Two Split-Time Artificial Insemination Programs in Suckled Beef Cows

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Abstract
Successful programs to manipulate estrus and ovulation to maximize pregnancy outcomes in suckled beef cattle have been developed to limit animal handling and to eliminate the need to detect estrus, thereby providing more opportunity to incorporate AI to start the breeding season. The most successful and consistent synchronization scheme employs an intravaginal progesterone insert (controlled internal drug release, CIDR) in place for 7 d concurrent with GnRH treatment, and, upon removal of the insert, injection of PGF\textsubscript{2α}, followed by timed AI at 60 to 66 h concurrent with a second dose of GnRH (CO-synch + CIDR).

Inseminating cows after expressed estrus increases pregnancy rate compared with cows that do not display estrus in a timed AI program. Identification of estrus can be accomplished easily by using estrus-detection patches, thus facilitating timing of AI to meet herd-specific situations. Varying AI timing has increased pregnancy rate in some, but not all studies.

Rather than using 1 planned fixed-time AI for all cows, we tested split-time AI intervals, and used detection of estrus to optimally time AI relative to detected estrus. In previous research, in 2 separate experiments, estrus was detected by using estrus-detection patches at either 60 or 65 h after PGF\textsubscript{2α} and AI was conducted for cows in estrus. Remaining cows received a fixed-time AI at either 75 or 84 h, respectively. Administering GnRH to cows already detected in estrus at 60 or 75 h did not improve pregnancy rate. Additionally, administering GnRH at 60 or 75 h to cows not yet in estrus only improved pregnancy rate in cows that did not come into estrus during the interim before timed AI occurred at 75 or 84 h, respectively.

Our objective was to determine which time combination for split-time AI would maximize pregnancy rate in suckled beef cows: 55 h (AI cows in estrus) + 75 h (receive GnRH at 55 h and AI at 75 h) or 65 h (AI cows in estrus) + 85 h (receive GnRH at 65 h and AI at 85 h).

Keywords
beef cattle, CIDR, estrus, split-time artificial insemination

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Two Split-Time Artificial Insemination Programs in Suckled Beef Cows

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Introduction
Successful programs to manipulate estrus and ovulation to maximize pregnancy outcomes in suckled beef cattle have been developed to limit animal handling and to eliminate the need to detect estrus, thereby providing more opportunity to incorporate AI to start the breeding season. The most successful and consistent synchronization scheme employs an intravaginal progesterone insert (controlled internal drug release, CIDR) in place for 7 d concurrent with GnRH treatment, and, upon removal of the insert, injection of PGF 2α, followed by timed AI at 60 to 66 h concurrent with a second dose of GnRH (CO-Synch + CIDR).

Inseminating cows after expressed estrus increases pregnancy rate compared with cows that do not display estrus in a timed AI program. Identification of estrus can be accomplished easily by using estrus-detection patches, thus facilitating timing of AI to meet herd-specific situations. Varying AI timing has increased pregnancy rate in some, but not all studies.

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GnRH at 55 h and AI at 75 h) or 65 h (AI cows in estrus) + 85 h (receive GnRH at 65 h and AI at 85 h).

**Experimental Procedures**

Suckled beef cows (n = 1,062) of mixed parity at 12 locations in 4 states were enrolled in the experiment. Characteristics by location including breed composition, proportion of primiparous cows, days postpartum at artificial insemination (AI), and body condition score (BCS) are summarized (Table 1). Cows grazed native pastures during the treatment and AI period.

Cows were placed in a CO-Synch program plus an intravaginal insert which was impregnated with progesterone (1.38 g) (CIDR; Zoetis, Florham Park, NJ): GnRH + CIDR on d -7, CIDR insert removal + PGF₂α on d 0 at which time estrus-detection patches (Estrotect, Spring Valley, WI) were affixed to the tail head of all cows according to manufacturer’s recommendations. The dose of GnRH was 100 μg (2 mL Factrel i.m.; Zoetis). The dose of PGF₂α was 25 mg (2 mL Lutalyse HighCon i.m.; Zoetis). Body condition scores (1 = thin; 9 = obese) were assigned on d −7 by trained evaluators in all but 2 locations.

The study was designed as a completely randomized experiment of 2 treatment combinations. Within location and balanced for parity (primiparous vs. multiparous), cows were assigned randomly to 2 treatment times (55 vs. 65 h after CIDR insert removal; Figure 1). At 55 or 65 h, estrus-detection patches were assessed. Estrus was defined to have occurred if patches were > 50% colored. Cows determined to be in estrus were inseminated at either 55 or 65 h, whereas the remaining nonestrous cows in both treatment times received GnRH at 55 or 65 h and were inseminated 20 h later at 75 or 85 h, respectively. To facilitate inseminations (AI based on estrus or at a fixed time) at only 2 times, CIDR inserts, PGF₂α injection, and application of estrus-detection patches in the 65-h treatment were removed 10 h before those in the 55-h treatment. The 2 treatment combinations, therefore, consisted of the 55 + 75-h and the 65 + 85-h combinations with inseminations based on estrus occurring at either 55 or 65 h and their peer herd mates not in estrus at 55 or 65 h were inseminated 20 h later at 75 or 85 h, respectively.

Activated patches were removed from cows in estrus at 55 or 65 h after the time of AI. Patches on remaining cows were assessed at 75 or 85 h when cows received their timed AI, thus allowing a determination of the number of cows showing estrus during the 20-h period after GnRH was administered compared to nonestrous cows before they were inseminated at 75 or 85 h.

No sooner than 1 wk after AI, cows were exposed to natural service sires for a total breeding season of 45 to 60 d. A positive pregnancy outcome via transrectal ultrasonography required presence of an embryo with a visible heart beat at 36 d after AI (mean = 36.0 ± 0.1 d; range of 32 to 45 d). A final pregnancy diagnosis was determined via transrectal ultrasonography or transrectal palpation no sooner than 35 d after the end of the breeding season. Pregnancy loss was defined as those cows pregnant at 36 d after AI, but not at the appropriate stage of pregnancy at the time of the final pregnancy diagnosis.
Results and Discussion

Location Characteristics
Summarized in Table 1 are location characteristics of 1,046 cows enrolled at the 12 locations in the 4 states where the experiment was conducted. The cows were Angus-based, including both purebreds and crosses of Angus. Cows at all locations calved during the spring, but at one location (CO-1), the spring-calving cows were moved to a fall-breeding program. As expected, resulting pregnancy rates varied ($P < 0.001$) among locations (Table 1). Actual insemination times were: 55 h (mean and range = 54.5 h; 53.6 to 55.8 h), 65 h (64.6 h; 63.7 to 66.0 h), 75 h (74.4 h; 73.6 to 75.5 h), and 85 h (84.7 h; 83.1 to 85.5 h).

Expression of Estrus
Expression of estrus was greater ($P = 0.001$) by 65 h after PGF$_{2\alpha}$ than by 55 h (Figure 2), and this proportion was influenced by parity (time × parity interaction; $P = 0.006$). By 55 h, fewer primiparous than multiparous cows were in estrus, whereas no differences in those proportions were detected between parity groups by 65 h. As a result, proportionally more ($P < 0.001$) cows received the timed AI at 75 than 85 h (59.4% vs. 40.6%). Similar proportions of cows not in estrus by 55 or 65 h were detected in estrus by 75 or 85 h (40.1% vs. 39.3%), respectively. As expected, the total proportion of cows in estrus by 75 h was less ($P < 0.001$) than that by 85 h (Figure 2). These proportions were affected by parity ($P = 0.045$), with more multiparous than primiparous cows showing estrus. No interaction of treatment and parity was detected ($P = 0.137$). Body condition did not affect ($P = 0.12$) expression of estrus in the current study.

Pregnancy Outcomes
Of cows in estrus (n = 500) by 55 or 65 h (inseminated at either 55 or 65 h), pregnancy rate was greater ($P < 0.001$; Table 2) compared with cows not in estrus (n = 549) by those times (inseminated at 75 or 85 h), respectively. Of cows not in estrus (n = 198) by 55 or 65 h, but showed estrus by 75 or 85 h, respectively, pregnancy rates were greater ($P < 0.001$) for cows expressing estrus than for cows not showing estrus (n = 302) during that 20-h period (66.7% vs. 42.7%), respectively. Overall, regardless of when estrus occurred, pregnancy rate was greater ($P < 0.001$) for cows that showed estrus (n = 747) than for those not showing estrus and receiving the timed AI (n = 302; 67.3% vs. 42.7%).

Both actual and adjusted mean proportions of cows pregnant at 36 d after AI, cows pregnant at the end of the breeding season (which included exposure to natural service sires), and intervening pregnancy loss are summarized in Table 2. Pregnancy rate at 36 d for estrous cows inseminated at 55 h did not differ from those estrous cows inseminated at 65 h. Although estrous cows inseminated at 55 h had a greater pregnancy rate than their nonestrous peers receiving the timed AI at 75 h, that did not hold true for estrous cows inseminated at 65 h compared with their nonestrous peers receiving the timed AI at 85 h (contrasts: 55 vs. 65 h [$P = 0.337$]; 55 vs. 75 h [$P = 0.029$]; 65 vs. 85 h [$P = 0.113$]; 75 vs. 85 h [$P = 0.127$]).
A more appropriate way to assess the programs is to examine the combined pregnancy rate for cows in the 55 + 75-h treatment combination with those in the 65 + 85-h treatment combination. Cows in the latter treatment combination had the greater pregnancy rate at 36 d than the former (Table 2). This difference in pregnancy rate is explained partly by the proportionally greater number of cows receiving the timed AI in the 55 + 75-h combination compared with those in the 65 + 85-h combination (59.4% vs. 40.6%), respectively. In other words, the greater pregnancy rate of the 65 + 85-h combination occurred because more cows were inseminated after having been detected in estrus and a tendency \( P = 0.127 \) for greater pregnancy rate for cows receiving the timed AI at 85 h compared with 75 h.

Pregnancy rates at 36 d tended \( P = 0.067 \) to be less in primiparous than multiparous cows, but no interaction was detected \( P = 0.192 \) between treatment and parity (Figure 3). In 10 locations in which BCS was assessed, BCS did not significantly influence pregnancy rate at 36 d. Cows with longer postpartum intervals to AI had greater pregnancy rate. For every 10-d increase in days postpartum at AI, pregnancy rate at 36 d increased by 3.1 ± 0.1% \( P = 0.014 \) or 3.5 ± 1.1% \( P = 0.026 \) depending whether the model included all 4 insemination times or the 2 treatment combinations, respectively.

Final pregnancy rates tended \( P = 0.058 \) to be less in cows that received the timed AI at either 75 or 85 h (Table 2). In contrast, when the treatment combinations were examined, final pregnancy outcomes did not differ. As expected, final pregnancy outcomes were less \( P = 0.001 \) in primiparous than multiparous cows (Figure 4). Final pregnancy rates for primiparous cows increased \( P = 0.001 \) by 3.4 ± 0.7% (4 AI times) or by 3.5 ± 0.7% (2 treatment combinations) for each 10-d increase in postpartum days to AI. Body condition score influenced final pregnancy risk in locations in which it was assessed. For every unit increase in BCS, final pregnancy rate increased \( P = 0.001 \) by 4.1 ± 1.5% (4 AI times) to 4.4 ± 1.5% (2 treatment combinations).

Although small, pregnancy losses between AI and the end of the breeding season tended \( P = 0.058 \) to differ among treatments, but they did not differ between the 2 treatment combinations (Table 2), averaging less than 4%. For every 10-d increase in days postpartum at AI, pregnancy loss decreased \( P < 0.001 \) by -1.1 ± 0.5% (4 AI times) to -1.3 ± 0.5% (2 treatment combinations).

**Implications**

We conclude that employing a split-time AI program at 65 + 85 h produced more pregnancies than the 55 + 75 h program because more cows were in estrus at the 65- and 85-h insemination times and fertility was greater in estrous than nonestrous cows.
Table 1. Selected characteristic of suckled beef cows enrolled in the experiment

<table>
<thead>
<tr>
<th>Location^1</th>
<th>Breed^2</th>
<th>n</th>
<th>2-year-old</th>
<th>Days post-partum at AI^3</th>
<th>BCS^3</th>
<th>Pregnancy rate^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO-1</td>
<td>A, H</td>
<td>75</td>
<td>31</td>
<td>156 ± 23</td>
<td>5.2 ± 0.8</td>
<td>62.7</td>
</tr>
<tr>
<td>CO-2</td>
<td>A</td>
<td>150</td>
<td>27</td>
<td>77 ± 9</td>
<td>-</td>
<td>61.3</td>
</tr>
<tr>
<td>CO-3</td>
<td>A</td>
<td>169</td>
<td>44</td>
<td>73 ± 24</td>
<td>-</td>
<td>63.3</td>
</tr>
<tr>
<td>KS-1</td>
<td>A × H</td>
<td>45</td>
<td>20</td>
<td>82 ± 16</td>
<td>5.7 ± 0.5</td>
<td>46.7</td>
</tr>
<tr>
<td>KS-2</td>
<td>A × H</td>
<td>44</td>
<td>27</td>
<td>83 ± 14</td>
<td>5.4 ± 0.4</td>
<td>29.5</td>
</tr>
<tr>
<td>KS-3</td>
<td>A × H</td>
<td>64</td>
<td>62</td>
<td>77 ± 15</td>
<td>6.0 ± 0.8</td>
<td>43.7</td>
</tr>
<tr>
<td>KS-4</td>
<td>A × H</td>
<td>75</td>
<td>16</td>
<td>69 ± 16</td>
<td>5.4 ± 0.5</td>
<td>65.8</td>
</tr>
<tr>
<td>KS-5</td>
<td>A × H</td>
<td>61</td>
<td>28</td>
<td>75 ± 17</td>
<td>5.1 ± 0.5</td>
<td>63.9</td>
</tr>
<tr>
<td>KS-6</td>
<td>A × H</td>
<td>101</td>
<td>0</td>
<td>75 ± 18</td>
<td>6.0 ± 0.8</td>
<td>54.3</td>
</tr>
<tr>
<td>MT-1</td>
<td>A</td>
<td>108</td>
<td>24</td>
<td>75 ± 15</td>
<td>4.8 ± 0.6</td>
<td>69.2</td>
</tr>
<tr>
<td>WA-1</td>
<td>A cross</td>
<td>70</td>
<td>0</td>
<td>59 ± 10</td>
<td>6.2 ± 1.1</td>
<td>71.4</td>
</tr>
<tr>
<td>WA-2</td>
<td>A</td>
<td>100</td>
<td>0</td>
<td>59 ± 10</td>
<td>6.2 ± 0.7</td>
<td>63.0</td>
</tr>
</tbody>
</table>

^1Cows at 12 locations in 4 states were enrolled.  ^2A = Angus and H = Hereford.  ^3Mean ± SD.  ^4Assessed at 33 to 45 d (average = 36 d) after AI.

Table 2. Pregnancy rate (PR) at 36 d after artificial insemination, final pregnancy rate at the end of the breeding season, and intervening pregnancy loss

<table>
<thead>
<tr>
<th>Treatment^1</th>
<th>PR at 36 d</th>
<th>Final PR^1</th>
<th>Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>55</td>
<td>65</td>
<td>75</td>
</tr>
<tr>
<td>n</td>
<td>223</td>
<td>326</td>
<td>302</td>
</tr>
<tr>
<td>Actual, %</td>
<td>66.8</td>
<td>68.6</td>
<td>49.7</td>
</tr>
<tr>
<td>Adjusted, %</td>
<td>58.8</td>
<td>64.7</td>
<td>46.3</td>
</tr>
<tr>
<td>n</td>
<td>223</td>
<td>326</td>
<td>307</td>
</tr>
<tr>
<td>Actual, %</td>
<td>94.8</td>
<td>92.8</td>
<td>87.9</td>
</tr>
<tr>
<td>Adjusted, %</td>
<td>92.3</td>
<td>93.9</td>
<td>86.8</td>
</tr>
<tr>
<td>n</td>
<td>153</td>
<td>217</td>
<td>149</td>
</tr>
<tr>
<td>Actual, %</td>
<td>2.0</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Adjusted, %</td>
<td>2.0</td>
<td>0.9</td>
<td>2.7</td>
</tr>
</tbody>
</table>

^1Cows were enrolled in a CO-Synch + controlled-internal drug release insert (CIDR) program (GnRH + CIDR on d −7, CIDR insert removal + PGF2α + fitted with estrus-detection patches on d 0). Cows were assigned to have their patches assessed (>50% activation) at either 55 or 65 h after CIDR insert removal. Cows with activated patches were then inseminated at either 55 or 65 h. The remaining nonestrous cows in both groups received GnRH at 55 or 65 h but were inseminated at 75 or 85 h, respectively.  ^2Unadjusted raw mean percentages.  ^3Adjusted mean percentages resulting from logistic regression analysis. Contrasts: 55 vs. 65 h (P = 0.337); 55 vs. 75 h (P = 0.029); 65 vs. 85 h (P = 0.113); and 75 vs. 85 h (P = 0.127).  ^4Six cows not present for the first pregnancy diagnosis (PR at 36 d) were present for the final pregnancy diagnosis.
Figure 1. Experimental design of treatments employed in the experiment. Cows were enrolled in a CO-Synch + CIDR program (GnRH + CIDR on d -7, CIDR insert removal + PGF$_{2\alpha}$ + fitted with estrus-detection patches on d 0). Cows assigned to have their patches “read” (<50% activation) at either 55 or 65 h after CIDR insert removal. Cows with activated patches were then inseminated at either 55 or 65 h. The remaining cows in both groups received GnRH at 55 or 65 h but were inseminated at 75 or 85 h, respectively. CIDR = controlled internal drug release insert containing 1.38 g of progesterone; GnRH = gonadotropin-releasing hormone; PGF = prostaglandin F$_{2\alpha}$; and TAI = timed artificial insemination.
Figure 2. Proportions of cows interpreted to be in estrus by 55, 65, 75, or 85 h after removal of CIDR insert and injection of PGF$_{2\alpha}$ in primiparous and multiparous suckled beef cows. Cows in the 75- and 85-h times were treated with GnRH 20 h earlier, respectively.
Figure 3. Pregnancy rate in primiparous and multiparous suckled beef cows at 36 d after artificial insemination by transrectal ultrasonography. Cows in the 55- and 65-h treatments were inseminated if estrus-detection patches were activated (>50%) by 55 or 65 h after removal of a CIDR insert and injection of PGF$_{2\alpha}$. The remaining nonestrous cows in both groups received GnRH at 55 or 65 h but were inseminated at 75 or 85 h, respectively. Thirteen cows were not present for this pregnancy diagnosis.
Figure 4. Final end of breeding season pregnancy rate in primiparous and multiparous suckled beef cows. Cows in the 55- and 65-h treatments were inseminated if estrus-detection patches were activated (>50%) by 55 or 65 h after removal of a CIDR insert and injection of PGF$_{2\alpha}$. The remaining nonestrous cows in both groups received GnRH at 55 or 65 h but were inseminated at 75 or 85 h, respectively. Cows were exposed to cleanup bulls beginning 10 d after AI.