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Estrous response and pregnancy percentages following use of a progesterone-based, split-time estrous synchronization treatment regimens in beef heifers

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ABSTRACT

Mean estrous response rate (%ERR) and pregnancy/AI percentages (%P/AI) were determined after imposing split-time AI (STAI) and fixed time AI (FTAI) following 14-d controlled internal drug release (CIDR) + PGF2α or 5-d Select Synch + CIDR regimens. In Experiment 1, 1152 heifers (five locations) were randomly assigned to 14- or 5-d and to 54 + 74- or 64 + 84-h STAI treatment combinations. Estrous detection patches were affixed at PGF2α administration (19 day after- and on day 5 at- CIDR removal for 14- and 5-d regimens, respectively), assessed at 54- or 64-h and again at 74- or 84-h after PGF2α. Heifers determined to be in estrus at respective times were inseminated and non-estrous heifers at 74- or 84-h were given GnRH and inseminated concomitantly. The %ERR between 54 + 74- and 64 + 84-h STAI combinations differed (73.2 % and 78.8 %, respectively; $P < 0.05$), but %P/AI did not. In Experiment 2, 2014 heifers (eight locations) were randomly assigned to 14- or 5-d regimens and were inseminated split-time (64 + 84-h combination, similar to Experiment 1) or at fixed time (72- or 56-h after PGF2α for 14- or 5-d regimens, respectively). There were differences ($P < 0.01$) between STAI and FTAI treatments for %ERR (81.3 % and 64.4 %) and %P/AI (61.2 % and 55.4 %). Estrous synchronization regimen by AI treatment interaction ($P < 0.05$) showed that the %ERR were 79.8 %, 82.6 %, 66.2 % and 62.8 % and the %P/AI were 58.9 %, 63.4 %, 56.5 % and 56.5 % (for 14-d/STAI, 5-d/STAI, 14-d/FTAI and 5-d/FTAI, respectively). In conclusion, the 5-d CIDR with 64 + 84-h STAI combination was the most effective because of the greater %P/AI when this regimen was imposed.

1. Introduction

Estrous synchronization and artificial insemination (AI) technologies are an option for improving genetic composition of beef herds in a timely and an economical means. These technologies can be readily applied to replacement beef heifers because there are not suckling calf effects that can confound the responses to the treatment regimens. Pregnancy per AI percentages (%P/AI) following estrous synchronization in beef heifers are considerably less and more unpredictable than in cows and consequently requires more intensive management for estrous detection (Lamb et al., 2001; Bader et al., 2005; Larson et al., 2006). An important reason for this

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difference is that response to treatment with GnRH is not as consistent for synchronizing the state of ovarian follicular waves among heifers as occurs with administration of GnRH for this purpose in cows. After GnRH treatment at random stages of the estrous cycle, ovulation occurred in 64 %–75 % of postpartum beef and dairy cows (Geary et al., 1998; Thompson et al., 1999; El-Zarkouny et al., 2004), but only 48 %–60 % of beef and dairy heifers (Macmillan and Thatcher, 1991; Pursley et al., 1995; Moreira et al., 2000). Due to variations in ovarian follicular dynamics, heifers in a herd can have ovulations that extend for a 2- to 5-d period after the last treatment imposed when there is imposing of estrous synchronization treatment regimens.

In several studies there has been use of varying combinations and timing of hormonal administrations in treatment regimens that vary in length in attempts to increase pregnancy rate outcomes (Kasimanickam et al., 2015; Thomas et al., 2014; Bishop et al., 2016; Bishop et., 2017a; Bishop et al., 2017b). Long-term [LT; 14-d controlled internal drug release insert (CIDR)-PGF2α-GnRH with FTAI at 72 h after PGF2α administration] and short-term (ST; 5-d CO-Synch + CIDR with AI being 56 h after PGF2α administration) progesterone-based treatment regimens have been studied to improve pregnancy rates in beef heifers (Kasimanickam et al., 2015).

There is increasing recognition of benefits of split-time AI (STAI) in beef heifers. The rationale is that pregnancy percentages are greater in beef females that express estrus before insemination (Perry et al., 2005; Thomas et al., 2014; Bishop et al., 2016; Richardson et al., 2016; Bishop et al., 2017a, b; Kasimanickam et al., 2020). In short, STAI involves not conducting AI in females that have not expressed estrus at the time of FTAI (Thomas et al., 2014; Markwood et al., 2014; Bishop et al., 2016; Hill et al., 2016; Nielson et al., 2016; Richardson et al., 2016; Bishop et al., 2017a,b; Stevenson et al., 2017). With the heifers that have not expressed estrus at the time of FTAI, therefore, there is an additional period of time for behavioral estrous expression to occur before AI, with all the heifers that have not expressed estrus at the time of FTAI being inseminated at the time of the second FTAI, with there also being administration of GnRH either at first or at second FTAI to heifers not detected in estrus (Bishop et al., 2016, 2017a, b; Stevenson et al., 2017).

Considering advantages following STAI, two experiments were conducted to determine the estrous response rate percentage [% ERR] and %P/AI following STAI when there was imposing of long-term (14-d CIDR- PGF2α) and short-term (5-d CO-Synch + CIDR) Select-Synch + CIDR hormonal treatment regimens. The objective of Experiment 1 was to determine effects of two combinations of STAI, at 54 and 74 or 64 and 84 h after administration of PGF2α, when there was imposing of 14- and 5-d CIDR treatment regimens (2 × 2 factorial) in heifers. It was hypothesized that for all four treatment groups there would be a similar %P/AI. The objective of Experiment 2 was to determine effects from imposing a STAI (AI at 64 and 84 h after PGF2α administration) as compared with a FTAI regimen when there were hormonal treatments using a 14-d CIDR- PGF2α and 5-d CO-Synch + CIDR estrous synchronization treatment regimen (2 × 2 factorial) on %ERR and %P/AI in beef heifers. It was hypothesized that P/AI percentage would be greater when there was STAI compared with FTAI.

2. Materials and methods

This study was performed in accordance with appropriate ethics, standard operating procedures, handling and use of animals, sample collection and use of biomaterials for research (https://www.adsa.org/Portals/_default/SiteContent/docs/AgGuide3rd/Ag_Guide_3rd_ed.pdf). All procedures involving the use of animals and tissues were conducted compliant with the guidelines for agricultural animal care by the Washington State University. Heifers were assigned in 2018 and 2019 in experiments for which there was a completely randomized arrangement of treatment combinations (Fig. 1).

2.1. Experiment 1

2.1.1. Cows

Angus-crossbred beef heifers (n = 1152) at five locations were randomly assigned to experimental groups. Heifers grazed native pastures during the treatment period, AI period and subsequent to the time of breeding. Body condition scores (BCS: 1, emaciated; 9, obese) (Bellows et al., 1982), reproductive tract score (RTS: 1, immature, anestrus; 5, mature, estrous cyclic) (Anderson et al., 1991) and temperament score (0, calm, 1, excitable) (Kasimanickam et al., 2014) were assigned on d -33 by trained evaluators at all locations.

2.1.2. Estrous synchronization treatment

Within location, heifers were assigned randomly to one of two estrous synchronization treatment regimens [14-d (n = 576) and 5-d (n = 576) CIDR) and to one of two STAI [54 + 74 (= 568) and 64 + 84 (n = 584) h after CIDR removal] regimens. Heifers assigned to the 14-d CIDR treatment regimen were administered a progesterone (1.38 g) impregnated intravaginal insert (CIDR; Zoetis, Parsippany, NJ, USA) on d -33, CIDR insert removal occurred on day -19 and PGF2α (2 mL Lutalyse HighCon, dinoprost tromethamine, im; Zoetis) was administered on day 0, at which time estrous-detection patches (Estrotect™, Spring Valley, WI, USA) were fastened to the tail head, as per manufacturer's recommendation. Heifers assigned to the 5-d CIDR treatment regimen were administered GnRH (2 mL Factrel, gonadorelin hydrochloride, im, Zoetis) and there was CIDR insertion on day -5, and CIDR insert removal and an administration of PGF2α on day 0, at which time estrous-detection patches were affixed to the tail head, as per the manufacturer's recommendation. All heifers assigned to the 5-d CIDR treatment regimen were administered a second dose of PGFα 6 h later. At 54 or 64 h after PGF2α administration (from first PGF2α for 5-d CIDR group), estrous-detection patches were assessed. Estrus was defined to have occurred if there was greater than 50 % of the paint no longer present on the patches. Heifers determined to be in estrus were inseminated at either 54 or 64 h and heifers that had not expressed estrus by 54 or 64 h but had expressed estrus 20 h later (74 or 84, respectively) were inseminated at that time. The remaining heifers that had not expressed estrus when there was

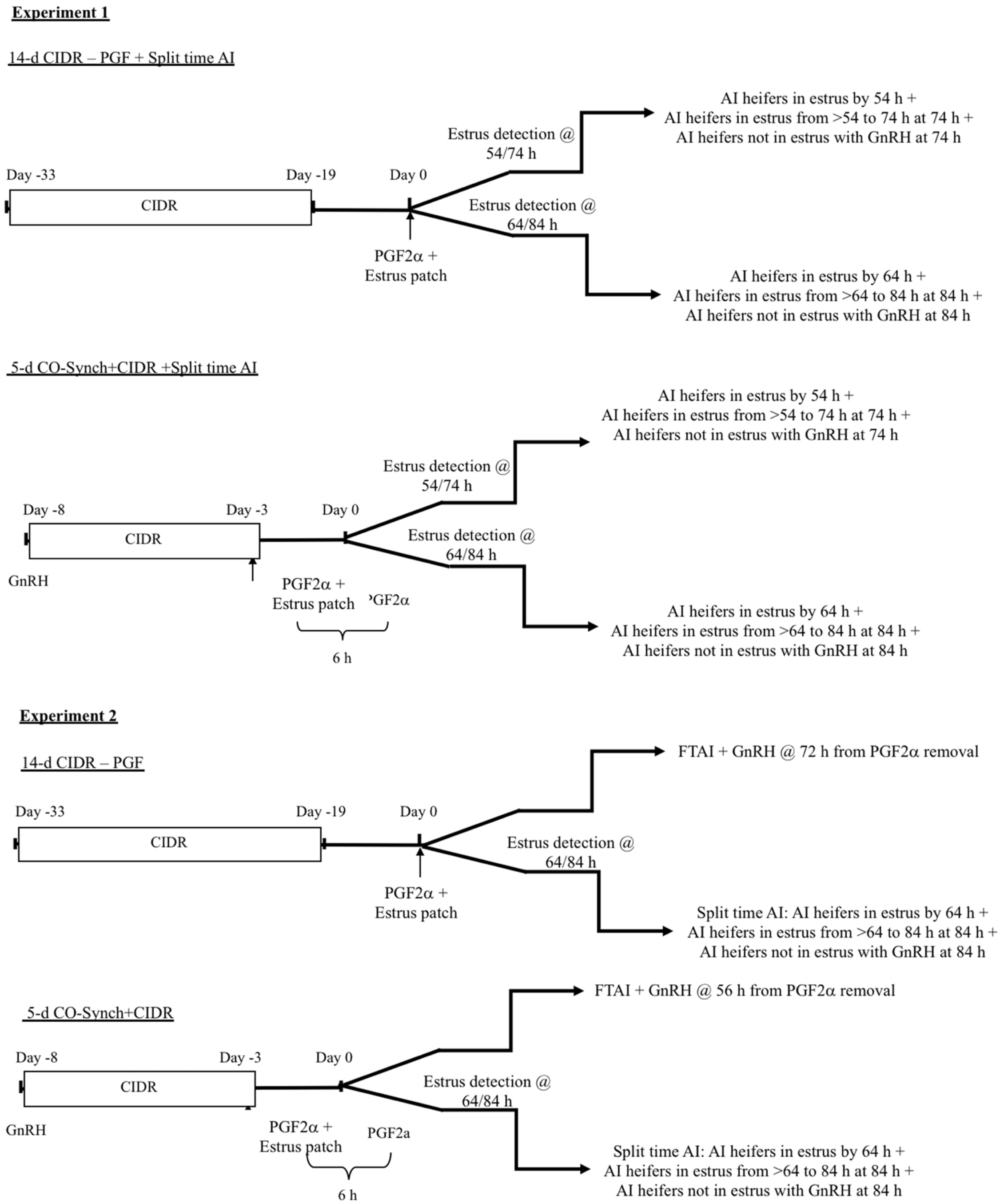

Fig. 1. Schematic depictions of treatment and AI regimens.
Two experiments were conducted to determine the effect of split-time (STAI) on estrous response rate and P/AI percentages in beef heifers; Experiment 1: timing of estrus among animals was synchronized in 1152 heifers at five locations using the 14-d CIDR-PGF2α or 5-d Select Synch + CIDR treatment regimens and estrous detection aids were applied at PGF2α on d 0; Heifers within each location were further randomly assigned (2 × 2 design) to one of two STAI treatments: 1) 54 + 74 h split-time: AI heifers in estrus by 54 h after PGF2α + AI heifers in estrus from > 54 to 74 h at 74 h + AI heifers not in estrus by 74 h with GnRH administration occurring at 74 h; and 2) 64 + 84 h split-time: AI heifers in estrus by 64 h after PGF2α + AI heifers in estrus from > 64 to 84 h at 84 h + AI heifers not in estrus by 84 h with GnRH at 84 h; Experiment 2: time of estrus was synchronized for 2014 heifers at eight locations using the 14-d CIDR- PGF2α or 5-d Select Synch + CIDR treatment regimens and estrous detection aids were applied at the time of PGF2α administration on d 0; Heifers at each location were further randomly assigned (2 × 2 design) to one of two AI treatments: 1) 64 + 84 STAI for heifers treated with both 14-d CIDR- PGF2α or 5-d Select Synch + CIDR regimens: AI heifers in estrus by 64 h after PGF2α + AI heifers in estrus from > 64 to 84 h at 84 h + AI heifers not in estrus by 84 h with GnRH at 84 h and 2) Fixed time AI (FTAI): AI at 72 h after PGF2α for heifers in 14-d CIDR- PGF2α treatment regimen or AI at 56 h for heifers in 5-d Select Synch + CIDR treatment regimen; Pregnancy diagnosis was performed 60 days after AI using transrectal ultrasonography.

estrous detection patch assessment at 74 or 84 h after administration of the initial PGF2α injection of the treatment regimen were administered GnRH at these times and were inseminated concomitantly. Activated patches (with greater than 50 % of the paint was no longer present) were removed from heifers in estrus at 54 or 64 h at the time of initial periods of AI. Patches on remaining heifers were assessed at 74 or 84 h when there was AI of heifers, thus allowing determination of the number of heifers expressing estrus during the 20 h interval. Artificial insemination sires (n = 12), and AI personnel (n = 9) and animal handlers (n = 19) were generally different individuals at each location; however, a few technicians and sires were the same at several locations. Bulls were placed in the pasture with the heifers starting 2 wk after AI, and bulls were removed approximately 70 d later, for a total breeding season of 85 d.

2.2. Experiment 2

2.2.1. Cows

Angus crossbred beef heifers (n = 2014) of at eight locations were assigned to treatment groups in this study. Heifers grazed native pastures during the treatment and AI periods. The BCS RTS and temperament score were assigned on d -33 by trained evaluators at all locations similar to Experiment 1.

2.2.2. Estrous synchronization treatment regimens

Estrous synchronization products and estrous detection aids used in Experiment 2 were similar as those in Experiment 1, except for the GnRH (2 mL Cystorelin, gonadorelin diacetate tetrahydrate, im, Boehringer Ingelheim Animal Health, Duluth, GA, USA). Within location, heifers were assigned randomly to one of two estrous synchronization treatment regimens [14-D-CIDR-PGF (n = 979) and 5-d CIDR + CO-Synch (n = 1035)] and one of two AI [STAI (n = 998), 64 + 84 h and FTAI (n = 1016), for 14-d CIDR regimens, AI occurred at 72 h and for 5-d CIDR treatment regimen, AI occurred at 56 h] (Kasimanickam et al., 2012, 2015). The 14- and 5-d protocols were imposed similar to Experiment 1. Heifers in STAI protocol were inseminated following 64 + 84 h protocol similar to Experiment 1. For FTAI, heifers were randomly assigned to either a group in which there was a 14-d CIDR-PGF2α treatment regimen, with AI occurring at 72 h after PGF2α administration or to a group in which there was a 5-d Select Synch + CIDR treatment regimen, with AI occurring at 56 h after first PGF2α administration. All heifers were administered GnRH at the time of insemination. Sire semen used for artificial insemination (n = 19), AI personnel (n = 15) and animal handlers (n = 28) were generally unique to the location; however, a few technicians and sire semen were the same at several locations. Bulls were placed with heifers for natural mating if there was return to estrus starting 2 wk after AI and removed approximately 70 d after placement of bulls with heifers, for a total breeding season of 85 d.

2.3. Pregnancy diagnosis

In both experiments, transrectal ultrasonography (Sonoscape S8, Universal Imaging, Bothell, WA, USA) was used to examine heifers for pregnancy at ~ 60 d after AI. Pregnancy was determined when there was visualization of the uterus and its contents (viable embryo/fetus). To differentiate pregnancies that occurred as a result of AI compared with natural-mating, gestational age was estimated based on sizes of embryo/fetus, amniotic vesicle and placentomes. Final pregnancy diagnosis procedures were conducted using transrectal ultrasonography no earlier than 35 d after the end of the breeding season. Only pregnancies that had occurred as a result of AI (at 60 days) was used in the analysis.

2.4. Statistical analyses

Data were analyzed using a statistical software program (SAS 9.4 version, SAS Institute Inc., Cary, NC, USA). For all analyses, there were considered to be mean differences when the $P < 0.05$.

Estrous response rate was defined as number of heifers that expressed estrus, divided by total number of heifers, whereas percentage conception (C)/AI rate was number of heifers pregnant as a result of AI divided by total number of heifers inseminated and % P/AI was number of heifers pregnant as a result of AI divided by total number of heifers.

2.4.1. Experiment 1

Data for RTS, BCS and age of heifers at different locations were analyzed using an ANOVA (PROC GLM), with a Bartlett test being used to assess homogeneity of variance. Because variances for means were heterogeneous, log10-transformed data were analyzed, with non-transformed values reported. Normality of data distribution was tested using the PROC UNIVARIATE procedure (Shapiro-Wilk test). Values for %ERR and %P/AI for location were tested using an ANOVA (PROC GLM).

The PROC GLIMMIX, logistic regression was conducted by applying the GLIMMIX procedure (METHOD = LAPLACE; ILINK = LOGIT; DIST = BINOMIAL SOLUTION ODDSRATIO) to determine differences in %ERR and %P/AI when there was imposing of the different hormonal treatment regimens and AI regimens. Fixed variables included in the analysis to determine differences in % ERR between treatments were: treatment regimen (14- and 5-d), STAI (54 + 74 and 64 + 84), RTS (2–5), BCS [< 5 (thin), 5–7 (moderate to good) and > 7 (obese)], temperament score (0 and 1), age (< 16 and ≥16 mo), treatment regimen by RTS, treatment regimen by BCS, treatment regimen by heifer age, treatment regimen by temperament score and treatment regimen by STAI interactions. Furthermore, location (n = 5) and animal handler (n = 19) nested in location (n = 5), were included as random variables. Fixed variables included in the analysis to determine differences in %P/AI were similar to %ERR. Furthermore, inseminator (n = 9) nested in location (n = 5), AI sire (n = 12) nested in location (n = 5) random variables were included. The final model included all fixed variable categories, treatment regimen by STAI interaction and all random variables. Other interactions were excluded from the model, because none were significant. Mean differences, including pairwise comparisons (class variable category with a lesser %ERR or %P/AI was used as reference), in %ERR or %P/AI for fixed variables were estimated.

2.4.2. Experiment 2

All statistical analyses were similar to Experiment 1, except the fixed variable STAI (54 + 74 and 64 + 84) was removed and AI methods (STAI, 64 + 84 and FTAI, 14-d CIDR-PGF2α, AI at 72 h after PGF2α and 5-d Select Synch + CIDR, AI at 56 h after PGF2α) were included. Similarly, treatment regimen by STAI interaction was removed and treatment regimen by AI method was included. Also, for estrous response random variables location (n = 5) and animal handler (n = 19) nested in location (n = 5) were removed and location (n = 8) and animal handler (n = 28) nested in location (n = 5) were included. For P/AI, random variables location (n = 5), inseminator (n = 9) nested in location (n = 5), AI sire (n = 12) nested in location (n = 5), and animal handler (n = 19) nested in location (n = 5) were removed and location (n = 8), inseminator (n = 15) nested in location (n = 8), AI sire (n = 19) nested in location (n = 8), and animal handler (n = 28) nested in location (n = 8) were included.

3. Results

3.1. Experiment 1

Mean (± SEM) RTS, BCS, age, %ERR and %P/AI for 14- and 5-d CIDR treatment regimens in different locations are included in Table 1. There were no differences between 14- and 5-d CIDR treatment regimens for RTS, BCS, and age of heifers for estrous response or P/AI percentage. Mean %ERR and %P/AI for 54 + 74 h and 64 + 84 h STAI are included in Table 1.

3.1.1. Estrous expression rate (%)

Mean %ERR did not differ ($P > 0.1$) when there was imposing of the 14- and 5-d CIDR estrous synchronization treatment regimens. Mean %ERR, however, differed ($P < 0.05$) between 54 + 74 and 64 + 84 h insemination times after PGF2α administration when there was use of the STAI regimen. The %ERR did not differ ($P > 0.1$): among RTS categories [2, 74.9 % (134/172); 3, 74.0 % (192/241); 4, 77.9 % (245/327) and 5, 79.7 (305/412)]; among BCS categories [thin, 75.5 % (114/151), moderate to good, 76.2 (714/937) and 70.3 % (45/64)]; between temperament categories [calm, 76.8 % (580/756) and excitable, 74.7 % (296/396)] and between age categories [< 16 mo, 74.5 % (319/428) and > 16 mo, 76.9 % (557/724). Furthermore, %ERR was not affected by interaction effects ($P > 0.1$). The %ERR at 54 + 64, at 64 + 84 h timepoints after PGF2α administration and percentages of heifers not detected in estrus are depicted in Fig. 2.

3.1.2. Pregnancy/AI (%)

Mean %P/AI did not differ ($P > 0.1$) when there was imposing of the 14- and 5-d CIDR treatment regimens, 60.9 % (351/576) and at the 54 + 74 and 64 + 84 h timepoints when there was imposing of the STAI regimen. The %P/AI was not affected by interaction effects ($P > 0.1$). The %P/AI did not differ ($P > 0.1$): among RTS categories [2, 58.7 % (101/172); 3, 60.2 % (145/241); 4, 63.0 % (205/327) and 5, 62.6 (258/412); among BCS categories [thin, 60.3 % (114/151), moderate to good, 61.6 % (714/937) and 64.1 % (45/64)]; between temperament categories [calm, 62.8 % (475/756) and excitable, 59.1 % (234/396)]; and between age categories [< 16 mo, 74.5 % (319/428) and > 16 mo, 76.9 % (557/724). Furthermore, %P/AI was not affected by an interaction effect ($P > 0.1$). The %P/AI for heifers that were detected to have expressed estrus at the 54 + 74, at 64 + 84 h timepoints and for percentage of heifers not detected in estrus are depicted in Fig. 3.

3.2. Experiment 2

There were no differences ($P > 0.1$) when there was imposing of the 14- and 5-d CIDR treatment regimens for RTS, BCS, age, %ERR and %P/AI (Table 2). Additionally, mean %ERR and %P/AI for STAI and FTAI are included in Table 2. The percentages for calm and excitable heifers were 67.7 % (1363/2014) and 32.3 % (651/2014), respectively. The percentages for thin, moderate to good and obese heifers were 14.3 % (288/2014), 72.5 % (1461/2014) and 13.2 % (265/2014), respectively. The percentage of heifers with RTS 2, 3, 4, and 5 were 10.4 % (210/2014), 21.9 % (441/2014), 25.4 % (512/2014) and 42.3 % (851/2014), respectively. The percentage of heifers in ≤ 16 and > 16 m age groups were 46.8 % and 53.2 %, respectively.

3.2.1. Estrous response (%)

Mean %ERR differed when the STAI [81.3 % (811/998) and FTAI 64.4 % (654/1016)] insemination regimens were imposed ($P < 0.001$). Mean %ERR, however, did not differ ($P > 0.1$) when there was imposing of the 14-d CIDR 73.0 % (715/979) and 5-d CIDR 72.5 % (750/1035) treatment regimens. The %ERR differed ($P < 0.001$): among RTS categories [2, 58.1 % (122/210); 3, 67.8 % (304/441); 4, 73.2 % (375/512) and 5, 78.6 % (669/851), among BCS categories [thin, 69.1 % (199/288), moderate to good, 78.4 % (714/1461) and 74.3 % (197/265)], between temperament categories [calm, 80.5 % (1069/1363) and excitable, 61.1 % (398/651)] and between age categories [< 16 mo, 68.3 % (643/942) and > 16 mo, 76.6 % (822/1073). Furthermore, the %ERR was affected AI protocol by estrous synchronization treatment regimen interaction ($P < 0.05$) but there were no other interaction effects. For 14-d/STAI, 5-d/STAI, 14-d/FTAI and 5-d/FTAI estrous synchronization combined with insemination regimens were imposed,

Table 1
Mean (± SEM) values for heifer characteristics and rates of estrous response and pregnancy/AI percentages when using 14-d CIDR-PGF2α or 5-d Select Synch + CIDR treatment regimens at five locations (Experiment 1).

Location	*n*	Age (mo)		BCS		RTS		Estrous response rate (%)				Pregnancy/AI (%)			
		14-d	5-d	14-d	5-d	14-d	5-d	14-d	5-d	54 + 74	64 + 84	14-d	5-d	5+/74	64 + 84
1	180	16.1 ± 1.42	15.8 ± 1.26	5.80 ± 1.21	5.77 ± 1.27	3.87 ± 1.03	3.79 ± 1.04	74.5	74.4	72.5	76.4	62.8	61.6	60.4	64.0
2	255	15.9 ± 1.29	16.1 ± 1.23	5.80 ± 1.18	5.84 ± 1.17	3.79 ± 1.09	3.92 ± 1.04	74.0	75.0	72.8	76.5	62.6	56.8	61.8	57.1
3	318	16.1 ± 1.23	16.1 ± 1.27	5.70 ± 1.21	5.84 ± 1.31	3.75 ± 1.08	3.91 ± 1.12	76.8	77.3	71.4	82.3	58.3	64.0	56.5	65.2
4	182	16.3 ± 1.32	16.1 ± 1.32	5.84 ± 1.20	5.76 ± 1.21	3.92 ± 1.07	3.78 ± 1.02	70.5	81.9	74.0	78.1	50.0	66.0	52.0	62.9
5	217	16.0 ± 1.35	16.2 ± 1.29	5.83 ± 1.27	5.94 ± 1.12	3.87 ± 1.10	3.91 ± 1.08	78.6	76.3	76.4	78.5	61.7	71.9	65.5	68.2

There were no differences in estrous response and P/AI percentage with use of 14- and 5-d treatment regimens and with use of the 54 + 74 and 64 + 74 split-time AI regimens.
[1]Refer Fig. 1 for treatment regimen.
BCS = body condition score (1, emaciated; 9, obese).
RTS = reproductive tract score (1, immature, anestrus; 5, mature, estrous cyclic).

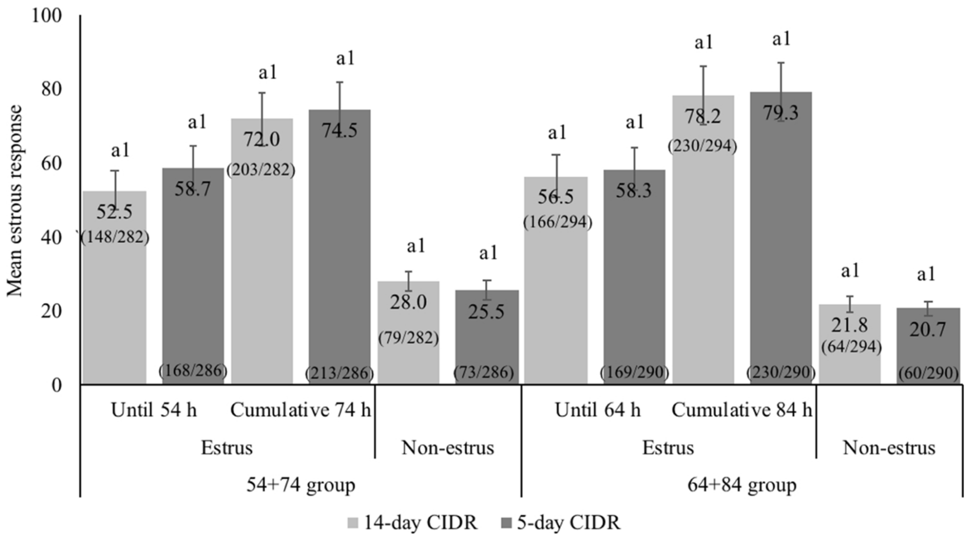

Fig. 2. Heifer estrous response rates (%) for split time AI at 54 + 74 and at 64 + 84 h and percentages of heifers not expressing estrus by 74 and 84 h after PGF2α administration when the 14-d CIDR-PGF2α or 5-d Select Synch + CIDR treatment regimens§ were imposed on Angus-cross beef heifers.
§ Refer Fig. 1, Experiment 1 for treatment regimens.
a, Between treatment regimens, within AI time, values indicate there was no difference ($P < 0.05$).
1, Between AI times, within treatment regimens, values indicate there was no difference ($P < 0.05$).

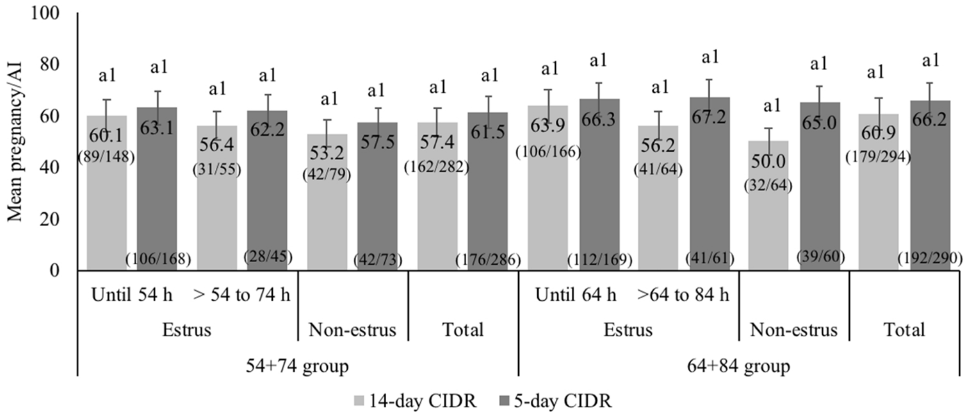

Fig. 3. Conception and pregnancy per AI percentages of Angus crossbred beef heifers inseminated following imposing of a 14-d CIDR-PGF2α or 5-d Select Synch + CIDR treatment regimens§ using two split-time insemination regimens, 54 + 74 and 64 + 84 h after PGF2α administration.
§ Refer Fig. 1, Experiment 1 for treatment regimens.
a, Between treatment regimens, within AI time, values indicate there was no difference ($P < 0.05$).
1, Between AI times, within treatment regimens, values indicate there was no difference ($P < 0.05$).

overall %ERR was 79.8 %, 82.6 %, 66.2 % and 62.8 %, respectively ($P < 0.05$). Mean %ERR for heifers in STAI and FTAI groups and mean percentages of heifers that failed to express estrus are depicted in Fig. 4.

3.2.2. Pregnancy/AI (%)

The mean %P/AI when imposing the STAI and FTAI regimens for insemination groups were different, being 61.2 % (611/998) and 55.4 % (574/1036), respectively ($P < 0.01$). The mean %P/AI when imposing the 14-d CIDR and 5-d CIDR treatment regimen did not differ, being 56.6 % (565/998) and 59.9 % (620/1035), respectively ($P > 0.1$). The %P/AI differed ($P < 0.001$) among BCS categories – thin, 45.8 % (132/288), moderate to good, 61.1 % (892/1461) and obese, 53.2 % (141/265). The %P/AI differed ($P < 0.001$) among heifers with RTS 2, 3, 4, and 5, 48.6 % (102/210), 51.2 % (226/441), 54.9 (281/512) and 65.3 % (556/851), respectively. The %P/AI differed for heifers in ≤ 16 and > 16 m age groups being 54.1 % (510/942) and 61.1 % (655/1073), respectively ($P < 0.01$). The %P/AI for calm and excitable heifers were different, 60.9 % (830/1363) and 54.5 % (335/651), respectively ($P < 0.001$). There was an AI protocol by synchronization treatment regimen interaction ($P < 0.05$). The %P/AI was greater for heifers on which there was imposed the 5-d/STAI compared to 14-d/FTAI and 5-d/FTAI treatment and insemination regimens. There was a trend for an increased %P/AI when there was imposing of the 5-d/STAI compared with14-d/STAI treatment

Table 2
Mean (± SEM) values for heifer characteristics and rates of estrous response and pregnancy/AI percentages with the split-time AI (STAI, 64 + 84 h) and fixed time AI (FTAI) regimens following 14-d CIDR-PGF2α or 5-d Select Synch + CIDR treatment regimens in Angus-cross heifers at eight locations (Experiment 2).

Location	*n*		Age		BCS		RTS		Estrous response rate (%)‡		Pregnancy/AI (%)‡	
	STAI	FTAI	STAI	FTAI	STAI	FTAI	STAI	FTAI	STAI	FTAI	STAI	FTAI
1	131	138	16.2 ± 1.5	16.3 ± 1.6	5.6 ± 1.5	5.7 ± 1.7	3.6 ± 1.3	3.7 ± 1.4	84.7 (111)^{a}	61.4 (88)^{b}	61.3 (80)^{a}	56.4 (78)^{a}
2	75	79	15.8 ± 1.9	15.8 ± 1.6	5.8 ± 1.8	5.6 ± 1.7	3.7 ± 1.8	3.9 ± 1.6	86.2 (65)^{a}	58.3 (48)^{b}	61.7 (47)^{a}	52.4 (42)^{a}
3	183	189	16.3 ± 1.2	16.1 ± 1.7	5.7 ± 1.2	5.8 ± 1.31	3.8 ± 1.8	3.9 ± 1.2	82.8 (151)^{a}	64.2 (123)^{b}	61.9 (113)^{a}	56.1 (107)^{a}
4	132	140	16.2 ± 1.3	16.1 ± 1.5	5.8 ± 1.6	5.6 ± 1.7	3.9 ± 1.4	3.9 ± 1.2	83.6 (110)^{a}	58.8 (85)^{b}	62.2 (82)^{a}	53.9 (76)^{a}
5	93	101	16.0 ± 1.5	16.2 ± 1.9	5.8 ± 1.7	5.9 ± 1.2	3.8 ± 1.1	3.9 ± 1.0	80.0 (75)^{a}	66.6 (69)^{a}	65.6 (61)^{a}	55.4 (56)^{a}
6	164	170	15.8 ± 1.5	15.9 ± 1.8	5.6 ± 1.5	5.5 ± 1.8	3.7 ± 1.3	3.9 ± 1.5	81.2 (133)^{b}	58.4 (101)^{a}	63.4 (104)^{a}	56.7 (96)^{a}
7	118	134	16.2 ± 1.2	15.9 ± 1.5	5.4 ± 1.7	5.6 ± 1.6	3.8 ± 1.6	3.9 ± 1.5	83.8 (99)^{a}	59.8 (82)^{b}	62.7 (74)^{a}	54.6 (73)^{a}
8	83	84	15.7 ± 1.1	16.0 ± 1.9	5.6 ± 1.6	5.8 ± 1.7	3.7 ± 1.4	3.8 ± 1.6	80.7 67)^{a}	65.5 (58)^{a}	60.2 (50)^{a}	55.3 (46)^{a}

[1]Refer Fig. 1 for treatment protocol.
No differences in age, BCS and RTS between STA and FTAI groups.
‡ Similar superscripts within location between treatment indicated there were not differences (P < 0.05).

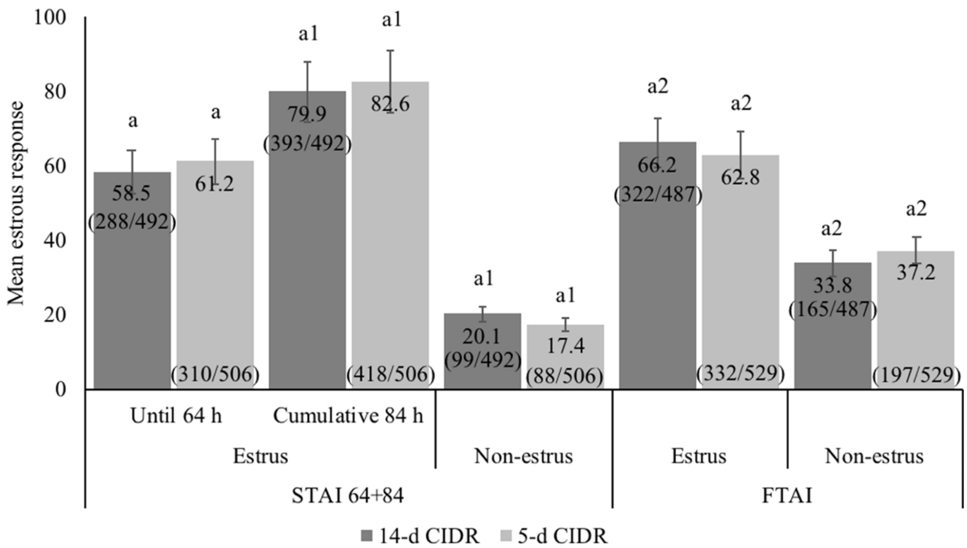

Fig. 4. Angus crossbred beef heifers that expressed or did not estrus following imposing a 14-d CIDR-PGF2α or 5-d Select Synch + CIDR treatment regimen§ and imposing of a split-time AI (STAI, 64 + 84 h after PGF2α) or fixed time AI (FTAI) regimen‡.
§ Refer Fig. 1, Experiment 2 for treatment regimens.
‡ AI at 56 h and AI at 72 h after CIDR insert removal for 5-d and 14-d protocol.
ab, Values associated with different superscripts between synchronization treatment regimen (within AI protocol) differed ($P < 0.05$).
12, Values associated with different superscripts between AI protocol (within synchronization treatment regimen) differed ($P < 0.05$).

and insemination regimens. Furthermore, there were not any other interaction effect on %P/AI ($P > 0.1$). The mean %P/AI for heifers that expressed or did not express estrus and total %P/AI are depicted in Fig. 5.

4. Discussion

In the present study, results from the Experiment 1 indicated that there were no differences in estrous response when imposing the 14-d and 5-d CIDR treatment regimens, however, there were differences when the 54 + 74 and 64 + 84 STAI insemination regimens were imposed. As hypothesized, there were no differences in %P/AI among the four groups. In Experiment 2, the total estrous response and %P/AI did not differ when there was imposing of the 14-d and 5-d CIDR treatment regimens. As hypothesized the %P/AI differed between STAI and FTAI insemination protocols. Considering results from both experiments the %ERR was less when there was imposing of the 54 + 74 STAI and FTAI insemination regimens, due to shorter time interval between PGF2α administration and AI, compared with use of the 64 + 84 STAI insemination regimen. Furthermore, the %P/AI was greatest for heifers in 5-d/STAI compared to 14-d/FTAI and 5-d/FTAI treatment and insemination regimens. Also, there was a trend for an increased %P/AI when the 5-d/STAI as compared with the 14-d/STAI treatment and insemination regimen was imposed.

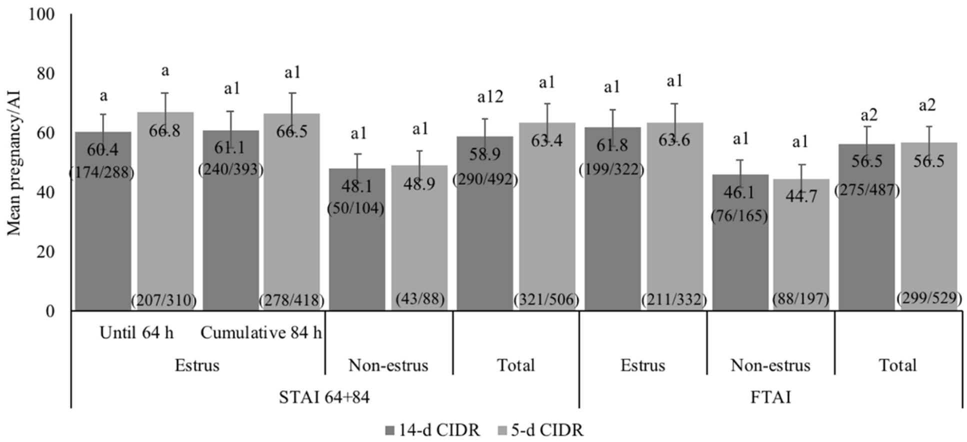

Fig. 5. Pregnancy per AI (%) for Angus crossbred beef heifers that expressed or did not express estrus following 14-d CIDR-PGF2α or 5-d Select Synch + CIDR treatment regimen[§] when there was a STAI (64 + 84 h after PGF2α) regimen or FTAI regimen[‡].
§ Refer Fig. 1, Experiment 2 for treatment regimens.
‡ AI at 56 h and AI at 72 h after CIDR insert removal for 5-d and 14-d protocol.
ab, Values with different superscripts between synchronization treatment regimen (within AI protocol) differed ($P < 0.05$).
12, Values with different superscripts between AI protocol (within synchronization treatment regimen) differed ($P < 0.05$).

The use of the split-time AI protocol provides the option for heifers to be managed on the basis of estrous status at the time of AI. From the combined results in the present study, the %ERR was 64.4 % when there was use of the FTAI which was less than with use of the STAI insemination regimen which was 78.4 % ($P < 0.0001$). In the present study, the estrous response by 74 and 84 h after PGF2α administration in Experiment 1 was 73.2 % and 78.8 %, respectively, and was 81.9 % by 84 h in Experiment 2 which was greater than previously reported. Bishop et al. (2016), reported that the overall estrous response for beef heifers by 66 h after the PGF2α administration was 69.5 % and at 90 h (from 66 to 90 h) was 54.4 % and Stevenson et al. (2017) reported a cumulative proportion of cows in estrus by 75 h of 66.7 % and by 85 h of 76.7 %.

Expression of estrus in cattle occurs after there is an increase in serum estradiol concentrations. Estrogen functions are important for processes involved with establishment of pregnancy because of actions on oocyte development that result in the capacity for fertilization (Martin et al., 1991; Arlotto et al., 1996; Driancourt et al., 1998; Pohler et al., 2012) and by having actions in decreasing uterine pH (Hawk, 1983; Perry and Perry, 2008a, b). It should be noted that that if there is expression of estrus in cattle this results in an increased P/AI percentage following AI because of a pH-mediated decrease in sperm motility that leads to an increase in sperm longevity (Perry and Perry, 2008a). In addition, increases in estradiol concentrations leads to the preparation of the uterus for pregnancy by regulating protein abundances in numerous uterine secretions and receptor populations for ligands that are favorable to production of a uterine milieu that is conducive to establishment of pregnancy (Bartol et al., 1981; Perry and Perry, 2008a, b). Collectively, these processes plausibly contributed to the increased %P/AI in the STAI programs because of increased %ERR following STAI programs.

Comparable %P/AI may be achieved by inseminating heifers using a STAI insemination regimen compared to detecting estrus and inseminating heifers during a 6-day period (Mallory et al., 2010; Wilson et al., 2010;). Improvements in %P/AI after STAI in beef heifers, however, have been attributed to an increase in overall estrous response before insemination (Thomas et al., 2014). In the current study, the overall %P/AI when there was use of the FTAI option (55.4 %) was less than that with use of the STAI (61.4 %) insemination regimen.

In a Bishop et al. (2016) study, %P/AI for beef heifers by 66 h after administration of PGF2α was 62.6 % and at 90 h (from 66 to 90 h) was 60 %. The %P/AI by 66 h in the previous study was similar to %P/AI when using the 54 + 74 h insemination regimen in the present study; whereas, the %P/AI by 90 h was less in a previous study compared to the % P/AI when the 64 + 84 h insemination regimen was imposed in the present study. In the present study, the %P/AI as a result of AI at 54 h was 61.7 % and at 74 h (from 54 to 74 h) was 59.0 %, and the overall P/AI percentage as a result of insemination at 64 h was 65.1 % and at 84 h (from 64 to 84 h) was 65.6 %. Stevenson et al. (2017) reported an overall %P/AI for cows when there was imposing of the 65 + 85 h STAI insemination regimen was greater at 36 d than for cows when there was use of the 55 + 75 h STAI insemination regimen (61.0 % and 51.4 %, respectively). In Experiment 1, the mean %P/AI did not differ with use of the 54 + 74 and 64 + 84 h STAI insemination regimen. It should be noted that in the present study, %P/AI did not differ between heifers that expressed or did not estrus when there was imposing of both the 54 + 74 and 64 + 84 h insemination regimens in Experiment 1.

In the preset study, there was no interaction between treatment and RTS for estrous response and %P/AI. There were no differences in estrous response or pregnancy rates as a result of AI between heifers that were classified before the breeding period as being pubertal or peripubertal prior to long-term progesterone treatment (Knickmeyer et al., 2019). These similarities may be attributed to successful induction of puberty that occurred in pre- or peri-pubertal heifers after administering progesterone (Gonzalez-Padilla et al., 1975; Patterson et al., 1990; Mallory et al., 2010; Kasimanickam et al., 2020). Because there were no differences in response to the treatments as a result of pre-treatment pubertal status of heifers in these previous studies, it is plausible that greater pregnancy rates for heifers on which the STAI insemination protocol was imposed can be largely attributed to the increased estrous response that occurred during the 20 h period between time of inseminations.

Thomas et al. (2014) compared the use of the FTAI and STAI insemination regimens when there was use of a 14-d CIDR-PGF2α treatment regimen in beef heifers for synchronization of time of estrus among heifers. Heifers on which the STAI insemination regimen was imposed had a greater ($P = 0.01$) AI pregnancy rate (AIPR) (54 %) than when there was FTAI (46 %). The greater AIPR was attributed to time of AI being later when heifers had not expressed estrus at the time of the first insemination period when the STAI treatment regimen is imposed, because the AIPR for the heifers that had not expressed estrus at this time of the first insemination period was greater ($P = 0.02$) when there was use of STAI insemination regimen (49 %) as compared with FTAI (34 %). The AIPR (56 % and 52 %) of heifers that had expressed estrus at the time of the first AI period when there was imposing of the STAI insemination did not differ from that of heifers in which there was FTAI. In Experiment 2 in the current study, the %P/AI did not differ between heifers that did or did not express estrus, when there was imposing of the STAI (by 84 h) and FTAI insemination regimens.

Experiments were conducted involving STAI (66 + 90 h combination) that were reported by Bishop et al. (2016) in which there was evaluation of effects of timing and administration of GnRH to anestrous heifers on %P/AI. Heifers were allocated to two treatments; GnRH at 66 h after PGF2α, irrespective of estrous response, and GnRH administered at the time of the second AI when the STAI regimen is imposed only to heifers not detected in estrus at 66 h after PGF2α administration. The results from the study indicated that GnRH was not required for induction of ovulation in heifers that were in estrus prior to imposing the STAI regimen. Furthermore, pregnancy rates were affected to a greater extent by expression of estrus during the 24 h period after the initial AI period when there is imposing of the STAI regimen than by altering the timing of GnRH after PGF2α. In each treatment, heifers that expressed estrus during the 24 h period after the initial AI period when there is imposing of the STAI regimen had pregnancy rates that were as much as 30 % greater than those that failed to express estrus during this 24 h period. It should be noted that if administering GnRH earlier was beneficial to heifers that were inseminated at the time of the second insemination when imposing the STAI regimen, greater pregnancy rates should have resulted among heifers administered GnRH at 66 h and inseminated at 90 h, specifically among those that failed to express estrus during the 24 h period. In the current study GnRH was administered only to heifers that had not expressed estrus by the time of second AI period when imposing the STAI regimen.

In an earlier study (Bridges et al., 2008), imposed either a 7- or 5-d CIDR + CO-Synch treatment regimen on cows with FTAI occurring concurrent with the time of GnRH administration at either 60 (7 d) or 72 (5 d) h after CIDR withdrawal. Pregnancy rates, as a result of FTAI, were 13.3 % (60 h; $P < 0.05$) and 9.1 % (72 h; $P < 0.05$) greater with use of the 5-d CIDR than 7d-CIDR treatment regimen. In Experiment 2 of the current study, there was a greater %P/AI for heifers when there was a 64 + 84 h STAI regimen was imposed after use of the 5-d Select Synch + CIDR treatment regimen for estrous synchronization. The %P/AI for heifers expressing estrus by 64 h was 66.8 %, cumulative %P/AI of heifers expressing estrus by 84 h was 66.5 % and %P/AI for heifers that did not express estrus by 84 h (AI plus GnRH) was 48.9 %, for an overall %P/AI of 63.4 %. It should be noted that the recommended time for AI for heifers on which there is imposed a 5-d CIDR treatment regimen is 60 ± 4 h after PGF2α administration. Results from studies indicate there are different P/AI percentages for heifers on which the 5-d CIDR treatment regimen is imposed for FTAI when AI was performed at 56 h after PGF2α administration (66.2 %, Kasimanickam et al., 2012), (63.6 %, White et al., 2016), (62.2 %, Peterson et al., 2011) and (55.5 %, Kasimanickam et al., 2015). Insemination at 56 h after PGF2α administration led to an improvement in pregnancy rate as a result of AI compared to insemination at 72 h after PGF2α administration, 66.2 % and 55.9 %, respectively ($P < 0.001$; Kasimanickam et al., 2012). It should be noted that insemination at 72 h after PGF2α administration when imposing the 5-d CIDR treatment regimen for estrous synchronization also resulted in an acceptable %P/AI in other studies (55.9 %, Kasimanickam et al., 2012; 57.8 %, Kasimanickam et al., 2015). Furthermore, when heifers have not expressed estrus at the time of first AI period when there is imposing of an STAI regimen not administrating GnRH at time of initial predetermined AI period results in an improvement in overall estrous response in these heifers.

5. Conclusions

In Experiment 1, though not different from other groups, the %P/AI was similar when there was imposing the 5-d CIDR combined with the Select Synch treatment regimen for STAI at 64 or 84 after PGF2α administration. In Experiment 2, %P/AI was greater for heifers on which the 5-d hormonal treatment regimen was imposed and there was an STAI regimen compared with when there was a 14-d hormonal treatment regimen imposed with there being FTAI and 5-d hormonal treatment imposed and there being FTAI. There was also a trend for an increased %P/AI when the 5-d/STAI as compared with when the14-d/STAI treatment/insemination regimen was imposed. In conclusion, imposing of the 5-d Select Synch + CIDR treatment regimen for conducting STAI at 64 + 84 h after PGF2α administration has the greatest efficacy of all the treatment regimens evaluated because of a greater %P/AI.

CRediT authorship contribution statement

Ramanathan Kasimanickam: Conceptualization, Resources, Methodology, Investigation, Formal analysis, Software, Supervision, Writing - review & editing, Funding acquisition. **Katriana Jorgensen-Muga:** Methodology, Investigation, Formal analysis. **Janey Beumeler:** Methodology, Investigation, Formal analysis. **Kamron Ratzburg:** . **Aliasgar Kapi:** Methodology, Investigation, Formal analysis. **Vanmathy Kasimanickam:** Conceptualization, Methodology, Resources, Investigation, Formal analysis, Software, Writing - review & editing. **John Kastelic:** Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest

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