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Administration of a GnRH analog on day 9 of a 14-day controlled internal drug release insert with timed artificial insemination in lactating beef cows[1]

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ABSTRACT: Many estrus synchronization protocols aim to induce a new follicular wave to improve response and enhance pregnancy rate. Our objectives were to determine the effectiveness of GnRH analog administered d 0 and 9 during an extended controlled internal drug release (CIDR) protocol to produce 2 follicular waves, induce cyclicity in anestrus cows, and evaluate the efficacy of a single 50-mg dose of $PGF_{2\alpha}$ to initiate luteal regression on CIDR removal. Lactating beef cows (n = 779) at 3 locations (n = 247, location 1; n = 395, location 2; n = 137, location 3) were randomly assigned to 1 of 3 treatments. Cows in the 14-d 50 PG treatment received a CIDR (1.38 g progesterone) with 100 μg GnRH analog intramuscularly (i.m.) on d 0, 100 μg GnRH analog i.m. on d 9, and CIDR removal concurrent with 50 mg $PGF_{2\alpha}$ i.m. on d 14. Cows in the 14-d 6-h PG treatment were assigned the same protocol as the 14-d 50 PG treatment except that 25 mg $PGF_{2\alpha}$ i.m. was given on d 14 plus 25 mg PGF2α i.m. 6 ± 1 h later. Cows in the control treatment, 5-d CO-Synch + CIDR (5-d CO-Synch), received a CIDR concurrent with 100 μg GnRH analog i.m. on d 9, CIDR removal concurrent with 25 mg $PGF_{2\alpha}$ i.m. on d 14, and 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h after first F2α injection. Cows in all treatments received 100 μg GnRH analog i.m. and timed AI (TAI) 72 ± 3 h after CIDR removal. Pregnancy status to TAI was determined by ultrasonography 37 to 40 d after TAI. Averaged over all locations, pregnancy rates to TAI for 14-d 50 PG, 14-d 6-h PG, and 5-d CO-Synch treatments were 58.2%, 46.8%, and 41.9%, respectively. Pregnancy rates to TAI were greater ($P < 0.05$) in 14-d 50 PG treatment than 14-d 6-h $PGF_{2\alpha}$ and 5-d CO-Synch treatments. Cycling status at 2 locations (n = 243, location 1; n = 391, location 2) was determined from blood collected on d –7 and 0; cows with serum progesterone concentrations >1 ng/mL at either (or both) bleeding date were considered cyclic. Averaged over the 2 locations, there was a tendency ($P = 0.06$) for a greater number of cyclic animals to become pregnant to TAI in the 14-d 50 PG treatment (64.4%) than 5-d CO-Synch treatment (50.2%). The 14-d CIDR with GnRH analog on d 0 and 9 and a single 50-mg dose of PG i.m. at CIDR removal was a more efficacious protocol to maximize TAI pregnancy rates than the standard 5-d CO-Synch.

Key Words: beef cows, controlled internal drug release, estrus synchronization, $PGF_{2\alpha}$, timed artificial insemination

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INTRODUCTION

Recent estrus synchronization protocols for timed AI (**TAI**) such as the 5-d CO-Synch + controlled internal drug release (**CIDR**) have reported pregnancy rates up to 70% in beef cows. This is an increase compared with other protocols (Bridges et al., 2008; Gunn et al., 2009). These protocols attempt to induce 1 new follicular wave by injecting GnRH at their initiation. This approach does not address the stage of the estrous cy-

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cle when cows are nonresponsive to GnRH. At the start of most protocols, on average, a maximum of 66% of cyclic beef cows are in a stage of the estrous cycle where they have an existing follicle that will respond to GnRH to trigger a surge of LH to cause ovulation and induce a new follicular wave. This phenomenon likely results in a reduction in pregnancy rate (Geary et al., 2000).

The use of progestins in synchronization protocols has been shown to induce cyclicity in anestrus cows (Smith et al., 1987; Perry et al., 2002, 2004) but can be labor and time intensive. When implementing long-term progestin protocols in cycling animals, the need to initiate a new follicular wave to produce a fertile ovulation after progestin removal lengthens these protocols because of reduced oocyte fertility in persistent follicles that may result from extended interestrous intervals with low progestin concentrations (Mihm et al., 1994; Patterson et al., 1989).

Our first objective was to assess the efficacy of adding an additional GnRH injection during a 14-d CIDR program, designed to induce 2 follicular waves during the influence of progesterone. We hypothesized that this would position more cows in a stage of the estrous cycle to be responsive to GnRH at progestin removal. This protocol also mimicked the 5-d CO-Synch + CIDR by giving the second GnRH injection 5 d before CIDR removal. Objective 2 was to evaluate a 14-d CIDR protocol to induce cyclicity in anestrus cows. Objective 3 was to assess the efficacy of a single 50-mg dose of $PGF_{2\alpha}$ at CIDR removal compared with two 25-mg doses 6 h apart.

MATERIALS AND METHODS

All experimental procedures with cows were approved by the Colorado State University Animal Care and Use Committee before initiation of the experiment.

Animals and Treatments

Multiparous and primiparous Angus, Angus cross, and Hereford cross beef cows (n = 779) at 3 ranches in Wyoming and Colorado (location 1, n = 247; location 2, n = 395; location 3, n = 137) were used in this experiment. Cows were assigned to 1 of 3 treatments on the basis of a preplanned randomization process. However, at location 1, there was a mistake in randomization, which led to the improper balancing of treatments at this location. All animals were evaluated for BCS by a single evaluator throughout the experiment on d 9 of treatments using a 1 to 9 BCS system (Richards et al., 1986). The day of initial GnRH analog (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) injection and CIDR (1.38 g progesterone, EAZI-BREED CIDR, Pfizer Animal Health, New York, NY) insertion was considered d 0 of the 14-d CIDR treatments. Cows in the **14-d 50 PG** treatment received a CIDR insert concurrent with 100 µg GnRH analog intramuscularly (**i.m.**) on d 0, 100 µg GnRH analog i.m. on d 9, and CIDR removal concurrent with a single 50-mg dose of $PGF_{2\alpha}$ i.m. (Lutalyse, Pfizer Animal Health) on d 14. Cows in the **14-d 6-h PG** treatment received a CIDR insert concurrent with 100 µg GnRH analog i.m. on d 0, 100 µg GnRH analog i.m. on d 9, and CIDR removal concurrent with 25 mg $PGF_{2\alpha}$ i.m. on d 14, with another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later. Cows in the control treatment, **5-d CO-Synch + CIDR**, received a CIDR insert concurrent with 100 µg GnRH analog i.m. on d 9; CIDR removal concurrent with 25 mg $PGF_{2\alpha}$ i.m. was on d14, with another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later (Fig. 1).This interval was used as the most efficacious interval for maximizing TAI pregnancy rates from our previous research using the 5-d CO-Synch + CIDR protocol (Peel et al., 2010). Cows in all treatments received 100 µg GnRH analog i.m. concurrent with TAI 72 ± 3 h after CIDR removal. For all treatments, estrus detection patches (Estrotect, Estrotect Inc., Spring Valley, WI) were placed on the tail head of each cow at CIDR removal to aid in visual detection of estrus. Estrus detection began 36 h after CIDR removal at 12 h intervals and continued until TAI at locations 1 and 2. Visual observa-

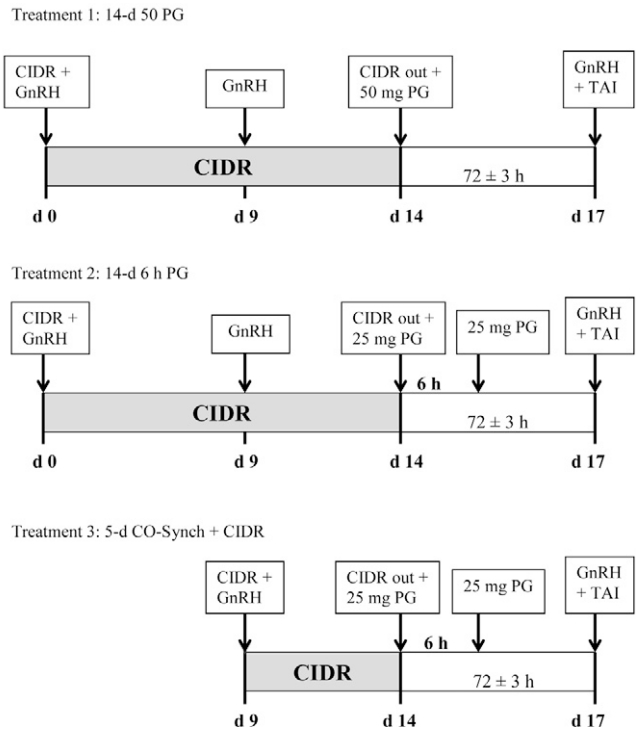

Figure 1. Estrus synchronization treatments administered to lactating beef cows. CIDR = controlled internal drug release device inserted intravaginally (1.38 g progesterone, EAZI-BREED CIDR, Pfizer Animal Health, New York, NY); GnRH = 100 µg GnRH analog administered intramuscularly (i.m.; Factrel, Fort Dodge Animal Health, Fort Dodge, IA); PG = $PGF_{2\alpha}$ administered i.m. (Lutalyse, Pfizer Animal Health); TAI = timed AI.

tion of estrus was not possible at location 3, but estrus detection patches were applied. Patches were scored at TAI on d 17 at all 3 locations using a 1 to 4 scale based on the amount of film on each patch that was removed (1 = completely untouched, 2 = approximately 50% removed, 3 = almost all or 100% removed, and 4 = missing patch).

Blood Collection

At locations 1 and 2, reproductive cyclicity status was determined from blood obtained by coccygeal venipuncture on d –7 and 0 for serum concentrations of progesterone. Blood was collected in 10-mL serum Vacutainer tubes (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ) and placed directly on ice within 10 min of collection. Samples were centrifuged at 2800 × *g* for 10 min at 5°C within 8 h after collection and were stored at −20°C. Concentrations of progesterone were determined using the Niswender procedure for RIA and conducted in the endocrinology laboratory at Colorado State University (Niswender, 1973). The assay included extraction of progesterone from serum and then competitive binding with a radiolabeled progesterone source (rabbit antisera). Once bound, progesterone concentrations of each cow were determined by a γ spectrometer. Cows with progesterone concentrations >1 ng/mL at either (or both) bleeding date were identified as cyclic at the initiation of treatments, and cows with progesterone concentrations <1 ng/mL for both bleeding dates were identified as anestrus at initiation of treatments. Interassay and intra-assay CV were 9.3% and 3.1%, respectively. Average sensitivity of assays was 0.03 ng/mL.

Pregnancy Diagnosis

Pregnancy status to TAI was diagnosed between 37 and 40 d after TAI using transrectal ultrasonography (3.5-MHz linear transducer GP-DV, E.I. Medical, Loveland, CO). Cows were exposed to intact bulls beginning 9 to 10 d after TAI for the remainder of the breeding season.

Statistical Analyses

Data were analyzed via logistic regression using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC). The initial model included location, treatment, parity (primiparous or multiparous), BCS, post-partum interval in days from calving to TAI (**PPI**), sire, AI technician, and location × treatment. However, the final model used only significant factors ($P < 0.10$), which were treatment, location × treatment, parity, BCS, and PPI. For cycling status data at locations 1 and 2, cycling and cycling × treatment were added to the final model.

RESULTS AND DISCUSSION

Numbers of cows, PPI, BCS, and parity are presented in Table 1. Mean BCS did not differ among treatments within location ($P > 0.10$; data not shown) but differed by location ($P < 0.05$). Similarly, mean PPI did not differ among treatments within location ($P > 0.10$; data not shown) but differed by location ($P < 0.05$).

The first objective was to evaluate the efficacy of 2 GnRH analog injections within a 14-d CIDR to increase TAI pregnancy rate compared with the 5-d CO-Synch + CIDR protocol. Overall, pregnancy rate to TAI did not differ between treatments ($P > 0.05$; Table 2) at locations 1 or 2. At location 3, pregnancy rate to TAI was greater ($P < 0.01$) in the 14-d 50 PG treatment (60.7%)

Table 1. Postpartum interval [PPI, day at timed AI (TAI)], BCS, and parity of lactating beef cows for 3 treatments at 3 locations (least squares mean ± SE)

				Parity,%	
Location and treatment[1]	*n*	PPI, d	BCS	Primiparous	Multiparous
Location					
1	247	81 ± 1.1[a]	5.0 ± 0.05[a]	25.1	74.9
2	395	75 ± 1.1[b]	4.5 ± .03[b]	14.9	85.1
3	137	72 ± 1.3[c]	5.6 ± .04[c]	11.7	88.3
Combined across locations					
14-d 50 PG	278	76 ± 1.1	4.8 ± 0.05	17.3	82.7
14-d 6-h PG	249	76 ± 1.2	4.9 ± 0.04	16.9	83.1
5-d CO-Synch + CIDR	252	76 ± 1.2	5.0 ± 0.05	18.7	81.3
Total for all treatments across locations	779	76 ± 0.7	4.9 ± 0.01	17.6	82.4

[a–c]Within a column, means without common superscripts differ ($P < 0.05$).

[1]Cows in all treatment groups received 100 µg GnRH analog intramuscularly (i.m.) concurrent with TAI 72 ± 3 h after controlled internal drug release device (CIDR) removal. Treatment: 14-d 50 PG = 14-d CIDR (1.38 g progesterone, EAZI-BREED CIDR, Pfizer Animal Health, New York, NY) with 100 **µg** GnRH analog i.m. (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) d 0 and 9 with 50 mg $PGF_{2\alpha}$ i.m. (Lutalyse, Pfizer Animal Health) on d 14 with CIDR removal; 14-d 6-h PG = same protocol as 14-d 50 PG treatment, except cows received 25 mg $PGF_{2\alpha}$ i.m. at CIDR removal and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later; 5-d CO-Synch + CIDR = 5-d CIDR with 100 **µg** GnRH analog i.m. d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ i.m. with CIDR removal, and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later.

than both the 14-d 6-h PG (32.0%) and 5-dCO-Synch + CIDR treatments (26.5%), but there was no difference ($P > 0.10$) between the 14-d 6-h PG and 5-d CO-Synch+ CIDR treatments. There was a treatment × location interaction ($P < 0.05$) for combined results across locations. Pregnancy rate to TAI was greater ($P < 0.05$) in the 14-d 50 PG treatment than both the 14-d 6-h $PGF_{2\alpha}$ and 5-d CO-Synch + CIDR treatments, and the 14-d 6-h $PGF_{2\alpha}$ treatment did not differ from the 5-d CO-Synch + CIDR treatment ($P > 0.10$; Table 2).

In the current study, the strategy to induce 2 follicular waves in the 14-d 50 PG treatment when a 50-mg dose of $PGF_{2\alpha}$ was given at CIDR removal resulted in an increased TAI pregnancy rate compared with the 5-d CO-Synch + CIDR protocol recommended by the Beef Reproduction Task Force (2012).

The second objective was to determine if extended (14-d) progesterone influence could induce cyclicity in anestrus animals; however, averaged over locations 1 and 2, pregnancy rate to TAI for both cycling and noncycling cows (Table 3) did not differ ($P > 0.05$) by treatment. However, there was a tendency ($P = 0.06$) for cycling animals to have a greater TAI pregnancy rate in the 14-d 50 PG treatment than the 5-d CO-Synch + CIDR treatment when data were combined across locations 1 and 2. Cyclicity data at location 3 were not collected because of logistical restraints.

Postpartum anestrus in beef cows results in infertility and poor response to certain estrus synchronization protocols (Short et al., 1990; Perry et al., 2004). However, at the 2 locations where cycling status was evaluated in the current study, pregnancies by TAI in anestrus cows, as determined at the initiation of the protocols, were similar ($P > 0.10$) among treatments. It bears emphasizing that approximately half of the anestrus cows at these 2 study locations became pregnant to TAI, regardless of treatment. This result in itself demonstrates the positive impact on the beef industry of using CIDR and GnRH in estrus synchronization protocols to manage anestrus beef cows for achieving AI pregnancies.

The extended (14-d) influence of progesterone on prepubertal beef heifers has been shown to induce cy-

Table 2. Least squares means (±SE) for timed AI (TAI) pregnancy rates (PR) of lactating beef cows by treatment and location and across locations

Location and treatment[1]	*n*	TAI PR, %
Location 1		
14-d 50 PG	103	54.7 ± 5.4[a]
14-d 6-h PG	70	60.5 ± 6.3[a]
5-d CO-Synch + CIDR	74	47.0 ± 6.1[a]
Location 2		
14-d 50 PG	128	59.1 ± 5.0[a]
14-d 6-h PG	133	48.6 ± 4.9[a]
5-d CO-Synch + CIDR	134	54.0 ± 4.9[a]
Location 3		
14-d 50 PG	47	60.7 ± 8.3[a]
14-d 6-h PG	46	32.0 ± 7.3[b]
5-d CO-Synch + CIDR	44	26.5 ± 6.8[b]
Combined across locations[2]		
14-d 50 PG	278	58.2 ± 3.9[a]
14-d 6-h PG	249	46.8 ± 4.2[b]
5-d CO-Synch + CIDR	252	41.9 ± 4.1[b]

[a,b]Within a location or combined across locations, means without common superscripts differ within location ($P < 0.05$).

[1]Cows in all treatment groups received 100 µg GnRH analog intramuscularly (i.m.) concurrent with TAI 72 ± 3 h after controlled internal drug release device (CIDR) removal. Treatment: 14-d 50 PG = 14-d CIDR (1.38 g progesterone, EAZI-BREED CIDR, Pfizer Animal Health, New York, NY) with 100 µg GnRH analog i.m. (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) d 0 and 9 with 50 mg $PGF_{2\alpha}$ i.m. (Lutalyse, Pfizer Animal Health) on d 14 with CIDR removal; 14-d 6-h PG = same protocol as 14-d 50 PG treatment, except cows received 25 mg $PGF_{2\alpha}$ i.m. at CIDR removal and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later; 5-d CO-Synch + CIDR = 5-d CIDR with 100 µg GnRH analog i.m. d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ i.m. with CIDR removal, and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later.

[2]There was a treatment × location interaction ($P < 0.05$).

Table 3. Least squares means (±SE) for timed AI (TAI) pregnancy rates (PR) of lactating beef cows by cycling status and treatment within 2 locations by location and combined across 2 locations

	Cycling cows[2]		Noncycling cows[3]	
Location and treatment[1]	*n*	TAI PR, %	*n*	TAI PR, %
Location 1				
14-d 50 PG	40	66.1 ± 7.9	61	48.8 ± 6.4
14-d 6-h PG	32	69.5 ± 8.8	37	55.6 ± 8.2
5-d CO-Synch + CIDR	26	49.2 ± 9.8	47	47.6 ± 7.3
Location 2				
14-d 50 PG	78	62.6 ± 5.7[a]	50	51.8 ± 7.1
14-d 6-h PG	69	45.4 ± 6.0[b]	62	50.3 ± 6.3
5-d CO-Synch + CIDR	73	51.1 ± 5.9[a,b]	59	55.6 ± 6.5
Combined across 2 locations[4]				
14-d 50 PG	118	64.4 ± 4.6[x]	111	50.3 ± 4.8
14-d 6-h PG	101	57.9 ± 5.3[x,y]	99	53.0 ± 5.1
5-d CO-Synch + CIDR	99	50.2 ± 5.1[y]	106	51.6 ± 4.9

[a,b]Within location and cycling status, means without common superscripts differ ($P < 0.05$).

[x,y]Timed AI pregnancy rates pooled across locations for the 14-d 50 PG treatment had a tendency ($P = 0.06$) to be greater than the 5-d CO-Synch + controlled internal drug release device (CIDR) treatment.

[1]Cows in all treatment groups received 100 µg GnRH analog intramuscularly (i.m.) concurrent with TAI 72 ± 3 h after CIDR removal. Treatment: 14-d 50 PG = 14-d CIDR (1.38 g progesterone, EAZI-BREED CIDR, Pfizer Animal Health, New York, NY) with 100 µg GnRH analog i.m. (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) d 0 and 9 with 50 mg $PGF_{2\alpha}$ i.m. (Lutalyse, Pfizer Animal Health) on d 14 with CIDR removal; 14-d 6-h PG = same protocol as 14-d 50 PG treatment, except cows received 25 mg $PGF_{2\alpha}$ i.m. at CIDR removal and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later; 5-d CO-Synch + CIDR = 5-d CIDR with 100 µg GnRH analog i.m. d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ i.m. with CIDR removal, and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later.

[2]Cycling cows = cows with progesterone concentration > 1 ng/ml on d –7 and/or 0.

[3]Noncycling cows = cows with progesterone concentration < 1 ng/mL on d –7 and 0.

[4]There was no treatment × location interaction ($P > 0.10$).

clicity because of the longer progesterone exposure needed to elicit an effect on follicular growth (Leitman et al., 2009), but this progesterone was given about 2 wk earlier relative to TAI than in the current study. Although the extended progesterone exposure is necessary to induce cyclicity in prepubertal heifers, the same length of exposure is not seen in lactating beef cows. Short-term pretreatment (5 to 8 d) with exogenous progesterone has induced cyclicity and ovarian response to GnRH administered to short-postpartum anestrus lactating beef cows (Troxel and Kesler, 1983). Normal GnRH-induced corpus luteum (**CL**) life span was also verified after progesterone pretreatment compared with the shorter GnRH-induced luteal phase without progesterone exposure (Walters et al., 1982; Smith et al., 1987) or spontaneously formed CL (Manns et al., 1983) seen in previous experiments. The shorter luteal phase also resulted in an overall shorter estrous cycle in the first postpartum cycle of the animals. Therefore, the ability to induce cyclicity was expected to be similar between 5- and 14-d CIDR protocols in the current experiment. However, the extra GnRH injection and 9 d of progesterone influence initiated on d 0 of the 14-d CIDR treatments could have added benefits. Although previous experiments have shown induced cyclicity with either exogenous GnRH and/or progesterone treatment, the pretreatment with both could induce both cyclicity and resumption of follicular growth to ensure response to the second GnRH administered on d 9. A similar response would not be expected in the 5-d CO-Synch + CIDR treatment because of only a single GnRH injection within the treatment at CIDR insertion.

Short-postpartum cows (n = 63; PPI at TAI of 38 to 45 d) had acceptable TAI pregnancy rates [14-d 50 PG: 13/24 (54.2%), 14-d 6-h PG: 10/19 (52.6%), 5-d CO-Synch + CIDR: 17/20 (85.0%)]. Although there were limited numbers of animals in this category, the ability of all 3 treatments to result in high TAI pregnancy rates is another significant benefit to progestin-based estrus synchronization protocols in short-postpartum lactating beef cows. Although it is not recommended to implement these protocols in cows with a shorter PPI than in the current experiment (38 d), the benefits of TAI pregnancy rates above 50% can help more animals to conceive earlier in the breeding season than without the use of such protocols.

The third objective was to evaluate the difference in effectiveness of a single 50-mg dose of $PGF_{2\alpha}$ i.m. to two 25-mg i.m. doses at 6-h intervals within the 14-d CIDR treatments for maximizing TAI pregnancy rates. A single 50-mg dose would reduce the number of times animals must go through the chute if effective at lysing the CL. At locations 1 and 2, there were no differences ($P > 0.10$) between the two 14-d CIDR treatments in pregnancy rate to TAI. However, at location 3, pregnancy rate to TAI was greater ($P < 0.05$) in the 14-d 50 PG treatment than the 14-d 6-h PG treatment. In combined data across locations, pregnancy rate to TAI was greater ($P < 0.05$) in the14-d 50 PGF treatment than the 14-d 6-h PG treatment. The ability of PG to induce luteal regression of a CL resulting from exogenous GnRH 5 d before progestin removal has been previously documented in both beef cows and heifers (Bridges et al., 2008; Kasimanickam et al., 2009; Peel et al., 2010). However, within these protocols it has still been recommended (Beef Reproduction Task Force, 2012) to give a second 25-mg $PGF_{2\alpha}$ injection 8 ± 2 h after progestin removal and initial $PGF_{2\alpha}$ injection to ensure complete luteal regression for effective timing of ovulation and successful conception to TAI. This can be a burden because of the need for processing animals through the chute an additional time. The 5-d protocol requires 4 processing events for animals, including 3 times gathering and sorting. The 14-d CIDR treatments also require extra labor because of either 4 times gathering and processing (14-d 50 PG treatment) or 4 times gathering and 5 times processing (14-d 6-h PG treatment). However, the extra labor required must be weighed against the added benefits of increased numbers of animals becoming pregnant to TAI within the 14-d 50 PG treatment.

In the present study, a single 50-mg dose of $PGF_{2\alpha}$ at CIDR removal for the 14-d 50 PG treatment appeared to be sufficient in inducing luteal regression and was verified indirectly from comparison of TAI pregnancy rates between the 14-d CIDR treatments. However, without ultrasonography data the true physiological response of luteal regression requires further verification. For example, it was not possible to determine numbers of CL present at $PGF_{2\alpha}$ injection with CIDR removal. Animals could have had no CL present or as many as 3 from the presence of a native CL on d 0 and response to GnRH analog administration on both d 0 and 9.

Acceptable TAI pregnancy rates in the 14-d 50 PG treatment seen in the current study were similar to those in a 5-d CO-Synch + CIDR protocol with two 25-mg doses of $PGF_{2\alpha}$ at CIDR removal in a multistate trial (Bridges et al., 2012). In both experiments, no differences in TAI pregnancy rates were seen between a single 50-mg dose of $PGF_{2\alpha}$ i.m. or two 25-mg i.m. doses at 6-h intervals. However, preceding research (Bridges et al., 2008; Kasimanickam et al., 2009) found that the 8-h interval between two 25-mg doses of $PGF_{2\alpha}$ was necessary for maximum success in TAI pregnancy rates. The d 5 bovine CL has been originally described as nonresponsive to $PGF_{2\alpha}$ (Lauderdale, 1972). However, research had determined that 2 injections of 25 mg of $PGF_{2\alpha}$ at 6-h intervals is as effective as 2 injections of 25 mg $PGF_{2\alpha}$ at 12-h intervals (Peel et al., 2010) and that 6-h intervals were more effective than 2- or 4-h intervals (Seabrook et al., 2010)

to achieve maximum TAI pregnancy rates within the 5-d CO-Synch + CIDR protocol.

At locations 1 and 2, few cows (n = 31) were observed in estrus at 36 and 48 h after CIDR removal before TAI. No cows at either location came into estrus by 36 h after CIDR removal and $PGF_{2\alpha}$ injection. Those animals observed in estrus at 48 h still had acceptable pregnancy rates to TAI 24 h later [14-d 50 PG: 6/11 (54.5%), 14-d 6-h PG: 8/12 (66.7%), 5-d CO-Synch + CIDR: 5/8 (62.5%)]. There were no detected differences in pregnancy rate to TAI among treatments ($P > 0.10$), perhaps because of limited cow numbers for adequate power to test this result.

Estrus response of treatments was determined by the amount of film removed from estrus detection patches on d of TAI and is presented in Table 4. There was a patch score × location interaction ($P < 0.05$) from the increased estrus response of the 14-d 50 $PGF_{2\alpha}$ treatment at location 3. A greater percentage of animals ($P < 0.05$) had a patch score of 3, indicating that estrus behavior occurred during the interval from CIDR removal to TAI, in both the 14-d 50 PG (58.3%) and 14-d 6-h PG (54.6%) treatments than the 5-d CO-Synch + CIDR treatment (44.0%) in combined data across locations. Similarly, a greater percentage of cows ($P < 0.05$) in the 5-d CO-Synch + CIDR treatment (36.5%) had a patch score of 1, indicating a lack of behavioral estrus, than both the 14-d 50 PG (24.1%) and 14-d 6-h PG (24.9%) treatments. There were no differences ($P > 0.10$) between 14-d 50 PG and 14-d 6-h PG treatments in either estrus response (patch score 1 and 3 comparisons). However, solely using estrus detection patch scores as an indicator of estrus response has limitations because it was not determined exactly when patches were rubbed off during the 72-h interval from CIDR removal to TAI. The increase in estrus response in the 14-d 50 PG treatment is consistent with increased TAI pregnancy rates when compared with the 5-d CO-Synch + CIDR treatment, but the increased estrus response in the 14-d 6-h treatment did not result in an increased TAI pregnancy rate vs. the 5-d CO-Synch + CIDR treatment.

The increased estrus response seen in the 14-d CIDR treatments could be explained by the different endocrine environments these cows were exposed to during the 9 d of progesterone influence before the second GnRH analog injection. In cycling animals, circulating concentrations of progesterone that resulted from the 14-d CIDR treatments during this period could have increased the amount of circulating estradiol-17β secreted by the dominant follicle compared with cows in the 5-d CO-Synch + CIDR treatment if a spontaneously formed CL was not present and there was a lack of response to the GnRH analog on d 0. This increased estradiol-17β could be explained by increased LH pulse frequencies and would agree with previous research investigating long-term progesterone estrus synchronization (Kinder et al., 1996). In the absence of a spontaneously formed CL, progesterone absorbed from a CIDR is high enough to prevent ovulation but low enough to mimic the high LH pulse frequency of the follicular phase rather than the luteal phase (Kinder et al., 1996). This environment could have occurred in the 14-d CIDR treatments and could explain the increased estrus response but is unlikely. Because of the lack of information on actual follicular turnover in response to GnRH analog injections, failure to ovulate in response to the initial GnRH analog injection on d 0, under the influence of progesterone, could have effectively produced follicles with extended follicular growth for 9 d or longer depending on what stage of growth the follicles were in on d 0. This would occur if a spontaneously formed CL were not present on d 0. Growth of persistent follicles has been proven to produce high concentrations of estradiol-17β (Savio et al., 1993; Revah and Butler, 1996), and early stages of this type of follicle could have been present in the current study from d 0 to 9 and contributed to increased estradiol-17β concentrations. However, the ovulatory capability of these follicles would not be diminished because of the potentially increased number of LH receptors present on these follicles from the high LH pulse frequency environment. Although they were not considered true persistent follicles, this theory would agree with research finding increased numbers of LH receptors on granulosa cells of persistent follicles compared with dominant follicles with normal follicular growth (Cupp et al., 1993). However, the second GnRH injection on d 9 would have avoided persistent follicles by forcing ovulation and would have

Table 4. Estrus response by treatment using estrus detection patches evaluated on day of timed AI (TAI) in lactating beef cows across all locations

		Patch score,[2] % of treatment			
Treatment[1]	n	1	2	3	4
14-d 50 PG	278	24.1[a]	13.3	58.3[a]	4.3
14-d 6-h PG	249	24.9[a]	16.9	54.6[a]	3.6
5-d CO-Synch + CIDR	252	36.5[b]	16.7	44.0[b]	2.8

[a,b]Within columns, patch score means without common superscripts differ ($P < 0.05$).

[1]Cows in all treatment groups received 100 μg GnRH analog intramuscularly (i.m.) concurrent with TAI 72 ± 3 h after controlled internal drug release device (CIDR) removal. Treatment: 14-d 50 PG = 14-d CIDR (1.38 g progesterone, EAZI-BREED CIDR, Pfizer Animal Health, New York, NY) with 100 μg GnRH analog i.m. (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) d 0 and 9 with 50 mg $PGF_{2\alpha}$ i.m. (Lutalyse, Pfizer Animal Health) on d 14 with CIDR removal; 14-d 6-h PG = same protocol as 14-d 50 PG treatment, except cows received 25 mg $PGF_{2\alpha}$ i.m. at CIDR removal and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later; 5-d CO-Synch + CIDR = 5-d CIDR with 100 μg GnRH analog i.m. d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ i.m. with CIDR removal, and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later.

[2]Patch score is based on amount of film removed from Estrotect patch on day of TAI: 1 = completely untouched, 2 = approximately 50% rubbed off, 3 = almost all or 100% rubbed off, and 4 = missing patch.

initiated a new wave of follicular growth. Thus, the idea of increased estradiol-17β concentrations in the 14-d CIDR treatments seem unlikely because of the multiple potential sources of progesterone throughout the treatments. Animals with a CL present on d 0 and/or response to GnRH analog on d 0 and/or 9 would diminish these greater concentrations of estradiol-17β from the elevated progesterone released from the GnRH-induced corpora lutea formed. Incorporation of ultrasonography into the 14-d CIDR treatments in future experiments could be beneficial to determine whether follicular turnover was induced in the 14-d CIDR treatments and that increased response to the d 9 GnRH analog occurred compared with the 5-d CO-Synch + CIDR treatment. Although it could not be verified, the likely cause for increased estrus response could have been due to overall increased response to treatments and synchrony of estrus after CIDR removal in the 14-d CIDR treatments compared with the 5-d CO-Synch + CIDR treatment. This would explain the high estrus response of the 14-d CIDR treatments and low TAI pregnancy rate of the 14-d 6-h $PGF_{2\alpha}$ treatment at location 3 (32%), but these low results were not seen at any other location in either of the two 14-d CIDR treatments. However, within location 3, there was a substantial increase in estrus response ($P < 0.05$) in the 14-d 50 $PGF_{2\alpha}$ treatment (87.2%) compared to 14-d 6-h $PGF_{2\alpha}$ (67.4%) and 5-d CO-Synch + CIDR (48.3%) treatments (estrus response by location not shown). With the low number of animals at location 3, this disparity in estrus response between the 14-d CIDR treatments could have led to the difference in TAI pregnancy rates. Increased estrus response was also seen with increased TAI pregnancy rates across locations. As patch score increased, TAI pregnancy rates increased (Table 5). However, there were no differences ($P > 0.10$) between treatments and TAI pregnancy rates by estrus response.

Table 5. Least squares means for timed AI (TAI) pregnancy rates (PR) by patch score and treatment using estrus detection patches evaluated on day of TAI in lactating beef cows across all locations

Patch score[1]	TAI PR by treatment,[2] %		
	14-d 50 PG	14-d 6-h PG	5-d CO-Synch + CIDR
1	52.2 (35/67)	43.5 (27/62)	42.4 (39/92)
2	48.6[a] (18/37)	35.7[ab] (15/42)	28.6[b] (12/42)
3	67.3 (109/162)	62.5 (85/136)	65.8 (73/111)
4	66.7 (8/12)	66.7 (6/9)	57.1 (4/7)

[a,b]Within row, means without common superscripts tend ($P = 0.06$) to differ.

[1]Patch score is based on the amount of film removed from estrus detection patch on day of TAI: 1 = completely untouched, 2 = approximately 50% rubbed off, 3 = almost all or 100% rubbed off, and 4 = missing patch.

[2]Cows in all treatment groups received a 100 **μg** GnRH analog intramuscularly (i.m.) concurrent with TAI 72 ± 3 h after controlled internal drug release device (CIDR) removal. Treatment: 14-d 50 PG = 14-d CIDR (1.38 g progesterone, EAZI-BREED CIDR, Pfizer Animal Health, New York, NY) with 100 **μg** GnRH analog i.m. (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) d 0 and 9 with 50 mg $PGF_{2\alpha}$ i.m. (Lutalyse, Pfizer Animal Health) on d 14 with CIDR removal; 14-d 6-h PG = same protocol as 14-d 50 PG treatment, except cows received 25 mg $PGF_{2\alpha}$ i.m. at CIDR removal and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later; 5-d CO-Synch + CIDR = 5-d CIDR with 100 **μg** GnRH analog i.m. d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ i.m. with CIDR removal, and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later. Fractions in parentheses denote actual numbers of animals and averages within each patch score group.

As previously mentioned, response to the initial GnRH injection in 5- to 7-d CIDR-based estrus synchronization protocols is a key factor in initiating a new follicular wave to ovulate a fertile oocyte after CIDR removal. The ability to increase the likelihood of cows responding to GnRH has been previously documented with presynchronization of estrous cycles (Stegner et al., 2004; Schafer et al., 2006; Leitman et al., 2009) and has been shown to increase pregnancy rates in both beef cows and heifers. That concept was used in the current experiment with a different approach, by inclusion of 2 GnRH injections concurrent with 14 d of progestin influence. Cows with a follicle responsive to GnRH on d 0 should ovulate and start a new follicular wave. As a result of this protocol, they would then have a new follicle that would be responsive to GnRH on d 9, and ovulating the induced follicle during progestin influence would initiate a second follicular wave until CIDR removal on d 14. We expected some cows that did not respond to the initial GnRH on d 0 to have a follicle that was responsive to GnRH by d 9 and thus initiate a new follicular wave to ovulate after CIDR removal. The GnRH given on d 9 addressed the issue of poor fertility of oocytes in follicles during extended (14-d) progestin influence by ovulating or luteinizing persistent follicles or follicles on the way to becoming persistent. Unfortunately, TAI pregnancy rates in noncycling animals were not increased using the 14-d CIDR protocols compared with the shorter 5-d CO-Synch + CIDR protocol. Therefore, additional cyclicity was not induced in the 14-d CIDR treatments compared with the 5-d CO-Synch + CIDR treatment. A single 50-mg dose of $PGF_{2\alpha}$ at CIDR removal produced similar TAI pregnancy rates to two 25-mg doses at 6-h intervals. Possible explanations for disparities in TAI pregnancy rates between the 14-d 50 PG and 14-d 6-h $PGF_{2\alpha}$ treatments at location 3 remain unclear. However, within this location, the increased estrus response in the 14-d 50 PG treatment compared to both 14-d 6-h PG and 5-d CO-Synch + CIDR treatments was likely the major cause of these greater pregnancy rates. The low number of animals in each treatment at this location could have also been a factor.

Pregnancy rates to TAI with 14-d CIDR estrus synchronization protocols using a GnRH analog on d 9 were promising. Further research involving ultrasonography is needed to quantify the rate that 2 follicular waves were induced within the 14-d CIDR protocols. However, the 14-d 50 PG treatment may increase TAI pregnancy rates compared with the beef industry accepted 5-d CO-

Synch + CIDR protocol, and the single 50-mg dose of $PGF_{2\alpha}$ i.m. was sufficient in the 14-d 50 PG treatment vs. two 25-mg doses of $PGF_{2\alpha}$ at 6-h intervals. Future studies to assess the ovulation/luteinization rate of persistent follicles potentially formed within this protocol are needed along with optimizing timing of the GnRH injection within the 14 d of progesterone influence.

LITERATURE CITED

Beef Reproduction Task Force. 2012. Estrus synchronization protocols for heifers and cows. http://beefrepro.unl.edu/resources.html. (Accessed 29 March 2013.)

Bridges, G. A., J. K. Ahola, C. Brauner, L. H. Cruppe, J. C. Currin, M. L. Day, P. J. Gunn, J. R. Jaeger, S. L. Lake, G. C. Lamb, G. H. L. Marquezini, R. K. Peel, A. E. Radunz, J. S. Stevenson, and W. D. Whittier. 2012. Determination of the appropriate delivery of postglandin $F_{2\alpha}$ in the 5-day CO-Synch + controlled intravaginal drug release protocol in suckled beef cows. J. Anim. Sci. 90:4814–4822.

Bridges, G. A., L. A. Helser, D. E. Grum, M. L. Mussard, C. L. Gasser, and M. L. Day. 2008. Decreasing the interval between GnRH and $PGF_{2\alpha}$ from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology 69:843–851.

Cupp, A., M. Garcia-Winder, A. Zamudio, V. Mariscal, M. Wehrman, N. Kojima, K. Peters, E. Bergfeld, P. Hernandez, T. Sanchez, R. Kittok, and J. Kinder. 1993. Concentration of progesterone (P4) in circulation has a differential effect on biochemical characteristics of dominant follicles in cows. J. Anim. Sci. 71(Suppl. 1):211. (Abstr.)

Geary, T. W., E. R. Downing, J. E. Bruemmer, and J. C. Whittier. 2000. Ovarian and estrous response of suckled beef cows to the Select Synch estrous synchronization protocol. Prof. Anim. Sci. 16:1–5.

Gunn, P. J., K. C. Culp, S. L. Lake, R. P. Arias, R. P. Lemenager, K. Heaton, and G. A. Bridges. 2009. Comparison of the CIDR Select and 5 day CO-Synch + CIDR protocols for synchronizing estrus in beef heifers. J. Anim. Sci. 87(Suppl. 1):217. (Abstr.)

Kasimanickam, R., M. L. Day, J. S. Rudolph, J. B. Hall, and W. D. Whittier. 2009. Two doses of prostaglandin improve pregnancy rates to timed-AI in a 5-day progesterone based synchronization protocol in beef cows. Theriogenology 71:762–767.

Kinder, J. E., F. N. Kojima, E. G. Bergfeld, M. E. Wehrman, and K. E. Fike. 1996. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. J. Anim. Sci. 74:1424–1440.

Lauderdale, J. W. 1972. Effects of $PGF_{2\alpha}$ on pregnancy and estrous cycles of cattle. J. Anim. Sci. 35(Suppl. 1):246. (Abstr.)

Leitman, N. R., D. C. Busch, D. A. Mallory, D. J. Wilson, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2009.Comparison of long-term CIDR-based protocols to synchronize estrus in beef heifers. Anim. Reprod. Sci. 114:345–355.

Manns, J. G., W. D. Humphrey, P. F. Flood, R. J. Mapletoft, N. Rawlings, and K. W. Cheng. 1983. Endocrine profiles and functional characteristics of corpora lutea following onset of postpartum ovarian activity in beef cows. Can. J. Anim. Sci. 63:331–347.

Mihm, M., A. Baguisi, M. P. Boland, and J. F. Roche. 1994. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. J. Reprod. Fertil. 102:123–130.

Niswender, G. D. 1973. Influence of the site of conjugation on specificity of antibodies to progesterone. Steroids. 22:413–424.

Patterson, D. J., L. R. Corah, G. H. Kiracofe, J. S. Stevenson, and J. R. Brethour. 1989. Conception rate in *Bos taurus* and *Bos indicus* crossbred heifers after postweaning energy manipulation and synchronization of estrus with melengestrol acetate and fenprostalene. J. Anim. Sci. 67:1138–1147.

Peel, R. K., J. C. Whittier, R. M. Enns, A. V. Grove, and G. E. Seidel Jr. 2010. Effect of 6 versus 12 hour interval between 2 prostaglandin F2α injections administered with 5 day CO-Synch + controlled internal drug release protocol on pregnancy rate in beef cows. Prof. Anim. Sci. 26:307–312.

Perry, G. A., M. F. Smith, and T. W. Geary. 2004. Ability of intravaginal progesterone inserts and melengestrol acetate to induce estrous cycles in postpartum beef cows. J. Anim. Sci. 82:695–704.

Perry, G. A., M. F. Smith, and D. J. Patterson. 2002. Evaluation of a fixed-time artificial insemination protocol for postpartum suckled beef cows. J. Anim. Sci. 80:3060–3064.

Revah, I. and W. R. Butler. 1996. Prolonged dominance of follicles and reduced viability of bovine oocytes. J. Reprod. Fertil. 106:39–47.

Richards, M. W., J. C. Spitzer, and M. B. Warner.1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. 62:300–306.

Savio, J. D., W. W. Thatcher, G. R. Morris, K. Entwistle, M. Drost, and M. R. Mattiacci. 1993. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. J. Reprod. Fertil. 98:77–84.

Schafer, D. J., D. C. Busch, M. F. Smith, and D. J. Patterson. 2006. Characterization of follicular dynamics, timing of estrus, and response to GnRH and PG in replacement beef heifers after presynchronization with a 14-day CIDR. J. Anim. Sci. 84(Suppl. 1):49. (Abstr.)

Seabrook, J. L., R. K. Peel, G. E. Seidel, and J. C. Whittier. 2010. Fixed-time AI conception rates in beef cows resulting from reduced 2-shot prostaglandin intervals on day 5 of a 5-d CIDR-Co-synch estrus synchronization. J. Anim. Sci. 88(Suppl. 1):742. (Abstr.)

Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J. Anim. Sci. 68:799–816.

Smith, V. G., J. R. Chenault, J. F. McAllister, and J. W. Lauderdale. 1987. Response of postpartum beef cows to exogenous progestin and gonadotropin releasing hormone. J. Anim. Sci. 64:540–551.

Stegner, J. E., F. N. Kojima, M. R. Ellersieck, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2004. A comparison of progestin-based protocols to synchronize estrus in postpartum beef cows. J. Anim. Sci. 82:1016–1021.

Troxel, T. R., and D. J. Kesler. 1983. Progestin pretreatment enhances the function of corpora lutea induced by gonadotropin releasing hormone treatment in postpartum suckled beef cows. J. Anim. Sci. 57(Suppl. 1):107. (Abstr.)

Walters, D. L., R. E. Short, E. M. Convey, R. B. Staigmiller, T. G. Dunn, and C. C. Kaltenbach. 1982. Pituitary and ovarian function in postpartum beef cows. III. Induction of estrus, ovulation, and luteal function with intermittent small-dose injection of GnRH. Biol. Reprod. 26:655–662.