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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


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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 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. 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 PGF$_2\alpha$ 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

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-
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 d 14, 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](https://www.journalofanimalscience.org/assets/images/estrus-synchronization-treatments-administered-to-lactating-beef-cows.png)

**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 and 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 anestruis 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 x treatment. However, the final model used only significant factors (P < 0.10), which were treatment, location x treatment, parity, BCS, and PPI. For cycling status data at locations 1 and 2, cycling and cycling x 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)**

<table>
<thead>
<tr>
<th>Location and treatment</th>
<th>n</th>
<th>PPI, d</th>
<th>BCS</th>
<th>Parity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Primiparous</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>247</td>
<td>81 ± 1.1a</td>
<td>5.0 ± 0.05b</td>
<td>25.1</td>
</tr>
<tr>
<td>2</td>
<td>395</td>
<td>75 ± 1.1b</td>
<td>4.5 ± 0.3b</td>
<td>14.9</td>
</tr>
<tr>
<td>3</td>
<td>137</td>
<td>72 ± 1.3c</td>
<td>5.6 ± 0.04c</td>
<td>11.7</td>
</tr>
<tr>
<td>Combined across locations</td>
<td>779</td>
<td>76 ± 0.7</td>
<td>4.9 ± 0.01</td>
<td>17.6</td>
</tr>
</tbody>
</table>

a-cWithin a column, means without common superscripts differ (P < 0.05).

1Cows 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_{2α,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_{2α,i.m.} at CIDR removal and another 25 mg PGF_{2α,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_{2α,i.m.} with CIDR removal, and another 25 mg PGF_{2α,i.m.} ± 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 \times 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 PG and 5-d CO-Synch + CIDR treatments, and the 14-d 6-h PGF2α treatment did not differ from the 5-d CO-Synch + CIDR treatment \((P > 0.10; \text{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 PGF2α 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 cyclicity.

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

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

\(^a,b\)Within a location or combined across locations, 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 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 PGF2α 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 PGF2α i.m. at CIDR removal and another 25 mg PGF2α i.m. 6 ± 1 h later; 5-d CO-Synch + CIDR = 5-d CIDR with 100 μg GnRH analog i.m. 9 d with CIDR insertion, 25 mg PGF2α i.m. with CIDR removal, and another 25 mg PGF2α i.m. 6 ± 1 h later.  
\(^2\)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 PGF2α 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 PGF2α i.m. at CIDR removal and another 25 mg PGF2α i.m. 6 ± 1 h later; 5-d CO-Synch + CIDR = 5-d CIDR with 100 μg GnRH analog i.m. 9 d with CIDR insertion, 25 mg PGF2α i.m. with CIDR removal, and another 25 mg PGF2α i.m. 6 ± 1 h later.  
\(^3\)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 PGF2α 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 PGF2α i.m. at CIDR removal and another 25 mg PGF2α i.m. 6 ± 1 h later; 5-d CO-Synch + CIDR = 5-d CIDR with 100 μg GnRH analog i.m. 9 d with CIDR insertion, 25 mg PGF2α i.m. with CIDR removal, and another 25 mg PGF2α i.m. 6 ± 1 h later.  
\(^4\)There was a treatment \times location interaction \((P < 0.05)\).  
\(^5\)There was no treatment \times 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 the 14-d 50 PGF$_{2\alpha}$ 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 has 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-17$\beta$ 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-17$\beta$ 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-17$\beta$ (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-17$\beta$ 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n$</th>
<th>Patch score, $%$ of treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-d 50 PG</td>
<td>278</td>
<td>24.1$^a$</td>
<td>13.3</td>
<td>58.3$^a$</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>14-d 6-h PG</td>
<td>249</td>
<td>24.9$^a$</td>
<td>16.9</td>
<td>54.6$^a$</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>5-d CO-Synch + CIDR</td>
<td>252</td>
<td>36.5$^b$</td>
<td>16.7</td>
<td>44.0$^b$</td>
<td>2.8</td>
<td></td>
</tr>
</tbody>
</table>

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

$^c$Cows in all treatment groups received 100 $\mu$g 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 $\mu$g 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 $\mu$g 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$_{2α}$ 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$_{2α}$ treatment (87.2%) compared to 14-d 6-h PGF$_{2α}$ (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.

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 (Steiger 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 by 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) progesterone 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$_{2α}$ 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$_{2α}$ 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-

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

<table>
<thead>
<tr>
<th>Patch score</th>
<th>TAI PR by treatment,$^2$ %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14-d 50 PG</td>
</tr>
<tr>
<td>1</td>
<td>52.2 (35/67)</td>
</tr>
<tr>
<td>2</td>
<td>48.6$^{a,b}$ (18/37)</td>
</tr>
<tr>
<td>3</td>
<td>67.3 (109/162)</td>
</tr>
<tr>
<td>4</td>
<td>66.7 (8/12)</td>
</tr>
</tbody>
</table>

$^a,b$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$_{2α}$ 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$_{2α}$ i.m. at CIDR removal and another 25 mg PGF$_{2α}$ 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$_{2α}$ i.m. with CIDR removal, and another 25 mg PGF$_{2α}$ i.m. 6 ± 1 h later. Fractions in parentheses denote actual numbers of animals and averages within each patch score group.
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


