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Gonadotropin-releasing hormone increased pregnancy risk in suckled beef cows not detected in estrus and subjected to a split-time artificial insemination program[1,2]

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ABSTRACT: We hypothesized that GnRH would increase pregnancy risk (PR) in a split-time AI program for cows in which estrus was not detected. A total of 1,236 suckled beef cows at 12 locations in 3 states (Colorado, Kansas, and North Dakota) were enrolled. Before applying the fixed-time AI program, BCS was assessed. Cows were treated on d −7 with a progesterone insert concurrent with 100 μg GnRH and on d 0 with 25 mg $PGF_{2\alpha}$ plus removal of the insert. Estrus-detection patches were affixed to cows at insert removal. Estrus was defined to have occurred when an estrus-detection patch was >50% colored (activated). Cows in estrus by 65 h (n = 758; 61.3% of all cows) were randomly allocated to 2 treatments: 1) 100 μg GnRH and early + GnRH (E+G; n = 373) or 2) AI only at 65 h (early – no GnRH [E-G]; n = 385). The remaining cows were randomly allocated to 2 treatments: 1) 5(L+G; n = 252) or 2) AI only at 84 h (late no GnRH [L–G]; n = 226). Pregnancy was determined 35 d after AI via transrectal ultrasound. Pregnancy risk did not differ (P = 0.68) between E+G and E–G cows (61.9 vs. 60.4%, respectively). Conversely, for cows inseminated at 84 h, PR was greater (P = 0.01) in cows that received GnRH (L+G) compared with their herd mates not receiving GnRH (L– G; 41.7 vs. 30.8%, respectively). Of those cows not detected in estrus by 65 h, 42.1% were detected by 84 h, for a total expression of estrus by all cows of 77.6%. Administration of GnRH increased (P < 0.01) PR in cows not detected in estrus by 84 h (+GnRH = 33.4% [n = 146] vs. no GnRH = 15.0% [n = 128]) but had no effect in cows expressing estrus by 84 h (+GnRH = 65.3% [n = 103] vs. no GnRH = 61.7% [n = 97]). Neither estrus expression by 65 or 84 h nor PR was influenced by BCS, parity, or days postpartum at AI. Cows had greater PR when they had been detected in estrus before AI, and PR was improved by administration of GnRH at 65 h after insert removal in cows that were not detected in estrus and inseminated at 84 h.

Key words: beef cattle, estrus detection, gonadotropin-releasing hormone, timed artificial insemination

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INTRODUCTION

Estrus-synchronization programs allow insemination of all females in a herd at one fixed time on the first day of the breeding season. Inseminating cows after they have expressed estrus increases pregnancy risk (**PR**) compared with cows that do not display estrus in a timed AI (**TAI**) program (Richardson et al., 2016). Identification of estrus status can be facilitated by using estrus-detection patches. Varying AI timing according to estrus status has increased PR in some (Markwood et al., 2014; Thomas et al., 2014a; Hill et al., 2016) but not

[1]We gratefully acknowledge the donations of Factrel, Lutalyse, and CIDR inserts from Roger Saltman, Zoetis, Florham Park, NJ, and Boyd Dingus, Estrotect, Spring Valley, WI, for supplying the estrus-detection patches. We acknowledge Wenjing Fausnett for analyzing progesterone concentrations in blood samples.

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all studies (Thomas et al., 2014b). Reducing the number of injections in a TAI program decreases labor requirements, stress on cows, and overall cost of the program. Previous studies have demonstrated that PR is not compromised in cows displaying estrus when the GnRH injection concurrent with AI is eliminated (Perry and Perry, 2009). A split-time AI program decreases the time between estrus expression and insemination compared with a single fixed-time AI when the first AI occurs before the recommended standard 60- to 66-h fixed time (Johnson et al., 2011). Delaying AI results in approximately 50% more cows displaying estrus when compared with a single insemination time (Hill et al., 2016). Eliminating the GnRH injection at AI for cows displaying estrus in a split TAI program can reduce the number of GnRH injections required and the program cost. The objective of this study was to test the hypothesis that GnRH injection concurrent with split TAI program improves PR only in cows not displaying estrus.

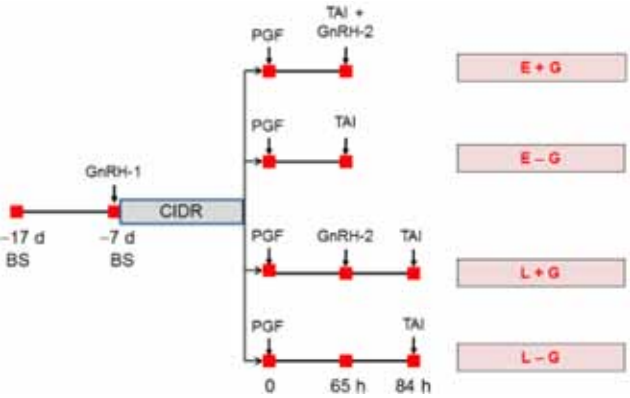

Figure 1. Experimental design of treatments. All cows (n = 1,236) received intramuscularly 100 µg GnRH (GnRH-1) and a controlled internal drug release (CIDR) insert containing 1.38 g of progesterone followed in 7 d by 25 mg $PGF_{2\alpha}$ (PGF) and CIDR removal (d 0). Cows with patches >50% activated were defined to be in estrus and treatment assignments were made at 65 h. The 100 µg GnRH and early AI at 65 h (E+G) cows (n = 373) received 100 µg GnRH (GnRH-2) and insemination at 65 h. The AI only at 65 h (E–G) cows (n = 385) received no GnRH and were inseminated at 65 h. The 100 µg GnRH at 65 h and late AI at 84 h (L+G) cows (n = 252) received GnRH-2 at 65 h and were inseminated at 84 h. The AI only at 84 h (L–G) cows (n = 226) received no GnRH and were inseminated at 84 h. Blood samples (BS) were collected on d −17 and −7 from a subset of cows (n = 427) at 8 of 12 locations. TAI = timed AI.

MATERIALS AND METHODS

Experimental Design

The Kansas State University Animal Care and Use Committee approved all experimental procedures (protocol number 3392). A total of 1,236 mixed-parity suckled beef cows at 12 locations in 3 states (Colorado, Kansas, and North Dakota) were enrolled in the experiment in 2015. Body condition scores (1 = thin and 9 = obese; Bellows et al., 1982) were assigned (d −17) before the start of the TAI program by a trained evaluator (Fig. 1). Characteristics of suckled beef cows enrolled by location including breed, parity, days postpartum at split TAI, and BCS at the onset of the synchronization program are summarized (Table 1). All cows were injected intramuscularly with 100 µg GnRH (2 mL Factrel; Zoetis Inc., Florham Park, NJ) 7 d before 25 mg $PGF_{2\alpha}$ on d 0 (5 mL Lutalyse; Zoetis Inc.). A new progesterone-impregnated controlled internal drug release (**CIDR**) insert (Zoetis Inc.) containing 1.38 g progesterone was placed intravaginally at the time of the GnRH injection (d −7). Progesterone inserts were removed and $PGF_{2\alpha}$ was injected at 1700 h on d 0 to allow for AI to begin 65 h later at 1000 h. The 84-h time was selected to begin the second AI time as early as daylight would allow (0700 h) and to allow insemination of cows approximately 5 to 13 h before ovulation induced by GnRH 19 h earlier. Ovulation occurs between 24 and 32 h after exogenous GnRH in cattle (Wiltbank and Pursley, 2014).

On d 0, concurrent with CIDR insert removal, estrus-detection patches (Estrotect, Spring Valley, WI) were affixed to the tail head of all cows according to the manufacturer's recommendation. Patches were evaluated at 65 h after CIDR insert removal, and estrus was defined to have occurred when an estrus-detection patch was >50% colored (activated). Cows with activated patches were assigned by random chute order to either receive 100 µg GnRH and early AI at 65 h (**E+G**) or AI only at 65 h (**E–G**). Remaining nonestrus cows received either 100 µg GnRH at 65 h and late AI at 84 h (**L+G**) or AI only at 84 h (**L–G**). An additional evaluation of patch activation status was also conducted at 84 h to determine if activation had occurred between 65 and 84 h.

Pregnancy Diagnosis

Cows were either observed for estrus and reinseminated on subsequent estrus or were exposed to cleanup bulls beginning 10 to 12 d after split TAI. At 35 d after split TAI, pregnancy risk (PR) was confirmed by transrectal ultrasonography (Aloka 500V, 5 MHz transrectal transducer, Wallingford, CT). A positive pregnancy outcome required the presence of an embryo with a visible heart beat. In rare exceptions, when an embryo was not observed, a corpus luteum and uterine fluid consistent with a d-35 pregnancy was evidence for a positive diagnosis. A final pregnancy diagnosis was determined via transrectal ultrasonography or palpation per rectum no sooner than 35 d after the end of the breeding season (range of 35 to 42 d). Pregnancy loss was defined as those cows pregnant 35 d after split TAI but not at the appropriate stage of pregnancy at the time of the final pregnancy diagnosis.

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

Location[1]	Breed[2]	*n*	2 yr old, %	Days postpartum at AI[3]	BCS[3]	Estrus cycle status,[4] %	Pregnancy risk,[5] %
CO-1	A	333	26	83 ± 1.0	4.6 ± 0.02	–[6]	58.9
CO-2	A	80	23	81 ± 1.9	5.5 ± 0.08	–[6]	68.8
KS-1	H	39	28	78 ± 2.9	5.6 ± 0.08	33	66.7
KS-2	A × H	40	25	82 ± 2.6	5.7 ± 0.10	53	45.0
KS-3	A × H	77	31	84 ± 1.7	5.4 ± 0.07	61	49.4
KS-4	A × H	61	26	83 ± 1.8	5.4 ± 0.09	49	55.7
KS-5	A × H	64	86	78 ± 2.6	5.5 ± 0.08	6	23.4
KS-6	A × H	98	0	69 ± 1.8	5.7 ± 0.06	18	46.9
KS-7	A × H	29	0	49 ± 3.8	5.8 ± 0.07	14	51.7
KS-8	A × H	19	0	69 ± 4.2	5.3 ± 0.18	5	21.1
ND-1	A × H	190	0	72 ± 1.4	4.4 ± 0.04	–[6]	68.9
ND-2	A × H	206	32	83 ± 1.2	4.3 ± 0.04	–[6]	62.6

[1]Cows at 12 locations in 3 states were enrolled. CO = Colorado; KS = Kansas; ND = North Dakota.

[2]A = Angus and H = Hereford.

[3]Mean ± SE.

[4]Based on progesterone concentrations measured in 2 blood samples collected 10 d apart before the onset of the experimental protocol in 427 cows (cut point for determining a functional corpus luteum was ≥1 ng/mL).

[5]Assessed at 35 d after AI.

[6]Blood samples were not collected to assess estrus cycle status.

Estrus-Cycle Status

Blood samples were collected via puncture of a caudal blood vessel from cows (n = 427) at 8 of the 12 locations on d −17 and −7. Concentrations of progesterone in blood serum were measured in all samples by direct quantitative (nonextracted) RIA using ImmuChem Double Antibody progesterone ^{125}I kits (catalog number 07-170105; MP Biomedicals LLC, Orangeburg, NY) and validated for bovine serum. The radioligand was ^{125}I-labeled progesterone (1,500 to 2,000 μCi/μg). The antiprogesterone antibody was generated in rabbits using 11α-hydroxyprogesterone-11α-hemisuccinate-human serum albumin as the antigen. Kit standards (0.2, 0.5, 2.0, 5.0, 10.0, 25.0, and 50.0 ng/mL), to which we added 2 more standards (0.05 and 0.1 ng/mL), unknowns, and assay pools were added (100 μL each) in duplicate to 12- by 75-mm plastic conical tubes. Next, 500 μL of antiprogesterone antibody and 200 μL of ^{125}I-labeled progesterone were added to each tube, vortexed for 5 s, and incubated for 1 h in a water bath at 37°C. Next, 500 μL of a precipitant solution (second antibody) were added to all tubes and thoroughly vortexed for 10 s before centrifuging at 5°C for 30 min at 5,000 × g. Tubes were then decanted and blotted on paper towels, and the radioactivity of each tube was quantified for 1 min in a γ counter. Recovery of added progesterone in triplicate to 100 μL of 4 different bovine serum samples (0.39, 1.38, 3.67, and 13.72 ng/mL) averaged 101.5%. Parallelism was demonstrated by assaying 50-, 75- and 100-μL aliquots of bovine serum at 2 different concentrations in quadruplicate. Recovery of added progesterone averaged 105.5%. Duplicate unknowns that failed to replicate within 10% were reassayed. Intra-assay CV for progesterone was 5.6%. Interassay CV for the low and high pool was 11.9 and 9.3%, respectively. Assay sensitivity was 53.4 ± 14.4 pg/mL.

Cows with a serum progesterone concentration ≥ 1.0 ng/mL at either d −17 or −7 were defined to have resumed estrous cycles (Ireland et al., 1980). All other sampled cows with concentrations of progesterone < 1.0 ng/mL were considered to have been anestrous at the onset of the ovulation synchronization program (Table 1).

Statistical Analyses

The median values of the continuous variables BCS (<5 vs. ≥5) and days postpartum (≤82 vs. >82 d) were used to create corresponding binomial variables. Each of the dependent variables (estrus cycle status, estrus expression at 3 time intervals [by 65 h, 65 through 84 h, and by 84 h], PR, final PR, and pregnancy loss) was regressed on the independent variables in the LOGISTIC procedure (SAS Inst. Inc., Cary, NC). A final model was produced using the stepwise selection method with a P-value < 0.30 for initial inclusion and P < 0.15 for retention in the model. The independent fixed variables—BCS, days postpartum, and parity (primiparous vs. multiparous)—and all interactions of these variables were initially included in the selection for estrus cycle status. A final model produced by backward stepwise selection of independent variables entered or retained in the model was based on a Wald statistic (P < 0.10).

A similar selection procedure was performed with the remaining outcome variables with the following inclusions: estrus cycle status was added as an independent variable in the remaining models, total estrus

expression was added to the models analyzing PR and pregnancy loss, and treatment was added to all models. The list of variables measured and the factors included in the final model are summarized in Table 2.

The GLIMMIX procedure (method = laplace, ilink = logit, and dist = binomial solution oddsratio) in SAS was used to analyze the binomial outcome variables. Differences of means calculated using unequal sample sizes were adjusted using the Bonferroni adjustment. An additional GLIMMIX model with PR as the dependent variable was constructed to examine the interaction of GnRH injection and estrus expression for the cows inseminated at 84 h. The location of each herd was included in all models as a random variable. Differences were considered significant at $P \leq 0.05$.

Table 2. Composition of statistical model selections

Dependent variable	Independent variable	Interactions
Estrus cycling status	Days[1] and parity	None[2]
Estrus by 65 h	None[3]	None[2]
Estrus from 65 to 84 h	None[3]	None[2]
Estrus by 84 h	BCS, days,[1] and parity	None[2]
PR[4] at 35 d	Days,[1] parity, and treatment	None[2]
PR 35 d after breeding season	Days,[1] parity, and treatment	Days × parity
Pregnancy loss	BCS, days,[1] parity, and treatment	None[2]

[1]Days postpartum at split timed AI.

[2]No interactions had a lesser *P*-value than the selection criteria ($P = 0.15$).

[3]No variables had a lesser *P*-value than the selection criteria ($P = 0.15$).

[4]PR = pregnancy risk.

RESULTS

Pregnancy Risk

Cows detected in estrus and inseminated at 65 h had greater PR than the cows inseminated at 84 h regardless of GnRH treatment (Fig. 2). Pregnancy risk was not improved ($P = 0.68$) by administration of GnRH in cows that were in estrus by 65 h (61.9 and 60.4% for E+G and E–G, respectively). For cows inseminated at 84 h, PR was greater ($P = 0.001$) in cows that received GnRH at 65 h (L+G) compared with their herd mates not receiving GnRH at 65 h (41.7 and 30.8% for L+G and L–G, respectively).

Four cows were eliminated from the analysis of PR because patch data were not available at 84 h. Administration of GnRH at 65 h increased ($P < 0.01$) PR in cows not detected in estrus by 84 h (Fig. 3). In contrast, administration of GnRH did not impact PR ($P = 0.60$) in cows expressing estrus during the interval from 65 to 84 h. Pregnancy risk for cows inseminated at either time was not affected ($P \geq 0.10$) by BCS, parity, or days postpartum at AI. Final PR assessed at least 35 d after the end of the breeding season for E+G, E–G, L+G, and L–G cows were 87.4, 89.0, 84.5, and 78%, respectively. Final PR of L–G cows differed from E+G ($P = 0.02$) and E–G cows ($P = 0.004$). Body condition score did not affect final PR. An interaction ($P = 0.05$) was detected between days postpartum and parity when considering the final PR. Primiparous cows that were ≤82 d postpartum had a lesser ($P = 0.003$) final PR than primiparous cows >82 d (70.9 vs. 87.6%, respectively). Final PR of primiparous cows ≤82 d postpartum also differed ($P = 0.01$) from that of multiparous cows ≤82 d and multiparous cows >82 d (87.8 and 89.7%, respectively).

Pregnancy loss (1.3%) between 35 d after TAI and final pregnancy status after the end of the breeding season did not differ among treatments ($P = 0.89$) and was not affected ($P \geq 0.14$) by BCS, days postpartum, or parity.

Occurrence of Estrus

Activated estrus-detection patches were observed in 61.3% (758/1,236) of cows at 65 h after insert removal. Of the remaining cows, 42.2% (200/474) had activated estrus-detection patches at 84 h, indicating estrus had occurred between 65 and 84 h. In total, 77.5% (958/1,236) of cows were observed with activated estrus-detection patches by 84 h.

The proportion of cows expressing estrus by 65 h was not impacted ($P > 0.10$) by BCS, parity, days postpartum (Table 3), or their respective interactions. Likewise, the proportion of cows expressing estrus during the interval from 65 to 84 h was not influenced ($P > 0.10$) by BCS, parity, days postpartum, or their respective interactions. A greater proportion of cows >82 d postpartum tended ($P = 0.09$) to express estrus by 84 h compared with cows ≤82 d postpartum (79.8 vs. 75.5%, respectively).

Estrus-cycle status based on concentrations of progesterone was examined for its effect on occurrence of estrus in the subset of 427 cows for which that information was available. Analysis of the impact of estrus-cycle status on estrus expression revealed that similar ($P > 0.26$) proportions of cycling and anestrous cows were detected in estrus in each of the 3 observation periods (51 vs. 58% by 65 h, 25 vs. 28% between 65 and 84 h, and 65 vs. 70% by 84 h) for cycling and anestrous cows, respectively. The proportion of cows that had resumed estrous cycles (32.3%; 138/427) was influenced by neither BCS nor days postpartum. Primiparous cows, however, were more ($P < 0.01$) likely to be anestrous than their multiparous herd mates (94.6 vs. 63.6%, respectively).

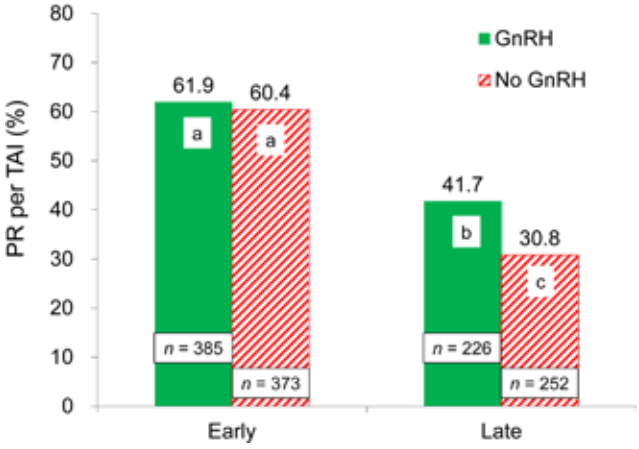

Figure 2. Pregnancy risk (PR) per timed AI (TAI) by treatment. The early cows were detected in estrus by 65 h, inseminated, and either received GnRH at 65 h (E+G) or did not receive GnRH (E–G). The remaining cows were allocated to 2 late treatments: 1) injected with GnRH at 65 h and inseminated at 84 h (L+G) or 2) no GnRH at 65 h and inseminated at 84 h (L-G). [a–c]Bars with different letters differ ($P < 0.05$). Values at the base of each bar represent the number of cows per treatment.

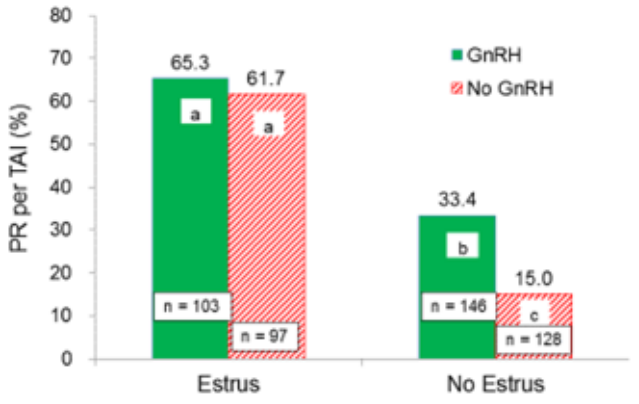

Figure 3. Pregnancy risk (PR) per timed AI (TAI) for cows inseminated at 84 h. Based on whether the estrus-detection patch with >50% activated between 65 and 84 h after controlled internal drug release insert removal, cows were classified as estrus or no estrus. [a–c]Bars with different letters differ ($P < 0.05$). Values at the base of each bar represent the number of cows per bar.

DISCUSSION

The current experiment demonstrates that GnRH is not essential to achieve acceptable PR (>60%) when cows had expressed estrus before AI at 65 h. More importantly, for cows not detected in estrus by 65 h, treatment with GnRH at 65 h was beneficial only to enhance PR in cows that did not express estrus by 84 h when they were inseminated. Treating nonestrual cows at 65 h after $PGF_{2\alpha}$ with GnRH resulted in more than a 2-fold greater PR compared with non-GnRH–treated contemporaries inseminated at 84 h. Cows observed in estrus by 65 h likely would have been exposed to an endogenous GnRH-induced LH surge and subsequently spontaneously ovulated 31 ± 0.6 h after the onset of estrus (White et al., 2002).

Programs that use 2 insemination times determined by occurrence of estrus allow for a closer alignment of the spontaneous LH surge, AI, and subsequent ovulation, negating the need for exogenous GnRH in cows that display estrus. Furthermore, use of estrus-detection patches to identify estrus accurately seems to be validated by the PR achieved (>60%) in cows not exposed to exogenous GnRH before TAI, regardless of time of AI.

In nonlactating beef cows that spontaneously display estrus, ovulation occurs 31 h after the initiation of estrus (White et al., 2002). Administering GnRH near the onset of estrus may induce the LH surge or augment the magnitude of the LH surge from the pituitary (Lucy and Stevenson, 1986); however, circulating concentrations of LH are only minimally influenced by exogenous GnRH after the spontaneous LH surge in dairy cows (Lucy and Stevenson, 1986; Pulley et al., 2015). In a previous experiment conducted in beef cows that were detected in estrus, no increase was detected in the proportion of cows becoming pregnant after AI when GnRH was administered at the time of AI (Perry and Perry, 2009). In that study, cows were subjected to AI between 6 and 18 h after estrus was first detected. In a TAI program, the interval from initiation of estrus until AI is variable, which may change the effectiveness of a GnRH injection concurrent with AI. Cows that ovulate after AI in a TAI program, and therefore have the opportunity to conceive, can be grouped into the classifications of those that spontaneously ovulate and those that are induced to ovulate after exogenous GnRH-induced LH release. Timing of the GnRH treatment in relationship to AI in cows that do not display estrus should be examined more closely. In the current study, cows that were detected in estrus before either the first (65 h) or the second (84 h) split-time AI had no improvement in PR after split TAI when treated with GnRH compared with untreated herd mates.

In split TAI programs, cows are treated differently based on the occurrence of estrus (Thomas et al., 2014a). The current study and previous research indicates that more than 50% of cows in a split TAI program displayed estrus by 60 to 65 h (Thomas et al., 2014b; Hill et al., 2016). In previous research (Hill et al., 2016), time to the first assessment of estrus was 60 h after CIDR insert removal, resulting in 46.3% of cows in estrus compared with 61.3% of cows detected by 65 h in the current study. Furthermore, for cows not detected in estrus by 60 h in the latter study, administration of GnRH at either 60 h (15 h before AI) or 75 h (concurrent with AI) produced a similar PR. For cows not detected in estrus between 60 and 75 h in the latter study, PR did not differ but was numerically greater at 46% ($n = 139$) compared with 39.1% ($n = 133$) when GnRH was administered at 60 h vs. at 75 h, respectively, and AI occurred at 75 h. An earlier time of the first split-time AI will likely result in a smaller proportion of cows in estrus and more cows requiring GnRH before

Table 3. Estrus expression by 65 h, between 65 and 84 h, and by 84 h after controlled internal drug release insert removal as affected by BCS, parity, days postpartum, and GnRH

	Estrus by 65 h		Estrus between 65 and 84 h		Estrus by 84 h	
Item	*n*	%	*n*	%	*n*	%
Days postpartum						
≤82	596	61.1	229	37.9	596	75.5[a]
>82	640	65.8	245	41.6	640	79.8[b]
BCS						
≥5	689	64.3	269	41.8	689	79.3
<5	547	62.6	205	37.7	547	76.1
Parity						
Primiparous	287	64.2	119	37.5	287	77.1
Multiparous	949	62.7	355	42.0	949	78.4
GnRH at 65 h	–	–	249	40.9	–	–
No GnRH at 65 h	–	–	225	38.6	–	–

[a,b]Means within estrus category with different superscript letters tend ($P < 0.10$) to differ.

the second split-time AI. Therefore, timing of estrus may be important relative to the selected first and second split time, so further examination of insemination timing in a split TAI program is warranted.

The initial studies using the Ovsynch and CO-Synch programs in beef cattle demonstrated the importance of inducing ovulation by administering GnRH either before or concurrently with insemination in a 7-d TAI program (Geary and Whittier, 1998). Interval from $PGF_{2\alpha}$ injection to spontaneous ovulation in TAI systems varies depending on the prior estrus-cycle status, parity, and the stage of the estrous cycle at the initiation of the procedure (Geary et al., 2000). Results of the current experiment indicate that the increase in PR from GnRH treatment was associated with cows that did not display estrus.

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 anestrous at the onset of TAI 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, previous estrus cycle status of the sampled subpopulation of cows in the present study did not influence the proportion of cycling and noncycling cows detected in estrus by 65 h after $PGF_{2\alpha}$. Cows with a lesser BCS, however, are less likely to display estrus than cows with a greater BCS (Richardson et al., 2016). Stage of the estrous cycle at the initiation of the synchronization program for cycling heifers can alter the timing of estrus (Atkins et al., 2008). Furthermore, variations in the response to GnRH have been identified in beef cows depending on the stage of their follicular wave and their prior estrus cycle status (Atkins et al., 2010a,b). In our previous research (Hill et al., 2016), we noted that more primiparous cows with BCS > 5 were in estrus by 60 h after $PGF_{2\alpha}$ than older and thinner cows. In contrast, in the current study, occurrence of estrus by 65 h after the removal of the CIDR insert was not affected by parity, BCS, or cycling status. It is possible that the different time after CIDR insert removal chosen to measure estrus masked some of these physiological causative factors. In our previous report (Hill et al., 2016), time to the first assessment of estrus was 60 h after CIDR insert removal, resulting in 46.3% of cows in estrus compared with 61.3% of cows detected by 65 h in the current study.

In summary, GnRH injection at AI improved PR only in those cows that were not detected in estrus before time of AI. Cows that exhibited estrus, regardless of GnRH treatment, had better PR than cows that did not display estrus. Insemination at a predetermined time in beef cows can reduce the time and labor associated with conventional single standard fixed-time AI program. The split-time AI program serves as a compromise between conventional AI after detection of estrus and a standard one fixed-time AI program. Depending on the cost of GnRH (range of US$2.22 to $3.10 per dose) and 60% of cows in estrus by 65 h, the economic trade-off of using estrus-detection patches in a split-time AI program is favorable and saved $0.33 to $0.86 per cow but does not account for the extra time and cow–calf handling invested to carry out the second AI at 84 h. Furthermore, the cost of semen and sire selection for cows detected in estrus having resulting greater PR compared with those not detected in estrus having lesser PR could provide other favorable options and economic advantages for using a split-time AI program.

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