Application of one injection of prostaglandin $F_{2\alpha}$ in the five-day Co-Synch + CIDR protocol for estrous synchronization and resynchronization of dairy heifers

M. B. Rabaglino,* C. A. Risco,* M.-J. Thatcher,* I. H. Kim,† J. E. P. Santos,‡ and W. W. Thatcher‡¹

*College of Veterinary Medicine, University of Florida, PO Box 100136, Gainesville 32610-0136

†Department of Veterinary Medicine, College of Veterinary Medicine, Chungbuk National University, 12 Gaeshin-dong Heungduk-gu, Chongju Chungbuk 361-763, South Korea

‡Department of Animal Sciences, University of Florida, PO Box 110910, Gainesville 32611-0910

ABSTRACT

The objective was to determine if the 5-d Co-Synch + CIDR (controlled internal drug releasing insert) protocol can be used in dairy heifers for a synchronized timed artificial insemination (TAI) with one injection of $PGF_{2\alpha}$ for first and second services. In experiment 1, heifers were assigned randomly to receive 1 ($n = 295$) or 2 ($n = 298$) injections of $PGF_{2\alpha}$ in the 5-d Co-Synch + CIDR protocol. Corpus luteum (CL) regression was measured in one replicate ($n = 218$). No difference in pregnancy per TAI (P/TAI; 46.1 and 48.6%) or CL regression (86.9 and 92.8%) was detected for 1 versus 2 injections of $PGF_{2\alpha}$, respectively. In experiment 2, nonpregnant heifers ($n = 86$) were assigned to a resynchronized 5-d Co-Synch + CIDR with 1 $PGF_{2\alpha}$ /TAI or insemination at detected estrus. There was no difference in P/TAI (52.2 and 55%) between groups. In experiment 3, nonpregnant heifers ($n = 110$) were assigned randomly to receive a CIDR ($n = 54$) or no CIDR insert ($n = 56$) in the 5-d Co-Synch protocol for resynchronization of TAI. Pregnancy per TAI was lower without the CIDR device (39.3 vs. 51.8%). In a commercial field evaluation, 416 heifers were synchronized for the first and resynchronized TAI with the 5-d Co-Synch + CIDR protocol with 1 injection of $PGF_{2\alpha}$. Pregnancy per TAI on d 60 was 58.2 and 47.5% for first and second TAI, respectively; there was a sire effect to the second TAI. In conclusion, the 5-d Co-Synch + CIDR protocol with 1 injection of $PGF_{2\alpha}$ is an effective reproductive management program for first and second TAI in dairy heifers.

Key words: dairy heifer, reproduction, synchronization, timed artificial insemination

INTRODUCTION

A major limitation for use of AI in dairy replacement heifers is the time and effort related to daily estrous detection (Erven and Arbaugh, 1987; Caraviello et al., 2006). Ovulation-synchronization protocols, such as Ovsynch, permit a fixed timed AI (TAI) without the need for estrous detection. This protocol consists of administering GnRH, followed 7 d later with an injection of $PGF_{2\alpha}$, and 48 h later, a second administration of GnRH and TAI 0 to 24 h later (Pursley et al., 1995; Burke et al., 1996). When the Ovsynch protocol is modified with TAI performed at the same time as the second GnRH injection, it is referred to as Co-Synch (Geary and Whittier, 1998). Such programs have been used successfully in lactating dairy cows but poorer results were obtained in dairy heifers (Pursley et al., 1995, 1997; Schmitt et al., 1996). Because of differences in follicular dynamics between cows and heifers (Savio et al., 1988; Pursley et al., 1997), dairy heifers could express estrus close to the $PGF_{2\alpha}$ injection, thereby causing asynchrony at TAI (Rivera et al., 2005).

Inclusion of controlled internal drug-release insert (CIDR, Eazi Breed CIDR, Pfizer Animal Health, New York, NY) containing progesterone in the Ovsynch or Co-Synch protocols suppresses ovulation during the days of CIDR insertion, thereby allowing 100% submission rate of heifers for TAI without affecting fertility to the TAI protocol (Rivera et al., 2005).

Bridges et al. (2008) modified the Co-Synch + CIDR protocol to a 5-d interval from the first GnRH to $PGF_{2\alpha}$ injection, with a final injection of GnRH and TAI 72 h from the $PGF_{2\alpha}$ injection. In lactating beef cows, this approach resulted in higher pregnancy per TAI (P/TAI) compared with a 7-d Co-Synch + CIDR with the second GnRH and TAI occurring concurrently at 60 h (80.0 vs. 66.7%, respectively, in experiment 1 and 65.3 vs. 56.2%, respectively, in experiment 2). Because of the shortened interval from the initial GnRH to $PGF_{2\alpha}$

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¹Corresponding author: Thatcher@ufl.edu

in the 5-d Co-Synch + CIDR protocol, it is not known whether regression of an induced accessory corpus luteum (CL) occurs with 1 injection of PGF_{2α}. Therefore, a second PGF_{2α} injection was applied approximately 12 h after the first PGF_{2α} injection, which results in additional animal handling and cost. However, 2 injections of PGF_{2α} appear to be necessary when this protocol is applied to beef cows (Kasimanickam et al., 2009) or lactating dairy cows (Chebel et al., 2008).

The hypothesis of the present experiments was that in dairy heifers, 1 injection of PGF_{2α} at the time of CIDR withdrawal in the 5-d Co-Synch + CIDR protocol would regress CL present in the ovary and result in an acceptable P/TAI. The 5-d Co-Synch + CIDR protocol with 1 injection of PGF_{2α} would simplify and reduce the cost of dairy heifer reproductive management to optimize P/TAI under commercial conditions.

Objectives were to determine 1) P/TAI to first TAI and CL regression in heifers receiving 1 or 2 injections of PGF_{2α} in the 5-d Co-Synch + CIDR protocol; 2) P/AI to the second service in heifers resynchronized with the 5-d CoSynch + CIDR protocol with 1 PGF_{2α} injection versus insemination at detected estrus; 3) whether inclusion of a CIDR device between the first GnRH and the single PGF_{2α} injection is required for an effective P/TAI to a resynchronized second TAI; and 4) field verification of the 5-d Co-Synch + CIDR program with 1 injection of PGF_{2α} for reproductive management of first and second services in dairy heifers.

MATERIALS AND METHODS

Heifers, Diets, and Housing

A total of 1,013 nulliparous Holstein heifers between 13 to 14 mo of age from 2 commercial dairy farms located in south Florida (SF) and north central Florida (NCF) were used in these studies. Heifers in both locations were managed on pasture, with access to portable shades and trees, and fed a TMR once daily that met or exceeded the nutritional requirements of Holstein heifers weighing 360 kg and gaining 0.8 kg/d (NRC, 2001). The diet for each farm was based on the following ingredients: 1) NCF location; lactation cow ration weighback, grass hay, brewers grain, and a mineral and vitamin supplement, 2) SF location; grass silage, lactation cow ration weighback, grass hay, distillers grain, citrus pulp, and a mineral and vitamin supplement. For implementation of synchronization protocol, insemination, blood collection, and pregnancy examination, heifers were handled in a barn in SF or in an open-sided shaded barn in NCF that contained self-locking stanchions.

Experiment 1: Pregnancy/First TAI and CL Regression in Dairy Heifers Receiving One or Two Injections of PGF_{2α}

From August 2007 to September 2008, heifers located in the SF location (first replicate: n = 176; second replicate: n = 218) or the NCF location (n = 199) were assigned randomly to receive 1 (n = 295) or 2 (n = 298) injections of PGF_{2α} in the 5-d Co-Synch + CIDR protocol. The protocol consisted of a first injection of GnRH (100 µg; Cystorelin, Merial Ltd., Iselin, NJ) and insertion of a CIDR containing 1.38 g of progesterone inserted at d 0; 5 d later (d 5) the CIDR was removed and 1 or 2 injections (12 h apart) of PGF_{2α} (25 mg i.m.; Lutalyse, Pfizer Animal Health) were administered; 3 d later (d 8) a second injection of GnRH was administered concurrently with TAI. Heifers were inseminated with a single sire in the NCF location. In the SF location, 2 sires and 4 sires were used for inseminations of heifers in the first and second replicates, respectively. Inseminations were performed by 4 technicians at each dairy location.

The first replicate at SF (n = 176) was used only to compare P/TAI between 1 (n = 88) or 2 (n = 88) PGF_{2α} injections. The second replicate of heifers from the SF location (n = 218) was also used to evaluate CL regression and P/TAI after 1 or 2 injections of PGF_{2α} in the 5-d Co-Synch + CIDR protocol. Heifers were assigned randomly to receive 1 (n = 109) or 2 (n = 109) injections of PGF_{2α} in the 5-d Co-Synch + CIDR protocol as described previously. In all heifers of the second replicate at SF, blood samples were collected 2 h after withdrawal of the CIDR insert and before the first injection of PGF_{2α}. Ovaries were scanned by ultrasonography to determine the number of CL. On d 6 (i.e., 24 h after the first PGF_{2α} injection), a second blood sample was collected. Blood samples were taken to characterize plasma progesterone concentrations before and after PGF_{2α} injections. Blood samples (~10 mL) were collected by puncture of the median coccygeal vein or artery using evacuated tubes (Becton Dickinson, Franklin Lakes, NJ) containing K₂ EDTA for plasma separation. Samples were placed immediately in ice and kept refrigerated until transported to the laboratory. Blood tubes were centrifuged at 3,000 × g for 15 min, and plasma was frozen at -25°C until analyses. Analysis of progesterone in plasma was determined using a Coat-A-Count Kit (DPC Diagnostic Products Corporation, Los Angeles, CA) radioimmunoassay designed for the quantitative measurement of progesterone in plasma. Samples with known low, medium, and high concentrations of progesterone were run in duplicate before the experimental samples. Intraassay CV for each concen-

tration was 3.95, 9.1, and 3.5% for low, medium, and high concentrations, respectively. Every sixth experimental sample was also duplicated. Duplicate plasma concentrations of progesterone were categorized into samples with progesterone ≥ 3.0 ng/mL and samples with progesterone ≥ 1.0 but < 3.0 ng/mL. Duplicate samples with progesterone > 3 ng/mL had intraassay CV of 8.4%, and samples with progesterone between 1.0 and 3.0 ng/mL had a CV of 12.3%. Regression of CL was defined when progesterone concentration was ≥ 1 ng/mL in the first sample (immediately before the first PGF_{2 α}) and ≤ 1 ng/mL 24 h later.

Experiment 2: Pregnancy Per TAI or AI to Second Service in Heifers Resynchronized with the 5-d Co-Synch + CIDR Protocol with One PGF_{2 α} Injection and TAI or Inseminated at Detected Estrus

Heifers from the NCF location ($n = 199$) were used for this experiment. Heifers were synchronized with the 5-d Co-Synch + CIDR protocol for the first TAI. After the first TAI, heifers were allocated randomly into 2 groups: resynchronized TAI after diagnosed as nonpregnant (TAI group; $n = 101$), and daily observation of estrus and insemination at detected estrus (IDE group; $n = 98$) plus resynchronization and TAI if diagnosed nonpregnant and not detected previously in estrus. Therefore, after the first TAI all heifers in both groups underwent daily estrous detection with the use of tail chalk. Heifers with rubbed-off chalk or that stood to be mounted were classified as being in estrus. Only those heifers seen in estrus in the IDE group ($n = 40$) were inseminated. At d 32 after the first TAI, pregnancy status was determined by ultrasonography in the TAI group and in those heifers not detected in estrus and not reinseminated in the IDE group. For the second TAI, nonpregnant heifers ($n = 46$) were resynchronized, beginning on d 32 at the time of pregnancy diagnosis, with the 5-d Co-Synch + CIDR protocol with 1 injection of PGF_{2 α} . These heifers were 43 from the TAI group plus 3 heifers nonpregnant and not seen in estrus from the IDE group. One sire was used and heifers were inseminated by 4 technicians.

Experiment 3: Inclusion of the CIDR Device in the 5-d Co-Synch Protocol with a Single Injection of PGF_{2 α} for Resynchronization of the Second TAI

Pregnancy status was determined at d 28 after the first TAI by ultrasonography and nonpregnant heifers from the SF location ($n = 110$) were assigned randomly to receive a CIDR device ($n = 54$) between the first GnRH and PGF_{2 α} injection or not ($n = 56$) in the 5-d Co-Synch protocol for resynchronization of the second

TAI. The protocols were initiated on the same day. Three sires were used for inseminations performed by 4 technicians.

Experiment 4: Field Verification of the 5-d Co-Synch + CIDR Protocol with One Injection of PGF_{2 α}

Two replicates of heifers from NCF location were synchronized for a first TAI with the 5-d Co-Synch + CIDR protocol with 1 injection of PGF_{2 α} ($n = 203$ and $n = 213$ for replicates 1 and 2, respectively). Nonpregnant heifers at 30 d after first TAI were resynchronized for a second TAI following the same protocol initiated on the same day when the diagnosis of nonpregnancy was performed. A single sire was used for the first TAI in both replicates and the second TAI of the first replicate, whereas 3 additional sires were used for the second TAI of the second replicate. Three and 2 technicians performed the AI for the first or second services, respectively.

Ultrasonography

In replicate 2 of experiment 1 at SF, ovaries were scanned by ultrasound to determine CL number in heifers receiving 1 versus 2 injections of PGF_{2 α} . Ultrasonography was also used for pregnancy diagnoses to first TAI in all experiments using a 5-MHz ultrasound unit (Easi-Scan, BCF Systems, Livingston, UK). Pregnancy diagnosis was determined between 28 and 32 d after first or second TAI and was based on the presence of an embryo with a heartbeat. Reconfirmation of pregnancy was performed at d 45 (SF location) or d 60 (NCF location) by transrectal palpation of the uterus and its contents. Pregnancy per timed AI was defined as the percentage of all animals receiving TAI that became pregnant.

Statistical Analysis

For the first experiment, P/TAI was analyzed by logistic regression using the GLIMMIX procedure of SAS (SAS Version 9.2 for Windows, SAS Institute, Cary, NC) with heifer treated as a random effect. The model for P/TAI as the dependent variable included treatment group (1 or 2 PGF_{2 α} injections), location, treatment group by location interaction, technician nested within location, sire nested within location, and technician by sire interaction nested within location.

In the second replicate at the SF location, where CL regression was measured, P/TAI and proportion of heifers that underwent CL regression were analyzed by logistic regression using the LOGISTIC procedure of SAS. Terms with $P > 0.20$ were removed from the

Table 1. Pregnancy per timed AI (P/TAI) for dairy heifers receiving 1 or 2 injections of PGF_{2α} in the 5-d Co-Synch + CIDR (controlled internal drug releasing insert) protocol in the first replicate of the south Florida (SF) location and in the north central Florida (NCF) location; experiment 1

Item	Location	
	SF (n = 176)	NCF (n = 199)
P/TAI (%) at 32 d		
One PGF _{2α} injection	43.2 (38/88) ^a	56.1 (55/98) ^b
Two PGF _{2α} injections	42.0 (37/88) ^a	57.4 (58/101) ^b
Total	42.6 (75/176)	56.8 (113/199)
P/TAI (%) at pregnancy reconfirmation ¹		
One PGF _{2α} injection	37.5 (33/88) ^a	54.0 (53/98) ^b
Two PGF _{2α} injections	38.6 (34/88) ^a	55.4 (56/101) ^b
Total	38.1 (67/176)	54.8 (109/199)

^{a,b}Means differ between locations ($P = 0.02$).

¹Pregnancy reconfirmation was performed at 45 d in the SF location and at 60 d in the NCF location.

complete model based on the Wald's statistics criterion in a stepwise manner to derive the final reduced model for each variable. The area under the receiver operating characteristic curve, also known as the c-statistic, was used to estimate the best cut-off plasma progesterone concentration in the second sample as predictor of pregnancy at first TAI. Once the best cut-off was obtained, cows were dichotomized as having progesterone concentration above or below that value as a response variable to model P/TAI for heifers in SF location. The independent variables in the model included treatment group (1 or 2 PGF_{2α} injections), number of CL present, technician, sire, and progesterone concentration 24 h after the PGF_{2α} injection as above or below the best cut-off, and interactions between these variables. Progesterone concentration was retained in the final model.

The model for the proportion of heifers that underwent CL regression included treatment, number of CL present, and interaction between these variables. There was no significant variable to be retained in a final model.

Progesterone concentration 24 h after PGF_{2α} was evaluated by ANOVA with the MIXED procedure of SAS. The model included the effects of treatment group (1 or 2 PGF_{2α} injections), number of CL present in each ovary, and the interaction between both terms, progesterone concentration in the sample obtained before PGF_{2α} as covariate, and the interaction treatment and CL number.

For experiments 2, 3, and 4, P/TAI was analyzed with the GLIMMIX procedure of SAS as described for the first experiment. Variables included in the model of the second experiment included treatment group, technician and the interaction between these variables. In the third experiment the model was treatment group, technician, sire, and the interactions between these variables. In experiment 4, the model for the first TAI

included replicate number, technician, and the interaction. For the second TAI, sire nested within replicate number was added to the model.

A value of $P \leq 0.05$ was considered significant, and a value of $P \leq 0.10$ and >0.05 was considered a tendency.

RESULTS

Experiment 1: Comparison of One Versus Two Injections of PGF_{2α} in the 5-d Co-Synch + CIDR Protocol

Pregnancy to First TAI. Overall P/TAI for heifers receiving 1 or 2 PGF_{2α} at 32 d were 48.8% (144/295) and 50.7% (151/298), respectively, and overall pregnancy losses between 32 and 45 (SF) or 60 (NCF) d of pregnancy were 5.5% (8/144) and 4.0% (6/151). Pregnancy per TAI for each PGF_{2α} treatment in the first replicate of the SF and NCF locations is shown in Table 1. Difference in P/TAI was significant [$P = 0.02$; odds ratio (OR) = 0.59; 95% CI: 0.37 to 0.94] between locations. There were no differences ($P = 0.99$) in P/TAI between 1 versus 2 PGF_{2α} treatments, and no interaction between treatment and location was detected ($P = 0.67$). Also, there were no significant effects of technician ($P = 0.72$), sire ($P = 0.52$), or technician by sire interaction ($P = 0.92$).

Determination of CL Regression. Proportions of heifers presenting 0, 1, or 2 CL in the ovary at the ultrasound scanning before PGF_{2α} injections were 3.7% (8/218), 73.4% (160/218), and 22.9% (50/218), respectively. Considering the quantitative analysis of progesterone concentration in plasma, heifers with a progesterone concentration in the first sample >1 ng/mL were considered to have an active CL (167/218). Using this criterion, the percentage of heifers that regressed their CL (i.e., <1 ng/mL in the second sample) after 1 or 2

Table 2. Proportion of corpus luteum (CL) regression and least squares means for plasma progesterone concentrations (ng/mL) in heifers with a progesterone concentration in the first sample >1 ng/mL, receiving 1 or 2 injections of PGF_{2α} in the 5-d Co-Synch + CIDR (controlled internal drug releasing insert) protocol¹

Item	PGF _{2α} injection	
	One (n = 81)	Two (n = 82)
CL regression (%)		
One CL (n = 116)	85.9 (55/64)	92.3 (48/52)
Two CL (n = 47)	88.2 (15/17)	93.3 (28/30)
Progesterone (ng/mL) 24 h after PGF _{2α}		
One CL	0.52 ± 0.03	0.40 ± 0.04
Two CL	0.54 ± 0.07	0.38 ± 0.05
Decrease in progesterone after PGF _{2α} (ng/mL)		
One CL	3.01 ± 0.24	3.37 ± 0.27
Two CL	3.03 ± 0.50	3.64 ± 0.40

¹Second replicate from south Florida location; experiment 1.

PGF_{2α} injections were 86.9 and 92.8%, respectively ($P = 0.21$). There were no effects of treatment ($P = 0.22$), number of CL ($P = 0.96$), or interaction between treatment and number of CL ($P = 0.99$) on the percentages of heifers undergoing luteolysis. For heifers with 1 and 2 CL, luteolysis were 88.8 and 91.5%, respectively ($P = 0.69$).

Progesterone concentrations according to treatment and CL number are presented in Table 2. There were no differences in progesterone concentrations in the second sample between PGF_{2α} groups ($P = 0.27$), number of CL ($P = 0.96$), or an interaction between PGF_{2α} and number of CL ($P = 0.63$).

Pregnancy per TAI with 1 injection of PGF_{2α} was 47.7% (52/109) at 30 d and 45.9% (50/109) at 45 d; with 2 injections of PGF_{2α}, P/TAI was 51.4% (56/109) at 30 d and 50.5% (55/109) at 45 d. There was no difference ($P = 0.49$) in P/TAI between 1 versus 2 PGF_{2α} treatments. For the number of CL in the ovary, P/TAI was 47.5% (76/160) at 30 d and 46.9% (75/160) at 45 d for heifers with 1 CL; and P/TAI was 52.0% (26/50) at 30 d and 50.0% (25/50) at 45 d for heifers with 2 CL. There was no difference ($P = 0.72$) in P/TAI between heifers with 1 or 2 CL in the ovary. According to the c-statistics, the progesterone concentration in plasma 24 h after PGF_{2α} that best predicted the probability of pregnancy and nonpregnancy was ≤0.2 ng/mL. At 0.2 ng/mL, the sensitivity was 37.4% and the specificity 78.4%. This means that 37.4% of the pregnant heifers had a progesterone concentration ≤0.2 ng/mL at the second sample, but 78.4% of the nonpregnant heifers had a progesterone concentration >0.2 ng/mL at the second sample. Using this cut-off to model P/TAI, we observed that the odds of pregnancy decreased ($P = 0.01$) for heifers with progesterone concentration >0.2 ng/mL (OR = 0.46; 95% CI: 0.25 to 0.83).

Experiment 2: Pregnancy to Second Service in Heifers Resynchronized with the 5-d Co-Synch + CIDR Protocol with One PGF_{2α} Injection and TAI or Inseminated at Detected Estrus

The distribution of detected estruses is shown in Figure 1. A total of 69 of 86 nonpregnant heifers from both the TAI and IDE groups were detected in estrus after the first TAI (69/86; 80.2%). Of these heifers, 79.7% (55/69) were detected between d 18 and 24 after the first TAI. Only 3 heifers in the IDE group that were not detected in estrus after the first TAI were diagnosed as nonpregnant at 32 d by ultrasound. These heifers were resynchronized and TAI with the TAI group, and one of them became pregnant after the second TAI. In the TAI group, P/TAI was 53.5% (23/43) at 32 d after the second TAI and did not differ ($P = 0.79$) from the P/AI for heifers in the IDE group following insemination at detected estrus (55%; 22/40). Overall P/TAI to the second TAI was 52.1% (24/46). At 60 d after insemination, P/AI to the second service was 52.1% (24/46) for heifers receiving TAI and 52.1% (21/40) for heifers in the IDE group ($P = 0.65$). There were no differences in P/TAI between technicians ($P = 0.70$) or significant effect of the interaction treatment group by technician ($P = 0.80$).

Experiment 3: Inclusion of the CIDR Device in the 5-d Co-Synch Protocol for Resynchronization of the Second TAI

Pregnancy per TAI at 32 d was 39.3% in the group without the CIDR device (22/56) and 51.8% (28/54) in the group with the CIDR device inserted between the first GnRH and the PGF_{2α} injection ($P = 0.07$). There were no pregnancy losses between 32 and 45 d of gesta-

Table 3. Pregnancy per timed AI (P/TAI) for the first and second TAI of dairy heifers in each replicate in the north central Florida location; experiment 4

Item	First TAI	Second TAI
P/TAI (%) at 32 d		
First replicate	61.5 (125/203)	60.2 (47/78) ^a
Second replicate	59.1 (126/213)	44.8 (39/87) ^b
Total	60.3 (251/416)	52.1 (86/165)
P/TAI (%) at 60 d		
First replicate	59.6 (121/203)	57.7 (45/78) ^a
Second replicate	56.8 (121/213)	39.0 (33/87) ^b
Total	58.2 (242/416)	47.2 (78/165)

^{a,b}Means differ between replicates ($P = 0.01$).

tion in either group. There were no significant effects of technician ($P = 0.16$) or sire ($P = 0.27$), and there were no interactions detected between technician and bull ($P = 0.22$), technician and treatment group ($P = 0.37$), or sire and treatment group ($P = 0.87$).

Experiment 4: Field Verification of the 5-d Co-Synch + CIDR Protocol with One Injection of PGF_{2α}

Pregnancy per TAI for the first and second TAI for each replicate is presented in Table 3. Considering both replicates, overall accumulative P/TAI to first and second TAI was 81.0 and 76.9% at 32 and 60 d, respectively, after TAI with the 5-d Co-Synch + CIDR program. For the first TAI service, P/TAI was not affected by replicate number ($P = 0.60$), technician ($P = 0.89$), or the interaction of replicate number by technician ($P = 0.31$). For the second service, difference in P/TAI was significant ($P = 0.01$; OR = 0.30; 95% CI: 0.12 to 0.76) between replicates. Sires tended to affect ($P = 0.10$) P/TAI to the second TAI: 11.1% (1/9), 50.0% (14/28), and 35.3% (18/51) at 60 d after AI for sires 1, 2, and 3, respectively. There were no differences in P/TAI between technicians ($P = 0.61$), and the interac-

tion of technician by replicate number ($P 0.16$) was not significant.

DISCUSSION

A single injection of 25 mg of PGF_{2α} was as effective as 2 injections when utilizing the 5-d Co-Synch + CIDR protocol in dairy heifers. Heifers receiving 1 injection of PGF_{2α} had a P/TAI of 48.8%, whereas heifers receiving 2 injections achieved 50.7%. Lack of differences in first-service P/TAI indicated equal effectiveness of 1 or 2 injections of PGF_{2α} to induce CL regression at the time of CIDR withdrawal. Of concern was whether 1 injection of PGF_{2α} would regress a newly formed CL in response to the injection of GnRH given at the time of CIDR insertion. Compared with lactating cows, heifers have a faster rate of follicular growth (Pursley et al., 1997) and a higher frequency of 3-wave follicular cycles (Savio et al., 1988). When the first GnRH of the protocol is given at the beginning of a follicular wave, a dominant follicle is not present, so ovulation frequency to the GnRH injection is expected to be low (Moreira et al., 2000). If heifers began the 5-d Co-Synch + CIDR protocol at random stages of the cycle and there were predominantly 3-wave follicular cycles, then approximately 43% of the estrous cycle would have a dominant follicle capable of undergoing an ovulation to form a new CL either in the presence (luteal phase) or absence (proestrus/estrus phases) of an original CL. Of the 298 heifers injected with GnRH at the time of CIDR insertion, 22.9% (50/218) had 2 CL. Indeed, the occurrence of CL regression and basal concentrations of progesterone 24 h after the first injection of PGF_{2α} did not differ between the 1 or 2 injections of PGF_{2α}. Even when heifers were presynchronized with GnRH 6 d before initiating the TAI protocol, ovulation to the first GnRH was still low (Rivera et al., 2006), because only 39.3% (63/160) of the heifers ovulated to the first GnRH of the TAI protocol regardless of presynchronization. Stevenson et al. (2008) observed that administration of GnRH at random stages of the estrous cycle only induced ovulation in 14.3 to 30.7% of the heifers. Collectively, the equivalent P/TAI and similar efficacy of CL regression indicate that 1 injection of PGF_{2α} is adequate when applying the 5-d Co-Synch + CIDR protocol to dairy heifers. The single injection of PGF_{2α} is sufficient to regress CL, even in the low proportion of heifers presenting more than one CL. Contrary to the results of the present study, a difference in CL regression was found when 1 versus 2 injections of PGF_{2α} was used in a 5-d Co-Synch 72-h protocol with lactating dairy cows (59.1 vs. 95.7%, respectively; Chebel et al., 2008). In this latter study, 57.6% of the lactating cows ovulated to the first GnRH injection. If lactating cows

Figure 1. Distribution of return to estrus after the first 5-d Co-Synch + CIDR (controlled internal drug releasing insert) protocol (experiment 2). All heifers underwent daily estrous detection with the use of tail chalk, and those heifers with rubbed off chalk or that stood to be mounted were classified as being in estrus. TAI = timed AI.

have a higher frequency of ovulation in response to the first GnRH injection, they will have a higher frequency of accessory CL. Therefore, if the period between the GnRH and PGF_{2α} is shortened to 5 d, the accessory CL will not be as responsive to a single injection of PGF_{2α}. Thus, an additional injection of PGF_{2α} 12 or 24 h (Chebel et al., 2008) after the first PGF_{2α} injection is needed to successfully induce luteolysis of the less responsive GnRH-induced accessory CL. In cows that ovulated to the first GnRH of the 5-d Co-Synch, the subsequent percentage CL regression was significantly less in cows that received 1 injection of PGF_{2α} compared with 2 injections of PGF_{2α} (51.7 vs. 96.0%). Luteal regression could be induced by an exogenous injection of PGF_{2α} given after d 7 of the normal estrous cycle, but the same luteolytic dose of PGF_{2α} did not induce luteolysis before d 5 (Schallenberger et al., 1984). In lactating dairy cows it has been shown that 2 PGF_{2α} injections at an 8-h interval were more effective in inducing luteolysis than a single injection (Archbald et al., 1993; Repasi et al., 2005). Although these studies were done with mature CL during mid-diestrus, similar mechanisms can induce luteolysis of an early CL, even 5 d after formation, when 2 injections of PGF_{2α} are administered 12 h apart. In beef cattle, it has been reported that in either the 5- or 7-d Select Synch + CIDR protocol with a single PGF_{2α} treatment, reproductive performance was similar between treatments for yearling beef heifers (Helser et al., 2006). For beef cows, it was concluded that a similar inadequate response to 1 injection of PGF_{2α} as observed in lactating cows may necessitate the use of a double injection of PGF_{2α} in the 5-d Co-Synch + CIDR protocol (Kasimanickam et al., 2009).

The use of a single injection of PGF_{2α} in the protocol represents a distinct advantage for the implementation of TAI in dairy heifers from an economic and practical point of view. Differences in P/TAI were found between farm locations. In the farm in SF with the lower P/TAI, TAI was performed during the summer season; therefore, heat stress could have affected fertility. A study from tropical Australia showed that fertility decreases significantly when temperature increases above 27.5°C (Orr et al., 1993). In another study conducted in Florida, heifers undergoing AI during the summer season were 76% less likely to become pregnant to the first AI than those inseminated during winter (Donovan et al., 2003). Despite a lower P/TAI in the SF location, no interaction was observed between location and number of PGF_{2α} treatments on P/TAI, thereby indicating that a single PGF_{2α} was as effective as 2 PGF_{2α} injections in both locations.

In experiment 2 involving resynchronization, no difference in P/AI was detected between TAI and AI at

detected estrus. Estrous detection requires more labor per animal and therefore can increase costs compared with a synchronization program (Olynk and Wolf, 2008). In an overall economic analysis of reproductive management strategies on United States commercial dairy farms, synchronization programs emerged as having greater expected net present values than visual estrous detection programs (Olynk and Wolf, 2008).

Most heifers came into estrus from d 18 to 24 after the first TAI, so the interovulatory interval was in agreement with the commonly accepted 21-d average. Consequently, nonpregnant heifers would be from d 4 to 14 of their estrous cycle when the second resynchronization protocol was initiated between 28 to 32 d after the first TAI. Most of these heifers could be in a stage of the cycle in which a potential ovulatory follicle would grow under high concentrations of progesterone (Moraes et al., 2000) with favorable pregnancy responses to the second programmed 5-d Co-Synch + CIDR TAI.

In the third experiment, resynchronization of nonpregnant heifers with the use of a CIDR device between the first GnRH and PGF_{2α} injections tended to increase P/TAI. Including the CIDR during the TAI protocol is expected to improve synchronization of ovulation by avoiding heifers from coming into estrus and ovulating before the day of TAI. Another possibility is that a certain number of nonpregnant heifers experienced either early or late embryonic mortality (Van Cleeff et al., 1996) and may benefit from a 5-d period of progesterone exposure to reset the hypothalamic-pituitary-reproductive tract axis in a manner that would improve P/TAI to the subsequent synchronized service. Within the context of a 5-d Co-Synch + CIDR program for resynchronization, insertion of a CIDR device appears necessary to improve P/TAI.

The field verification studies indicated that the 5-d Co-Synch + CIDR protocol can be successfully used with a single injection of PGF_{2α} either for the synchronization of first TAI or resynchronization for second TAI. The P/TAI obtained for the first TAI was 60.3% at 32 d, with a low (3.6%) embryonic loss between d 32 and 60 of pregnancy. This result is comparable to the overall P/TAI obtained by Rabaglino et al. [M. B. Rabaglino, C. A. Risco, M.-J. Thatcher, F. Lima (Department of Animal Sciences, University of Florida, Gainesville, FL), J. E. P. Santos, and W. W. Thatcher; unpublished data, article in press], in which 2 injections of PGF_{2α} were used in the 5-d Co-Synch + CIDR program (58.3% at d 32 and 57.6% at d 46). However, the P/TAI to the second TAI was lower (52.5%) compared with the results of the first TAI. Fertility in the second TAI tended to be affected by sire. Herd-level or animal-level factors affect fertility in dairy heifers (Donovan et al., 2003), and most herd-level variation in P/AI in

heifers is caused by variation among inseminators and service sires (Ron et al., 1984).

The effects of service sires on P/TAI in the field studies may be attributed to differences in post-thaw sperm viability, progression of spermatozoa in the female internal genital tract affecting the resultant sperm reservoir, capacitation, acrosome reaction, and fertilizing capacity (Januskauskas et al., 1999). Seminal differences that decrease pregnancy rates can be categorized as compensable or uncompensable. Males with compensable deficiencies require a higher number of sperm to increase pregnancy, because these defects affect sperm transport and function in the female reproductive tract, including initiation of the fertilization process and the block to polyspermy. When differences in fertility among males or inseminates are independent of sperm dosage, the seminal deficiencies are known as uncompensable. Such deficiencies are important to the maintenance of the fertilization event and subsequent embryogenesis (Saacke et al., 2000; Saacke, 2008). Because of compensable deficiencies there are differences among bulls in response to time of insemination (Dalton et al., 2001). If the bull has few or no compensable deficiencies it would easily meet the threshold numbers of sperm to the cow by AI, perform as well at low sperm dosages as at normal, and be less vulnerable than other bulls to inseminator error in semen placement and handling. Therefore, it would perform well over a broad time span relative to ovulation time. On the other hand, if seminal compensable deficiencies were high, bulls would be more vulnerable to dilution rates, inseminator competence, and timing of insemination, requiring later breeding to optimize their efficiency in sperm access to the ovum (Saacke, 2008). Our results, along with these studies, highlight the importance of semen quality in a TAI protocol to optimize P/TAI.

CONCLUSIONS

In dairy heifers, the modified 5-d Co-Synch + CIDR protocol with 1 injection of PGF_{2α} at the time of the CIDR withdrawal and GnRH/TAI 72 h later was an efficient reproductive management program to achieve acceptable P/TAI in dairy heifers. This is an alternative program for managing reproduction in dairy heifers without the need for detection of estrus.

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