Theriogenology 146 (2020) 80–87

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

# Presynchronization with CIDR, with or without GnRH, prior to CO-Synch in beef heifers

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ARTICLE INFO

*Article history:*
Received 16 November 2019
Received in revised form
3 February 2020
Accepted 4 February 2020
Available online 6 February 2020

*Keywords:*
Presynchronization
Progesterone
CO-Synch
Artificial insemination
Pregnancy rate
Cattle

ABSTRACT

Objectives were to compare ovarian responses and pregnancy per AI (P/AI) in Angus-cross beef heifers (n = 521; 4 locations) synchronized with CIDR–CO–Synch (CCOS) versus CIDR-GnRH–CO–Synch (CGCOS) protocols. Heifers were assigned a reproductive tract score (RTS: 1, immature, acyclic; 5, mature, cyclic), body condition score (BCS: 1, emaciated; 9, obese) and temperament score (0, calm, 1, excitable). Heifers in the CCOS (n = 261) group received a CIDR on Day −20 (removed on Day −13), 100 μg GnRH on Day −10, 25 mg PGF2α on Day −3 and were timed inseminated 60 h later, with concomitant GnRH (Day 0). Heifers in the CGCOS (n = 260) group received a CIDR on Day −26 (removed on Day −19), 100 μg of GnRH on days −16 and −10, 25 mg of PGF2α on Day −3 and were timed inseminated 60 h later, with concomitant GnRH (Day 0). Ovarian ultrasonography was done in a subset of heifers (n = 60; 30 in each group) to determine number and size of ovarian follicles and presence of corpus luteum (CL). There was increased ($P < 0.05$) percentage of heifers with CL in CGCOS group compared to heifers in CCOS group on Day −10 (82.3 vs 68.2%) and on Day −3 (88.3 vs 75.1%). Average size of the largest ovarian follicle on Day 0 was greater for heifers in CGCOS group compared to CCOS group ($P < 0.05$). However, P/AI did not differ between CCOS and CGCOS groups, 55.0% (143/260) and 59.8% (156/261), respectively ($P > 0.1$). In conclusion, CIDR presynchronization with or without GnRH (CCOS and CGCOS protocols) in beef heifers resulted in similar P/AI. Adding GnRH to presynchronization with CIDR resulted in more heifers with a CL at PGF2α and increased preovulatory follicle diameter at AI. Future studies are needed with bigger sample size and CIDR + CO-Synch treatment as control to determine economic benefit.

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## 1. Introduction

Synchronizing follicular wave emergence with GnRH, followed by inducing luteolysis with PGF2α 7 d later, is widely used in dairy cattle breeding programs [1–4]. A second dose of GnRH 60 h after PGF2α is often given to synchronize an LH surge and ovulation in beef heifers and beef cows that have not expressed estrus, facilitating concomitant fixed-timed artificial insemination (FTAI) and eliminating the need for estrus detection [1,5,6]. Notwithstanding, heifers expressing estrus before FTAI usually ovulate in response to a spontaneous GnRH/LH surge in response to endogenous estradiol from the dominant follicle.

Percentage of beef heifers cycling at initiation of a synchronization protocol is highly variable [7,8]. However, giving prepubertal beef heifers a controlled internal drug (progesterone) release (CIDR) vaginal insert and GnRH increases both cyclicity and fertility to FTAI [6]. Progesterone intravaginal inserts are approved by the US Food and Drug Administration for advancement of first pubertal estrus in replacement beef heifers [9]. Progesterone supplementation prior to the breeding season increased AI pregnancy rates in pre- and peri-pubertal beef heifers [6,8,10]. In addition, in cycling heifers, it resulted in better ovarian synchrony.

Presynchronization is synchronization of cycles prior to synchronization for FTAI. There are various approaches, including one dose of PGF2α (10 d before initiation of protocol) in dairy cows [11] or two doses of PGF2α 10–14 d apart, with the second dose 10–14 d before protocol initiation in dairy cows [12], GnRH alone [12] or combined with PGF2α [12,13] in cattle [12–16], or a CIDR for 7, 9, 14

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https://doi.org/10.1016/j.theriogenology.2020.02.005
0093-691X/Published by Elsevier Inc.

or 18 d [14–16] before protocol initiation [10–18] in beef heifers, and beef and dairy cows. Beef herds with a high percentage of pre- or peri-pubertal heifers at the start of the breeding season may benefit from presynchronization.

Treatments such as CIDR and/or GnRH before initiating a FTAI program may promote pubertal status in beef heifers [8,18–20]. Follicles are present in pre- and peri-pubertal heifers and may be induced to ovulate with exogenous GnRH to initiate cyclicity. The response is depending on the size of the follicle in those heifers at the time of GnRH administration. Presence of smaller follicles (<11 mm) at the GnRH administration less likely to induce cyclicity than presence of larger follicles (11–16 mm) [21–25]. It is shown that progesterone supplementation to pre- and peri-pubertal beef heifer hastens cyclicity in supplemented heifers.

Poorer P/AI in heifers following FTAI are largely attributed to less synchronization of follicular waves in beef heifers versus cows [26]. Ovulatory response to GnRH in beef heifers is influenced by lack of progesterone priming; therefore, presynchronization with progestin before GnRH may increase ovulatory response to GnRH and synchronization of ovarian follicular waves [27,28]. The CIDR Select protocol improved synchrony of estrus and ovulation compared to Select Synch + CIDR, CIDR-PGF2α, and Select Synch protocols in cycling beef heifers [29]. In addition, progesterone presynchronization before GnRH and PGF2α improved P/AI following FTAI in beef heifers compared to a 7-d CO-Synch + CIDR protocol [30]. However, protocols for beef heifers involving short-term CIDR treatment have produced inconsistent results to FTAI [23] due to variable ability of GnRH to synchronize follicular waves, as only 43–60% of beef and dairy heifers ovulated in response to GnRH [6,14,27].

Ovarian follicular dynamics, timing of estrus, and response to GnRH in yearling beef heifers after treatment with a 14-day CIDR protocol were reported [31]. That study provided a descriptive comparison of response to presynchronization with a CIDR prior to GnRH and PGF2α in pubertal and prepubertal beef heifers. Response to GnRH was higher among heifers with dominant follicles ≥10.0 mm (64/71, 90%), but lower among heifers with follicles < 10 mm (4/8, 44%). Serum progesterone concentrations at PGF2α were higher among pubertal versus prepubertal heifers (7.9 versus 6.9 ng/mL, respectively. Estrous response after PGF2α did not differ among pubertal and prepubertal heifers and peaked between 48 and 60 h. Interval from CIDR removal to estrus was not different ($P > 0.05$) in pubertal versus prepubertal heifers (50.0 ± 27.3 and 48.1 ± 28.3 h, respectively).

We tested the hypothesis that CGCOS treatment with GnRH 3 d after 7-d CIDR treatment and 6 d before initiation of a CO-Synch protocol improves pregnancy per AI (P/AI) compared to CCOS, 7-d CIDR treatment and initiation of a CO-Synch protocol 3 d later. Our objectives were to compare effects of CCOS and CGCOS protocols in beef heifers (presynchronization with a CIDR, with or without GnRH), on ovulatory response and P/AI.

## 2. Materials and methods

This study was performed in accordance with the ethics, standard operating procedure, handling and use of animals, collection and use of biomaterials for research.

### 2.1. Heifers and treatments

Angus cross beef heifers (n = 521; mean (±SD) age, 15.9 ± 1.2 mo; Angus × Simmental, Angus × Hereford, Angus × Simmental × Hereford) were used at four locations. At study onset, heifers were assigned a reproductive tract score (RTS; 1 to 5; 1, immature, acyclic; 5, mature, cyclic), body condition score (BCS: 1 to 9; 1, emaciated; 9, obese) and temperament score (0, calm, slow chute exit; walk, 1, excitable, fast chute exit; jump, trot or run). A schematic presentation of synchronization protocol is shown (Fig. 1). Briefly, within location, heifers were randomly allocated to CCOS or CGCOS groups. On Day −20, heifers in CCOS (n = 261) group received a CIDR (1.38 g of progesterone; Eazi-Breed™ CIDR® Cattle Insert; Zoetis Animal Health, Kalamazoo, MI, USA); it was removed on Day −13. They were given 100 μg of gonadorelin hydrochloride (GnRH; Factrel®, 2 mL im, Zoetis Animal Health) on Day −10, 25 mg of dinoprost (PGF2α; Lutalyse®, 5 mL, im, Zoetis Animal Health) on Day −3 and were inseminated 60 h later [32], with a second dose of GnRH given concomitantly (Day 0). Heifers in CGCOS (n = 260) group received a CIDR on Day −26, with subsequent removal on Day −19. They were given 100 μg of GnRH on Days −16 and −10, with 25 mg of PGF2α on Day −3 and insemination 60 h later, with a second dose of GnRH given concomitantly (Day 0). At PGF2α administration, all heifers in both groups were fitted with Estrus Alert patches (Western Point Inc., Apple Valley, MN, USA) and were observed thrice daily for standing estrus and estrus detection aid status until time of AI. A heifer was designated in estrus if she was observed to stand for mounting by other herd mates or if she had an activated (>90% of grey patch was red colored), lost (with mount marks) or partially-activated (50–90% of grey patch was red colored) estrus-detection aid.

One clinician conducted transrectal palpation on all heifers and assigned RTS. In addition, the same clinician assigned BCS and temperament scores. The inseminators (n = 6), AI sires (n = 7) and animal handlers (n = 10) differed among locations. The AI sires were selected based on sire traits and assigned to heifers to avoid inbreeding. Starting 2 wk after AI, heifers were exposed to natural service sires for a total breeding season of 85 d.

### 2.2. Ovarian ultrasonography

Ovarian ultrasonography (Sonoscape S8, Universal Imaging, Bothell, WA, USA) with 5 MHz linear-array transducer was performed by one clinician in a subset of heifers (n = 60; 30 in each group) on the day of CIDR insertion and on Days −10, −3 and 0. Size of dominant follicle respectively) and presence of CL were recorded [33,34].

### 2.3. Pregnancy diagnosis

Approximately 60 d after AI and again 30 days after removal of bull, one clinician examined heifers for pregnancy using ultrasonography (Sonoscape S8). Pregnancy was confirmed by visualization of the uterus and its contents (viable embryo/fetus). To differentiate AI versus natural-mating pregnancies, gestational age was estimated based on sizes of embryo/fetus, amniotic vesicle and placentomes. Only pregnancy to AI was used in the analysis.

### 2.4. Statistical analyses

To determine the size of the effect [12% difference (52 vs 64%) in P/AI] at 5% significance and 80% power, the study needed 262 heifers per group. Average pregnancy rate following progesterone presynchronization to GnRH based timed AI ranged from 40 to 64% [16,18], whereas pregnancy rates in MGA (with PGF2α administered 19 days after MGA removal) or a long-term CIDR (with PGF2α administered 16 days after MGA removal) protocols have been reported to range from 60 to 75% [10,28,35–38]. Based on these results from these studies, it was hypothesized that the difference in P/AI would be 12% points.

Estrus expression rate was number of heifers that expressed estrus divided by total number of heifers, whereas P/AI was number

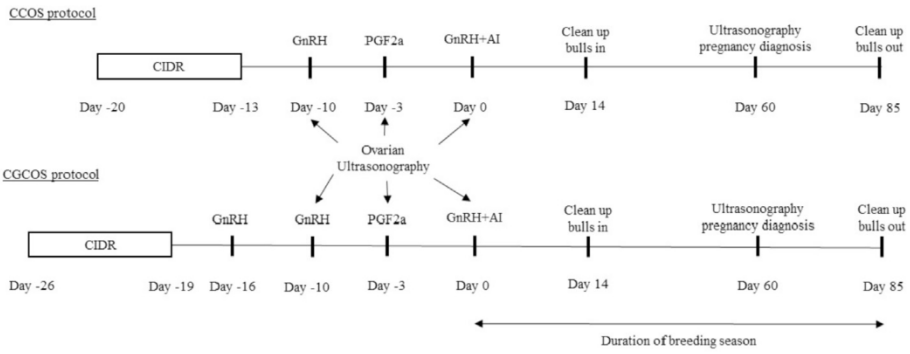


**Fig. 1.** Schematic presentation of synchronization protocol.
Briefly, on the day of initiation of synchronization, Angus cross beef heifers (n = 521) heifers were assigned a reproductive tract score (RTS; 1 to 5; 1, immature; 5, mature, cyclic), body condition score (BCS: 1 to 9; 1, emaciated; 9, obese) and temperament score (0, calm; 1, excitable). Within location, heifers were randomly allocated to CIDR–CO–Synch (CCOS) or CIDR-GnRH–CO–Synch (CGCOS) groups. Heifers in CCOS (n = 261) group received a CIDR (1.38 g of progesterone) on Day −20 which was removed on Day −13. Heifers received 100 μg of gonadorelin hydrochloride (GnRH) on Day −10, 25 mg of dinoprost (PGF2α) on Day −3 and were inseminated 60 h later and a second dose of GnRH was administered concomitantly (Day 0). Heifers in CGCOS (n = 260) group received a CIDR on Day −26 which was removed on Day −19. Heifers received 100 μg of GnRH on Day −16 and on Day −10, 25 mg of PGF2α on Day −3 and were inseminated 60 h later and a third dose of GnRH was administered concomitantly (Day 0). At the time of PGF2α administration, all heifers in both groups were fitted with Estrus Alert patches and were observed thrice daily for standing estrus and estrus detection aid status until the time of AI. Ovarian ultrasonography (Sonoscape S8, 5 MHz linear-array transducer; Universal Imaging, Bothell, WA, USA) was performed by one clinician in a subset of heifers (n = 60; 30 in each group) on Days −10, −3 and 0, and dominant follicle size and presence of CL were recorded. Heifers were exposed to natural service sires 2 wks after AI, and bulls remained with heifers for a total breeding season of 85 d. Heifers were examined for pregnancy status approximately 60 d after AI using ultrasonography.

of heifers pregnant to AI divided by total number of heifers inseminated.

Data were analyzed using a statistical software program (SAS Version 9.4, SAS Institute, Cary, NC, USA). For all analyses, the differences were considered as significant when $P < 0.05$.

Differences between treatments in mean RTS, BCS and age of heifers were analyzed using ANOVA (PROC GLM), with a Bartlett test used to assess homogeneity of variance. Because variances for means were heterogeneous, log10-transformed data were analyzed, with non-transformed values reported. Normality was tested by PROC UNIVARIATE (Shapiro-Wilk test). Mean differences in P/AI for location, treatment and treatment by location were tested using ANOVA (PROC GLM).

PROC GLIMMIX was used to determine the mean differences in follicle size for treatment, age, BCS, and RTS (main effects), and BCS by treatment, age by treatment, RTS by treatment (interaction effects). Further location was used as random effect. Final model included fixed effects treatment, age, BCS, and random effect location.

PROC GLIMMIX of SAS was used to examine treatment effects on estrus expression rate and P/AI. Fixed variables included in the analysis to determine differences in P/AI between treatments were: treatment (CCOS vs CGCOS), RTS (2–5), BCS (<5, 5, 6 and 7 and > 7), temperament score (0 and 1), estrus expression at or before AI (yes or no), age (<16 vs ≥ 16 mo), treatment by RTS, treatment by BCS, treatment by heifer age, treatment by temperament score and treatment by estrus expression at or before AI interactions. Further, location (n = 4), inseminator (n = 6) nested in location (n = 4), AI sire (n = 7) nested in location (n = 4), and animal handler (n = 10) nested in location (n = 4), were included as random variables. The final model included all fixed variables categories (interaction was excluded as there was no significance) and all random variables. Mean differences, including pairwise comparisons (class variable category with lower P/AI was used as reference), in P/AI for fixed variables were estimated.

## 3. Results

Within location, mean age, BCS and time interval from PGF2α to AI did not differ between treatment groups ($P > 0.1$; Table 1). Percentages of heifers cycling at CIDR insertion were 53.3 and 46.7% for CCOS and CGCOS groups, respectively ($P > 0.1$; Table 2). The BCS, age of heifers, and treatment influenced follicle size on Day 0 ($P < 0.05$; Table 3). Mean (SEM) follicle size for BCS categories were: <5, 12.5 ± 1.8; 5, 12.3 ± 0.8, 6 and 7, 17.9 ± 1.3 and >7, 14.7 ± 1.6. Mean (SEM) follicle size for age of heifers: <16 mo 14.2 ± 0.4 and ≥16 mo 16.3 ± 0.6 ($P < 0.05$). Mean (SEM) follicle size CCOS and CGCOS were given in Table 2. Heifers with a CL at first GnRH on Day −10 were 14.1% percentage points (pp) greater for heifers in CGCOS group compared to those in CCOS group ($P < 0.05$; Table 2). Similarly, heifers with a CL at PGF2α on Day −3 were 13.2% percentage points (pp) greater for heifers in CGCOS group compared to heifers in CCOS group. Follicle size at AI on Day 0 were 1.15 times greater for heifers in CGCOS group compared to CCOS group ($P < 0.05$; Table 2). The P/AI was not affected by a location, treatment and location by synchronization treatment interaction ($P > 0.1$; Table 1).

In the mixed model, the P/AI did not differ between CCOS versus CGCOS, 55.0% (143/260) and 59.8% (156/261), respectively ($P > 0.1$; Table 4). Temperament, RTS, BCS, age of heifer and estrus expression did not influence P/AI ($P > 0.1$; Table 4). Furthermore, P/AI was not affected by treatment by RTS ($P > 0.1$), treatment by BCS ($P > 0.1$), treatment by heifer age ($P > 0.1$), treatment by temperament score ($P > 0.1$), and treatment by estrus expression at or before AI interactions ($P > 0.1$; Fig. 2). Estimates for covariance parameters location, and AI sires, inseminators and animal handlers nested in location are given in Table 4.

## 4. Discussion

Reproductive physiological state usually varies among heifers at the beginning of a breeding season. An advantage of a progestin-based estrous synchronization protocol is that progestins hasten cyclicity in prepubertal heifers [8,19–21]. In the present study, presynchronization with progestin and GnRH may have reduced variation that is inherent in having a mixture of prepuberal, peripuberal and puberal heifers, thereby increasing the probability that a majority would have a potentially fertile ovulation during the synchronization period. In the current study, CIDR presynchronization with or without GnRH prior to CO-Synch protocol resulted in

**Table 1**
Mean ± SEM differences between two estrous synchronization protocols for Angus-cross heifers (n = 521) at four locations.

| Location | Synchronization protocol[a] | No. | Age (mo) | BCS[b] | Interval from PGF2α to AI (h) | P/AI (%) |
|---|---|---|---|---|---|---|
| 1 | CGCOS[c] | 56 | 15.8 ± 1.3 | 5.71 ± 0.09 | 60.8 ± 0.11 | 53.6 |
| | CCOS[d] | 68 | 15.7 ± 1.0 | 5.65 ± 0.07 | 59.4 ± 0.09 | 61.8 |
| 2 | CGCOS | 70 | 15.9 ± 1.2 | 5.82 ± 0.11 | 58.9 ± 0.17 | 58.6 |
| | CCOS | 52 | 16.0 ± 1.1 | 5.69 ± 0.09 | 59.6 ± 0.16 | 55.8 |
| 3 | CGCOS | 78 | 15.7 ± 1.4 | 5.92 ± 0.12 | 60.4 ± 0.07 | 60.3 |
| | CCOS | 75 | 15.9 ± 1.2 | 5.78 ± 0.08 | 59.8 ± 0.08 | 50.7 |
| 4 | CGCOS | 57 | 15.9 ± 1.3 | 5.99 ± 0.13 | 60.8 ± 0.10 | 66.7 |
| | CCOS | 65 | 16.1 ± 1.5 | 5.91 ± 0.11 | 61.2 ± 0.08 | 52.3 |

[a] Refer Fig. 1 for treatment protocol.
[b] Body condition score: 1 to 9; 1, emaciated; 9, obese.
[c] CCOS, CIDR preceding CO-Synch protocol.
[d] CGCOS, CIDR-GnRH preceding modified CO-Synch protocol.

similar P/AI in beef heifers. To our knowledge, this was the first report describing effects of short-term (7 d) CIDR presynchronization with or without GnRH prior to 7-d CO-Synch protocol.

Presynchronization with a progestin before GnRH and PGF2α should be more effective in successfully synchronizing estrus and ovulation than either short-term CIDR-based or GnRH-PGF2α estrus synchronization protocols. A previous study [30] compared estrus and ovulatory responses to long- and short-term CIDR based protocols to evaluate their potential to facilitate FTAI in beef heifers. A greater proportion of prepubertal heifers presynchronized with CIDR (86%) ovulated in response to GnRH compared to prepubertal heifers synchronized with Select Synch + CIDR (36%). Addition of GnRH on Day −16 may plausibly resulted in an increased number of heifers with CL on Day −10 for heifers in CGCOS group compared to heifers in CCOS group (82.3 vs. 68.2%, respectively). It is conceivable that the greater response to GnRH in heifers treated with a CIDR was attributed to greater synchrony from CIDR-GnRH presynchronization. In addition, 75.1% of heifers in CCOS group, compared to 88.3% of heifers in CGCOS group had CL on Day −3 plausibly due to response to the additional GnRH. Interestingly, the average size of the largest follicle on Day 0 was greater for heifers in CGCOS group compared to heifers in CCOS group. It is plausible that synchrony of follicular wave initiation occurred earlier in CGCOS group. In the current study, treatment, age of heifers and BCS influenced the preovulatory follicle size. It should be noted that preovulatory follicle size was influenced by-pubertal status of beef heifers (diameter of the dominant follicle in pre-pubertal heifers varied between 8 and 12 mm diameter) [39], effect of dietary intake in beef heifers (low dietary intake reduced the diameter and persistence of dominant follicles during the estrous cycle of beef heifers) [40], days of estrous cycle at first GnRH in beef cows [41,42], number of waves in a cycle in heifers (diameter on day before ovulation - 16.5 ± 0.4 and 13.9 ± 0.4 mm for heifers with 2 vs 3 waves, respectively), and increased LH during dominant follicle growth phase, expression of LH receptors on granulosa cells and low FSH mileu [43].

Appropriate progesterone concentrations are important to promote healthy follicular growth during the luteal phase [44–49]. Follicles grown under a high-progesterone milieu are more responsive to LH, more fertile and more likely to have physiological luteal phase lengths [50–52]. In contrast, Colazo et al. (2008) claimed the ovulatory response (and presumably fertility) in GnRH-based TAI protocols may be improved by ensuring reduced blood progesterone concentrations at the first GnRH treatment [53]. It should be noted that P/AI did not differ between CCOS versus CGCOS in the current study. It is plausible that additional GnRH resulted in accessary CLs and resulted in high progesterone and suppression of LH needed for growth of dominant follicle during the growth phase in the current study. In addition, presence of accessary CLs may have resulted in failure of or reduction in the luteolytic response to PGF2α. The combination of high progesterone, reduced LH concentrations and reduced luteolytic response to PGF2α due to accessary CLs may have mitigated the P/AI in CGCOS group.

Inclusion of GnRH at initiation of a synchronization protocol facilitates synchronized ovulation of most large dominant follicles, thereby synchronizing emergence of a new follicular wave [2,4,54]. In a previous study, GnRH improved synchrony of follicular growth and estrous response, dependent on pubertal status before treatment initiation [28]. Interval to estrus was more variable among prepubertal heifers compared to heifers that had reached puberty prior to initiation of treatment. However, there was failure in increasing synchrony of estrus, due to an inconsistent ovulatory response after inclusion of GnRH in a 14- to 19-d melengestrol acetate (MGA)-PGF2α, a similar progestin-based protocol [55]. Several studies replaced oral MGA supplementation with a CIDR for 14 d [29,56–58]. In comparisons of 14-d CIDR protocols with and without GnRH on Day 23, there were no differences in estrous response [29] or interaction between GnRH and interval to cyclicity [56]. However, variance in interval to estrus was increased when GnRH was included in the treatment schedule of the long-term protocol. It should be noted that the results noted in the current study may have been due to differences in responses to short- versus long-term (7 vs 14-d) CIDR treatment.

Previously, we compared P/AI in heifers with or without first GnRH on Day 23 (day of initiation of CO-Synch), in a 14-d CIDR-

**Table 2**
Mean ± SEM differences in ovarian characteristics between two estrous synchronization protocols for Angus-cross heifers.

| Protocols[b] | No. | Cycling at CIDR insertion[a] (%) | CL on Day- 3 (%) | CL on Day −10 (%) | Largest follicle on Day 0 (mm) |
|---|---|---|---|---|---|
| CCOS | 30 | 53.3 | 68.2 ± 3.4[a] | 75.1 ± 2.8[a] | 15.8 ± 1.9[a] |
| CGCOS | 30 | 46.7 | 82.3 ± 2.2[b] | 88.3 ± 2.2[b] | 18.1 ± 1.2[b] |

[ab]Within a column, numbers without a common superscript differed ($P < 0.05$).
CL, Corpus luteum.
[a] Based on presence of CL.
[b] Refer Fig. 1 for protocol.

**Table 3**
Explanatory variables influencing follicle size at the time of insemination following synchronization treatment.

| Variable | df | "F" value | "P" value |
|---|---|---|---|
| Synchronization[a] (CCOS[b] vs. CGCOS[c]) | 1 | 3.17 | 0.02 |
| Age of heifers[d] | 1 | 2.87 | 0.04 |
| Body condition score[e] | 3 | 3.04 | 0.03 |

df, Degrees of freedom.
Covariance parameter estimates: Location, 0.00914 ± 0.000732; Residual 0.0738 ± 0.00611; Fit statistics - BIC = 532.28; −2 Res log likelihood = 529.91.
[a] Refer Fig. 1 for treatment protocol.
[b] CCOS, CIDR preceding CO-Synch protocol.
[c] CGCOS, CIDR-GnRH preceding modified CO-Synch protocol.
[d] Age of heifers (mo) - <16 and ≥ 16.
[e] Body condition score - 1 to 9; 1, emaciated; 9, obese; Categories: Thin, <5; Moderate, 5; Good, 6 and 7; Obese, >7).

GnRH-PGF2α-GnRH and CIDR-PGF2α-GnRH synchronization protocol FTAI at 56 or 72 h after PGF2α) [54]. In that study, to increase P/AI, inclusion of GnRH on Day 23 was needed for FTAI at 56 h after PGF2α.

Presynchronization based solely on PGF2α has limited efficacy in prepubertal heifers, as response to PGF2α depends on presence of a responsive CL. That prevalence of prepubertal heifers that varies from 20 to >50% can compromise responses to PGF2α-based presynchronization programs [6,7,26,54]. Beef females classified as prepubertal immediately before enrollment in an AI program have decreased reproductive performance, due to decreases in expression of estrus and pregnancy rate [6,7,26,54] and increases in embryonic and fetal losses [59]. Furthermore, failure to sustain cycles after inducing ovulation in prepubertal heifers limits efficacy of synchronization programs and reduces reproductive efficiency [60].

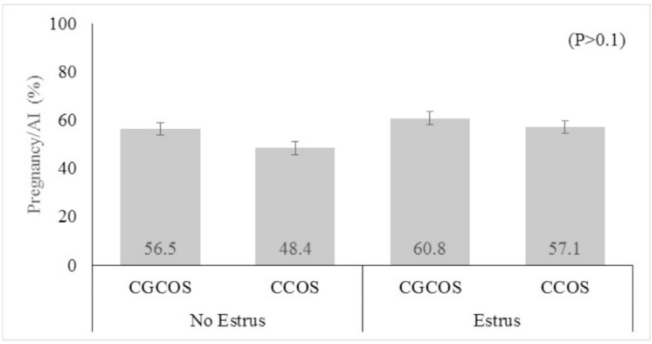


**Fig. 2.** Effect of synchronization treatment[§] by estrus expression[‡] on mean pregnancy per AI in Angus-cross heifers.
[§]Refer Fig. 1 for treatment protocol.
[‡]A heifer was designated in estrus if observed to stand for mounting by other herd mates or if heifer had an activated (>90% of grey patch was red colored), lost (with mount marks) or partially-activated (50–90% of grey patch was red colored) estrus detection aid.
CCOS, CIDR preceding CO-Synch protocol.
CGCOS, CIDR-GnRH preceding modified CO-Synch protocol.

**Table 4**
Explanatory variables influencing pregnancy per AI in Angus-cross heifers (n = 521).

| Variables | No. pregnant | Total no. | P/AI | P value |
|---|---|---|---|---|
| Synchronization treatment[a] | | | | |
| CGCOS[b] | 156 | 261 | 59.8 | Ref |
| CCOS[c] | 143 | 260 | 55.0 | 0.27 |
| Temperament[d] | | | | |
| Excitable | 95 | 175 | 54.3 | Ref |
| Calm | 204 | 346 | 59.0 | 0.31 |
| Reproductive tract score[e] | | | | |
| 2 | 35 | 71 | 49.3 | Ref |
| 3 | 63 | 113 | 55.8 | 0.39 |
| 4 | 90 | 152 | 59.2 | 0.16 |
| 5 | 111 | 185 | 60.0 | 0.12 |
| Body condition score[f] | | | | |
| Thin (<5) | 35 | 62 | 56.5 | Ref |
| Moderate (5) | 76 | 129 | 58.9 | 0.75 |
| Good (6 and 7) | 167 | 269 | 62.1 | 0.41 |
| Obese (>7) | 21 | 34 | 61.8 | 0.61 |
| Age of heifers (mo)[g] | | | | |
| <16 | 109 | 195 | 55.9 | Ref |
| ≥16 | 190 | 326 | 58.3 | 0.59 |
| Estrus expression[h] | | | | |
| No | 65 | 124 | 52.4 | Ref |
| Yes | 234 | 397 | 58.9 | 0.20 |

Covariance parameter estimates: Location, 0.002491 ± 0.003428; AI sires, 0.06954 ± 0.03347, Inseminators, 0.001974 ± 0.002044; Animal handlers, 0.01988 ± 0.03173; Residual 0.1834 ± 0.01148; Fit statistics - BIC = 749.82; −2 Res log likelihood = 745.19.
[a] Refer Fig. 1 for treatment protocol.
[b] CCOS, CIDR preceding CO-Synch protocol.
[c] CGCOS, CIDR-GnRH preceding modified CO-Synch protocol.
[d] Temperament score - Calm versus excitable.
[e] Reproductive tract score - 1 to 5; 1, acyclic, immature; 5, cyclic, mature.
[f] Body condition score - 1 to 9; 1, emaciated; 9, obese; Categories: Thin, <5; Moderate, 5; Good, 6 and 7; Obese, >7).
[g] Age of heifers (mo) - <16 and ≥ 16.
[h] A heifer was designated in estrus if observed to stand for mounting by other herd mates or if she had an activated (>90% of grey patch was red colored), lost (with mount marks) or partially-activated (50–90% of grey patch was red colored) estrus detection aid.

Intravaginal progesterone inserts can be used to induce cyclicity in anestrous beef heifers and cows [8,19–21,61]. It is plausible that CIDR treatment for 7 d may hastened induction of puberty in the current study. In prepubertal heifers, dominant follicles reach variable sizes, although ovulation does not occur, as follicles secrete insufficient estradiol due to increased negative feedback of estradiol on GnRH or LH secretion, or lack of hypothalamic responsiveness to estradiol [62]. The mechanism of action of exogenous progesterone in establishing cyclicity is not completely elucidated, but progesterone likely upregulates number of estrogen receptors in the medial basal hypothalamus, which re-establishes responsiveness to estradiol, resulting in a preovulatory LH surge [63]. Furthermore, dairy cows that received a CIDR insert initially had lesser LH pulse frequency, which could lead to increased LH storage in the anterior pituitary [64,65]. After progesterone withdrawal, beef cows have increased pulse frequency and mean concentrations of LH, increased LH receptors in granulosa and theca cells [66], increased estradiol production, and an estradiol-stimulated LH surge and ovulation [61,63]. Because progesterone from a CIDR increased induction of cyclicity prepubertal heifers [8,19–21,61], use of a CIDR during presynchronization tended to increase number of heifers inseminated on detection of estrus. Furthermore, use of a CIDR during presynchronization could have decreased estrus expression. Giving GnRH after CIDR removal would facilitate synchronizing estrus, ovulation and pregnancy [8,54,67]. However, in the current study, although GnRH increased ovulation [14.1 pp difference in ovulation (presence of CL on Day 3) between CCOS (68.2%) and CGCOS (82.3%)], it failed to improve P/AI.

If the first GnRH does not synchronize follicular wave emergence, ovulation following the second GnRH may be poorly synchronized resulting in poor P/AI following FTAI. It should be noted that in the absence of any presynchronization treatment, synchronization of follicle growth by inducing ovulation in response to the first GnRH treatment of the FTAI program occurs in only 45–50% in beef cows [16]. So, presynchronization may improve

response to GnRH. Presynchronization with a progesterone for 7 or 15 d increased the proportion of cows ovulating to the first GnRH treatment (77% versus 55% or 75% versus 49%, respectively) (Small). The amount of LH released following a GnRH treatment is regulated by the level of circulating progesterone at the time of GnRH administration [67,68]. It has been shown that ovulation following GnRH administration is significantly lower in heifers with high progesterone concentrations compared to heifers with low progesterone concentration in beef heifers [68]. In addition, immunosuppression by progesterone mediates uterine immune function [69] may lead to persistent infection [70] and plausibly reduce fertility.

Further, limitation to success of FTAI programs is the inability of a single dose of PGF2α to induce complete luteolysis. It is conceivable that immature CLs non responsive to PGF2α may not or prepubertal heifers with no CL will not respond. To achieve high P/AI, concentrations of progesterone on the day of FTAI must reach basal level. Administering PGF2α as a single dose on d 7 after GnRH usually results in 80% of cows [49] with low progesterone on the day of the FTAI. Interestingly, greater P4 concentrations at the time of PGF were associated with greater probability of luteolysis after PGF treatment and greater fertility (50 vs. 28%). It should be noted that incomplete lutolysis may plausibly influences the features of GnRH-induced LH release when progesterone concentrations are at or near baseline at the final GnRH treatment and subsequent P/AI.

Temperament, RTS, BCS, age and estrus expression did not influence P/AI ($P > 0.1$), in contrast to previous studies [58,71,72]. Furthermore, P/AI did not differ among locations ($P > 0.1$)] and there was no location by synchronization treatment and estrus expression by synchronization treatment effect on P/AI. Even though the protocols used required more heifer handling (five vs six handlings for CCOS versus CIDR-GnRH–CO–Synch, respectively) compared to a CO-Synch protocol (three handlings), temperament did not influence P/AI, perhaps due to a smaller sample size.

In the current study, age of heifers was grouped as $< 16$ and $\geq 16$ mo in the statistical analysis (instead of continuous variable). In this study, mean age of heifers was 15.9 mo. It was reported that odds of pregnancy increased by 20% for every 1 mo increase in heifer age at the start of the breeding period [73]. Further, conception rate of heifers increased by approximately 21% from the first ovulation to their third estrous cycle [74]. Thus, heifers' age was categorized as $< 16$ and $\geq 16$ mo groups.

In general, beef heifers that have experienced several estrous cycles before the onset of the breeding season have greater likelihood of conceiving early in the first breeding season [74]. The most effective method to induce puberty in heifers involves administration of a progestin [44,75]. That progesterone supplementation before the beginning of the breeding season in prepubertal and peripubertal beef heifers increased P/AI [10] was likely an important contributor to no significant group effect in the current study. Based on post-hoc analysis, 1663 heifers were required to determine 4.8% point difference in P/AI between CCOS and CGCOS groups (55.0 vs. 59.8%) at 5% significance and 80% power. In a future experiment, we plan to include more heifers to treatment groups, use ovarian ultrasonography and study estrus response with inclusion of CIDR + CO-Synch as a control. Further, Sartori et al. (2003) reported treatment with CIDR for 13 days, PGF2α injection 8 days after CIDR insertion and GnRH treatment 1 day after CIDR removal resulted in 90% synchronization rate [76]. It would be interesting to test administration of GnRH or PGF2a alone or along with CIDR as presycnhronization treatments.

In conclusion, CIDR presynchronization with or without GnRH (CCOS and CGCOS protocols) in beef heifers resulted in similar P/AI. Addition of GnRH to presynchronization with CIDR prior to CO-Synch helped more heifers with CL at PGF2α and increased preovulatory follicle size. Even though the P/AI was similar between CGCOS and CCOS protocols, longer duration and extra handling may limit its use. Future study should include CIDR + CO-Synch as a control to better assess economic benefits.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Declaration of competing interest

None of the authors have any conflict of interest to declare.

## CRediT authorship contribution statement

**Kamron Ratzburg:** Conceptualization, Data curation, Formal analysis. **Katriana Jorgensen-Muga:** Data curation. **Jeeviya Murugesan:** Data curation, Formal analysis, Writing - original draft. **John Kastelic:** Writing - review & editing. **Vanmathy Kasimanickam:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Ramanathan Kasimanickam:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

## Acknowledgements

The authors thank participating beef cattle producers and express their gratitude to Zoetis Animal Health for donation of synchronization products and Kreps/Hecomovich WSU large animal (Beef Cattle) travel fund (#2530-4362) for partial financial support.

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