**Effect of timing of insemination following CO-Synch protocol with or without progesterone on pregnancy in beef heifers**

Rabie L. Abdel Aziz,[a,b] Ramanathan K. Kasimanickam[b]
[a]Department of Theriogenology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt; [b]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA

## Abstract

The objective of the study was to determine the effect of 56 or 72 hr insemination following CO-Synch protocol with or without progesterone on pregnancy per AI (P/AI) in beef heifers. Angus cross beef heifers (n=1,314) were randomly assigned to CIDR-CO-Synch (n=633) and CO-Synch (n=681) synchronization protocol groups. All heifers were given a body condition score (BCS: 1, emaciated; 9, obese) and a temperament score (0, calm, slow chute exit; walk, 1, excited, fast chute exit; jump, trot or run). Briefly, heifers in CIDR-CO-Synch group received a 1.3 g progesterone intravaginal insert (CIDR) and 100µg of gonadorelin diacetate tetrahydrate (GnRH) im, on day 0. On day 7, the CIDRs were removed and a single dose of 25 mg dinoprost tromethamine (PGF2α) was administered im. Heifers in CO-Synch were given 100µg of GnRH im on Day 0 and 25 mg of PGF2α im on Day 7. Heifers in both synchronization groups were randomly assigned to AI56 (n=657) or AI72(n=657) groups and were inseminated at 56 h or 72 h after PGF2α administration, respectively. All heifers received 100µg of GnRH im concurrently at the time of AI. On Day 7 at the time of CIDR removal, all heifers were fitted with estrus detection aids and were observed for estrus, twice daily, from PGF2α administration to AI. Heifers were examined for pregnancy status between 50 and 70 d after AI using ultrasonography. A partial farm budget-type analysis for projected economic outcome was calculated based on mean P/AI differences for each protocol. Accounting for BCS ($P<0.01$), estrus expression ($P<0.01$), and estrus expression by synchronization treatment ($P<0.05$) and time of insemination by synchronization treatment interactions ($P<0.001$), the P/AI differed between the time of insemination ($P<0.01$). The P/AI for heifers inseminated at 56 h after PGF2α administration was greater [59.7%, (378/633)] compared to heifers inseminated at 72 h after PGF2α administration [52.1%, (355/681)]. The P/AI did not differ between CO-Synch and CO-Synch+CIDR groups, 54.8 (373/681) and 56.7% (359/633), respectively ($P>0.1$). The P/AI for CO-Synch-56, CIDR-CO-Synch-56, CO-Synch-72 and CIDR-CO-Synch-72 were 56.9, 61.3, 50.1 and 55.3%, respectively. Given the mean P/AI differences among different protocols from this study, the economic sensitivity analysis indicated that benefit for implementing the CO-Synch synchronization protocol could be as great as $979 (Table 3) compared to implementing CIDR-CO-Synch protocol. In conclusion, the P/AI were greater for CIDR-CO-Synch-56 compared to CO-Synch-72. However, the economic analysis revealed CO-Synch protocols without CIDR were cost effective.

**Keywords:** Beef heifers, ovulation synchronization, artificial insemination, insemination time, pregnancy

## Introduction

Estrus synchronization and artificial insemination (AI) can be used as an effective management tools to maximize the reproductive efficiency of heifers and to incorporate superior genetics into beef operations.[1] Several synchronization protocols have been used effectively to synchronize estrus and ovulation in beef heifers, facilitating the use of AI with or without the need for estrus detection.[2-5] In spite of these facts, adoption of AI into beef industry is still limited, lowering the rate of genetic improvement.[6] Labor concerns and complications of the protocols are reported to be the major constrains stated by beef producers.

Estrus synchronization and fixed time AI (FTAI) involve control of the luteal phase with progesterone and PGF2α, synchronization of follicular wave emergence, and synchronization of prevoulatory luteinizing hormone (LH) surge and ovulation with GnRH, and AI at a fixed time.

Timing of insemination in FTAI was evaluated for highest fertility. Beef heifers inseminated between 4 and 24 h after estrus onset had greater pregnancy per AI (P/AI; 63.7%) compared to the 0 to 4 h (48.1%) and >24 h (55.9%) groups.[7] However, insemination between 8 and 12 h after the onset of estrus yielded 69.2% P/AI in that study. Several studies reported P/AI with the CIDR-CO-Synch protocol: 65%[8] and 68% at 48 h[9], 54% at 52 h[10], 48%[11], and 55%[12] at 54 h, and 53% at 60 h[13] after PGF2α administration and CIDR removal. We reported insemination at 56 h after CIDR insert removal improved AI pregnancy rate compared to insemination at 72 h in 5-day CIDR-CO-Synch protocol (66.2 vs. 55.9%).[14] However, the effect of timing of insemination (56 vs. 72h) following CO-Synch protocol with or without progesterone on P/AI in beef heifers was not evaluated. The objective of this study was to determine the effect of AI at 56 or 72 hr following PGF2α administration in CO-Synch protocol with or without progesterone on P/AI in beef heifers.

## Materials and methods

### Animals and treatments

Eight beef farms in Washington State that used synchronization protocols as part of their breeding strategies during spring breeding season were included in the study. Angus cross beef heifers (n=1,314) were randomly assigned to CIDR-CO-Synch (n=633) and CO-Synch (n=681) synchronization protocol groups. All heifers were assigned body condition score (BCS: 1, emaciated; 9, obese) and temperament score (0, calm, slow chute exit; walk, 1, excited, fast chute exit; jump, trot or run). Briefly, heifers in CIDR-CO-Synch group received a 1.3 g progesterone intravaginal insert (CIDR, Eazi-Breed™ CIDR® Cattle Insert; Zoetis Animal Health, New York, NY) and 100µg of gonadorelin diacetate tetrahydrate (GnRH; 2 mL; Cystorelin®, Merial Inc., Duluth, GA) im, on Day 0. On Day 7, the CIDRs were removed and a single dose of 25 mg dinoprost tromethamine (PGF2α; 5mL; Lutalyse® sterile solution; Zoetis Animal Health) was administered im to all heifers. Heifers in CO-Synch group were given 100µg of GnRH im on Day 0 and 25 mg PGF2α im on Day 7. Heifers in both experiments were randomly assigned to AI56 (n=657) or AI72 (n=657) groups and were inseminated at 56 h or 72 h after PGF2α administration, respectively. All heifers received 100µg of GnRH im concurrently at the time of AI.

On day 7 at the time of CIDR removal, all heifers in both groups were fitted with estrus detection aids [Kamar® Heatmount detector patches (Kamar, Inc., Steamboat Springs, CO) or Estrus Alert patches (Western Point Inc., Apple Valley, MN) or chalk]. Heifers were observed twice daily for standing estrus and estrus detector aid status. A heifer was determined to be in estrus if the heifer was visually observed to stand for mounting or if the heifer had an activated, lost (with mount marks) or partially-activated heat detector aids. The time of insertion and removal of CIDR, and PGF2α administration and the time of AI was recorded for each animal.

The inseminators (n=7), AI sires (n=10) and animal handlers (n=16) differed among locations. In six locations, the ranch used AI technicians from stud companies and in the other two locations one clinician performed the inseminations. The AI sires were selected and assigned to heifers based on sire traits and to avoid inbreeding. Two weeks later, Angus bulls [classified as "satisfactory potential breeder" on breeding soundness evaluation and trichomonas negative (on PCR test)] were placed with heifers (approximately 1:30 to 1:40, bull: cow ratio) across treatments, for the remainder of the 60 to70 day breeding season.

### Diets

The heifers were fed to meet NRC recommendations (2016).[15] Beef heifers were either allocated to mixed alfalfa or grass hay from 3 months before and 2 weeks after AI and then turned out to pasture or they grazed Bermuda grass supplemented with corn silage and a corn soybean meal supplement. Heifers on pasture were provided with tree shade.

### Pregnancy diagnosis

Heifers were examined for pregnancy status between 50 and 70 d after AI using transrectal ultrasonography (Sonoscape S8 with 5 MHz linear-array transducer, Universal imaging, Bedford Hills, NY) of the uterus and its contents, including visualization of viable embryo/fetus and/or by per-rectal palpation of uterus and its contents. The gestational age was determined by size of the embryo, amniotic vesicle, fetus and/or placentomes. The pregnancy diagnosis was performed by an experienced clinician.

Statistical analyses

Data were analyzed with a statistical software program (SAS Version 9.4 for Windows, SAS Institute, Cary, NC). Differences in the mean body condition score and age of heifers between treatments were analyzed using one-way ANOVA (PROC GLM of SAS). Differences in mean interval (h) from CIDR insertion and removal, and CIDR removal and time of insemination between groups were analyzed by ANOVA. The Bartlett test was used to assess homogeneity of variance (PROC GLM of SAS). Because variances for the mean interval were heterogeneous, a log10 transformation was performed. All values are presented with non-transformed values.

The estrus expression rate was measured by number of heifers that expressed estrus divided by total number of heifers. The P/AI was measured by number of heifers pregnant to AI divided by total number of heifers inseminated.

PROC GLIMMIX of SAS was used to examine the effect of treatments on P/AI differences. Variables included in the model to determine the difference in P/AI between treatment groups were, time of insemination (56 vs 72 h), synchronization treatments (CIDR-CO-Synch vs. CO-Synch), BCS (≤ 5 vs > 5), temperament score (0, calm, slow chute exit; walk, 1, excited, fast chute exit; jump, trot or run), estrus expression at or before AI (yes or no), age (≤15 vs. >15), treatment by time of insemination, time of insemination by estrus expression at or before AI interactions. Locations (n=8), AI sires (n=10) and inseminators (n=7) were included as random variables in the model. Treatment was retained during model reduction and in the final models.

It was hypothesized that the P/AI difference between treatment groups will be 8.0% (59 vs 51%). To detect similar difference in the P/AI, with adequate statistical power (1-β=0.8) and statistical significance (α=0.05), the study needed a sample size of 604 heifers per treatment group. For all analysis, *P* value at 0.05 was considered significant. For model reduction, the *P* value was set at ≤ 0.1 for inclusion and > 0.1 for exclusion until the model contained appropriate significant main and interaction effects. Treatment was retained in the model during model reduction and in the final model.

Economic analysis

A partial farm budget-type analysis for projected economic outcome was calculated based on mean P/AI differences between protocols. Because of the considerable variation in outcomes for these protocols, this analysis becomes essentially a sensitivity analysis (how different AI pregnancy outcomes will impact economic outcomes under our conditions vs. a typical sensitivity analysis where regular intervals in outcomes are typically tested) for potential profit or loss for using the CO-Synch with or without progesterone synchronization protocols. Mean P/AI difference was calculated by subtracting the mean AI pregnancy rate for the CIDR-CO-Synch-56 protocol from the mean pregnancy rate for the other protocols. In addition, economic benefit / heifer for implementing CIDR-CO-Synch-56 compared to other protocols in beef heifers were calculated.

The assumptions were

1. $49.14 advantage per AI pregnancy for the CIDR-CO-Synch protocol compared with a pregnancy from natural service.[16]
2. For heifers in CO-Synch protocol decreased additional cost of $11.95 (reduced CIDR cost = $11.70/heifer plus reduced cost for insertion of CIDR = $0.25/heifer).

## Results

The mean (± SEM) age (mo), interval (h) from CIDR insertion to removal, interval from PGF2α administration to time of insemination, and body condition scores are given in Table 1. The mean age varied from 15.2 ± 0.39 to 15.6 ± 0.11 among the groups. There were no differences in mean age between treatment groups and between time of insemination within treatment groups (*P*>0.1). The mean BCS varied from 5.61 ± 0.13 to 5.32 ± 0.16 among treatment groups. The mean BCS was not different between treatment groups and between time of insemination within treatment groups (*P*>0.1). The intervals from PGF2α administration to time of insemination was similar for the AI time groups (*P*>0.1). Percentages of heifers with BCS ≤5, and >5 were 62.8 (825/1314) and 37.2% (489/1314), respectively. The percentage of heifers with excitable temperament was 68.8% and varied across locations (16.7 to 78.2%).

Effect of treatment on AI pregnancy

Accounting for BCS (P<0.01), estrus expression (P<0.01), and estrus expression by synchronization treatment (P<0.05) and time of insemination by synchronization treatment interactions (P<0.001), the P/AI for heifers inseminated at 56 h after PGF2α administration was greater (59.7%, 378/633) compared to heifers inseminated at 72 h after PGF2α administration (52.1%, 355/681) (Table 2). The P/AI for heifers with BCS ≤ 5 was 53.4% (443/830); whereas the P/AI for heifers with BCS >5 was 60.1% (291/484) (P<0.01). The P/AI for the heifers that expressed estrus and did not express estrus from PGF2α administration to AI were 59.0% (419/710) and 52.2% (315/604), respectively (P<0.01). The P/AI for synchronization treatment by estrus expression is given in Fig. 2. The P/AI for synchronization treatment by time of insemination is given in Fig. 3. The P/AI did not differ between CO-Synch and CO-Synch+CIDR groups, 54.8 (373/681) and 56.7% (359/633) (P=0.48). The P/AI for heifers with excited and calm temperament did not differ, 56.9 (514/904) and 53.7% (220/410), respectively (P=0.28). The P/AI for RTS 2, 3, 4 and 5 categories were 49.3 (72/146), 55.6 (155/279), 58.4 (93/159), and 56.7% (414/730), respectively (P=0.36). The P/AI for different location is given in Fig. 4.

Economic analysis

Mean P/AI differences between timing of insemination by synchronization protocols are shown in Fig. 3. The analysis of projected economic outcomes for 1 heifer for implementing CIDR-CO-Synch-56 protocol for heifers based on the mean P/AI difference compared to other protocols are presented in Table 3. Based on assumptions and results from this study, CO-Synch protocols without CIDR were economical even though they resulted in reduced P/AI (Table 4).

**Discussion**

The results from the current study showed that the P/AI for heifers inseminated at 56 h after PGF2α administration was greater compared to heifers inseminated at 72 h after PGF2α administration. The P/AI did not differ between CO-Synch and CO-Synch+CIDR groups. The P/AI were greater for CO-Synch-56, CIDR-CO-Synch-56, compared to CO-Synch-72; the P/AI for CIDR-CO-Synch-72 was intermediate.

Similar results were observed in a previous study where heifers synchronized with 5-day CO-Synch+CIDR protocol.[14] Evidence supports the fact that P/AI was improved in beef cows[17] and heifers[10,13] inseminated at 56 h after PGF2α in experiments involving longer periods of CIDR inserts. It is important to notice that Jersey heifers received a progesterone implant (1.38 g progesterone) for 8 days ovulated 66.5 ± 4.2 h after device removal as reported by Sales et al. (2011) who used ultrasonography to monitor ovulation every 12 h.[18] In addition, a shorter interval from progesterone implant removal to estrus (32.11 ± 1.6 vs. 38.5 ± 2.1 h) was observed in beef heifers pregnant to first service as compared to those which failed to become pregnant.[19] According to Kasimanickam et al. (2012),[14] higher P/AI would be expected in heifers inseminated 56 h after PGF2α considering that AI is recommended to occur 15 to 32 h before ovulation and that ovulation occurs 66 h after progesterone implant removal.[18]

Interestingly, heifers expressing estrus in 72 h and 56 h groups were similar, 51.6 and 52.1%, respectively. It should be noted that there was a thunderstorm between 56 and 72 h time interval plausibly resulting in reduction of estrus expression in 72 h group. However, the P/AI for heifers expressing estrus were greater in both 56 and 72 h groups, 59.1 and 64.0%, respectively. The P/AI for heifers that did not express estrus in 72 h group was 51.6%. However, heifers in 56 h group that did not express estrus had only 47.9% P/AI. Administration of the CO-Synch+CIDR protocol was associated with higher P/AI in beef cattle expressing estrus prior to FTAI compared to those that did not.[20-23] Heifers that expressed estrus prior to FTAI might have had greater serum estradiol concentrations. This is necessary for the effective preparation of follicular cells for luteinization and inducing an adequate number of uterine progesterone receptors.[24] This provides adequate uterine environment necessary for the establishment and maintenance of pregnancy. Progesterone supplementation plausibly stimulated an increase in follicular growth that results subsequently in an increased production of estrogen by ovarian follicles.[25-28]

In this study, heifers with BCS >5 achieved higher (P<0.05) P/AI, compared to heifers with BCS ≤5. Gutierrez et al. (2014) reported that P/AI was significantly affected by BCS in beef heifers assigned to AI and natural service or heifers assigned to natural service only.[22] Additionally, the BCS at the beginning of the breeding season and BCS loss from calving to breeding affected reproductive outcomes in beef.[29,30] Conversely, BCS was not associated with reproductive outcomes in dairy[31] or in beef heifers.[32] It is well known that heavier heifers attain puberty earlier than lighter herd-mates. Accordingly, it is plausible that higher percentage of heifers with lower BCS (< 5) was prepubertal at the time they were submitted to synchronization, thus achieved lower P/AI.

Excitable temperament in beef cattle is linked to poor fertility through nutritional inadequacies or stress-related response.[33-37] Lower pregnancy rates and calving rates in beef cattle with excitable temperament compared to those with calm temperament has been reported.[33,34,36,37] However, in the current study, heifer temperament did not affect P/AI. Variation of the results in these studies may be due to improved handling and management of heifers.

Given the mean differences in P/AI among different protocols from this study, the economic sensitivity analysis indicated that substantial differences in profit outcomes would result (Table 4). Insemination of heifers either at 56 or at 72h following CO-Synch protocol was beneficial even with reduced P/AI compared to CIDR-CO-Synch protocols. This is mainly because of additional cost associated with the use of CIDRs in CIDR-CO-Synch protocols. The mean P/AI difference between CIDR-CO-Synch-56 and CO-Synch-56 was 4.4%; however, the loss incurred by the use of CIDR-CO-Synch-56 compared to CO-Synch-56 was $9.79/heifer. Similarly, the mean P/AI difference between CIDR-CO-Synch-56 and CO-Synch-72 was 11.2%; however, the loss incurred by the use of CIDR-CO-Synch-56 compared to CO-Synch-56 was $6.45/heifer.

It should be noted that for a producer to implement the CO-Synch synchronization protocol without progesterone supplementation in 100 cows, the economic benefit could be as great as $979 (Table 3) compared to implementing CIDR-CO-Synch protocol. It should be noted that in all locations CIDR-CO-Synch protocol resulted in numerically greater P/AI compared to CO-Synch protocol (Fig. 4).

In conclusion, the P/AI were greater for CIDR-CO-Synch-56 compared to CO-Synch-72. However, the economic analysis revealed CO-Synch protocols without CIDR were cost effective.


## Acknowledgements

The authors thank all participating beef cattle producers. Dr. Rabie Abdel Aziz, Beni-Suef University, Beni-Suef, Egypt was financially supported by Egyptian Government Research Scholarship (SAB 2086), Egypt Cultural and Educational Bureau, The Arab Republic of Egypt. The authors thank Merial Inc., Duluth, GA for their partial support for this study.


## Conflict of interest

The authors declare that there is no conflict of interest.

## References


1. Leitman NR, Busch DC, Wilson DJ, et al: 2009. Comparison of controlled internal drug release insert-based protocols to synchronize estrus in prepubertal and estrus-cycling beef heifers. J Anim Sci 2009;87:3976-3982.
2. Patterson DJ, Kojima FN, Smith, MF: A review of methods to synchronize estrus in replacement beef heifers and postpartum cows. J Anim Sci 2003;81(E. Suppl. 2):E166-177.
3. Kasimanickam R, Schroeder S, Hall JB, et al: Fertility after implementation of long- and short-term progesterone-based ovulation synchronization protocols for fixed-time artificial insemination in beef heifers. Theriogenology 2015;83:1226-1232.
4. Bridges GA, Helser LA, Grum DE, et al: Decreasing the interval between GnRH and PGF2alpha from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology 2008;69:843-851.

5. Bishop BE, Thomas JM, Abel JM, et al; Split-time artificial insemination in beef cattle: I–Using estrus response to determine the optimal time(s) at which to administer GnRH in beef heifers and postpartum cows. Theriogenology 2016:86:1102-1110.
6. Lamb GC, Mercadante VR: Synchronization and artificial insemination strategies in beef cattle. Vet Clin North Am Food Anim Pract 2016;32:335-347.
7. Dorsey BR, Kasimanickam R, Whittier WD, et al: Effect of time from estrus to AI on pregnancy rates in estrus synchronized beef heifers. Anim Reprod Sci 2011;127:1-6.
8. Martinez MF, Kastelic JP, Adams GP, et al: The use of progestins in regimens for fixed-time artificial insemination in beef cattle. Theriogenology 2002;57:1049-1059.
9. Martinez MF, Kastelic JP, Adams GP, et al: The use of a progesterone-releasing device (CIDR-B) or melengestrol acetate with GnRH, LH, or estradiol benzoate for fixed-time AI in beef heifers. J Anim Sci 2002;80:1746-1751.
10. Colazo MG, Kastelic JP, Martínez MF, et al: Fertility following fixed-time AI in CIDR treated beef heifers given GnRH or estradiol cypionate and fed diets supplemented with flax seed or sunflower seed. Theriogenology 2004;61:1115-1124.
11. Martinez MF, Kastelic JP, Adams GP, et al: Estrus synchronization and pregnancy rates in beef cattle given CIDR-B, prostaglandin and estradiol, or GnRH. Can Vet J 2000;41:786-790.
12. Lamb GC, Larson JE, Geary TW, et al: Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. J Anim Sci 2006;84:3000-3009.
13. Walker RS, Enns RM, Geary TW, et al: Evaluation of gonadotropin-releasing hormone at fixed-time artificial insemination in beef heifers synchronized using a modified CO-synch plus controlled internal device release protocol. Prof Anim Sci 2005;21:449-454.
14. Kasimanickam R, Asay M, Firth P, et al: Artificial insemination at 56 h after intravaginal progesterone device removal improved AI pregnancy rate in beef heifers synchronized with five day CO-Synch controlled internal drug release (CIDR) protocol. Theriogenology 2012;77:1624-1631.
15. Nutrient Requirements of Beef Cattle, 8th Revised Edition. National Academic Press. 2016
16. Rodgers JC, Bird SL, Larson JE, et al: An economic evaluation of estrus synchronization and timed artificial insemination in suckled beef cows. J Anim Sci 2012;90:4055-4062.
17. Dobbins CA, Eborn DR, Tenhouse DE et al:. Insemination timing affects pregnancy rates in beef cows treated with CO-synch protocol including an intravaginal progesterone insert. Theriogenology 2009:72:1009-1016.
18. Sales JN, Neves KA, Souza AH, et al: Timing of insemination and fertility in dairy and beef cattle receiving timed artificial insemination using sex-sorted sperm. Theriogenology 2011;76:427-435.
19. Rae DO, Chenoweth PJ, Giangreco MA, et al: Assessment of estrus detection by visual observation and electronic detection methods and characterization of factors associated with estrus and pregnancy in beef heifers. Theriogenology 1999:51:1121-1132.
20. Perry GA, Smith MF, Lucy MC, et al: Relationship between follicle size at insemination and pregnancy success. Proc Natl Acad Sci USA 2005;102:5268-5273.
21. Busch DC, Wilson DJ, Schafer DJ, et al: Comparison of CIDR-based estrus synchronization protocols prior to fixed-time AI on pregnancy rate in beef heifers. J Anim Sci 2007:85:1933-1939.
22. Gutierrez K, Kasimanickam R, Tibary A, et al: Effect of reproductive tract scoring on reproductive efficiency in beef heifers bred by timed insemination and natural service versus only natural service. Theriogenology 2014;81:918-924.
23. Kasimanickam RK, Whittier WD, Hall JB, et al: Estrus synchronization strategies to optimize beef heifer reproductive performance after reproductive tract scoring. Theriogenology 2016;86: 831-838.
24. Zelinski MB, Hirota NA, Keenan EJ, et al: Influence of exogenous estradiol-17 beta on endometrial progesterone and estrogen receptors during the luteal phase of the ovine estrus cycle. Biol Reprod 1980;23:743-751.
25. Henricks DM, Hill JR, Dickey JF: Plasma ovarian hormone levels and fertility in beef heifers treated with melengestrol acetate (MGA). J Anim Sci 1973;37:1169-1175.
26. Wetteman RP, Hafs HD:Pituitary and gonadal hormones associated with fertile and nonfertile inseminations at synchronized and control estrus. J Anim Sci 1973;36:716-721.
27. Sheffel CE, Pratt BR, Ferrell WL, et al: Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. J Anim Sci 1982;54:830-836.
28. Garcia-Winder M, Lewis PE, Deaver DR, et al: Endocrine profiles associated with the life span of induced corpora lutea in postpartum beef cows. J Anim Sci986;62:1353-1362.
29. Kasimanickam R, Whittier WD, Currin JF, et al: Effect of body condition at initiation of synchronization on estrus expression, pregnancy rates to AI and breeding season in beef cows. Clin Therio 2011;3:29-41.
30. Kasimanickam R, Firth P, Asay M, et al: Impact of body condition change post-breeding on reproductive performance of beef cows. Clin Therio 2012;4:469-476
31. Stevenson JL, Rodrigues JL, Braga FA, et al: Effect of breeding protocols and reproductive tract score on reproductive performance of dairy heifers and economic outcomes of breeding programs. J Dairy Sci 2008;91:3424-3438.
32. DeRouen SM, Franke DE: Effects of sire breed, breed type and age and weight at breeding on calving rate and date in beef heifers first exposed at three ages. J Anim Sci 1989:67:1128-1137.

33. Cooke RF, Arthington JD, Austin BR, et al: Effects of acclimation to handling on performance, reproductive, and physiological responses of Brahman-crossbred heifers. J Anim Sci 2009;87:3403-3412.
34. Cooke RF, Bohnert DW, Cappellozza BI, et al: Effects of temperament and acclimation to handling on reproductive performance of Bos taurus beef females. J Anim Sci 2012;90:3547-3555.
35. Echternkamp SE: Relationship between LH and cortisol in acutely stressed beef cows. Theriogenology 1984;22:305-311.
36. Kasimanickam R, Asay M, Schroeder S, et al: Calm temperament improves reproductive performance in beef cows. Reprod Domest Anim 2014;49:1063-1067.
37. Kasimanickam R, Schroeder S, Asay M, et al: Influence of modified 2-point temperament score on A.I. pregnancy in beef heifers. Reprod Domest Anim 2014:49:775-782.

Table 1. The mean (± SEM) age (mo), interval (h) from CIDR insertion to removal, and PGF2α administration to time of insemination, and body condition scores for the synchronization groups‡

| Parameter | CO-Synch | | CIDR-CO-Synch | |
|---|---|---|---|---|
| | 56 | 72 | 56 | 72 |
| N | 342 | 339 | 315 | 318 |
| Age (mo) | 14.9±0.29 | 15.3±0.14 | 15.1 ± 0.20 | 15.0 ± 0.24 |
| Interval from CIDR insertion to removal (h) | - | - | 170±0.11 | 167±0.28 |
| Interval from PGF2α administration to AI (h) | 56.18 ± 0.12 | 71.92 ± 0.19 | 55.98 ± 0.14 | 72.26 ± 0.16 |
| Body condition score | 5.62 ± 0.19 | 5.41 ± 0.21 | 5.53 ± 0.19 | 5.49 ± 0.17 |

‡Refer to Fig. 1 for the synchronization protocol; CIDR, Controlled internal drug release; PGF2α, Prostaglandin F2α; Body condition score, 1 to 9; 1, emaciated; 9, obese;

Table 2. The effects of explanatory variables; synchronization protocol, time of insemination, body condition score, estrus expression at or before AI, synchronization protocol by estrus expression influencing AI pregnancy in Angus cross beef heifers (n=1,314) synchronized with CO-Synch or CIDR-CO-Synch protocol and inseminated at 56 or 72h after PGF2α administration.

| Variables | DF | *F* Value | *P* > *F* |
|---|---|---|---|
| Synchronization treatment[1] | 1 | 0.43 | 0.5119 |
| Time of insemination[2] | 1 | 10.16 | 0.0015 |
| Body condition categories[3] | 1 | 6.08 | 0.0138 |
| Estrus expression at or before AI | 1 | 7.66 | 0.0057 |
| Synchronization treatment × estrus expression | 1 | 10.03 | 0.0016 |
| Synchronization treatment × time of insemination | 1 | 9.61 | 0.0071 |

[1]Refer to Fig. 1 for synchronization protocol;
[2]Insemination at 56 or 72 h from PGF2α administration;
[3]Body condition Score (1 to 9) -1, emaciated; 9, obese; Categories, (<4, 5 to 6 and >6);
DF, Degrees of freedom;
Covariance parameter estimates: Location, 0.01486±0.01042; A.I. sire, 0.051836±0.03596; Inseminators, 0.01567±0.01197; Residual 0.2052±0.008104; Fit statistics - BIC =1730.14; -2 Res log likelihood =1721.82;

Table 3. The economic analysis[a] per heifer (based on analysis for 100 heifers) for implementing the CIDR-CO-Synch-56 h FTAI protocol compared to other protocols

| Protocols | Mean P/AI (%) | Mean P/AI (%) for CIDR-CO-Synch-56 | Mean P/AI (%) difference[a] | Loss per pregnancy[b] ($) | Saved CIDR cost[c] ($)/heifer | Benefit per heifer[d] ($) |
|---|---|---|---|---|---|---|
| CO-Synch -56 | 56.9 | 61.3 | 4.4 | -2.16 | 11.95 | 9.79 |
| CIDR-CO-Synch-72 | 55.3 | 61.3 | 6.0 | -2.95 | 0 | -2.95 |
| CO-Synch -72 | 50.1 | 61.3 | 11.2 | -5.50 | 11.95 | 6.40 |

CIDR, controlled internal drug releasing devise; P/AI, pregnancy per AI;
[a]How different P/AI will impact economic outcomes under a given set of parameters (the difference in mean P/AI for CIDR-CO-Synch-56 protocol minus mean P/AI for other protocols);
[b] Loss per pregnancy = (Mean P/AI difference × 49.14)/100; $49.14 US advantage per P/AI in the CIDR-CO-Synch treatments compared with a pregnancy from natural service [32];
[c]Additional cost, CIDR = $11.70; 25c/heifer for insertion of CIDR;
[d]Benefit per heifer = Loss per AI pregnancy – Saved CIDR cost per heifer.

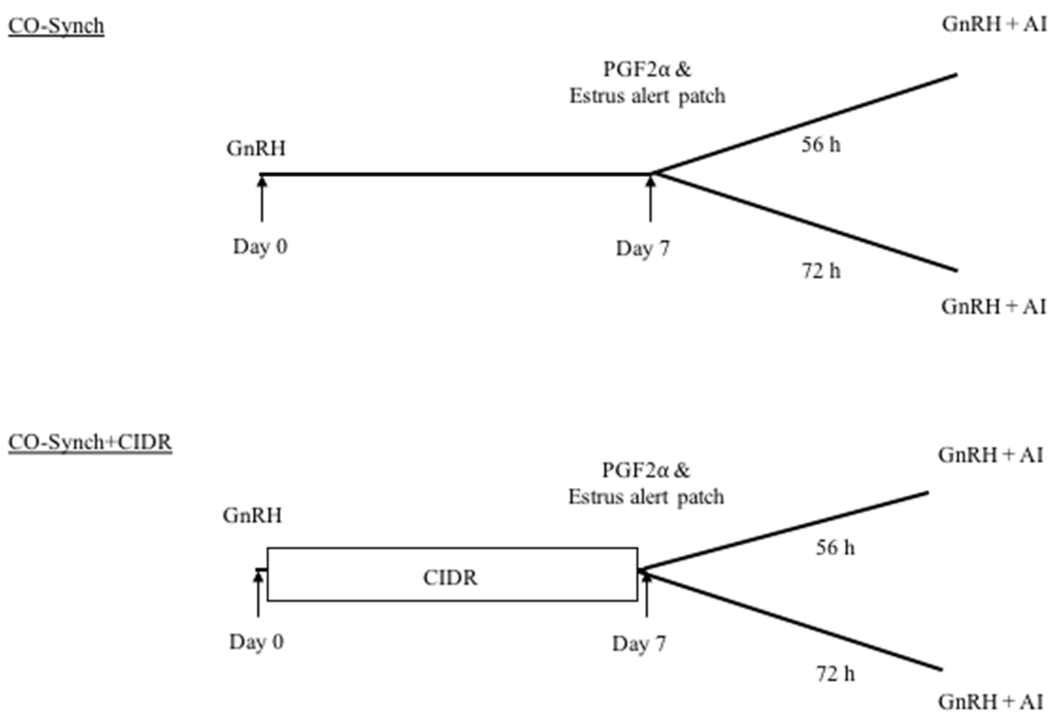
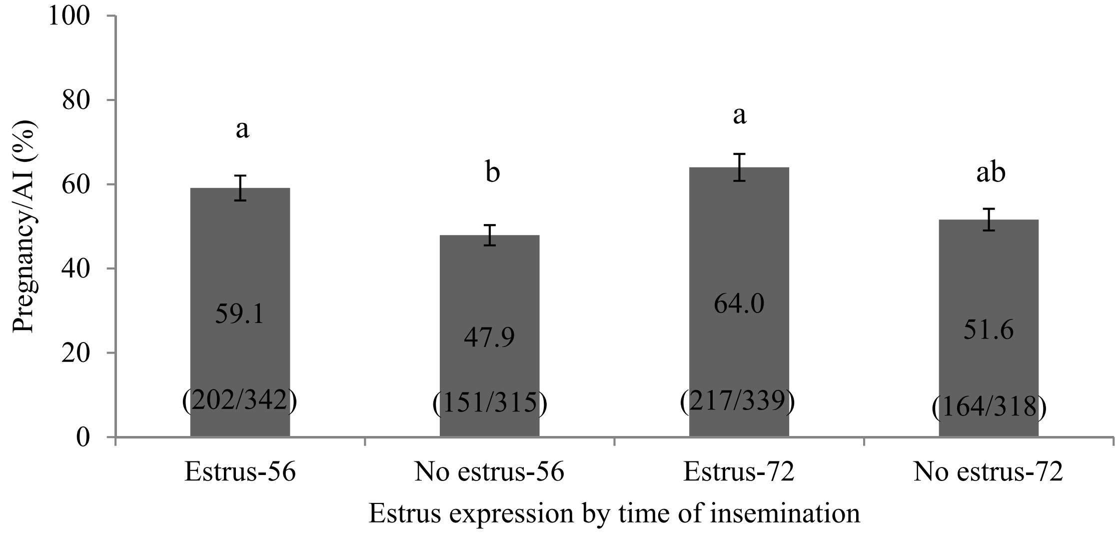
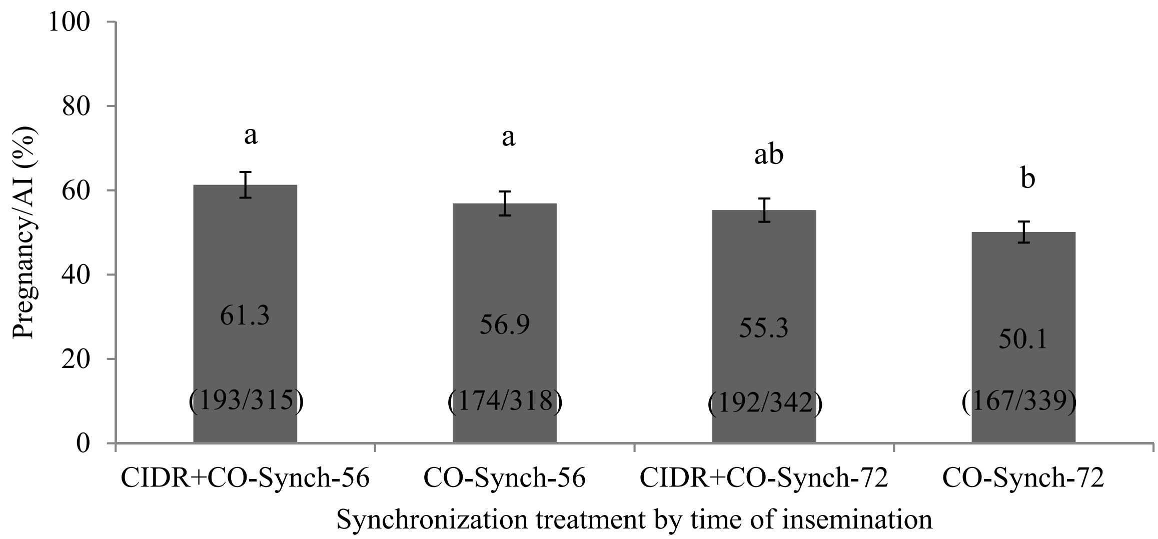



Fig. 1. Schematic presentaiton of ovulation sycnhronization protocol
Angus cross beef heifers (n=1,314) were randomly assigned to CIDR-CO-Synch (n=633) and CO-Synch (n=681) synchronization protocol groups. All heifers were given body condition score (BCS: 1, emaciated; 9, obese) and A temperament score (0, calm, slow chute exit; walk, 1, excited, fast chute exit; jump, trot or run). Briefly, heifers in CIDR-CO-Synch group received a 1.3 g progesterone intravaginal insert (CIDR) and 100µg of gonadorelin diacetate tetrahydrate (GnRH) im, on day 0 and on day 7, the CIDRs were removed and a single dose of 25 mg dinoprost tromethamine (PGF2α) im was administered. Heifers in CO-Synch were given 100µg of GnRH im on Day 0 and on Day 7, 25 mg PGF2α im. Heifers in both experiments were randomly assigned to AI56 (n=657) or AI72 (n=657) groups and were inseminated at 56 h or 72 h after PGF2α administration, respectively. All heifers received 100µg of GnRH im concurrently at the time of AI. On Day 7 at the time of CIDR removal, all heifers were fitted with estrus detection aids and were observed for estrus, twice daily, from PGF2α administration to AI for estrus. Heifers were examined for pregnancy status between 50 and 70 d after AI using ultrasonography.



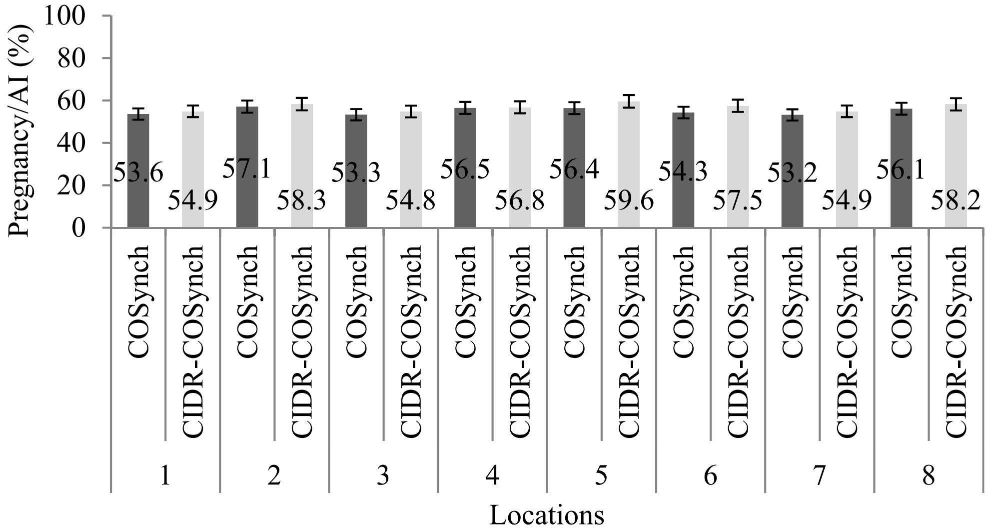

Figue 2. The pregnancy per AI (%) to time of artificial insmeination in beef heifers that expressed estrus or not following synchronization* of beef hefiers with CO-Synch protocols with or without progesterone supplementaiton and insemianted at 56 or 72h after PGF2α administration.

[1]Refer to Fig. 1 for synchronization protocol;




Figue 3. The pregnancy per AI (%) of beef heifers following CO-Synch ovulation synchronization[1] with or without progesterone that were inseminated at 56 or 72 h after PGF2α adminstration.

[1]Refer to Fig. 1 for synchronization protocol;




Figue 4. The pregnancy per AI (%) of beef heifers following ovulation synchronization[1] that were housed in different locations.
[1]Refer to Fig. 1 for synchronization protocol