BCS. A total of 2,327 lactating dairy cows were initially enrolled in experiment 2 seven days before NPD. A cow could be enrolled multiple times if continuously found nonpregnant at NPD. A total of 1,252 multiparous and 1,075 primiparous lactating dairy cows were enrolled, of which 458 were assigned to the GGPG protocol, 940 were assigned to the P7GPG protocol, and 929 were assigned to the P11GPG protocol. The average milk yield at experiment enrollment was not different across resynchronization programs (GGPG = 35.5 ± 0.35 , P7GPG = 35.9 ± 0.24 , and P11GPG = 35.6 ± 0.26 kg/d). The BCS at enrollment did not differ among resynchronization programs and within each program, the median BCS for multiparous and primiparous cows were 2.75 and 3.00, respectively.

Estrus Detection and Median Days to AI. Type of resynchronization program affected ($P = 0.002$) the proportion of cows inseminated upon estrus detection. Cows assigned to the GGPG resynchronization program had the lowest proportion of cows being identified in estrus and, therefore, the largest proportion of cows submitted to TAI (Table 3). Estrus detection was affected by parity ($P = 0.002$) but no interaction between parity and type of reproductive program was detected

Figure 3. Kaplan-Meier survival analysis illustrating the effect of synchronization programs (experiment 1) on the interval between the voluntary waiting period (VWP) and AI for 1,521 cows enrolled in 3 synchronization programs for first AI (OVS = Presynch-Ovsynch synchronization program; GGPG = GnRH-based synchronization program; P7GPG = PGF_{2α}-based synchronization program). A difference existed in median days between VWP and first AI (OVS = 22, GGPG = 23, and P7GPG = 11 d, $P < 0.001$).

(multiparous = 68.9 and primiparous = 61.6%; AOR = 1.62; 95% CI = 1.18 to 2.21).

The interval between experiment enrollment and re-insemination was different ($P < 0.001$) and identified across resynchronization programs. The median-day interval between experiment enrollment and subsequent AI was 11 d for cows assigned to the P7GPG protocol, whereas it was 14 and 17 d for the P11GPG and GGPG cows, respectively (Figure 4). The median days between NPD and subsequent AI was 5 d for the P7GPG protocol, whereas for P11GPG and GGPG were 8- and 10-d intervals.

Fertility Responses. Overall, P/AI at 36 and 66 d after first service were not different between types of resynchronization programs (Table 3). However, cows inseminated upon estrus detection had greater ($P < 0.01$) P/AI than TAI cows at 36 d (32.2 vs. 25.2%; AOR = 1.59; 95% CI = 1.12 to 2.26) and also at 66 d after AI (30.5 vs. 22.6%; AOR = 1.66; 95% CI = 1.16 to 2.38). Parity also affected P/AI at 36 and 66 d after AI. Primiparous cows had greater P/AI ($P < 0.01$) than multiparous cows at both 36 (33.6 vs. 26.83%; AOR = 1.45; 95% CI = 1.09 to 1.93) and 66 d after AI (32.0 vs. 24.4%; AOR = 1.53; 95% CI = 1.14 to 2.05).

Table 1. Overall outcomes of experiment 1

Item [% (no./total no.)]	Treatment ¹			P-value
	OVS	GGPG	P7GPG	
Estrus detection				
After PS ²	34.8 (192/552)	34.4 (138/402)	34.4 (195/567)	0.47
After TRT ³	24.4 (88/360) ^b	18.9 (50/264) ^b	35.5 (132/372) ^a	<0.01
Total ED ⁴	50.7 (280/552) ^b	46.8 (188/402) ^b	57.7 (327/567) ^a	0.002
TAI ⁵	49.3 (272/552) ^a	53.2 (214/402) ^a	42.3 (240/567) ^b	0.002
P/AI, 36 d				
After PS	42.2 (81/192)	35.5 (49/138)	39.0 (76/195)	0.47
After TRT	29.6 (26/88)	34.0 (17/50)	39.4 (52/132)	0.32
ED	38.2 (107/280)	35.1 (66/188)	39.1 (128/327)	0.65
TAI	29.8 (81/272)	28.0 (60/214)	28.3 (68/240)	0.89
Overall	34.1 (188/552)	31.3 (126/402)	34.6 (196/567)	0.71
P/AI, 66 d				
After PS	41.2 (79/192)	32.6 (45/138)	33.3 (65/195)	0.17
After TRT	27.3 (24/88)	32.0 (16/50)	38.6 (51/132)	0.21
ED	36.8 (103/280)	32.5 (61/188)	35.2 (116/327)	0.62
TAI	27.6 (75/272)	24.3 (52/214)	27.1 (65/240)	0.69
Overall	32.3 (178/552)	28.1 (113/402)	31.9 (181/567)	0.46
Pregnancy loss ⁶	5.3 (10/188)	10.3 (13/126)	7.7 (15/196)	0.54
Pregnancy rate at 66 d				
From 0 to 7 d	14.3 (79/552)	11.2 (45/402)	11.5 (65/567)	0.24
From 8 to 14 d	6.7 (24/360) ^b	6.1 (16/264) ^b	13.7 (51/372) ^a	<0.01
From 15 to 24 d	27.6 (75/272)	24.3 (52/214)	27.1 (65/240)	0.69

^{a,b}Within a row, values with different superscripts differ ($P < 0.05$).

¹OVS = Presynch-Ovsynch synchronization program; GGPG = GnRH-based synchronization program; P7GPG = PGF_{2α}-based synchronization program.

²After PS = after receiving 2 PGF_{2α} injections for presynchronization.

³After TRT = after receiving GnRH or PGF_{2α} injection 7 d after presynchronization.

⁴ED = estrus detection.

⁵TAI = timed AI.

⁶Pregnancy loss between 36 and 66 d after previous AI.

Table 2. Ovarian responses in experiment 1

Item	Treatment ¹			<i>P</i> -value
	OVS	GGPG	P7GPG	
At first GnRH injection of TAI ²				
High P ₄ ³ (%)	46.3 ^b	71.6 ^a	32.6 ^c	<0.01
Cows with CL ⁴ (%)	54.4 ^b	71.6 ^a	44.7 ^b	<0.01
At PGF _{2α} injection of TAI				
High P ₄ (%)	71.3	73.9	75.0	0.61
Cows with CL (%)	86.8	92.1	89.3	0.45
Ovulation to first GnRH injection of TAI (%)	51.9	51.1	59.9	0.31

^{a-c}Within a row, values with different superscripts differ ($P < 0.05$).

¹OVS = Presynch-Ovsynch synchronization program; GGPG = GnRH-based synchronization program; P7GPG = PGF_{2α}-based synchronization program.

²TAI = timed AI.

³Percentage of cows with progesterone (P₄) >1 ng/mL.

⁴CL = corpus luteum.

Table 3. Overall outcomes of experiment 2

Item	Treatment ¹			<i>P</i> -value
	GGPG	P7GPG	P11GPG	
Enrollment (no.)	458	940	929	
Nonpregnant 7 d after enrollment	42.0 (192/458)	40.6 (382/940)	37.5 (348/929)	
ED ² [% (n/n)]	21.9 (42/192) ^b	75.9 (290/382) ^a	78.2 (272/348) ^a	<0.01
TAI ³ [% (n/n)]	78.1 (150/192) ^a	24.1 (92/382) ^b	21.8 (76/348) ^b	<0.01
P/AI, 36 d [% (n/n)]				
From 0 to 7 d ⁴	44.4 (8/18)	31.6 (25/79)	23.1 (15/65)	0.18
From 8 to 14 d	16.7 (4/24) ^b	28.1 (55/196) ^a	39.3 (59/150) ^a	0.02
From 15 to 21 d	29.3 (44/150)	40.0 (6/15)	43.7 (21/48)	0.15
From 22 to 28 d	—	23.9 (22/92)	22.2 (2/9)	0.38
From 29 to 35 d	—	—	18.4 (14/76)	—
Overall for ED (%)	28.6 (12/42)	29.7 (86/290)	35.7 (97/272)	0.23
Overall for TAI (%)	29.3 (44/150)	23.9 (22/92)	18.4 (14/76)	0.19
Overall (%)	29.2 (56/192)	28.3 (108/382)	31.9 (111/348)	0.28
P/AI, 66 d [% (n/n)]				
From 0 to 7 d	44.4 (8/18)	31.6 (24/79)	23.1 (14/65)	0.14
From 8 to 14 d	12.5 (3/24) ^b	26.7 (52/195) ^a	38.3 (57/149) ^a	<0.01
From 15 to 21 d	25.7 (38/148)	40.0 (6/15)	37.5 (18/48)	0.19
From 22 to 28 d	—	20.1 (19/91)	22.2 (2/9)	0.36
From 29 to 35 d	—	—	18.4 (14/76)	—
Overall for ED (%)	26.2 (11/42) ^b	28.4 (82/289) ^a	33.6 (91/271) ^a	0.31
Overall for TAI (%)	25.7 (38/148)	20.1 (19/91)	18.4 (14/76)	0.41
Overall (%)	25.7 (49/190)	26.6 (101/380)	30.3 (105/347)	0.38
Pregnancy loss ⁵ [% (n/n)]	7.1 (4/56) ⁶	6.5 (7/108)	5.4 (6/111)	0.71
Pregnancy rate, 66 d [% (n/n)]				
From 0 to 7 d	4.2 (8/190) ^b	6.3 (24/380) ^a	4.3 (14/347) ^b	<0.01
From 8 to 14 d	1.7 (3/172) ^b	17.3 (52/301) ^a	20.2 (57/282) ^a	<0.01
From 15 to 21 d	25.7 (38/148) ^a	5.6 (6/106) ^c	13.5 (18/133) ^b	<0.01
From 22 to 28 d	—	20.9 (19/91) ^a	2.4 (2/85) ^b	<0.01
From 29 to 35 d	—	—	18.4 (14/76)	—
Overall for ED (%)	5.7 (11/190) ^b	21.6 (82/380) ^a	26.2 (91/347) ^a	<0.01
Overall for TAI (%)	25.7 (38/147)	20.9 (19/91)	18.4 (14/76)	0.40
Overall (%)	25.7 (49/190)	26.6 (101/380)	30.3 (105/347)	0.38

^{a-c}Within a row, values with different superscripts differ ($P < 0.05$).

¹GGPG = GnRH-based synchronization program; P7GPG = PGF_{2α}-based synchronization program; P11GPG = resynchronization program starting with PGF_{2α} 3 d after nonpregnancy diagnosis, followed by Ovsynch 11 d later.

²ED = estrus detection.

³TAI = timed AI.

⁴Days after experiment enrollment.

⁵Pregnancy loss between 36 and 66 d after previous AI.

⁶Three cows from the GGPG treatment were culled before pregnancy reconfirmation at 66 d after AI.

Figure 4. Kaplan-Meier survival analysis illustrating the effect of resynchronization programs (experiment 2) on interval between experiment enrollment and subsequent AI for 922 cows enrolled in 3 resynchronization programs (P11GPG = resynchronization program starting with PGF_{2α} 3 d after nonpregnancy diagnosis, followed by Ovsynch 11 d later; GGPG = GnRH-based synchronization program; P7GPG = PGF_{2α}-based synchronization program). A difference existed in median days between experiment enrollment and AI (P7GPG = 11, P11GPG = 14, and GGPG = 17 d, $P < 0.001$).

Three cows enrolled in the GGPG resynchronization program left the herd before pregnancy reexamination at 66 d after AI. The PR on d 66 was affected by treatments at all weekly time points measured after experiment enrollment (Table 3). Interestingly, in the first week after experiment enrollment, P7GPG had an increased ($P < 0.01$) PR compared with the other 2 treatments. This was unexpected, as no treatments were applied in the P7GPG or P11GPG groups, which can only be explained by random chance. However, during the second week, P7GPG and P11GPG protocols had similar PR and both were greater ($P < 0.01$) than that of the GGPG protocol. On the remaining weeks of the

protocols, the group which had the fixed AI scheduled for that specific week had the highest PR compared with the other treatments (Table 3).

Ovarian Responses and Circulating Progesterone. Ovulation to first GnRH of the OVS TAI protocol did not differ across resynchronization programs and averaged 39.5% (Table 4). Plasma P₄ concentration on day of the OVS TAI protocol initiation was affected by resynchronization program ($P < 0.001$). A higher proportion of cows assigned to the GGPG protocol had a P₄ concentration >1 ng/mL at first GnRH injection of the OVS TAI protocol compared with P7GPG but did not differ from the P11GPG protocol (Table 4). Nevertheless, the proportion of cows with a plasma P₄ concentration >1 ng/mL at time of PGF_{2α} injection of the OVS TAI protocol did not differ across protocols (Table 4). At both time points, parity affected ($P \leq 0.03$) the proportion of cows with a P₄ concentration >1 ng/mL (at GnRH injection, 46.0 vs. 61.2%, $P = 0.03$; and at PGF_{2α} injection, 64.8 vs. 82.8%, $P < 0.001$ for primiparous and multiparous, respectively).

DISCUSSION

These studies were designed to evaluate strategies to improve reproductive performance at the first (experiment 1) and subsequent (experiment 2) AI in lactating dairy cows. Our goal was to evaluate not only the TAI protocol but the entire reproductive program. For both experiments, we chose reproductive programs commonly used in dairy operations. In experiment 1, we selected the Presynch-OVS program as the control group and compared this program with the other 2 programs in which a GnRH or PGF_{2α} injection was added between the Presynch and OVS programs; in experiment 2, we selected the GGPG protocol as the

Table 4. Ovarian responses in experiment 2

Item	Treatment ¹			P-value
	GGPG	P7GPG	P11GPG	
At first GnRH injection of TAI ²				
High P ₄ ³ (%)	69.9 ^a	29.4 ^c	53.9 ^b	<0.01
Cows with CL ⁴ (%)	86.8 ^a	50.7 ^b	60.0 ^b	<0.01
At PGF _{2α} injection of TAI				
High P ₄ (%)	75.6	75.3	67.7	0.33
Cows with CL (%)	92.9 ^a	83.1 ^b	79.3 ^b	0.05
Ovulation to first GnRH injection of TAI (%)	43.7	33.3	42.0	0.42

^{a-c}Within a row, values with different superscripts differ ($P < 0.05$).

¹GGPG = GnRH-based synchronization program; P7GPG = PGF_{2α}-based synchronization program; P11GPG = resynchronization program starting with PGF_{2α} 3 d after nonpregnancy diagnosis, followed by Ovsynch 11 d later.

²TAI = timed AI.

³Percentage of cows with progesterone (P₄) >1 ng/mL.

⁴CL = corpus luteum.

control group and compared this with 2 other programs that used a PGF_{2α} injection as a PS strategy before the resynchronization program.

In both experiments, GnRH-based programs reduced the percentage of cows artificially inseminated upon estrus detection, which led to more cows being artificially inseminated after completing the TAI protocol. Furthermore, the GGPG program increased the percentage of cows with a CL and also with high P₄ blood levels (≥ 1 ng/mL) at the initiation of the TAI protocol. These effects of the GnRH on estrus expression, ovarian structure, and on blood P₄ profile has been reported by others. Recently, data from our laboratory illustrated fewer cows displaying estrus when the resynchronization protocol started with a GnRH injection 17 d after previous AI (Bruno et al., 2011). Dewey et al. (2010) reported an increased proportion of cows with a CL and Alkar et al. (2011) reported an increased proportion of cows with high levels of P₄ at the initiation of the resynchronization TAI protocol when a GnRH injection was given 7 d before TAI. These results suggest that the negative effect of GnRH on estrus expression might be associated with the injection of GnRH given 1 wk before the initiation of the TAI protocol, which induced ovulation in cows with follicles ≥ 10 mm in diameter, the threshold for ovulatory capacity in dairy cows (Sartori et al., 2001), resulting in the formation of new CL and a new follicular wave recruitment.

Estrus expression did not differ across reproductive programs in the first week after the PS protocol, as expected, as all programs received 2 injections of PGF_{2α} and the treatments were not yet applied (experiment 1). However, when a PGF_{2α} injection was given 7 d after PS, a higher proportion of cows were artificially inseminated upon estrus detection; therefore, the interval between parturition and first AI was reduced. Stevenson and Phatak (2005) reported an increased estrus expression and higher P/AI for cows receiving 2 presynchronizing PGF_{2α} injections compared with cows receiving only 1. In this experiment, a PGF_{2α} injection given 7 d after PS was able to induce more cows to express estrus than the regular 2 PGF_{2α} injections for PS and waiting 14 d until beginning the TAI protocol. Cows responding to the third PGF_{2α} injection are more likely to be cows that did not have an active CL at the end of the PS, which are probably cows that were in proestrus or metestrus. For resynchronization (experiment 2), we were able to significantly increase the number of cows artificially inseminated upon estrus detection when the resynchronization protocol started with a PGF_{2α} injection. Although ovarian structures were not examined at the time of the PGF_{2α} injection before the TAI program, it was expected that cows with normal

estrus cycle would have an active CL at the time of the NPD, thereby increasing the number of cows displaying estrus after the PGF_{2α} injection. Several studies have reported a high prevalence of cows with a CL at the time of NPD (McDougall, 2003; McArt et al., 2010). McArt et al. (2010) evaluated different resynchronization programs and reported that 43% of cows had a CL larger than 23 mm at the time of NPD, which has been indicated to be the threshold diameter to define an active CL (Bicalho et al., 2008).

Adding a PGF_{2α} injection 7 d before the initiation of the TAI protocol may not be the optimal time before initiation of a TAI protocol and could affect responses to the injections given during the TAI protocol. This may be true in herds in which cows would respond to the PGF_{2α} injection but, due to poor estrus detection, prevented them from receiving AI. In this case, cows would be starting the TAI protocol around d 3 and 4 of the estrous cycle, which is not the optimum time for improved fertility at TAI (Vasconcelos et al., 1999). Cows treated with GnRH before d 5 of the estrous cycle were less likely to respond to injections of the TAI protocol (Vasconcelos et al., 1999) and more likely to have reduced embryo quality (grades >2) and increased number of degenerate and degenerate-unfertilized structures (Cerri et al., 2009). However, in herds with good estrus detection, it is likely that estrus would be detected in most cycling cows and only cows not responding to the previous PGF_{2α} injections or cows in anestrus would be submitted to the TAI protocol.

Pregnancy per AI was not affected by type of synchronization or resynchronization program for cows artificially inseminated upon estrus detection or TAI. However, in both experiments, we observed higher fertility when cows were artificially inseminated upon estrus detection, similar to those commonly observed in commercial dairy herds. Increased fertility for cows artificially inseminated upon estrus detection has been reported by others (Jordan et al., 2002; Kasimanickam et al., 2005) and may be due to the cows completing the TAI protocol being cows that were not identified in estrus because they experienced some issue such as silent estrus, negative energy balance, uterine or metabolic diseases, lameness, asynchrony to the reproductive program, or other issue affecting estrus expression, in which its negative effect on fertility has been extensively reported.

Silva et al. (2007) reported a 10-percentage unit improvement in P/AI assessed 66 d after AI, when a PGF_{2α} injection was given 12 d before the initiation of the TAI protocol compared with a regular OVS resynchronization protocol starting on the day after NPD (35.2 vs. 25.6%, respectively). Interestingly, in our ex-

periment, when a PGF_{2α} injection was given 7 or 11 d before the initiation of the TAI program, neither cows artificially inseminated upon estrus detection nor cows receiving the TAI protocol achieved higher P/AI compared with those reported by Silva et al. (2007) when the interval between PGF_{2α} injection and TAI was 12 d. This slightly decreased P/AI observed in the current experiment compared with those reported by Silva et al., (2007) might be associated with different intervals between PGF_{2α} injection and the TAI protocol. In addition, the study by Silva et al. (2007) did not use estrus detection, so all cows underwent TAI; however, within the current experiment, all cows not observed in estrus underwent TAI. Moreover, this difference in P/AI may be related to heat stress effects, as our experiment was performed between March and October, whereas Silva et al. (2007) enrolled cows between September and May during a cooler climate.

Another factor that may have contributed to decreased P/AI in cows receiving the P7GPG protocol compared with results of Silva et al. (2007) is that few cows on this resynchronization protocol had blood P₄ levels >1 ng/mL at initiation of the TAI protocol. Cows initiating the TAI protocol with blood P₄ concentration <1 ng/mL are less likely to conceive than cows that have P₄ ≥1 ng/mL (Silva et al., 2007; Lima et al., 2009).

In both experiments, the GGPG program increased the percentage of cows initiating the TAI protocol under high P₄ levels (>1 ng/mL) compared with PGF_{2α}-based programs. This result is similar to results reported by Dewey et al. (2010) and might be due to an increased ovulation rate in response to the GnRH injection given 7 d before the initiation of the TAI protocol. It is expected that the plasma P₄ concentration during the development of the ovulatory follicle affects follicle growth rate and maturation, as P₄ accounts for approximately 37% of the variation in LH concentration (Inskeep, 2004). In addition, Denicol et al. (2012), comparing the fertility of the first follicular wave with or without P₄ supplementation and fertility of the second follicular wave, established a negative correlation between ovulatory follicle diameter and P₄ concentration during follicle development. Moreover, when the first follicular wave was superstimulated, results showed a reduction in embryo quality. However, embryo quality was improved when exogenous P₄ was supplemented during the first follicular wave but did not differ from the embryos obtained in the second follicular wave stimulation (Rivera et al., 2011). In this experiment, cows completing the GGPG resynchronization protocol had numerically higher P/AI than PGF_{2α}-based programs. However, the small sample size of cows completing the TAI protocol may

have contributed to the inability to detect a significant difference across reproductive programs.

The benefit of incorporating a PGF_{2α} injection between the Presynch and OVS program or starting the resynchronization program with an injection of PGF can be observed by the increased PR achieved during the weeks before the TAI. This improvement in PR observed mainly in the second week of the experiment enrollment in both experiments is a consequence of an increased number of cows being artificially inseminated upon estrus detection for PGF_{2α}-based TAI programs and increased P/AI in cows artificially inseminated upon estrus detection.

Although PGF_{2α}-based synchronization and resynchronization programs yielded a higher proportion of cows inseminated upon estrus detection and GnRH-based programs generated more AI at fixed-time AI, we were unable to detect a significant difference in overall fertility. However, PGF_{2α}-based PS programs for synchronization and resynchronization shortened both intervals between voluntary waiting period and first AI and interval between AI, with no difference in P/AI, which can have an economic advantage to herds using a systematic breeding program. Caution should be taken when using a PGF_{2α} injection 7 d before a TAI program if no estrus detection (i.e., 100% TAI) is used, as this could reduce TAI fertility because cows would be in the incorrect stage of the estrous cycle when initiating TAI.

CONCLUSIONS

Reproductive programs for first or subsequent AI must be selected by evaluating overall fertility outcomes, which includes both cows detected in estrus and TAI cows if the dairy uses estrus detection as part of their reproductive program. When a GnRH or PGF_{2α} injection was added in between the Presynch and OVS program for first AI, P/AI was not altered, but resulted in increased number of cows being artificially inseminated upon estrus detection when a PGF_{2α} injection was added and increased the number of cows completing the TAI protocol when a GnRH injection was added 7 d after PS. Similar results were observed at resynchronization in which starting the resynchronization program with a PGF_{2α} injection at the time of the NPD increased the number of cows being artificially inseminated upon estrus detection and shortened the interval between AI. Dairy farms with good estrus detection and conception rates to those found in estrus should take advantage of PGF_{2α}-based synchronization and resynchronization programs, as a higher percentage of cows would be artificially inseminated upon estrus

detection, decreasing the cost and labor associated with TAI protocols.

ACKNOWLEDGMENTS

This research was partly supported by the Kilgore Research Center Competitive Grants Program from the West Texas A&M University (Canyon). The authors thank Pfizer Animal Health (Madison, NJ) for providing the Lutalyse sterile solution. Our gratitude is also extended to the owners and personnel of the collaborating dairy for allowing us access to their cows and facilities to conduct this research.

REFERENCES

- Alkar, A., A. Tibary, J. R. Wenz, R. L. Nebel, and R. Kasimanickam. 2011. Presynchronization with GnRH 7 days prior to resynchronization with CO-Synch did not improve pregnancy rate in lactating dairy cows. *Theriogenology* 76:1036–1041.
- Allison, P. D. 1999. *Logistic Regression Using the SAS System: Theory and Application*. SAS Institute Inc., Cary, NC.
- Bicalho, R. C., K. N. Galvão, C. L. Guard, and J. E. Santos. 2008. Optimizing the accuracy of detecting a functional corpus luteum in dairy cows. *Theriogenology* 70:199–207.
- Bruno, R. G. S., J. G. N. Moraes, J. A. Hernández-Rivera, K. J. Lager, P. R. B. Silva, A. L. A. Scanavez, L. G. D. Mendonça, R. C. Chebel, and T. R. Bilby. 2011. Comparison of two resynchronization protocols initiated at different intervals after insemination on fertility in lactating dairy cows. *J. Dairy Sci.* 94(Suppl. 1):64 (Abstr.).
- Bruno, R. G. S., H. Rutigliano, R. L. Cerri, P. H. Robinson, and J. E. P. Santos. 2009. Effect of feeding yeast culture on reproduction and lameness in dairy cows under heat stress. *Anim. Reprod. Sci.* 113:11–21.
- Cerri, R. L. A., H. M. Rutigliano, R. C. Chebel, and J. E. P. Santos. 2009. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. *Reproduction* 137:813–823.
- Denicol, A. C., G. Lopes Jr., L. G. D. Mendonça, F. A. Rivera, F. Guagnini, R. V. Perez, J. R. Lima, R. G. S. Bruno, J. E. P. Santos, and R. C. Chebel. 2012. Low progesterone concentration during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows. *J. Dairy Sci.* 95:1794–1806.
- Dewey, S. T., L. G. D. Mendonça, G. Lopes Jr., F. A. Rivera, F. Guagnini, R. C. Chebel, and T. R. Bilby. 2010. Resynchronization strategies to improve fertility in lactating dairy cows utilizing a presynchronization injection of GnRH or supplemental progesterone: I. Pregnancy rates and ovarian responses. *J. Dairy Sci.* 93:4086–4095.
- Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* 77:2695–2703.
- Galvão, K. N., M. F. Sá Filho, and J. E. P. Santos. 2007. Reducing the interval from presynchronization to initiation of timed artificial insemination improves fertility in dairy cows. *J. Dairy Sci.* 90:4212–4218.
- Inskip, E. K. 2004. Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. *J. Anim. Sci.* 82(E-Suppl.):E24–E39.
- Jordan, E. R., M. J. Schouten, J. W. Quast, A. P. Belschner, and M. A. Tomaszewski. 2002. Comparison of two timed artificial insemination (TAI) protocols for management of first insemination postpartum. *J. Dairy Sci.* 85:1002–1008.
- Kaplan, E. L., and P. Meier. 1958. Nonparametric estimation from incomplete observations. *J. Am. Statist. Assoc.* 53:457–481.
- Kasimanickam, R., J. M. Cornwell, and R. L. Nebel. 2005. Fertility following fixed-time AI or insemination at observed estrus in Ovsynch and Heatsynch programs in lactating dairy cows. *Theriogenology* 63:2550–2559.
- Kuehl, R. 1994. Complete block designs. Pages 256–305 in *Statistical Principles of Research Design and Analysis*. Wadsworth Publishing Co., Belmont, CA.
- 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.
- McArt, J. A. A., L. S. Caixeta, V. S. Machado, C. L. Guard, K. N. Galvão, O. G. Sá Filho, and R. C. Bicalho. 2010. Ovsynch versus Ultrasynd: Reproductive efficiency of a dairy cattle synchronization protocol incorporating corpus luteum function. *J. Dairy Sci.* 93:2525–2532.
- McDougall, S. 2003. Resynchrony of previously anoestrous cows and treatment of cows not detected in oestrus that had a palpable corpus luteum with prostaglandin F_{2α}. *N. Z. Vet. J.* 51:117–124.
- Minitab Inc. 2000. MINITAB Reference Manual. Release 13.32. Minitab Inc., State College, PA.
- 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.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th ed. Natl. Acad. Press, Washington, DC.
- Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. *J. Dairy Sci.* 80:301–306.
- Ribeiro, E. S., R. L. A. Cerri, R. S. Bisinotto, F. S. Lima, F. T. Silvestre, L. F. Greco, W. W. Thatcher, and J. E. P. Santos. 2011. Reproductive performance of grazing dairy cows following presynchronization and resynchronization protocols. *J. Dairy Sci.* 94:4984–4996.
- Rivera, F. A., L. G. Mendonça, G. Lopes Jr., J. E. Santos, R. V. Perez, M. Amstalden, A. Correa-Calderón, 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.
- Sartori, R., P. M. Fricke, J. C. P. Ferreira, O. J. Ginther, and M. C. Wiltbank. 2001. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. *Biol. Reprod.* 65:1403–1409.
- SAS Institute. 2008. SAS 9.2, Version 3. SAS Institute Inc., Cary, NC.
- Silva, E., R. A. Sterry, D. Kolb, M. C. Wiltbank, and P. M. Fricke. 2007. Effect of prostaglandin F_{2α} before resynchronization of ovulation on fertility of lactating dairy cows. *J. Dairy Sci.* 90:5509–5517.
- Stevenson, J. S., and A. P. Phatak. 2005. Inseminations at estrus induced by presynchronization before application of synchronized estrus and ovulation. *J. Dairy Sci.* 88:399–405.
- USDA (US Department of Agriculture). 2009. Dairy 2007, Part IV: Reference of Dairy Cattle Health and Management Practices in the United States, 2007. USDA:Animal and Plant Health Inspection Service:Veterinary Services:Centers for Epidemiology and Animal Health (USDA:APHIS:VS:CEAH), Fort Collins, CO.
- 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.