Running Head: Synchronized AI breeding in suckled *Bos indicus* × *Bos taurus* cows

Select Synch and Co-Synch protocols using a CIDR yield similar pregnancy rates after a fixed-time insemination in suckled *Bos indicus* × *Bos taurus* cows

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Abstract

Primiparous and multiparous suckled *Bos indicus* × *Bos taurus* cows were used to compare a Select Synch + CIDR and timed-AI protocol (SSC) to a modified CO-Synch + CIDR protocol (COS). Five separate groups of cows were utilized (n = 659) and within a group cows were allotted to two treatments based on body condition score (BCS) taken 10 d prior to and days postpartum (DPP) at CIDR insertion (Day 0). Blood samples were collected on Day -10 and 0 for assessment of circulating concentrations of progesterone to determine estrous cycling status. Cows in both treatments received GnRH (100 µg im) and a CIDR inserted on Day 0. On Day 7, the SSC treatment had CIDR removed and received PGF_{2α} (25 mg im); whereas, COS treatment had CIDR removed and received PGF_{2α} (25 mg im) on Day 7.5. In the SSC treatment, estrus was detected at 0700 and 1700 h daily for 72 h post-PGF_{2α} and cows were AI 8 to 12 h after a detected estrus. Cows failing to exhibit estrus were timed-AI and received GnRH (100 µg im) 76 ± 4 h post-PGF_{2α}. In the COS treatment, cows received GnRH (100 µg im) and were fixed timed-AI at 64 ± 4 h post-PGF_{2α}. Although group differences ($P < 0.05$) existed for synchronized AI pregnancy rates, there were no significant interactions of group with any variable analyzed; hence, groups were pooled for further evaluation. Within the SSC treatment, the 72 h estrous response was 50.6% (168/332). Conception rate of cows in estrus was 66.1% (111/168), and timed-AI pregnancy rate for non-responders was 32.3% (53/164). Overall synchronized AI pregnancy rates were similar ($P > 0.05$) for SSC (49.4%; n = 164/332) and COS (47.1%; n = 154/327). Estrous cycling status and DPP did not ($P > 0.05$) influence synchronized AI pregnancy rates but BCS did ($P < 0.05$). Cows with a BCS ≥ 5 had synchronized AI pregnancy rates of 50% compared to 39% for cows with BCS < 5.0. In summary, the modified COS protocol yielded similar synchronized AI pregnancy rates compared to the SSC protocol

and it appears that the modified COS protocol has the potential to be an effective timed-AI protocol in suckled *Bos indicus* × *Bos taurus* cows.

Keywords: artificial insemination, beef, *Bos indicus*, CIDR, estrous synchronization

1. Introduction

Development of a synchronization protocol that achieves acceptable and repeatable pregnancy rates in *Bos indicus* × *Bos taurus* cattle is important in subtropical regions of the world where a majority of cattle contain some *Bos indicus* genetics. Numerous synchronization protocols have been developed in *Bos taurus* cattle that yield acceptable AI pregnancy rates while minimizing the number of cattle workings and days needed to detect estrus. One of the more consistent protocols in *Bos taurus* cattle is the Select Synch + controlled internal drug release (CIDR) and timed-AI protocol [1]. This protocol includes GnRH administration at the initiation of a CIDR insert followed 7 d later with PGF_{2α} with estrous detection and AI for 72 h plus a timed-AI and GnRH 72 - 84 h post-PGF_{2α} for cattle not exhibiting estrus by 72 h. In a multi-site field study, Larson et al. [1] reported synchronized AI pregnancy rates of 58% in suckled *Bos taurus* cows. In contrast, there are only a few studies with limited cattle numbers in suckled *Bos indicus* × *Bos taurus* cows synchronized with the Select Synch + CIDR and timed-AI protocol where synchronized AI pregnancy rates range from 37 to 54.4% [2,3].

Because estrus can be difficult to detect in cattle of *Bos indicus* breeding [4,5] an additional approach to synchronization is to eliminate estrous detection and conduct a fixed timed-AI. A common fixed timed-AI protocol in *Bos taurus* cattle is the CO-Synch + CIDR protocol with fixed timed-AI is conducted either 48 [6] or 60 h [1] post-PGF_{2α} resulting in AI pregnancy rates of 44% and 54%, respectively. In contrast, synchronization of *Bos indicus* × *Bos taurus* cows

with the CO-Synch + CIDR protocol with a fixed timed-AI at 48 h yielded pregnancy rates between 33 to 39% [7,8]. In *Bos indicus* × *Bos taurus* cows synchronized with the Select Synch + CIDR protocol, [8] reported that the average interval from CIDR removal to the onset of estrus was 70 h, which was considerably greater than the mean interval to estrus of 55 h observed in *Bos taurus* cattle [9,10]. Zalauga et al. [11] further demonstrated that cows of *Bos indicus* × *Bos taurus* breeding synchronized with a Co-Synch + CIDR protocol with a fixed timed-AI at 66 h had a greater follicle diameter at the timed-AI, which was associated with greater pregnancy rates. Therefore, in *Bos indicus* × *Bos taurus* cattle, developing protocols to increase follicular diameter at timed-AI appears to be critical for maximizing AI pregnancy success. Potential methods to accomplish this include moving the timed-AI from 48 to 60 h post-PGF_{2α} and(or) extending the duration of CIDR placement and thereby extending the duration of follicular growth prior to an induced ovulation.

Therefore, the objective of this experiment was to evaluate the effectiveness of a Select Synch + CIDR and timed-AI protocol compared to a modified CO-Synch + CIDR protocol with the duration between CIDR insertion and removal extended from 7 to 7.5 days and the interval from PGF_{2α} to timed-AI extended to approximately 60 h in suckled *Bos indicus* × *Bos taurus* cows.

2. Materials & Methods

2.1 Animals

The experiment was conducted from January to May during a single year at two locations in northern Florida. All procedures during this experiment were approved by the University of

Florida's Institutional Animal Care and Use Committee (IACUC). Five groups of primiparous and multiparous suckled *Bos indicus* × *Bos taurus* cows (n = 659) were used. Cow age, estrous cycling status, BW, body condition score (BCS: 1 = severely emaciated, 5 = moderate, 9 = very obese) [12], and days postpartum (DPP) at controlled internal drug release (CIDR) insertion for each group are presented in Table 1. Groups 1 (n = 173) and 3 (n = 193) contained 100% multiparous cows. Group 2 contained 93 primiparous cows (first calf at 2 yr) and 39 multiparous cows, group 4 contained 26 primiparous cows (first calf at 3 yr) and 68 multiparous cows, and group 5 contained 12 primiparous cows (first calf at 3 yr) 35 multiparous cows. The degree of *Bos indicus* (Brahman based) breeding was approximately 10 to 40% for groups 1, 2 and 3 with the remainder being *Bos taurus* (Angus, Charolais) breeding. For groups 4 and 5, breed composition was known and it was 25 to 75% *Bos indicus* (Brahman based) with the remainder being *Bos taurus* (Angus based). Groups 1, 2, and 3 were started on the experimental protocol in three consecutive weeks and groups 4 and 5 were started on the experimental protocol three weeks after group 3 with 3 weeks between the start of each group. Calves remained with cows throughout the experiment and were only temporarily separated from cows when cattle were worked through the handling facilities during the synchronization protocol.

2.2 Experimental Design

Ten days prior to CIDR insertion (Day -10) and at CIDR insertion (Day 0), blood samples were collected by jugular venipuncture into evacuated tubes with an anticoagulant (EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ) for the determination of circulating concentrations of progesterone to evaluate estrous cycling status. After collection, blood samples were immediately placed on ice and transported to the laboratory where they were

centrifuged ($3000 \times g$ for 15 min) and plasma was separated and stored at -20°C until further analysis. A cow was determined to be estrous cycling if either sample had concentrations of progesterone ≥ 1 ng/mL, and non-estrous cycling (anestrus) if progesterone concentrations were < 1 ng/mL at both samples. Plasma concentrations of progesterone were determined in multiple assays by direct solid-phase RIA (Coat-A-Count, Siemens Healthcare Diagnostics Inc., Los Angeles, CA) without extraction as described by Seals et al. [13] with intra- and interassay CV of 8.3 and 21.5%, respectively. Sensitivity of the assay was 0.10 ng/mL.

Body weight and BCS were recorded on Day -10 and at the start of synchronization (Day 0), cows within each group were equally distributed by age, DPP, BCS, and breed when known to one of two treatments, which included a Select Synch + CIDR and timed-AI (SSC) protocol and a modified CO-Synch + CIDR (COS) protocol. Cows in both treatments received GnRH (100 μg im; Cystorelin, Merial, Athens, GA) at CIDR (1.38 g progesterone; Eazi-Breed CIDR, Zoetis, Florham Park, NJ) insertion on Day 0. On Day 7, SSC cows CIDR removed and cows received $\text{PGF}_{2\alpha}$ (25 mg im; Lutalyse, Zoetis, Florham Park, NJ). On Day 7.5, CIDRs were removed from COS cows and cows received $\text{PGF}_{2\alpha}$ (25 mg im; Lutalyse). The CIDRs were removed on Day 7.5 to allow additional time for follicular development in order to increase follicle size at timed-AI and so timed-AI in both the SSC and COS treatments coincided. Cows in both treatments received an estrous detection patch (Estroject, Rockway, Inc., Spring Valley, WI) at CIDR removal.

Estrus was visually detected in both treatments two times daily at 0700 and 1700 h for approximately 1 h during the 72 h following $\text{PGF}_{2\alpha}$. Estrus was defined as a cow standing to be mounted by another cow and/or a half to fully activated Estroject patch. Cows in the SSC treatment were inseminated 8 to 12 h after an observed estrus and cows that had not exhibited

estrus by 72 h after PGF_{2α} were AI and administered GnRH (100 µg im; Cystorelin) 76 ± 4 h post-PGF_{2α}. Cows in estrus at the 72 h observation were inseminated 8 to 12 h later, and not included in the timed-AI. Cows in the COS treatment were fixed timed-AI and received GnRH (100 µg im; Cystorelin) 64 ± 4 h post-PGF_{2α}.

Frozen-thawed semen was used in all groups. In groups 1 and 2, all cows were inseminated by a single AI technician and bred to a single AI sire. In group 3, all cows were inseminated by two AI technicians and bred to a single AI sire. In groups 4 and 5, cows were bred to 20 pre-assigned AI sires within each group as dictated by the farm's breeding program. In group 4, two AI technicians inseminated cows and in group 5 a single AI technician inseminated all cows. In groups 1, 2, 3, and 5, natural service sires were placed with cows 10 d after the timed-AI. In group 4, estrous detection continued for 30 d and cows displaying estrus were AI a second time. Pregnancy was diagnosed approximately 55 d after AI using a real-time B-mode ultrasonography machine (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 5.0 MHz transducer. Since there was a 10 d period in which no cows were bred by natural service sires in groups 1, 2, 3, and 5, differences in fetal size [14] as determined by ultrasonography were used to determine whether a pregnancy resulted from the synchronized AI breeding (fetal size approximately 45 mm in length) or natural service sire (fetal size approximately 25 mm or less in length) when needed.

2.3 Definitions

Estrous response was defined as the number of cows detected in estrus within 72 h after PGF_{2α} and inseminated divided by the total number of cows treated. Conception rate was defined as the number of cows that displayed estrus, were inseminated and became pregnant,

divided by the number of cows that displayed estrus and were inseminated. Timed-AI pregnancy rate was defined as the number of cows that failed to exhibit estrus and were timed-AI and became pregnant, divided by the total number of cows that were timed-AI. Synchronized AI pregnancy rate was defined as the total number of cows that were inseminated and became pregnant, divided by the total number of cows treated. Within the COS treatment, synchronized AI pregnancy rate was defined as the number of cows that were timed-AI and became pregnant divided by the total number of cows that were timed-AI. Thirty-day pregnancy rate was the number of cows pregnant during the first 30 d of the breeding season divided by the total number of cows treated.

2.3 Statistical Analysis

The GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) was used for the statistical analysis of this experiment. The effects of treatment, group, estrous cycling status, and all two and three way interactions were evaluated for synchronized pregnancy, and thirty-day pregnancy rates. Within groups that had multiparous and primiparous cows, the effects of treatment, cow age, estrous cycling status and appropriate interactions were analyzed and there were no significant cow age effects or interactions on any variables analyzed so groups were combined for all further analysis. The effects of AI sire and AI technician were nested within groups and evaluated for synchronized pregnancy rates. Cow age, DPP, and BCS were included as covariates in the final analysis. When a covariate was significant ($P < 0.05$) it was treated as an independent variable. The only significant covariate was BCS and it was divided into four categories (≤ 4.0 , 4.5, 5.0, ≥ 5.5) for additional analysis. The effect of interval from PGF_{2α} to the onset of estrus and its effect on AI pregnancy rates were evaluated within each treatment.

Adjusted odds ratios and 95% confidence intervals were calculated for synchronized AI pregnancy rates between treatments.

3. Results

There was a group effect ($P < 0.05$) on synchronized AI pregnancy rates (Figure 1), but there was not a group by treatment interaction so groups were combined for final analysis. Across groups, synchronized AI pregnancy rates were similar for SSC (49.4%) and COS (47.1%) treatments (Table 2). Synchronized AI pregnancy rates ranged from 28% to 63.1% (Figure 1). Estrous cycling status did not influence synchronized pregnancy rate and there was no treatment $\times$ estrous cycling status effect (SSC estrous cycling: $96/185 = 51.9\%$; COS estrous cycling: $82/182 = 45.1\%$; SSC anestrus: $68/147 = 46.3\%$; COS anestrus: $72/145 = 49.7\%$). Cow age and DPP did not influence synchronized AI pregnancy rate. Body condition score influenced ($P < 0.05$) synchronized AI pregnancy rate (Table 2), but there was no treatment $\times$ BCS effect. Cows with a BCS ≥ 5 (50%) had a greater ($P < 0.01$) synchronized AI pregnancy rate compared to cows with a BCS < 5 (39%). When nested within group, there were no effects of AI sire or AI technician on synchronized AI pregnancy rates.

The estrous response for the 72 h following PGF_{2 α} for the SSC treatment was 50.6% (168/332) and estrous response rates were different ($P < 0.05$) between groups (group 1: $46/89 = 51.7\%$; group 2: $29/75 = 38.7\%$; group 3: $84/97 = 55.7\%$; group 4: $29/46 = 63.0\%$; group 5: $10/25 = 40.0\%$). Estrous response for SSC cows was influenced ($P < 0.05$) by estrous cycling status at CIDR insertion, with a greater number of anestrous cows (59.2%; $87/147$) displaying estrus compared to estrous cycling cows (43.8%; $81/185$). For SSC cows that exhibited estrus

within the 72 h after PGF_{2α}, the mean interval from PGF_{2α} to the onset of estrus was 59.1 ± 0.7 h. Cow age, DPP, and BCS did not influence estrous response for the SSC treatment.

Even though cows in the COS treatment were fixed timed-AI, 52.6% (172/327) of the cows exhibited estrus during the 60 h before they were timed-AI. Estrous response rates were similar between groups (group 1: 52/84 = 61.9%; group 2: 37/77 = 48.1%; group 3: 51/96 = 53.1%; group 4: 25/48 = 52.1%; group 5: 7/22 = 31.8%). Additionally, estrous response prior to timed-AI was similar between anestrus (57.2%; 83/145) and estrous cycling (48.9%; 89/182) cows. The mean interval from PGF_{2α} to the onset of estrus was 51.0 ± 0.7 h. Cow age, DPP, and BCS did not influence estrous response for the SSC treatment.

Conception rate for SSC treatment was 66.1% (111/168) not affected by group (group 1: 34/46 = 73.9%; group 2: 20/29 = 69.0%; group 3: 32/54 = 59.3%; group 4: 19/29 = 65.5%; group 5: 6/10 = 60.0%) or estrous cycling status (cycling: 54/81 = 66.7%; anestrus: 57/87 = 65.5%). Interval from PGF_{2α} to the onset of estrus did not affect conception rates, which were 74.6% (50/63), 64.8% (35/54), and 56.9% (29/51) for cows that exhibited estrus at 48, 60, and 72 h post PGF_{2α}, respectively. Cow age, BCS, and DPP did not affect conception rate. For SSC cows that did not display estrus, mean timed-AI pregnancy rate across all groups was 32.3% (53/164). Timed-AI pregnancy rate differed ($P < 0.05$) across groups (group 1: 19/43 = 44.2%; group 2: 16/46 = 34.8%; group 3: 12/43 = 27.9%; group 4: 5/17 = 29.4%; group 5: 1/15 = 6.7%) and estrous cycling status ($P < 0.05$; cycling: 42/104 = 40.4%; anestrus: 11/60 = 18.3%). Timed-AI pregnancy rate was not affected by cow age, DPP, or BCS.

For COS cows that displayed estrus prior to the fixed timed-AI, AI pregnancy rate was 59.3% (102/172), which was not influenced by cow age, DPP, or BCS. However, interval from PGF_{2α} to the onset of estrus affected ($P < 0.05$) synchronized pregnancy rate in the COS

treatment. Timed-AI pregnancy rates were similar for cows displaying estrus at 36 h (25/34 = 73.5%) and 48 h (39/61 = 63.9%), which were both greater ($P < 0.05$) compared to cows that displayed estrus at 60 h (38/77 = 49.4%). Cows in the COS treatment that failed to display estrus prior to the fixed timed-AI had a synchronized AI pregnancy rate of 33.6% (52/155). The AI pregnancy rates for COS cows that did not display estrus were not affected by cow age, BCS, or DPP.

Thirty-day pregnancy rates were similar for SSC (209/261 = 80.1%) and COS (210/257 = 81.7%) treatments. There were no group (data not shown) or treatment $\times$ group effects on thirty-day pregnancy rates. Estrous cycling status did not influence thirty-day pregnancy rates and there was no treatment $\times$ estrous cycling status effect. Cow age, DPP, and BCS did not affect thirty-day pregnancy rate.

4. Discussion

Results of this experiment indicate that similar synchronized AI pregnancy rates can be achieved using the modified COS protocol when fixed timed-AI is performed 60 to 68 h post-PGF_{2 α} compared to the more labor-intensive SSC synchronization protocol in suckled cows of *Bos indicus* $\times$ *Bos taurus* breeding. The COS protocol allows producers the opportunity to eliminate the labor cost associated with estrus detection required of the SSC protocol without a significant reduction in synchronized pregnancy rate. Also, the COS protocol also minimizes the number of times cattle are handled, which should decrease the amount of stress imposed on cows and calves during frequent handling of cattle during the heat detection and AI portions of the SSC protocol. By decreasing the numbers of times cattle are handled it could reduce the amount of stress cattle are exposed to during the synchronization process. This is critical in *Bos indicus*

influenced cattle that are typically more excitable than *Bos taurus* cattle [15,16] and cattle with excitable temperaments have the potential for decreased reproductive performance [17,18].

Synchronized AI pregnancy rate of SSC (49%) treatment is less compared with a report in suckled *Bos indicus* × *Bos taurus* cows (98/157=62.4%)[2] and suckled *Bos taurus* cows (289/498=58%)[1] synchronized with a Select Synch + CIDR and timed-AI protocol. However, there was considerable group variation in synchronized AI pregnancy rates ranging from 28% to 59% in the SSC treatment. Similarly, Larson et al. [1] reported synchronized AI pregnancy rates ranging from 37 to 67% in suckled *Bos taurus* cows synchronized with Select Synch and timed-AI and CO-Synch protocols either with or without a CIDR.

Relative to the COS protocol, Zaluaga et al. [11] synchronized suckled *Bos indicus* × *Bos taurus* cows with CO-Synch + CIDR protocol with fixed timed-AI 66 h after PGF_{2α} and reported pregnancy rates of 45%, which is similar to the modified COS treatment in the present experiment. Previous studies in *Bos indicus* × *Bos taurus* cows using a traditional CO-Synch + CIDR protocol with fixed timed-AI at 48 h yielded considerably lower pregnancy rates (33 to 39%) [7,8]. Therefore, moving the fixed time-AI from 48 h to 64 ± 4 h post-PGF_{2α} in the modified COS treatment may be a more appropriate time to conduct the fixed timed-AI in suckled *Bos indicus* × *Bos taurus* cows. The fixed timed-AI pregnancy rates of the COS treatment are similar to suckled *Bos taurus* cows synchronized with CO-Synch + CIDR with fixed-timed-AI at 48 h (48%) [6] but still less when fixed-timed-AI is conducted at 60 h with pregnancy rates of 58.6% [19] and 54% [1]. Similar to the SCC protocol, there was considerable variation in synchronized AI pregnancy rates for the COS protocol, which ranged from 32 to 63% in the current study.

It is important to point out that acceptable synchronized pregnancy rates (> 50%) can be achieved in suckled *Bos indicus* × *Bos taurus* cows with the SCC and COS protocols. However, the resulting synchronized pregnancy rates are highly variable for both the SSC and COS protocols in suckled *Bos indicus* × *Bos taurus* cows and they appear to be more variable than synchronized pregnancy rates observed in suckled *Bos taurus* cows [1] synchronized with similar protocols. Moreover, regardless of breed composition, producers should still expect considerable variation in response to synchronization protocols as measured by AI pregnancy rates among groups of cattle based on location and (or) management differences. There are numerous factors that can affect synchronized AI pregnancy rates including breed, cows BCS, estrous cycling status, postpartum interval, and primiparous vs. multiparous status, AI technician and semen fertility.

In the current study, the only significant factor evaluated that affected synchronized AI pregnancy rates was BCS, which is consistent with other reports stressing the importance of BCS on response to a synchronized AI breeding and subsequent pregnancy rates [19-21]. Body condition score is an accurate measure of nutritional status in cattle [22] and nutritional status has a direct influence on reproductive function and estrous cycling status of suckled beef cows [23]. As expected, cows with lower BCS (< 5.0) had decreased synchronized AI pregnancy rates compared to cows with greater BCS (≥ 5.0) regardless of synchronization treatment.

One reason why the modified COS protocol resulted in greater synchronized AI pregnancy rates compared to the original Co-Synch + CIDR with fixed-timed-AI at 48 h protocol (33 to 39%) [7,8] could be due to enhanced follicle development. By extending the duration of CIDR retention for an additional 12 h, the extra time probably allowed for additional follicular growth and development resulting in larger follicles at CIDR removal. In *Bos indicus* × *Bos taurus* cows

synchronized with a CO-Synch + CIDR protocol with a fixed-TAI at 66 h, Zalauga et al. [11] reported that cows with larger follicles had greater pregnancy rates. Although we did not measure follicle diameter or circulating estrogen concentrations in the present study, extending the CIDR by 12 h as well as waiting until 60 - 68 h post-PGF to perform the fixed-timed-AI may have allowed for growth of larger follicles with greater steroidogenic activity at the fixed timed-AI. This is supported by the observation that estrous responses were similar between SSC (50.6%) and COS (52.6%) treatments even though the SSC treatment had an additional 12 h to exhibit estrus. Furthermore, 20% of the COS cows exhibited estrus 36 h after CIDR removal; whereas, no SSC cows exhibited estrus by 36 h post-CIDR removal.

Another reason the modified COS protocol was successful could be due to the timing of the second GnRH and the fact that all cows received GnRH regardless of whether or not they displayed estrus. Additional GnRH causes a LH surge and a dominant follicle exposed to greater LH pulse frequency may have greater final maturation [24] and improved estradiol production prior to ovulation. Preovulatory estradiol is essential for preovulatory LH secretion [25], and preparation of ideal oviduct and uterine environments for proper embryo development [26,27]. Therefore it is possible that the additional GnRH at fixed timed-AI may have had indirect effects on follicle development as well as the uterine environment to provide for a more conducive environment for fertilization and subsequent embryo development. Additional research will be needed to validate this hypothesis.

One concern with delaying the fixed timed-AI to 60 h in the COS treatment was the potential for reduced AI pregnancy rates of the cows displaying estrus 36 h post PGF_{2α}. In the COS treatment, 10.4% exhibited estrus at 36 h and 29.1% exhibited estrus at 48 h after PGF_{2α}. The resulting timed-AI pregnancy rates were 75.3% and 64.9%, respectively. Both of these timed-AI

pregnancy rates were greater compared to cows exhibiting estrus at 60 h (49.4%). From the time of insemination, it takes between 4 to 8 h for sperm to capacitate and reach the site of fertilization [28,29], which results in sperm arriving at the site of fertilization 24 h after estrus in cows which exhibited estrus 36 h post PGF_{2α}. Studies using the HeatWatch estrous detection system and frequent ultrasonography after the onset of estrus indicate that ovulation occurs 30 to 31 h after the onset of estrus or 13 to 16 h after the end of estrus in *Bos taurus* beef cows [30], which is similar to a report in Brahman cows where ovulation occurred 31 h after the onset of estrus [31]. However, in traditional visual estrus detection systems similar to the present study, it is difficult to determine the precise initiation, end, and duration of estrus as cows are typically observed twice daily. Hence, it is unclear if duration of estrus was extended in these cows and resulting in a delayed LH surge and subsequent ovulation with greater fertility. It was suggested by Saacke et al. [32] that late insemination (> 12 h after onset of estrus) was preferable to early insemination (< 12 h after onset of estrus), as late insemination resulted in greater fertilization rates, but overall poorer embryo quality. In contrast, early insemination resulted in decreased fertilization rates, but greater embryo quality. However, we did not characterize this in the current study. It is unclear why cows that exhibited estrus 24 h before timed-AI had greater timed-AI pregnancy rates in the current study. Additional studies will need to be completed to better characterize follicle development patterns in cattle synchronized with an extended CIDR treatment in combination with a longer interval from CIDR removal to timed-AI.

One of the major obstacles in success to a synchronization protocol in lactating postpartum beef cows is estrous cycling status and having a large number of cows estrous cycling prior to the start of the synchronization period. Estrous cycling status in this study ranged from 38 to 66% (mean 55.7%), although 4 of the 5 groups were between 59% to 66% estrous cycling. The

variation observed in this study is considerably less than reports of 16 - 65% in *Bos indicus* × *Bos taurus* cows [33] and 23 - 100% in *Bos taurus* cows [1,6]. As indicated previously, estrous cycling status did not influence synchronized AI pregnancy rates in the present study similar to a report by Saldarriaga et al. [8] in *Bos indicus* × *Bos taurus* cows but contrary to reports in *Bos indicus* × *Bos taurus* [33] and *Bos taurus* cows [1,6]. The reduced variation observed in estrous cycling status between groups may be one reason for that lack of an estrous cycling status effect in the present experiment. Additionally, use of a CIDR in the SSC and COS protocols likely aided in reinitiating estrous cycles in cows deemed anestrus at the start of the experiment since 48% of those cows became pregnant to the synchronized breeding. Therefore, inclusion of the CIDR, which has been demonstrated to initiate estrous cycles [1,20,34], combined with the mean DPP of 65 d, and adequate BCS (5.3) at the start of the synchronization, estrous cyclic status not impacting AI pregnancy rates may have been expected in the current study.

For the SSC treatment, 50.6% of cows exhibited estrus during the first 72 h after PGF_{2α}, which is similar to a report in suckled *Bos indicus* × *Bos taurus* cows (53.1%)[2] and only slightly less than *Bos taurus* cows (57.0%) [1] synchronized with a Select Synch + CIDR and timed-AI program. Estrus is difficult to detect in cattle of *Bos indicus* breeding [4,6]. Cows of *Bos indicus* breeding exhibit a shorter and less intense estrus [4,35,36], may exhibit more subtle signs of estrus [37,38], have fewer mounts during estrus [39], and often initiate estrus during the evening hours [36, 40]. However, the Estrotect patches probably aided in detecting estrus by helping to identify cows that were most likely in estrus during the early evening and night-time hours when a majority of *Bos indicus* type cows exhibit estrus [36, 40].

Conception rate for SSC cows exhibiting estrus during the 72 h post-PGF_{2α} was 66.1% similar to reports in suckled *Bos indicus* × *Bos taurus* (77%) [2] and *Bos taurus* cows (67.0%) [1] synchronized with a Select Synch + CIDR and timed-AI protocol. This is an interesting observation because of all of the differences observed between how *Bos indicus* × *Bos taurus* and *Bos taurus* cattle respond to synchronization protocols, conception rates to an observed estrus are very similar between breed types synchronized with the Select Synch + CIDR and timed-AI protocol. Consequently, estrus expression improvement post-CIDR removal should be considered to increase synchronized AI pregnancy rates.

In conclusion, there was no significant difference in the pregnancy rates achieved with the SSC and COS systems. However, the effectiveness of both protocols was significantly influenced by cows body condition as the initiation of the synchronization protocol. This suggests that the less labor intensive modified COS protocol may be a useful alternative to the SSC protocol in suckled cows of *Bos indicus* × *Bos taurus* breeding.

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Table 1. Physical description (Mean $\pm$ SD) and estrous cycling status for suckled *Bos indicus* x *Bos taurus* cows in each group used in the study.

Group	n	Estrous cycling status (%) ^a	Cow age (range)	Body weight, kg (range)	Body condition score (range) ^b	Days postpartum (range)
1	173	59.0	6.5 $\pm$ 1.9 (3 - 10)	557 $\pm$ 55 (418 - 705)	5.4 $\pm$ 0.7 (3.5 - 7.0)	74.0 $\pm$ 17.5 (48 - 128)
2	152	38.2	2.4 $\pm$ 0.6 (2 - 7)	463 $\pm$ 49 (367 - 630)	5.2 $\pm$ 0.5 (4.0 - 7.0)	75.4 $\pm$ 16.3 (47 - 102)
3	193	59.6	6.6 $\pm$ 2.0 (4 - 10)	562 $\pm$ 57 (432 - 730)	5.5 $\pm$ 0.6 (4.0 - 7.0)	62.8 $\pm$ 5.9 (54 - 99)
4	94	64.9	5.2 $\pm$ 2.2 (3 - 12)	561 $\pm$ 61 (427 - 736)	5.1 $\pm$ 0.6 (3.5 - 6.5)	71.3 $\pm$ 13.2 (50 - 99)
5	47	66.0	5.1 $\pm$ 1.8 (3 - 12)	557 $\pm$ 67 (397 - 736)	5.1 $\pm$ 0.7 (3.5 - 6.5)	53.8 $\pm$ 8.4 (41 - 69)

^a Blood samples were collected on Day -10 and 0 (CIDR insertion) of the experiment to determine estrous cycling status. A cow was defined as estrous cycling if either sample had progesterone concentrations $\geq$ 1 ng/mL, and anestrus if progesterone concentrations were $<$ 1 ng/mL at both samples.

^b Body condition score (Scale 1-9): 1 = severely emaciated, 5 = moderate; 9 = very obese.

Table 2. Synchronized AI pregnancy rates by treatment and body condition score (BCS) in *Bos indicus* x *Bos taurus* cows synchronized with either a Select Synch + CIDR and timed-AI (SSC) or modified CO-Synch + CIDR protocol.^a

Variable	n	Pregnancy rate, %	Odds ratio	95% confidence interval	P-Value
Treatment					
SSC	332	49.4	1.10	0.80 - 1.50	0.56
COS	327	47.1	Referent		
BCS ^b					
≤ 4.0	24	25.0	Referent		
4.5	94	42.6	2.22	0.81 - 6.12	0.12
5.0	186	46.7	2.64	1.00 - 6.95	0.05
≥ 5.5	355	52.1	3.27	1.26 - 8.43	0.01

^a All cows received GnRH at initiation of the 7 d CIDR treatment for SSC and 7.5 d CIDR treatment for COS, with PGF_{2α} administered at CIDR removal. For SSC cows, estrus was detected for 72 h with AI 8 to 12 h after being detected in estrus and cows not displaying estrus were timed-AI and received GnRH 76 ± 4 h post-PGF_{2α}. All COS cows were fixed timed-AI 64 ± 4 h post-PGF_{2α}.

^b BCS (Scale 1-9): 1 = severely emaciated, 5 = moderate; 9 = very obese.

Figure 1. Synchronized AI pregnancy rates of suckled *Bos indicus* × *Bos taurus* cows synchronized with either a Select Synch + CIDR and timed-AI (SSC) or modified CO-Synch + CIDR + protocol. Synchronized pregnancy rates were similar for SSC and COS, but differed ($P < 0.05$) between groups as shown in Table 1. Synchronized AI pregnancy rate means for the main effect of group were 61.4, 59.0, 41.5, 44.9, 30.0 % for groups 1, 2, 3, 4, and 5, respectively. There was no treatment × group effect. Numbers listed within each column are number of cows within a group and treatment..

Figure 1