Comparison of controlled internal drug release insert-based protocols to synchronize estrus in prepubertal and estrous-cycling beef heifers

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ABSTRACT: The objective of the experiment was to examine the necessity of adding a GnRH injection to a 14-d controlled internal drug release (CIDR)-based protocol for synchronization of estrus in beef heifers that were prepubertal or estrous-cycling at the initiation of treatment. The hypothesis tested was that the addition of GnRH in a CIDR-based estrus synchronization protocol would increase the synchrony of estrus after PGF2α (PG). Beef heifers (n = 285) were assigned to 1 of 2 treatments within reproductive tract scores (2 or 3 = prepubertal; 4 or 5 = estrous-cycling) by age and BW. Heifers assigned to CIDR Select received a CIDR insert (1.38 g of progesterone) from d 0 to 14 followed by GnRH (100 µg, intramuscularly) on d 23 and PG (25 mg intramuscularly) on d 30. Heifers assigned to CIDR-PG received a CIDR insert from d 0 to 14 and PG on d 30. Heifers were fitted with a HeatWatch estrus detection system transmitter at the time of PG administration for continuous estrus detection during the synchronized period (0 to 144 h after PG); AI was performed 12 h after estrus onset. Estrous response did not differ (P = 0.43) between treatments (94% CIDR Select, 98% CIDR-PG). Mean interval to estrus after PG was 7 h shorter (P = 0.01) and variance for interval to estrus was reduced (P < 0.01) among CIDR-PG-treated compared with CIDR Select-treated heifers. Conception rate to AI tended (P = 0.09) to be greater for CIDR-PG heifers (67%) compared with CIDR Select heifers (58%), and AI pregnancy rate was greater (P = 0.05) for CIDR-PG heifers (66%) compared with CIDR Select heifers (55%). Final pregnancy rate at the end of the breeding season was similar for the 2 treatments (81% for both; P = 0.94). We conclude that the administration of GnRH 9 d after CIDR removal in the CIDR Select protocol is not required to facilitate an improvement in the synchrony of estrus in beef heifers.

Key words: artificial insemination, beef heifer, controlled internal drug release insert, estrus synchronization

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INTRODUCTION

The use of fixed-time AI (FTAI) is appealing to many beef producers because it eliminates the time and labor required for estrus detection. The controlled internal drug release (CIDR) Select protocol has been used successfully to facilitate FTAI in beef heifers (Busch et al., 2007); however, a drawback to its use is that heifers must be handled through working facilities 5 separate times. The CIDR Select protocol yields significantly greater FTAI pregnancy rates compared with the CO-Synch + CIDR protocol (62 vs. 47%, respectively; Busch et al., 2007), which requires 2 fewer handling times.

The inclusion of GnRH in estrus synchronization protocols facilitates synchronized ovulation or luteinization of most large dominant follicles and initiates a new wave of follicular growth (Twagiramungu et al., 1995). Wood et al. (2001) reported that inclusion of GnRH within a 14- to 19-d melengestrol acetate (MGA)-PGF2α (PG) protocol improved the synchrony of follicular growth, although estrous response was dependent on the pubertal status of heifers before treatment initiation (Wood-Follis et al., 2004). In contrast, Johnson and Day (2004) reported that the inclusion of GnRH in the MGA-PG protocol failed to increase the synchrony of estrus, possibly because of an inconsistent ovulatory response to GnRH in heifers. When administered at random stages...
of the estrous cycle, only 48 to 60% of beef and dairy heifers ovulated in response to GnRH (Macmillan and Thatcher, 1991; Pursley et al., 1995; Moreira et al., 2000).

Given these considerations, the objective herein was to examine the necessity of adding a GnRH injection to a 14-d CIDR protocol (CIDR Select vs. CIDR-PG) for synchronization of estrus in beef heifers that were prepubertal or estrous-cycling at the initiation of treatment. Treatments were compared on the basis of estrous response and distribution of estrus after PG, and of synchronized AI conception and pregnancy rates. The hypothesis tested was that the addition of GnRH on d 23 (9 d after CIDR removal) in the CIDR Select protocol would result in a more synchronized estrus compared with the CIDR-PG protocol.

MATERIALS AND METHODS

The experimental procedures were approved by the University of Missouri-Columbia Animal Care and Use Committee.

Animals

Angus beef heifers (n = 285) were randomly assigned to 1 of 2 treatments within reproductive tract score (RTS; 1 = immature to 5 = luteal phase; Anderson et al., 1991) by age and BW. Evaluations of RTS were made 4 d before treatment initiation. Heifers assigned a RTS of 2 or 3 were designated as prepubertal and heifers assigned a RTS of 4 or 5 were designated as estrous-cycling (Patterson et al., 1999; Rosenkraus and Hardin, 2003; Holm et al., 2009). Heifers assigned to the CIDR Select protocol (n = 144) received an Eazi-Breed CIDR insert (1.38 g of progesterone; Pfizer Animal Health, New York, NY) from d 0 to 14 followed by GnRH (100 µg, intramuscularly; Cystorelin, Merial, Athens, GA) on d 23 and PG (Lutalyse, Pfizer Animal Health; 25 mg, intramuscularly) on d 30. Heifers assigned to CIDR-PG received a CIDR insert from d 0 to 14 and PG on d 30.

Estrus Detection and AI

Each heifer was fitted with a HeatWatch estrus detection transmitter (DDX Inc., Denver, CO) at the time of PG administration for continuous estrus detection during the synchronized period (0 to 144 h after PG administration). Estrus was defined as heifers receiving ≥3 mounts, each of which were ≥2 s in duration, within a 4-h period. Artificial insemination was performed by 1 experienced technician approximately 12 h after the onset of standing estrus with semen from 1 of 11 sires. Sires were assigned equally to heifers in each treatment. HeatWatch transmitters were removed at AI. Heifers were exposed to fertile bulls for 40 d beginning 10 d after the synchronized period began.

Definitions

Synchronized estrous response was defined as the number of heifers that exhibited estrus within 144 h (synchronized AI period) after PG injection, divided by the total number of heifers in each treatment. Synchronized AI conception rate was defined as the number of heifers that conceived to AI during the synchronization period, divided by the number of heifers inseminated during the same period. Synchronized AI pregnancy rate was defined as the number of heifers that became pregnant to AI during the synchronized period divided
Table 1. Number, age, BW, and reproductive tract score (RTS) of heifers before initiation of treatment
(least squares means ± SE)

<table>
<thead>
<tr>
<th>Item</th>
<th>CIDR Select</th>
<th>CIDR-PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of heifers</td>
<td>144</td>
<td>141</td>
</tr>
<tr>
<td>Estrous-cycling heifers</td>
<td>69</td>
<td>67</td>
</tr>
<tr>
<td>Prepubertal heifers</td>
<td>75</td>
<td>74</td>
</tr>
<tr>
<td>Age, d</td>
<td>462 ± 1.7</td>
<td>462 ± 1.7</td>
</tr>
<tr>
<td>BW, kg</td>
<td>340 ± 2.3</td>
<td>340 ± 2.3</td>
</tr>
<tr>
<td>RTS</td>
<td>4 ± 0.10</td>
<td>4 ± 0.10</td>
</tr>
</tbody>
</table>

1 Heifers assigned to controlled internal drug release (CIDR) Select received an Eazi-Breed CIDR insert (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, GnRH (Cystorelin, Merial, Athens, GA; 100 µg, intramuscularly) on d 23, and PG (Lutalyse, Pfizer Animal Health; 25 mg, intramuscularly) on d 30. Heifers assigned to CIDR-PG received a CIDR insert from d 0 to 14 and PG on d 30.
2 Estrous-cycling heifers = heifers assigned a RTS of 4 or 5.
3 Prepubertal heifers = heifers assigned a RTS of 2 or 3.
4 Age (d) of the heifers at the initiation of the treatments.
5 BW of the heifers at the initiation of the treatments.
6 RTS of the heifers evaluated 4 d before initiation of the treatments (1 to 5 scale, where 1 = immature and 5 = luteal phase).

by the total number of heifers. Pregnancy rate at the end of the breeding season was defined as the proportion of heifers diagnosed as pregnant of those assigned to each treatment.

Pregnancy Diagnosis

Conception rates to AI and final pregnancy rates were determined by transrectal ultrasonography (Aloka 500V equipped with a 5.0-MHz linear array transducer, Aloka, Wallingford, CT) 67 d after the end of the 40-d breeding season. Pregnancy data were unavailable for 2 heifers (1 heifer per treatment). However, because both heifers exhibited estrus after PG, the estrus data were included when analyzing estrous response and synchrony of estrus.

Statistical Analyses

Differences in age, BW, and RTS between treatments were analyzed by one-way ANOVA (PROC GLM, SAS Inst. Inc., Cary, NC). Differences in mean interval to estrus between treatments were analyzed by ANOVA using the linear statistical model of treatment, estrous cyclicity status, and the 2-way interaction; the Bartlett test was used to assess homogeneity of variance (PROC GLM of SAS). Because variances for the mean interval to estrus were heterogeneous between treatments, a log10 transformation was performed for testing differences. However, all tables and figures are presented with nontransformed values. Variances associated with the interval to estrus were compared by F-test (greater variance divided by the smaller variance; Snedecor and Cochran, 1989). These variances were calculated to provide comparisons of the degree of synchrony of estrus after treatment. Estrous response after PG, AI conception rate, AI pregnancy rate, and final pregnancy rate at the end of the breeding season were analyzed using a GLM method (PROC GLIMMIX of SAS) for a binomial distribution and the link function of logit. The model included the main effects of treatment, estrous cyclicity status, and the 2-way interaction. Conception rate to AI was reanalyzed (PROC GLIMMIX of SAS), adjusting for the effect of sire (this P-value appears in the text and figures, unless noted otherwise). All reported means are least squares means ± SE.

RESULTS

The number, mean age, BW, and RTS of heifers before the initiation of treatments are shown in Table 1. There was no difference between treatments for age (P = 0.94), BW (P = 0.91), or RTS (P = 0.98). Figure 2 illustrates the distribution of estrus after PG administration. Estrous response was not influenced by treatment (P = 0.43), but tended to be influenced by estrous cyclicity status (P = 0.08). The mean interval from PG administration to estrus was not affected by estrous cyclicity status (P = 0.14); however, the mean interval to estrus was 7 h shorter (P = 0.01; Table 2) for heifers assigned to the CIDR-PG protocol compared with the CIDR Select protocol.

For purposes of the current experiment, variance associated with the interval to estrus is a more descriptive variable for use in comparing treatments because it more accurately describes differences in synchrony of estrus after treatment and is more relevant to the development of an effective protocol for FTAI. Differences in the variance for interval to estrus were detected based on the main effects of treatment (Table 2) and estrous cyclicity status, as well as their interaction (Table 3). Overall, heifers assigned to the CIDR-PG protocol had a greater (P < 0.01) synchronization of estrus compared with heifers assigned to the CIDR Select protocol, and regardless of treatment, the prepubertal heifers had a greater (P < 0.01) synchronization of estrus compared with the estrous-cycling heifers. Both the estrous-cycling (P < 0.02) and prepubertal (P < 0.01) beef heifers assigned to the CIDR-PG protocol had a greater synchronization of estrus compared with their counterparts assigned to the CIDR Select protocol. Although the synchrony of estrus was similar (P = 0.10) between the estrous-cycling and prepubertal heifers assigned to the CIDR Select protocol, the prepubertal heifers assigned to the CIDR-PG protocol had a more synchronized (P < 0.05) estrus compared with the estrous-cycling heifers assigned to the CIDR-PG protocol.

Conception rate to AI tended to be greater for heifers assigned to CIDR-PG compared with CIDR Select (P = 0.10, no adjustment for sire effects; P = 0.09, adjusting for sire effects in the analysis), but was not influenced by estrous cyclicity status (P = 0.44, no adjustment for sire effects; P = 0.43, adjusting for sire effects in the analysis; Table 2). The odds of heifers assigned to CIDR-PG conceiving to AI were 2.6 times greater...
than not conceiving ($P < 0.01$), whereas the odds of heifers assigned to CIDR Select conceiving to AI were 1.7 times greater than not conceiving ($P = 0.04$). Based on the odds ratio, heifers assigned to CIDR-PG were 1.5 times more likely to conceive to AI compared with heifers assigned to CIDR Select ($P = 0.09$).

Perhaps a more important factor to evaluate than AI conception rate is the AI pregnancy rate because it more accurately describes the projected AI pregnancy outcome of all heifers treated. Heifers assigned to CIDR-PG had a greater ($P = 0.05$) pregnancy rate to AI compared with heifers assigned to CIDR Select.

### Table 2. Estrous response and interval to estrus after PGF$_{2a}$ (PG), and AI conception rates and pregnancy rates for heifers assigned to controlled internal drug release (CIDR) Select or CIDR-PG

<table>
<thead>
<tr>
<th>Item</th>
<th>CIDR Select</th>
<th>CIDR-PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrous response after PGF$_{2a}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>136/144</td>
<td>138/141</td>
</tr>
<tr>
<td>Interval from PGF$_{2a}$ to estrus, h (least squares mean ± SE)</td>
<td>61.5 ± 1.7$^a$</td>
<td>54.4 ± 1.7$^b$</td>
</tr>
<tr>
<td>Variance for interval to estrus after PGF$_{2a}$</td>
<td>508$^a$</td>
<td>262$^b$</td>
</tr>
<tr>
<td>Conception rate to AI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>78/135$^c$</td>
<td>92/137$^d$</td>
</tr>
<tr>
<td>Pregnancy rate to AI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>78/143$^c$</td>
<td>92/140$^d$</td>
</tr>
<tr>
<td>Pregnancy rate at the end of the breeding season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>116/143</td>
<td>113/140</td>
</tr>
</tbody>
</table>

*Means or variances within rows with different superscripts are different ($P \leq 0.01$).

$^a,b$Means within rows with different superscripts tend to differ ($P = 0.09$).

$^c,d$Means within rows with different superscripts are different ($P = 0.05$).

1Heifers assigned to CIDR Select received an Eazi-Breed CIDR insert (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, GnRH (Cystorelin, Merial, Athens, GA; 100 µg, intramuscularly) on d 23, and PG (Lutalyse, Pfizer Animal Health; 25 mg, intramuscularly) on d 30. Heifers assigned to CIDR-PG received a CIDR insert from d 0 to 14 and PG on d 30.

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**Figure 2.** Percentage of heifers in the controlled internal drug release (CIDR) Select and CIDR-PG$_{2a}$ (PG) treatments that exhibited estrus after PG: CIDR Select (black bar) and CIDR-PG (gray bar). NR = no estrous response. Heifers assigned to CIDR Select received an Eazi-Breed CIDR insert (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, GnRH (Cystorelin, Merial, Athens, GA; 100 µg, intramuscularly) on d 23, and PG (Lutalyse, Pfizer Animal Health; 25 mg, intramuscularly) on d 30. Heifers assigned to CIDR-PG received a CIDR insert from d 0 to 14 and PG on d 30.
(Table 2). Pregnancy rate to AI was not influenced by estrous cyclicity status \((P = 0.20)\). The odds of heifers assigned to CIDR-PG having an AI pregnancy were 1.9 times greater than not \((P < 0.01)\), whereas the odds of heifers assigned to CIDR Select having an AI pregnancy were 1.2 times greater than not \((P = 0.31)\). Based on the odds ratio, heifers assigned to CIDR-PG were 1.6 times more likely to have an AI pregnancy compared with heifers assigned to CIDR Select \((P = 0.05)\). Final pregnancy rates at the end of the breeding season were not affected by treatment \((P = 0.94)\) or estrous cyclicity status \((P = 0.67; \text{Table 2})\).

**DISCUSSION**

The ability to synchronize follicular waves after injection of GnRH has facilitated the development of protocols that precisely control the time of ovulation in beef cattle and has resulted in pregnancy rates to FTAI that are similar to insemination after detection of estrus in beef cows (Patterson et al., 2007). However, the requirement for inclusion of GnRH in estrus synchronization protocols for beef heifers has been questioned (Lamb et al., 2006). In beef heifers, inclusion of GnRH at the beginning of a FTAI protocol did not substantially increase pregnancy rates at several locations; however, the SD of pregnancy rates was increased when GnRH was not included. Therefore, incorporation of GnRH in a FTAI protocol may increase the uniformity of pregnancy rates in beef heifers among locations compared with protocols based on estrus detection only (Lamb et al., 2006).

The rationale for conducting the present experiment was based on a recent study by Leitman et al. (2009), who reported an interaction between treatment and pubertal status for interval to estrus and the variance associated with interval to estrus. Leitman et al. (2009) conducted 2 experiments to determine whether schedule modification of the CIDR Select protocol (Busch et al., 2007) would result in a greater synchronization of estrus potentially to facilitate FTAI. Leitman et al. (2009) reported an interaction between GnRH and estrous cyclicity status for mean interval to estrus and variance for interval to estrus. Among prepubertal heifers treated with the CIDR Select protocol, variance for interval to estrus was reduced for heifers receiving GnRH compared with those that did not receive GnRH. However, in estrous-cycling heifers treated with the CIDR Select protocol, variance for interval to estrus was reduced for heifers that did not receive GnRH compared with those that did. This observation was first reported by Wood-Follis et al. (2004) in studies involving MGA administration. The preceding study indicated that the decision to add GnRH to an MGA-PG protocol (MGA Select) should be based on the pubertal status of the heifer at the time treatments are initiated.

In the present experiment, differences in the variance for interval to estrus were detected based on the main effects of treatment and estrous cyclicity status as well as their interaction. Heifers assigned to the CIDR-PG protocol had a greater synchronization of estrus compared with heifers assigned to the CIDR Select protocol, and regardless of treatment, the prepubertal heifers had a more highly synchronized estrus compared with the estrous-cycling heifers. The improved synchrony of estrus observed among prepubertal heifers may be a result of a greater synchronization of estrus after CIDR removal compared with the estrous-cycling heifers. Stage of cycle differences among estrous-cycling heifers at CIDR insertion would perhaps explain the potential for reduced synchrony of estrus after CIDR removal compared with prepubertal heifers. Both the estrous-cycling and prepubertal heifers assigned to the CIDR-PG protocol had a greater synchronization of estrus compared with their counterparts assigned to the CIDR Select protocol. Although the synchrony of estrus was similar between the estrous-cycling and prepubertal heifers assigned to the CIDR Select protocol, prepubertal heifers assigned to the CIDR-PG protocol had a more synchronized estrus compared with estrous-cycling heifers assigned to the CIDR-PG protocol.

<table>
<thead>
<tr>
<th>Item</th>
<th>CIDR Select</th>
<th>CIDR-PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrous-cycling heifers(^2)</td>
<td>596(^a)</td>
<td>332(^{b,x})</td>
</tr>
<tr>
<td>Prepubertal heifers(^3)</td>
<td>392(^a)</td>
<td>186(^{b,y})</td>
</tr>
</tbody>
</table>

\(^{a\text{x}}\) Variances within rows with different superscripts are different \((P < 0.02)\).

\(^{b\text{x}}\) Variances within columns with different superscripts are different \((P < 0.05)\).

\(^{1}\) Heifers assigned to CIDR Select received an Eazi-Breed CIDR insert (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, GnRH (Cystorelin, Merial, Athens, GA; 100 µg, intramuscularly) on d 23, and PG (Lutalyse, Pfizer Animal Health; 25 mg, intramuscularly) on d 30. Heifers assigned to CIDR-PG received a CIDR insert from d 0 to 14 and PG on d 30.

\(^{2}\) Estrous-cycling heifers = heifers assigned a reproductive tract score of 4 or 5.

\(^{3}\) Prepubertal heifers = heifers assigned a reproductive tract score of 2 or 3.
treatment for 14 d was similar between estrous-cycling and prepubertal heifers. Additionally, more than 88% of the heifers (estrous-cycling and prepubertal) were on d 7 or 8 of their estrous cycles 9 d after CIDR removal, coincident with the time at which GnRH was administered on d 23 of treatment of the CIDR Select protocol.

Arguably, given what we know regarding the length of follicular waves (Savio et al., 1988; Sirois and Fortune, 1988), one might assume that a proportion of heifers may turn dominant follicles over on their own, before GnRH administration, independent of the need for GnRH to accomplish the same. More recently, Jaiswal et al. (2009) reported differences in 2-wave vs. 3-wave patterns of ovarian follicular development in Bos taurus heifers. The prevalence of 2-wave vs. 3-wave patterns was influenced by heifer age, maturity, or both (Jaiswal et al., 2009). Those authors (Jaiswal et al., 2009) suggested that more precise determination of predictive factors controlling patterns of follicular development in heifers would lead to the development of protocols that facilitate improvements in estrous cycle control and would enhance opportunities to expand the use of FTAI.

These considerations may relate to the current study, but fail to explain the significant improvement in synchrony of estrus for CIDR-PG-treated compared with CIDR Select-treated heifers. Although response to GnRH in heifers is reported to be inconsistent when compared with cows (Macmillan and Thatcher, 1991; Pursley et al., 1995; Moreira et al., 2000), these data indicate that the addition of GnRH to a 14-d CIDR-PG protocol reduced the synchrony of estrus, despite similarities between treatments in estrous response. Schafer et al. (2006) and Leitman et al. (2009) reported that the majority of heifers are on d 7 or 8 of the estrous cycle at the time GnRH is administered on d 23 of the CIDR Select protocol; therefore, the question arises regarding the potential subsequent effect of administering GnRH to heifers at a point in their follicular wave at or during the time emergence of a new follicular wave begins. Given the fact that the interval to estrus after PG was longer among CIDR Select-treated vs. CIDR-PG-treated heifers, the effect of GnRH on subsequent follicular dynamics is in question.

Conception rate to AI tended to be greater for heifers assigned to CIDR-PG compared with CIDR Select, but was not influenced by estrous cyclicity status. Heifers assigned to CIDR-PG, however, had a greater pregnancy rate to AI compared with heifers assigned to CIDR Select. Pregnancy rate to AI was not influenced by estrous cyclicity status. These data confirm the effectiveness of both protocols in inducing cyclicity in prepubertal heifers and successfully preparing heifers for breeding and subsequent pregnancy.

Perry et al. (2007) reported that the use of protocols that control or manipulate follicular growth and development and increase the likelihood of ovulating optimally sized follicles may result in positive benefits on pregnancy rates in beef heifers. This is an important consideration based on studies that have shown a relationship between ovulatory follicle size and pregnancy success in heifers (Perry et al., 2007) and cows (Lamb et al., 2001; Vasconcelos et al., 2001). Collectively, these reports support the concept that presynchronization is an effective means of manipulating follicle growth and development before a synchronized estrous period.

In summary, the similarities in estrous response after PG indicate that each of these CIDR-based protocols was effective in synchronizing estrus in prepubertal and estrous-cycling beef heifers. The results from this experiment, however, failed to confirm the hypothesis that the addition of GnRH on d 23 of the CIDR Select protocol would result in greater synchronization of estrus compared with CIDR-PG. Differences between treatments in the interval to estrus after PG, synchrony of estrus, and AI pregnancy rates during the synchronized period clearly indicate that further evaluation of these 2 CIDR-based protocols is required with and without the addition of GnRH and on the basis of estrous cyclicity status to determine the efficacy of these protocols for use in facilitating FTAI.

LITERATURE CITED


