Two split-time artificial insemination programs in suckled beef cows^{1,2}

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ABSTRACT: Our objective was to determine which of 2 split-time AI programs applied to suckled beef cows would result in greater pregnancy risk. Suckled beef cows ($n = 1,062$) at 12 locations in 4 states (CO, KS, MY, and WA) were enrolled. Cows were treated on d -7 with a progesterone insert concurrent with 100 μ g GnRH and on d 0 with 25 mg PGF_{2 α} plus removal of the insert. Estrus-detection patches were affixed to cows at insert removal. 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) at which time estrus-detection patches were assessed. Estrus was defined to have occurred when an estrus-detection patch was > 50% colored (activated). Cows determined to be in estrus were inseminated at either 55 or 65 h, whereas the residual nonestrous cows in both treatment times received GnRH at 55 or 65 h but were inseminated 20 h later at 75 or 85 h, respectively. Pregnancy outcomes were determined at 36 d after AI and at the end of the breeding season. Thus, pregnancy outcomes of interest were compared between the 55 +

75-h treatment combination and that of the 65+85-h combination. Expression of estrus was greater ($P = 0.001$) by 65 h after PGF_{2 α} than by 55 h (62.0% vs. 41.9%), respectively, and this proportion was influenced by parity (time $\times$ parity interaction; $P = 0.006$). 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. The cumulative proportion of cows in estrus by 75 h was less ($P < 0.001$) than that by 85 h (66.7% vs. 76.7%), respectively. Pregnancy risks at 36 d differed among treatments, with cows detected in estrus and inseminated at 55 or 65 h having greater pregnancy risks than their time-inseminated herd mates at 75 or 85 h (62.3% vs. 49.7%), respectively. Overall pregnancy risk for cows in the 65+85-h treatment combination was greater at 36 d than for cows in the 55 + 75-h treatment combination (61.0% vs. 51.4%), respectively. We conclude that the 65 + 85-h treatment combination produced more pregnancies than the 55 + 75-h combination, but its implementation may be somewhat less convenient in terms of cow handling times.

Key words: beef cattle, CIDR, estrus, split-time AI

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INTRODUCTION

Successful programs to manipulate estrus and ovulation to maximize pregnancy outcomes in suckled beef cattle have been developed (Lamb and Mercadante, 2016) to limit animal handling and eliminate detection of estrus to provide opportunities to incorporate AI to start the breeding season. The most successful and consistent scheme employs an intravaginal progesterone insert (control internal drug release or CIDR) for 7 d concurrent with GnRH treatment, and on removal

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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; Lamb and Mercadante, 2016).

Inseminating cows after expressed estrus increases pregnancy risk compared with cows that do not display estrus in a timed AI program (Richardson et al., 2016; Hill et al., 2016a,b). Identification of estrus can be accomplished easily by using estrus-detection patches (Hill et al., 2016a), thus facilitating timing of AI to meet herd-specific situations. Varying AI timing has increased pregnancy risk in some (Markwood et al., 2014; Thomas et al., 2014a; Hill et al., 2016a,b), but not all studies (Thomas et al., 2014b).

Employing detection of estrus to optimally time AI relative to detected estrus rather than using 1 planned fixed-time AI for all cows was tested (i.e., split-time AI). In 2 separate experiments, estrus was detected by using estrus-detection patches at either 60 or 65 h after PGF_{2α} and AI was conducted for cows in estrus. Residual cows received a fixed-time AI at either 75 or 84 h, respectively (Hill et al. 2016a,b). Administering GnRH to cows already detected in estrus at 60 or 75 h did not improve pregnancy risk, and administering GnRH at 60 or 75 h to cows not yet in estrus only improved pregnancy risk in cows that did not come into estrus during the interim before timed AI occurred at 75 or 84 h, respectively (Hill et al., 2016b).

Our objective was to determine in 1 experiment which time combination for split-time AI would maximize pregnancy risk 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).

MATERIALS AND METHODS

The Kansas State University Animal Care and Use Committee approved all experimental procedures (protocol 3392). Suckled beef cows ($n = 1,062$) of mixed parity at 12 locations in 4 states were enrolled in the experiment in 2016. Characteristics by location including breed composition, percentage of primiparous cows, days postpartum at AI, and BCS are summarized (Table 1). Cows grazed native pastures during the treatment and AI period.

Cows were enrolled in a CO-Synch program plus a progesterone (1.38 g)-impregnated intravaginally placed insert (CIDR; Zoetis, Florham Park, NJ): GnRH + CIDR on d -7, CIDR insert removal + PGF_{2α} on d 0 at which time estrus-detection patches (Estroject, Spring Valley, WI) were affixed to the tail head of all cows according to manufacturer's recommendation. Dose of GnRH (2 mL Factrel i.m.; Zoetis) was 100 µg. Dose of PGF_{2α} was 25 mg (2 mL Lutalyse

Table 1. Selected characteristics of suckled beef cows enrolled in the experiment

Location ¹	Breed ²	<i>n</i>	2 yr. old (%)	Days postpartum at AI ³	BCS ³	Pregnancy risk ⁴ , %
CO-1	A, H	75	31	156 ± 23	5.2 ± 0.8	62.7
CO-2	A	150	27	77 ± 9	—	61.3
CO-3	A	169	44	73 ± 24	—	63.3
KS-1	A cross	45	20	82 ± 16	5.7 ± 0.5	46.7
KS-2	A cross	44	27	83 ± 14	5.4 ± 0.4	29.5
KS-3	A x H	64	62	77 ± 15	6.0 ± 0.8	43.7
KS-4	A cross	75	16	69 ± 16	5.4 ± 0.5	65.8
KS-5	A cross	61	28	75 ± 17	5.1 ± 0.5	63.9
KS-6	A x H	101	0	75 ± 18	6.0 ± 0.8	54.3
MT-1	A	108	24	75 ± 15	4.8 ± 0.6	69.2
WA-1	A cross	70	0	59 ± 10	6.2 ± 1.1	71.4
WA-2	A	100	0	59 ± 10	6.2 ± 0.7	63.0

¹Cows at 12 locations in 4 states were enrolled.

²A = Angus and H = Hereford.

³Mean ± SD.

⁴Assessed at 33 to 45 d (median = 36 d) after AI.

HighCon i.m.; Zoetis). Body condition scores (1 = thin; 9 = obese; Bellows et al., 1982) 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 (Fig. 1). 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). 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 residual 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 were removed, PGF_{2α} 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 inseminated 20 h later at 75 or 85 h, respectively.

Activated patches were removed from cows in estrus at 55 or 65 h at 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 to nonestrous cows before they were inseminated at 75 or 85 h. Artificial insemination sires ($n = 43$) and technicians ($n = 14$) were generally

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_{2α} + 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 residual nonestrous 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_{2α}; TAI = timed artificial insemination.

unique to their location; however, a few technicians and sires were common to several locations within state.

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 d 36 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.

Statistical Analyses

All binomial variables were analyzed using logistic regression by applying procedure GLIMMIX (METHOD = LAPLACE; ILINK = LOGIT; DIST = BINOMIAL SOLUTION ODDSRATIO; SAS Inst. Inc., Cary, NC). Proportions of cows detected in estrus by 55 or 65 h, during the 20-h interim until 75 or 85 h, respectively, and total detected in estrus were analyzed. Estrus-detection risk at the times of 55 vs. 65 h were compared in one model and that for the times of 75 vs. 85 h were compared in a second model. The model included the fixed effects of time (55 vs. 65 h or 75 vs. 85 h), parity (primiparous vs. multiparous), interaction of time and parity, days postpartum as a continuous random variable, and location as a random effect. A second set of models was applied including cows from 10 locations that also included the continuous variable of BCS.

The binomial outcomes of pregnancy at 36 d after AI, final pregnancy risk at the end of the breeding season, and intervening pregnancy loss were analyzed using logistic regression in procedure GLIMMIX as described previously. Two models were constructed: one that included AI at all 4 times (55, 65, 75, and 85 h) and a second with

the treatment combinations of 55 + 75 vs. 65 + 85 h. Included in the models were the fixed effects of parity, interaction of time and parity or treatment combination and parity, days postpartum as a continuous random variable, and location as a random effect. Subsequent similar models also were constructed to include the continuous variable of BCS at 10 locations.

RESULTS

Location Characteristics

Summarized in Table 1 are location characteristics of 1,046 cows enrolled at the 12 locations in 4 states at which 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 risks 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_{2α} than by 55 h (Fig. 2), and this proportion was influenced by parity (time x 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

Figure 2. Proportions of cows interpreted to be in estrus by 55, 65, 75, and 85 h after removal of a CIDR insert and injection of PGF_{2α} in primiparous and multiparous suckled beef cows. Cows in the 75- and 85-h times were treated with GnRH 20 h earlier, respectively.

of cows in estrus by 75 h was less ($P < 0.001$) than that by 85 h (Fig. 2). These proportions were affected by parity ($P = 0.045$), with more multiparous than primiparous cows showing estrus. No interaction of treatment and parity, however, was detected ($P = 0.137$) in the previous analysis. 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 risk 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 risks 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 risk 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, at the end of the breeding season, which included exposure to natural service sires, and intervening pregnancy loss are summarized in Table 2. Pregnancy risk 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 greater pregnancy risk 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 [$P = 0.127$]).

Figure 3. Pregnancy risk 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_{2α}. The residual 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.

A more appropriate way to assess the programs is to examine the combined pregnancy risk 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 greatest pregnancy risk at 36 d than the former (Table 2). This difference in pregnancy risk 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 risk 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 risk for cows receiving the timed AI at 85 h compared with 75 h.

Pregnancy risks 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 (Fig. 3). In 10 locations in which BCS was assessed, BCS did not significantly influence pregnancy risk at 36 d. Cows with longer postpartum intervals to AI had greater pregnancy risk. For every 10-d increase in days postpartum at AI, pregnancy risk at 36 d increased by $3.1 \pm 0.1\%$ ($P = 0.014$) or $3.5 \pm 1.1\%$ ($P = 0.026$) depending whether the model included all 4 insemination times or the 2 treatment combinations, respectively.

Final pregnancy outcomes 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 (Fig. 4). Final pregnancy risks increased ($P = 0.001$) by $3.4 \pm 0.7\%$ (4 AI times) or by $3.5 \pm 0.7\%$ (2 treatment combinations) for each 10-d increase in

Table 2. Pregnancy risk (PR) at 36 d after artificial insemination, final pregnancy risk at the end of the breeding season, and intervening pregnancy loss¹

		Treatment ²				P-value	Program ²		P-value
		55	65	75	85		55 + 75	65 + 85	
PR at 36 d	<i>n</i>	223	326	302	198		525	524	
	Actual, ³ %	66.8	68.6	49.7	56.1		57.7	62.8	
	Adjusted, ⁴ %	58.8	64.7	46.3	55.0	0.002	51.4	61.0	0.015
Final PR ⁵	<i>n</i>	223	326	307	199		530	525	
	Actual %	94.8	92.8	87.9	87.4		90.0	92.0	
	Adjusted %	92.3	93.9	86.8	88.0	0.058	88.7	91.7	0.185
Loss	<i>n</i>	153	217	149	110		302	327	
	Actual %	2.0	0.9	2.7	8.2		2.3	3.4	
	Adjusted %	2.0	0.9	2.7	8.2	0.068	2.3	3.4	0.599

¹Mean percentages with different superscript letters differ ($P < 0.05$).

²Cows were enrolled in a CO-Synch + progesterone-impregnated insert (CIDR) program (GnRH + CIDR on d -7, CIDR insert removal + PGF_{2α} + fitted with estrus-detection patches on d 0). Cows 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 residual nonestrous cows in both groups received GnRH at 55 or 65 h but were inseminated at 75 or 85 h, respectively.

³Unadjusted raw mean percentages.

⁴Adjusted mean percentages resulting from logistic regression analyses. 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 ($P = 0.127$).

⁵Six cows not present for the first pregnancy diagnosis (PR at 36 d) were present for the final pregnancy diagnosis.

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 risk increased by ($P = 0.001$) $4.1 \pm 1.5\%$ (4 AI times) to $4.4 \pm 1.5\%$ (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 $-1.1 \pm 0.5\%$ (4 AI times) to $-1.3 \pm 0.5\%$ (2 treatment combinations).

DISCUSSION

Split-time AI programs that employ 2 insemination times determined by occurrence of estrus allow for a closer alignment of the LH surge (spontaneous or GnRH-induced), AI, and ovulation. Furthermore, cows that show estrus at either AI time have no need for exogenous GnRH because fertility is not improved when GnRH is administered to cows already in estrus (Hill et al., 2016b). This fact validates the accuracy of employing estrus-detection patches because pregnancy risk achieved is > 60%, regardless of the time of AI. Occurrence of estrus assessed by patch readings increases as time after CIDR insert removal and PGF_{2α} increases: 55 h (42%; current study), 60 h (46.3%; Hill et al., 2016a), 65 to 66 h (69.4%; current study; Hill et al., 2016b; Bishop et al., 2016; 2017), 75 h (69%; current study; Hill et al., 2016a), 85 h (77.2%; current

study; Hill et al., 2016b), and 90 h (87%; Bishop et al., 2016; 2017).

The current study demonstrated that 39% to 40% of cows in a split-time AI program displayed estrus between 55 and 75 h or between 65 and 85 h. Previous studies demonstrated that between 60 and 75 h, an additional 49% showed estrus (Hill et al., 2016a) and 40% more showed estrus between 65 and 85 h (Hill et al., 2016b) after CIDR insert removal. These responses are consistent with other studies where 45% to 61% more cows showed estrus between 66 and 90 h (Bishop et al., 2016, 2017).

Split-time AI inconsistently improves overall pregnancy risk. We previously demonstrated that cows identified in estrus by 60 h and inseminated at that time had greater pregnancy risk than herd mates not in estrus by 60 h but inseminated at 60 h concurrent with GnRH treatment (64.2% vs. 41.7%; Hill et al., 2016a). In contrast, for cows not in estrus at 60 h but inseminated at 75 h, regardless of whether GnRH was injected at 60 or 75 h, pregnancy risk was 52.8% and 50%, respectively; both of which were greater than that for nonestrous cows inseminated at 60 h. In another study (Thomas et al., 2014b), comparing nonestrous cows inseminated at 66 h with nonestrous cows inseminated at 86 h (20 h after GnRH), no difference in pregnancy risk was detected (45% vs. 50%, respectively). In a similar study (Bishop et al., 2016), for cows not in estrus by 66 h (received GnRH at 66 h and were inseminated at 90 h) compared with cows not in estrus by 66 h (received GnRH and AI at 90 h), pregnancy risk (30%) did not differ unless cows showed estrus between 66 and 90 h,

Figure 4. Final end of breeding season pregnancy risk 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_{2α}. The residual 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.

in which case pregnancy risk was 61%. Similar results occurred in a third study (Bishop et al., 2017) in which pregnancy risk was 35% for nonestrous cows at 66 h (inseminated and treated with GnRH at 66 h) compared with 38% for cows not detected in estrus but receiving GnRH and inseminated at 90 h.

In all studies, however, when cows had shown estrus before AI, pregnancy risk was exceptional based on hours after CIDR insert removal and PGF_{2α}: (1) estrus by 55 h (67%; current study); (2) by 60 h (64.2%; Hill et al., 2016a); (3) by 65 to 66 h (69%, current study; 61%, Hill et al., 2016b; 71%, Thomas et al., 2014a,b; 60 to 63%, Bishop et al., 2016; 58%, Bishop et al., 2017); (4), by 75 h (67%, current study; 65%, Hill et al., 2016a); (5) by 85 h (67%, current study; 65%, Hill et al., 2016b; 63%; Thomas et al., 2014b); or (6) by 90 h (60%, Bishop et al., 2016; 63%, Bishop et al., 2017).

Previous studies have identified conditions that alter the proportion of cows that display estrus at a given time or in total. Postpartum suckled cows that are anestrus at the onset of timed AI programs seem to display estrus earlier than their estrus-cycling herd mates (Geary et al., 2000; Stevenson et al., 2000; Busch et al., 2008). In contrast, cycling status of cows in a previous study did not influence the proportion of cycling and noncycling cows detected in estrus by 65 h after PGF_{2α} (Hill et al., 2016a). Although cows with a lesser BCS may be less likely to display estrus than cows with a greater BCS (Richardson et al., 2016), no effect of BCS was detected in the present study. In our previous study (Hill et al., 2016a), we noted that more primiparous cows with BCS > 5 were in estrus by 60 h after PGF_{2α} than older and thinner cows. In contrast, in a second study, occurrence of estrus by 65 h after

the removal of the CIDR insert was neither affected by parity, BCS, nor cycling status (Hill et al., 2016b).

Although other split-time combinations have been tested (e.g., 60 + 75 h, Hill et al., 2016a; 65 + 85 h, Hill et al., 2016b; 66 + 86 h, Thomas et al., 2014b; or 66 + 90 h, Bishop et al., 2016; 2017), our study is the first to test concurrently 2 split-time programs in the same experiment at multiple sites. The 65 + 85-h combination produced the greatest number of pregnancies compared with the 55 + 75-h program. Cows not detected in estrus at 65 h compared with 55 h, which received the timed AI at 85 h compared with 75 h, tended to have greater pregnancy risk suggesting that timing of the second split-time AI was more optimal for cows not showing estrus by 65 h and inseminated at 85 h. This improvement in pregnancy risk at 85 h likely occurred because 49% more cows that had shown estrus by 85 compared with 75 h.

Advantages and disadvantages exist for applying a split-time AI program, so those choosing to employ such a scheme should consider their options. Employing split-time AI can reduce the risk associated with inseminating cows at 1 fixed time when conditions are less than ideal for optimal fertility because of inadequate holding facilities, weather conditions, inadequate body condition, and nutritional status of cows. Using split-time AI options requires more labor, the expense of using estrus-detection patches, and extra animal handling. In contrast, potential benefits include eliminating GnRH treatments to induce ovulation in cows already detected in estrus before AI (Hill et al., 2016b). Because pregnancy risk was consistently greater in cows that expressed estrus in the current study as well as in others (Richardson et al., 2016; Hill et al., 2016a,b) compared with those cows receiving the timed AI and not in estrus, sire selection might be altered to include sires with more desirable traits or those with greater trait accuracy compared with their peers bearing minimal desired traits to meet selection objectives, but of lesser cost for cows in which estrus status is not known at AI. Furthermore, when sire choice is of great importance to the user, utilizing estrus-detection patches for a timed AI program offers the advantage of different sire selection for those cows with activated vs. nonactivated patches when presented for the single scheduled timed AI at 60 to 66 h. When using sex-sorted semen, use of split-time AI facilitates increased pregnancy risk in suckled beef cows that received the delayed insemination time (Thomas et al., 2014a). In addition, knowledge of when estrus occurred at the 2 response times (e.g., 55 + 75 h or 60 + 85 h after CIDR insert removal) may be useful in cases to troubleshoot an unsuccessful breeding program (Bishop et al., 2017).

Easily employing split-time AI programs requires workable facilities to hold cow-calf pairs overnight un-

less gathering cows the next day for the split-time AI is equally feasible. A small pasture trap or large pen to accommodate pairs is ideal for holding cow-calf pairs overnight. Having an available feed source, water, and shade during the period between split-AI times is optional, but may reduce some stress to cow-calf pairs. Location, pasture topography, daily temperatures, or ease of gathering pairs can limit or facilitate a split-time AI program. Although the 65 + 85 h treatment program resulted in more pregnancies, it may have its disadvantages in execution because of elevated ambient temperatures and gathering difficulties in afternoons rather than during cooler morning hours before either 55 or 65 h.

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 in total were in estrus at the 65- and 85-h insemination times and fertility was greater in estrous than nonestrous cows.

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