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SYNCHRONIZATION OF *BOS INDICUS*-INFLUENCED CATTLE FOR TIMED ARTIFICIAL INSEMINATION

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Introduction

Developing successful methods for synchronizing estrus and ovulation in cattle has been a major research interest for at least the last 35 yr. Early objectives focused on the control of the corpus luteum (CL) and artificial insemination (AI) relative to estrus. Ultimately, the goal has been to achieve precise synchronization of ovulation so that cattle can be inseminated without regard to estrus. However, for most treatments developed before 1995, variation in intervals from CL regression to ovulation has resulted in highly variable timed AI (TAI) conception rates (Odde, 1990).

In recent years, owing to a better understanding of ovarian follicular dynamics, treatments have been designed to control both the CL and the timing of follicular wave emergence (Pursley et al., 1995). Most recently, the CO-Synch protocol (COS; combination of GnRH and prostaglandin F2α; PGF) has been combined with an exogenous source of progesterone, the controlled internal drug release device (CIDR), to produce timed AI (TAI) pregnancy rates that are consistently greater than 50% in *Bos taurus* females (Lamb et al., 2001; Larson et al., 2004 a, b). Thus, the CO-Synch + CIDR protocol appears to offer much greater potential for achieving highly-successful TAI in beef cattle than in the past. However, in the southern regions of the U.S and in many other locations around the world where the environment is predominantly subtropical to tropical, the need to use cows with *Bos indicus*-influenced genetics may create additional challenges. Important deficiencies in conception rates have been reported when synchronization and TAI have been employed in these types of cattle (Hiers et al., 2003), and results using the CO-Synch + CIDR protocol have been particularly disappointing (Saldarriaga et al., 2005 a, b).

The objective of this review is to summarize results of TAI programs in *Bos taurus x Bos indicus* cattle in South Texas. This will be approached by first reviewing the performance of older progestin-based protocols (Syncro-Mate-B; SMB) that were established in the 1970's (Spitzer et al. 1978) and in conjunction with different methods of calf manipulation. This will be followed by a review of recent TAI results using the more-recently developed techniques, Ovsynch, CO-Synch and Co-Synch + CIDR.

Timed AI Fertility in Brahman-Influenced Cattle: Historical Perspectives from South Texas

Conception and Pregnancy Rates in Cows Synchronized with Syncro-Mate-B: Synchronized Breeding with TAI vs Natural Service

Table 1 summarizes historical data that provides a perspective of typical TAI conception rates in Brahman-influenced cows synchronized with SMB (Williams et al., 1987; Williams, 1988). In the first study, TAI was compared to the use of bulls for synchronized breeding. Cows were stratified by BW, body condition score (BCS), age and d postpartum and either left untreated (**Control**) or synchronized with SMB followed by TAI at 48-54 h (**SMB-AI**) or turned with fertile bulls for natural service in confinement at a bull:cow ratio of 1:15 to 1:20 (**SMB-NS**) in pens measuring 25.6 x 9.6 m . Calves were removed from cows in all groups for 48 h beginning at the time of SMB implant removal. Based on serum progesterone concentrations determined in blood samples collected before and at the start of synchronization, only 49% of all cows in these studies were cycling at the time of treatment onset. Cows averaged 71 d postpartum at the time of entry into the synchronization program. Both TAI (Day 2) and d-30 pregnancy rates favored SMB-AI over both SMB-NS and non-synchronized controls (**Table 2**). Average d of birth of calves also favored SMB-AI by 5 d over controls and by 11 d over SMB-NS.

Table 1. Effect of synchronization treatments and TAI or natural service on cumulative pregnancy rates in suckled cows on d 2, 30 and 90 of the breeding season [Adapted from Williams et al., (1987) and Williams (1988) with permission]

		Cumulative Pregnancy (%)		
Treatment	No. Cows	Day 2	Day 30	Day 90
Control	97	3.0[a]	57.7[a]	85.6
SMB-AI	98	38.8[b]	74.5[b, c]	84.0
SMB-NS	95	29.5[b]	64.2[d]	85.3

[a,b] $P < 0.05$
[c, d] $P < 0.10$

Bulls used in these studies, both for NS during the synchronization period and for clean–up, were Brahman-influenced (Beefmaster, Simbrah). For a complete summary of the physiology and behavior of bulls associated with this work, the reader is referred to Williams (1988). However, in brief, bulls in the SMB-NS group averaged 23.6 services during 33 h of cow exposure, with a range of 11-41 services. The average interval between services over the entire breeding period was 54.4 min. Approximately 80-90% of this activity occurred between 24 and 36 h after implant removal. One bull was observed to service a female approximately every 15 min from 1800 to 2400 h. Many females were serviced multiple times (3-10 times), and some were not serviced at all. Therefore, the average number and percentage of estrus females serviced per bull (8.6; 72%) was markedly less than the average number of estrus females available, and represented slightly more than half of all females in each pen. Although conception rate (number pregnant/number serviced) for SMB-NS was acceptable (57.3%), total pregnancy rate (number pregnant/number available) was low (32.6%).

Table 2. Effect of synchronization treatments on average d of birth of calves during the calving season in mature Brahman x Hereford, F_1 cows (From Williams et al., 1987)

Treatment	No. calves	Average d of birth
Control	97	$36.2^{b,c}$
SMB-AI	98	$31.0^{a,c}$
SMB-NS	95	42.9^{d}

^{a,b} $P < 0.10$
^{c,d} $P < 0.05$

Effects of Temporary Calf Removal and Alien Cohabitation for Timed AI of Cows Synchronized with SMB

Synchronization protocols developed in the 1970's, such as SMB, have historically benefited when cows were temporarily weaned (usually 48 h) before targeted insemination. Temporary weaning increases both the synchrony of estrus and conception rates at TAI. However, of all the procedures involved in estrus synchronization and AI, the process of temporary weaning seems to create the greatest concern for cattlemen. This aversion to temporary weaning is not without some degree of justification, since stress and weather conditions can result in morbidity in some groups of calves. Based on studies conducted at Beeville and in other laboratories (see reviews, Williams and Griffith, 1992; 1995), we know that calf association and suckling by a calf other than a cow's own calf does not inhibit estrus and ovulation. It appears that the maternal bond must be present in order for a calf to suppress ovarian and sexual activity. Based on this information, we proposed that sets of calves could be effectively switched among groups of cows kept temporarily in pens for estrus synchronization. We proposed that 1) this process would not attenuate the positive effects of temporary weaning of "own" calves, and 2) could result in some degree of suckling and/or nurturing of alien calves by the caretaker group. Therefore, objectives were to determine the effect of alien cohabitation vs temporary weaning in conjunction with SMB treatment on estrus synchronization, conception to timed AI, behavioral characteristics of cows and calves, and weight changes of calves. A control group (SMB-S) was included in which synchronized cows were maintained with their own calves throughout.

Procedures. Two-hundred sixty-eight mature, predominantly Brahman x Hereford cows were used. Cows at Beeville were maintained on tame pastures (Coastal bermudagrass and kleingrass, except during the synchronization/AI process. Cows at the La Copita Research Area, Alice, TX, were maintained on open range except during the synchronization/AI process. Cows at each location and each year were stratified by age, BW, body condition score, and date of calving, then assigned randomly to one of three groups: 1) **SMB-W;** standard SMB treatment plus 48-h calf removal, 2) **SMB-A**; Standard SMB treatment plus alien cohabitation 3) **SMB-S**; standard SMB treatment and ad libitum suckling by the cows' own calves.

SMB treatments were begun 12 d prior to targeted AI. Calves were removed from cows for 48 h in the SMB-W group at the time of implant removal. Calves weaned from SMB-W cows were placed with SMB-A cows to serve as "aliens" for the 48-h period. Calves removed from SMB-A cows were placed in separate pens 25 to 50 yards away. They were fed hay and creep feed and provided with water during this period. Cows were maintained in dry-lot and provided free access to hay and water, and were fed 3 lb (2.36 kg) of a supplemental concentrate daily during the 48-h period. Cows were inseminated at 48 h after SMB implant removal with semen from three different bulls and three AI technicians distributed evenly among groups at each trial. All calves were returned to their own dams immediately after AI. Pairs were then returned to pasture. Three d after AI, Angus or Red Angus clean-up bulls of known fertility were placed with the cows at a bull:cow ratio of approximately 1:25 for 90 d. Forty-five to 50 d after AI, cows were examined for pregnancy by palpation and transrectal ultrasound to determine conception at TAI and during the subsequent 3-wk period. Cows were palpated again approximately 45 d after bulls were removed to determine final pregnancy rates.

Results. **Table 3** summarizes the number and percentage of SMB-A cows that allowed suckling, number and percentage of calves allowed to suckle, and average total suckling time per calf for the 48-h period. Approximately 30% of cows allowed some suckling during both years. However, only 24 and 43% of alien calves in yr 1 and 2, respectively, suckled for 15 min or more during the 2-d period. There was no advantage to SMB-A vs SMB-W with regard to calf weight loss during the temporary weaning/alien cohabitation period (data not shown).

Table 3. Behavioral characteristics of temporarily weaned cows and alien calves maintained together for the 48-h weaning period during trials at Beeville

Year	No. Cows allowing suckling ≥ 5 min. (%)	No. Diff. Calves suckling ≥ 5 min. (%)	No. Calves allowed to suckle ≥ 15 min. (%)	Average attempts/ calf	Average total suckling/calf, min.
1	8/24 (33)	13/29 (44.8)	7/29 (24)	9	14.7
2	12/41 (29)	25/41 (61.0)	18/41 (43.9)	13.1	24.0

Reproductive performance is summarized in **Table 4**. Both SMB-W and SMB-A groups had higher timed AI conception rates than SMB-S groups both years and at both locations, with one exception. During yr 2 at Beeville, conception rates to timed AI in SMB-S were unexpectedly high and similar to SMB-W. Overall, a greater number of cows in the SMB-W and SMB-A groups became pregnant at timed-AI and cumulative pregnancy rates after 3 wk of breeding favored these groups over SMB-S. Timed AI conception in SMB-W and SMB-A was 15% greater than in SMB-S.

Table 4. Conception rates to TAI and cumulative pregnancy rates in SMB-S, SMB-W, and SMB-A cows over a 2-yr period at Beeville and La Copita

		Percent Pregnant		
Group	No. Cows	Timed AI	3 wk	90 d
SMB-S	88	40.9[b]	80.6	92.0
SMB-A	90	55.5[a]	86.6	92.2
SMB-W	90	54.4[a]	87.7	95.5

[a,b] $P < 0.05$
SMB-S = SMB suckled
SMB-A = SMB alien cohabitation
SMB-W = SMB and 48-h calf removal

Comparison of Ovsynch to Syncro-Mate-B and Norgestomet-Prostaglandin

The three most important factors affecting the relative value of a synchronization protocol is the number of times cattle must be worked, the cost of hormones, and pregnancy rates. For seedstock, a wider array of protocols, including those that utilize estrus detection, can be profitably exploited. However, for commercial cows, we believe that TAI must be possible with conception rates consistently of 50% or greater. Our long-term timed AI conception rate using SMB in mature, *Bos indicus x Bos taurus* cows in combination with 48-h calf removal has averaged about 48%, but has ranged from 30 to 60%. Hence, although the SMB protocol did not allow us to consistently achieve 50% TAI conception rates, dissatisfaction with the protocol probably resided more with the variation in results than with the average. Moreover, SMB has been removed from the U. S. market, further predicating a need for alternatives.

A protocol that controlled both CL function and follicular wave emergence using a combination of GnRH and PG (Ovsynch) was first introduced in dairy cattle in the mid 1990's (Pursley et al., 1995). When applied to beef cattle, conception rates using Ovsynch and TAI were reported to be greater than with SMB in cycling *Bos taurus* cattle (Geary et al., 1998). Much earlier, another approach had been to combine a progestin (SMB implant; norgestomet) with an injection of PG 2 d before implant removal (termed herein as NP; Hansel and Beal, 1979) to achieve better control of the CL. This protocol does not address synchronization of follicular wave emergence, but was used successfully in dairy heifers, particularly in combination with estrus detection and was later used in beef cattle as well (Beal et al., 1984). Neither of these methods represented a commercially-available package, but could be utilized by combining various parts of other commercially-available treatments. Objectives of the study summarized below were to compare the relative efficacies of SMB, Ovsynch and NP to synchronize estrus and ovulation for TAI in *Bos indicus x Bos taurus* beef cows.

Procedures. In Experiment 1, 273 Brahman x Hereford (F_1) cows at 3 locations were stratified by BW, body condition score (BCS), age, and d postpartum, and assigned randomly to one of three treatment groups: 1) **SMB**; SYNCRO-MATE-B, 2) **NP**; Norgestomet-prostaglandin, and 3) **Ovsynch**. The management approach for Experiment 1 required that cows have a minimum BCS of 5 and be at least 36 d postpartum at treatment onset. In Experiment 2, a total of 286 pubertal beef heifers were stratified by weight and BCS and allocated randomly to the three treatments. Heifers were predominantly Brahman crossbred or composites (n = 239; Brahman x Hereford, F_1; Santa Cruz, and Santa Gertrudis) with a smaller proportion (n = 42) of Hereford heifers used. Syncro-Mate-B treatment consisted of the standard 9-d norgestomet ear implant plus an estradiol valerate/norgestomet injection on d 0. NP females were implanted with the same 9-d norgestomet implant as in SMB, but received 25 mg prostaglandin $F2_{\alpha}$ i.m. 2 d before implant removal and did not receive the norgestomet-estradiol valerate injection at the time of implantation. OvSynch consisted of 100 μg GnRH i.m. on d 1, 25 mg PG i.m. on d 8, and a second GnRH injection on d 10. Beginning on d 9, calves were removed for 48 h in suckled cows. Cattle in both experiments were inseminated 48-54 h after implant removal (SMB; NP) and at 12-15 h after the second GnRH injection (Ovsynch), with the exception of a small group of cattle in the first trials. Those cattle were inseminated 18-24 h after the second GnRH injection, but this did not influence conception rates.

Results. Overall mean (± SEM) BW and BCS of heifers was 350 ± 2.8 kg and 5.5 ± .03, respectively and did not differ among groups. In this experiment, all heifers had a minimum BCS of 5.0 and were confirmed pubertal based on determination of twice weekly serum progesterone concentrations. The timed AI conception rate was greatest ($P < 0.056$) in NP-treated heifers compared to SMB- and Ovsynch-treated heifers (**Table 5**). During yr-3 of our study, 52 heifers that were allotted to the OvSynch/TAI treatment were also observed for estrus throughout the 9-d treatment period. Fifteen of 52 (28.9%) exhibited a natural estrus during the treatment period. We inseminated these heifers at the natural estrus, with 8 of 15 (53.3%) conceiving. Timed AI conception rate for these heifers was considered to be 0. When conception data for OvSynch heifers inseminated after a detected estrus during the synchronization period was combined with that obtained in the balance of the Ovsynch heifers at timed AI, conception rates increased from 42.4% (timed AI alone) to 57.7% (TAI + insemination at estrus), comparable to those achieved with NP (57.7%).

Overall mean (± SEM) BW, BCS, and d postpartum for cows was 554 ± 3.5 kg, 5.9 ± .06, and 61.4 ± .8 d. Timed AI conception rates did not differ among SMB, NP, and Ovsynch, groups (**Table 5**). However, there was a tendency for NP-treated cows to have a lower ($P < 0.13$) overall conception rate than those treated with SMB or OvSynch. When cows in each treatment were categorized into late (36-59 PP, n = 126), middle (60-79 d PP, n = 116), and early (80-99 d PP, n = 31) calving groups, late calving cows in the NP treatment had a lower ($P < 0.05$) conception rate than SMB and OvSynch. Since more cows would be expected to be anovulatory in the late-calving group, we can speculate that the lack of estradiol or GnRH treatment in the NP protocol resulted in a lower induction of ovulation in anovulatory cows compared to the other treatments.

Table 5. Timed AI conception rates in nulliparous heifers and suckled cows treated with Syncro-Mate-B (SMB), norgestomet-prostaglandin (NP) or Ovsynch (adapted with permission from Williams et al., 2002)

Age	Treatment	No.	TAI conception rate, %
Heifers	SMB	99	40.4
	NP	95	54.7*
	Ovsynch	92	39.1
Cows	SMB	91	45.1
	NP	90	31.1
	Ovsynch	92	42.4

* P < 0. 056
Calves were removed from all cows for 48 h beginning on d 9

Synchronization and TAI Conception Rates in *Bos taurus* x *Bos indicus* Cattle using GnRH, PGF and CIDR Combinations

Timed AI in Brahman-Influenced Cattle using CO-Synch + CIDR: Field Trials

Recently the CO-Synch protocol (Geary and Whittier, 1998), which involves the combined use of GnRH and PGF, has been coupled with an exogenous source of progesterone, the CIDR. This combination (Co-Synch + CIDR) appears capable of producing TAI conception rates that average consistently above 50% (Lamb et al., 2001, Larson et al., 2004 a, b) in *Bos taurus* females, which are greater than those reported previously using other traditional methods (Stevenson et al., 2003a). Improved outcomes have been linked in part to the ability of exogenous progesterone to induce ovulation in a high proportion of anestrous cows (Stevenson et al., 2000) and to reduce the occurrence of estrus before TAI (DeJarnette et al., 2001; Martinez et al., 2002). However, in environments that are predominantly subtropical to tropical, the need to utilize *Bos indicus*-influenced females may reduce the efficiency of synchronization and TAI conception rates compared to *Bos taurus* females (Lemaster et al., 2001; Hiers et al., 2003). Although not well-characterized, this may occur due to increased excitability and stress in *Bos indicus*-influenced cattle when subjected to intense management and (or) differences in timing of ovarian events. Reports specifically evaluating the CO-Synch + CIDR for TAI in *Bos indicus*-influenced cattle are limited.

Objectives of studies reported herein were to 1) evaluate the use of the CO-Synch + CIDR protocol for synchronization of ovulation and TAI in *Bos indicus*-influenced cattle, 2) compare cumulative pregnancy rates after CO-Synch + CIDR synchronization and TAI to those in a traditional management (TM) scheme 3) evaluate specific ovarian, hormonal, and estrual events associated with the use of CO-Synch + CIDR and related protocols to identify aspects of the system that may contribute to reductions or improvements in efficiency of the protocol in *Bos indicus*-influenced cattle.

Procedures. All cattle in this experiment were required to have a minimum BCS of 4.8 (1-9 scale), and if suckled, be at least 50 d postpartum. Cows were stratified by parity and BCS at each location, and assigned randomly in groups of not less than 25 to either a **TM** control or a synchronized, TAI group (**CO-Synch + CIDR**). The regimen included the insertion of a CIDR (Pfizer Animal Health, New York, NY) and an injection of GnRH (GnRH-1; 100 µg Cystorelin, Merial, Iselin, NJ) on d 0, removal of the CIDR and injection of PGF (25 mg Lutalyse; Pfizer Animal Health, New York, NY) on d 7, and an injection of GnRH (100 µg GnRH-2) and TAI at 48 h after PGF and CIDR removal (d 9). Cows in TM were managed as normal for each location, with both groups placed with fertile bulls for at least 60 d beginning 5 to 7 d after TAI. Pregnancy rates to TAI were determined in both groups by transrectal ultrasonography 30 d after TAI in the CO-Synch + CIDR group. Final pregnancy rates were assessed by palpation per rectum 45 d after the end of the breeding season. Control (TM) cattle were not available at all locations for management comparisons to CO-Synch + CIDR. Therefore, while there were 266 cows and heifers synchronized for TAI, only 170 were managed with a contemporary set of TM females (n = 165) for comparison.

Results. Timed AI pregnancy rates in all females synchronized with CO-Synch + CIDR are summarized in **Table 6**. Pregnancy rates using the CO-Synch + CIDR protocol and TAI at 48 h after CIDR removal averaged about 39%. Pregnancy rates were not affected by location (n = 4), yr (n = 2), BCS, d postpartum, parity, sire or AI technician. **Table 7** summarizes cumulative pregnancy rates after 30 and 60 d of breeding (TAI and/or natural service) for the CO-Synch + CIDR group and the contemporary control groups (TM). Overall, cumulative pregnancy rates were greater ($P < 0.05$) in synchronized cows at 30 and 60 d of the breeding season than in the TM group.

Table 6. Timed AI (TAI) pregnancy rates in nulliparous heifers, postpartum primiparous heifers, and pluriparous cows synchronized with CO-Synch + CIDR

Source	N	TAI Pregnancy Rate, %
Nulliparous	89	39.3
Primiparous	34	35.3
Pluriparous	143	39.9
Total	266	39.1

Table 7. Cumulative pregnancy rates after 30 and 60 d of breeding in nulliparous heifers, primiparous heifers, and pluriparous suckled cows synchronized with CO-Synch + CIDR followed by timed AI (TAI) or managed using traditional methods (TM)

		Cumulative Pregnancy Rate[a], %		
Source	Treatment	N	30 Days	60 Days
Nulliparous	CO-Synch + CIDR	62	75.8	95.2
	TM	71	71.8	88.7
Primiparous	CO-Synch + CIDR	34	67.6	100.0
	TM	28	60.7	89.3
Pluriparous	CO-Synch + CIDR	74	75.7[b]	94.6
	TM	66	51.5[c]	90.9
Total	CO-Synch + CIDR	170	74.1[b]	95.9[b]
	TM	165	61.8[c]	89.7[c]

[b,c] Percentages in columns with uncommon superscripts differ $P < 0.05$.

Follicular, Luteal and Hormonal Characteristics of CO-Synch and CO-Synch + CIDR Synchronization

Timed artificial insemination at 48 h after CIDR removal with CO-Synch + CIDR synchronization failed to produce acceptable (≥ 50%) TAI conception rates in *Bos indicus* influenced cattle. We hypothesize that CO-Synch + CIDR produced such results due to failure of one or more aspects of the procedure, which could potentially include: failure to 1) optimize the frequency of ovulation or regression of follicles on d 0; 2) cause optimally timed emergence of a new follicular wave between days 1 to 4; 3) efficiently regress the CL at the time of PGF; 4) produce an optimally-receptive preovulatory follicle at the time of the second GnRH injection.

Procedures. To gain further insight into ovarian and hormonal events associated with CO-Synch + CIDR synchronization in *Bos indicus*-influenced cattle, 100 postpartum Brahman x Hereford (F_1) cows were divided into four replicates of 25 females each. Criteria for inclusion in the study and stratification procedures were similar to the field trials discussed above. Cattle were placed in pens measuring 25.6 x 9.6 m 8 d before the onset of treatments, with five cow-calf pairs per pen, and fed according to National Research Council (NRC) recommendations for lactating beef cows. Half of the cows within each replicate (n = 12-13) received the CO-Synch + CIDR treatment and half the CO-Synch treatment alone without the CIDR. The CO-Synch + CIDR treatment was as described previously in Experiment 1.

Transrectal ultrasonography was performed every other d from d -8 to d 0, and then daily from d 0 until ovulation or d 12, whichever occurred first. Blood samples were collected via puncture of a coccygeal tail vessel following the same schedule as for transrectal ultrasonography. Serum was assayed by RIA for progesterone. Concentrations of

LH were also determined in blood samples collected during the first replicate at 0, 30, 60 and 120 min relative to GnRH injections on d 0 (GnRH-1) and 9 (GnRH-2). Cows were observed for estrus 3x daily from d 0 until ovulation or d 12, whichever occurred first, with the aid of androgenized cows. On d 12, all cows were returned to their pasture with clean-up bulls for a 60-d breeding period. Pregnancy determination was performed by transrectal ultrasonography at 30-32 d post AI, and re-confirmed by palpation per rectum 45 d after bulls were removed.

Results. Mean (± SEM) age, BCS, BW, and d postpartum were 8.8 ± 0.3 yr, 5.3 ± 0.07 (range 4-8), 543 ± 7.4 kg, and 77 ± 0.66 d, respectively. Ovarian and reproductive variables are summarized in **Table 8**. No differences in the major ovarian and reproductive endpoints were observed between CO-Synch + CIDR and CO-Synch. Therefore, data for both treatments are presented as pooled means. Data are also presented relative to cyclic status at the onset of treatments. The number of non-cyclic cows ovulating after GnRH-1 was greater ($P < 0.01$) than for cyclic cows. The number ovulating in response to GnRH-2 also differed between cyclic and non-cyclic cows; however, in this case, cyclic cows had the greater ($P < 0.05$) response. Mean follicular diameters are presented in **Table 9**. Non-cyclic cows had greater ($P < 0.05$) mean follicular size at PGF than cyclic cows, and therefore a greater ($P < 0.05$) follicular growth rate. Follicular sizes were not different at the subsequent stages.

Data were also summarized relative to presence or absence of ovulation after GnRH-1 to evaluate their effects on subsequent ovarian responses (**Table 10**). More ($P < 0.01$) cows that ovulated after GnRH-1 developed a synchronized follicular wave compared to cows that did not ovulate. Moreover, there was a trend ($P = 0.15$) for ovulation rates after GnRH-2 to be greater in cows that ovulated in response to GnRH-1 than cows that did not. Also, ovulation and TAI pregnancy rates after GnRH-2 were increased ($P < 0.01$) in cows that developed a synchronized follicular wave after GnRH-1 compared to cows that did not develop a new wave (**Table 11**).

Mean serum concentrations of progesterone are illustrated in **Figure 1**. As expected, concentrations of progesterone from d -8 to 0 relative to GnRH-1 differed between cyclic and non cyclic cows. After CIDR insertion (d 0), serum progesterone increased ($P < 0.001$) acutely for both cyclic and non-cyclic cows that received the CO-Synch + CIDR treatment. Serum concentrations of progesterone on d 1 were highest ($P < 0.05$) for cyclic cows receiving CO-Synch + CIDR compared to all other groups. Mean concentrations of progesterone did not differ between cyclic cows treated with CO-Synch + CIDR and non-cyclic cows treated with CO-Synch + CIDR. Mean concentrations of progesterone were lowest ($P < 0.01$) for the non-cyclic CO-Synch group compared to all others, and mean serum concentrations of progesterone never exceeded 1 ng/ml during the treatment period. After injection of PGF and CIDR removal (d 7), progesterone decreased below 1 ng/ml within 24 h in all groups and remained low until d 12 when mean progesterone exhibited a slight increase ($P = 0.09$) in cyclic, CO-Synch treated cows. The latter was caused by two cows that ovulated asynchronously before d 9.

Table 8. Ovarian and reproductive outcomes in postpartum suckled cows synchronized with CO-Synch and CO-Synch + CIDR and for cycling and non-cycling cows (Treatments did not differ; therefore, data are presented as pooled means.)

Variable	All Cows	Ovarian Status	
		Cycling	Non-cycling
No. Cows	100	78	22
Estrous cycling, %	78	-	-
Response to GnRH-1, %			
Ovulating	40	33[c]	64[d]
Follicle regression	39	40	36
Not responding	21	27[c]	0[d]
New follicular wave after GnRH-1, %			
Synchronized[a]	60	56	73
Not synchronized[b]	31	35	18
No emergence	9	9	9
Day of emergence	2.5 ± 0.12	2.4 ± 0.15	2.75 ± 0.23
CL regression, % (No.)	92 (75/81)	91(61/67)	100(14/14)
Ovulatory Response to GnRH-2, %			
0-24 h after TAI	15	14.1	36.3
24-48 h after TAI	57	62.8	18.2
Total	72	76.9[c]	54.5[d]
TAI pregnancy, %			
Ovulation 0-24 h after AI	9	10.3	4.5
Ovulation 24-48 h after AI	24	23	27.3
Total	33	33.3	31.8

[a] Cows that developed a follicular wave from d 1 to d 4 after GnRH-1.
[b] Cows that developed a follicular wave before day 1 and after day 4.
[c,d] Percentages within row with uncommon superscripts letters differ ($P < 0.01$).

Table 9. Mean follicular diameters in postpartum suckled cows synchronized with CO-Synch and CO-Synch + CIDR at different stages of the experiment (Treatments did not differ; therefore, data are presented as pooled means.)

Variable	All Cows	Ovarian Status	
		Cyclic	Anestrous
Diameter of the largest Follicle, mm (range)			
GnRH-1	9.6 ± 0.2	9.4 ± 0.2	10.2 ± 0.3
	(4.0 - 12.95)	(4.0 - 12.95)	(6.8 - 12.3)
PGF	9.8 ± 0.2	9.6 ± 0.2[a]	10.5 ± 0.2[b]
	(6.3 - 15.4)	(6.3 – 13.9)	(7.0 - 15.4)
GnRH-2	11.1 ± 0.2	11 ± 0.3	11.4 ± 0.5
	(6.0 - 15.4)	(6.0 – 15.4)	(7.5 – 14.5)
Before ovulation	11.6 ± 0.2	11.4 ± 0.2	12.2 ± 0.5
	(8.1-15.4)	(8.1 – 15.4)	(9.1 – 14.7)
Follicular growth rate, mm/day	1.4 ± 0.06	1.3 ±0.07[a]	1.7 ± 0.1[b]

[a,b] Percentages within row with uncommon superscripts letters differ ($P < 0.05$).

Table 10. Effects of the response to the first GnRH injection (GnRH-1) on subsequent ovarian and reproductive outcomes in cows synchronized with CO-Synch and CO-Synch + CIDR (Treatments did not differ; therefore, data are presented as pooled means.)

Variable	Ovulatory Response to GnRH-1	
	Ovulating No. (%)	Not Ovulating No. (%)
No of cows	40	60
Synchronized follicular wave		
Yes	35 (88)[a]	25 (42)[b]
No	5 (12)	35 (58)
Ovulated after GnRH-2		
Yes	32 (80)	40 (67)
No	8 (20)	20 (33)
TAI pregnancy	15 (37)	18 (30)

[a,b] Percentages within rows with uncommon superscripts differ ($P < 0.01$).

Table 11. Effects of synchronized follicular wave emergence after GnRH-1 on subsequent ovarian and reproductive outcomes in cows synchronized with CO-Synch and CO-Synch + CIDR (Treatments did not differ; therefore, data are presented as pooled means.)

	Occurrence of Synchronized Follicular Wave after GnRH-1	
	Yes	No
Variable	No. (%)	No. (%)
No of cows	60	40
Ovulation after GnRH-2		
Yes	51 (85)[a]	21 (52)[b]
No	9 (15)	19 (48)
TAI pregnancy	26 (43)[a]	7 (17)[b]

[a,b] Percentage within row with uncommon superscripts letters differ ($P < 0.01$).

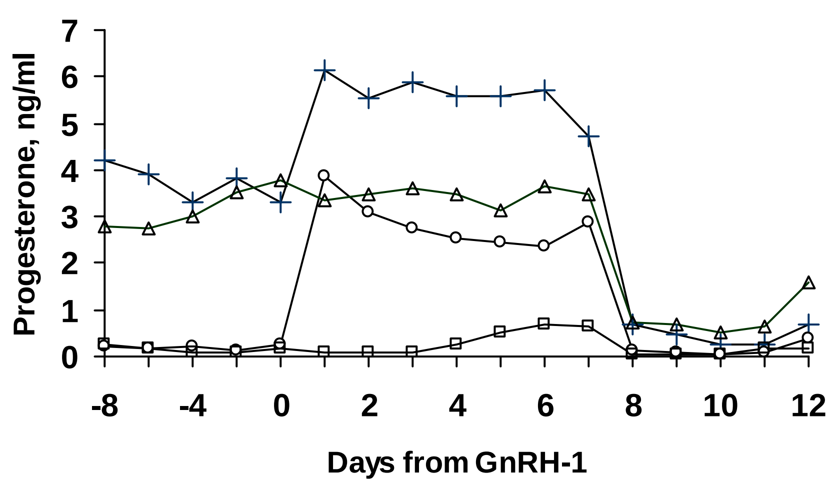

Figure 1. Concentrations of progesterone in serum of cycling (+; n = 39) and non-cycling (○; n = 11) cows treated with CO-Synch + CIDR, and cycling (Δ; n = 39) and non-cycling (□; n = 11) cows treated with CO-Synch only

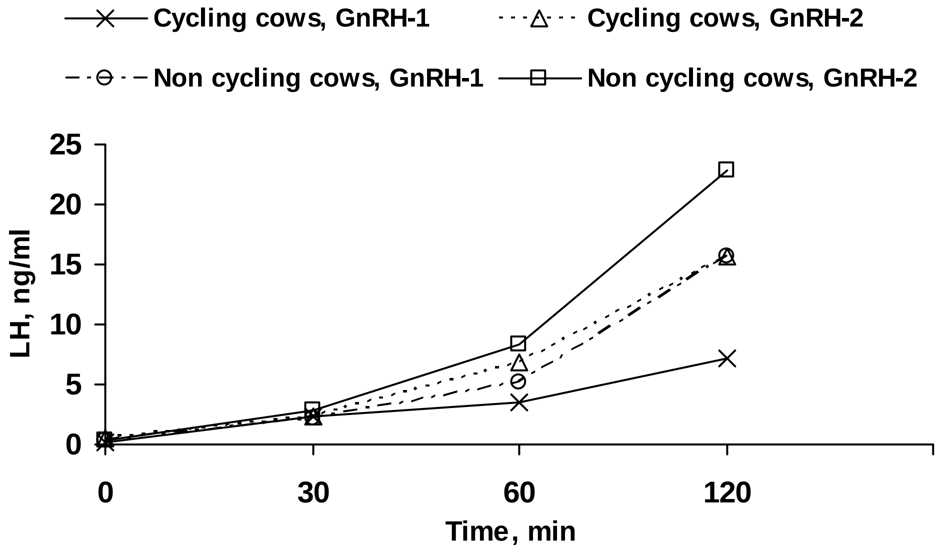

Figure 2. Mean serum concentrations of LH after GnRH-1 in cows that were cycling (n = 15) and not cycling (n = 10) before treatment onset, and in cyclic (n = 14) and non-cyclic (n = 9) cows after GnRH-2. Cows not cycling before treatment onset had greater ($P < 0.05$) induced release of LH after GnRH-1 than cycling cows, but not after GnRH-2 (cycling status x time, $P < 0.05$).

Release of LH induced by GnRH was considered to have occurred when an increment in the concentration of LH of at least 2 SD above the baseline was observed. Two cows had an endogenous LH surge before GnRH-2 and were excluded from further analysis in relation to this variable. The latter conclusion was based on the fact that concentrations of LH during the sampling period were in a declining mode. All other cows (n = 23) in replicate 1 exhibited increases ($P < 0.01$; **Figure 2**) in LH after both GnRH-1 and 2. Magnitude of release did not differ between treatments (CO-Synch + CIDR vs CO-Synch). Non-cyclic cows had an induced LH release greater ($P < 0.05$; Fig 3) than cyclic cows after GnRH-1, but concentrations of LH did not differ between cyclic and non-cyclic cows after GnRH-2. A time x cyclic status interaction ($P < 0.05$) associated with GnRH-induced LH release was observed after GnRH-2. Also, overall mean concentrations of LH were greater ($P < 0.01$) after GnRH-2 than after GnRH-1 (7.2 ± 0.71 and 4.3 ± 1.1, respectively).

Distribution of Estrus and Ovulation in Cows Programmed with Select-Synch + CIDR

Timing of insemination relative to ovulation is one of the most important factors affecting the outcome of synchronized, timed breeding in cattle. This becomes even more critical when TAI is combined with an injection of GnRH (GnRH-2) as in the CO-Synch + CIDR protocol. Based upon previously published reports, we employed TAI at 48 h after CIDR removal/PGF injection in the experiments summarized above. However, since TAI pregnancy rates were low and not similar to those reported for *Bos taurus* cattle, a third experiment was performed to determine when GnRH-2 and TAI would be most appropriate in our animal model.

Procedures. Fifty postpartum, suckled Brahman x Hereford (F-1) females were used. Criteria for inclusion were the same as for Experiments 1 and 2. Cows in the study were primiparous heifers (n = 32), and pluriparous cows (n = 18). Females were placed in pens as in Experiment 1, with 8 cow-calf pairs per pen, and fed according to NRC recommendations (1996). All cows received the same synchronization regimen as described in Experiment 1, but the second GnRH injection (GnRH-2) was not administered. Transrectal ultrasonography was performed the day of CIDR removal, and then every 12 h until ovulation or d 11, whichever occurred first. Estrus detection was performed by visual observation every 3 hours from CIDR removal through d 11. Blood samples were collected on d -21, -11, 0 (CIDR insertion), 7, 8 and 9 following the same procedures described in Experiment 2. Serum was assayed by RIA for progesterone in all samples collected as described in Experiment 2 to retrospectively estimate cyclicity and luteal regression.

Results. Neither ovarian cyclic status (cyclic 60%, non-cyclic 40%) nor parity affected the number of cows exhibiting estrus or ovulating. Mean age (± SEM), BCS, BW, and d postpartum were 5.81 ± 0.5, 5.6 ± 0.1, 565 ± 10.2 kg and 60 ± 1.1 d, respectively. On d 7, a majority of cows (72%) had a visible CL at ultrasound and 97% of those exhibited CL regression after PGF, as evidenced by a reduction in ultrasonographic size and morphology of the CL and a reduction in serum concentrations of progesterone to less than 1 ng/mL. No cows were observed in estrus during the first 48 h after CIDR removal. The majority (75 %) of estrual events was observed between 60 and 82 h after CIDR removal (**Figure 3**). Mean size of the largest follicle at CIDR removal and 48 h after removal were 9.45 ± 0.26 and 11.65 ± 0.26 mm, respectively (**Table 12**). Follicular diameter was greater for cows showing standing estrus than for cows showing only non-standing estrous behavior or no estrous behavior at both CIDR removal ($P < 0.05$) and 48 h after removal ($P < 0.01$). Cows that showed standing estrus had more ($P < 0.01$) ovulations than cows not standing.

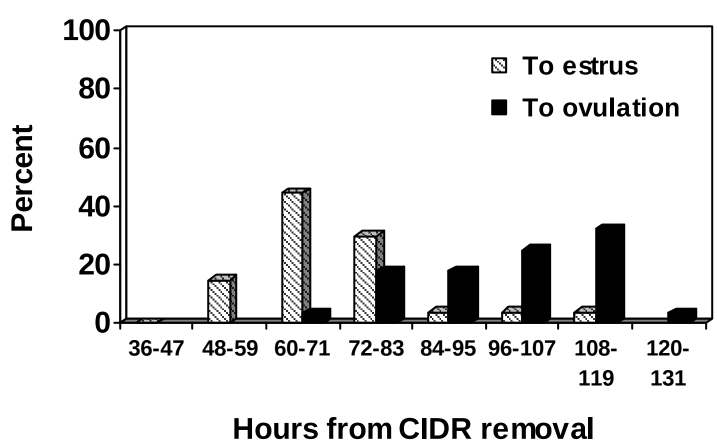

Figure 3. Distribution of estrus (n = 27) and ovulation (n=28) in suckled *Bos indicus x Bos taurus*, F_1 cows treated with Select Synch + CIDR

Table 12. Follicular, Estrual, and ovulatory events in suckled *Bos indicus x Bos taurus,* F_1 cows programmed with Select Synch + CIDR

Variable	All Cows	Estrus: Standing	Estrus: Non-Standing	Estrus: None
Number of Cows	50	27	14	9
Mean follicle size, mm				
At CIDR removal		10.1 ± 0.4^{a}	8.8 ± 0.6^{b}	8.62 ± 0.3^{b}
(range)		(6.1 - 13.5)	(6.0 - 12.8)	(7.2 - 10.2)
48 h after CIDR removal (range)		12.6 ± 0.4^{c} (9.6 - 14.7)	10.4 ± 0.4^{d} (8.3 - 13.8)	10.83 ± 0.4^{d} (9.7 - 13.9)
Ovulating, %	56	93^{c}	21^{d}	0^{d}
Mean ovulatory follicle Size, mm (range)	12.9 ± 0.3 (9.4 - 15.1)	12.9 ± 0.3 (9.4 - 15.1)	13.5 ± 0.5 (12.5- 14.1)	-
Mean interval from CIDR removal to:				
Standing estrus, h (range)		70 ± 2.9 (49 - 108)	-	-
Ovulation, h (range)	99 ± 2.8 (68 - 127)	99 ± 3 (68-127)	104 ± 11 (82 - 117)	-
Mean interval from estrus to ovulation, h (range)		29 ± 2.2 (5 - 55)	-	-

[a,b] Percentage within row with uncommon superscripts letters differ ($P < 0.05$).
[c,d] Percentage within row with uncommon superscripts letters differ ($P < 0.01$).

SUMMARY AND CONCLUSIONS

Ovsynch vs SMB. Synchronization of ovulation in *Bos indicus*-influenced beef cows managed in a subtropical environment using an earlier-generation, progestin-based (SMB) or GnRH/prostaglandin-based (Ovsynch) synchronization protocol resulted in TAI conception rates comparable to each other (Williams et al., 2002) and similar to those reported in *Bos taurus* cattle (Geary and Whittier, 1998). The progestin-based treatment utilizing only the norgestomet implant and a prostaglandin (NP) tended to give lower results than both SMB and Ovsynch in suckled cows, and this difference was observed primarily in cows less than 60 d PP.

In nulliparous heifers confirmed pubertal, the NP protocol yielded TAI conception rates greater than both SMB and Ovsynch (Williams et al., 2002). The lower conception rate in Ovsynch-treated heifers compared to SMB can only be obviated by inseminating heifers observed in estrus (non-synchronized females) for 9 d during the synchronization period in combination with timed AI of the balance of heifers on d 11. In the current study, and in previous reports, failure of heifers to respond to the first GnRH injection results in a reduced number of heifers with a functional CL. Therefore, these heifers are not synchronized and exhibit estrus before the targeted insemination period.

CO-Synch + CIDR. From these series of experiments summarized above (Saldarriaga et al., 2005 a, b), we concluded that the CO-Synch + CIDR protocol in which GnRH-2 and TAI are employed at 48 h after CIDR removal/PGF fails to synchronize ovulation and optimize TAI pregnancy rates in *Bos indicus x Bos taurus* females. This appeared to occur in the current experiments primarily because the proportion of cows that exhibited a synchronized follicular wave after GnRH-1 was only 60%. The remaining 40% of females, those without a synchronized follicular wave, introduced marked variability into the system relative to follicular maturity and health, and oocyte fertility at the time of GnRH-2. In addition, the timing of GnRH-2 for inducing ovulation does not appear to be optimal relative to follicular maturity in the 60% of cows that developed what appeared to be a synchronized follicular wave after GnRH-1.

In order to optimize TAI pregnancy rates utilizing CO-Synch + CIDR or similar approaches, it will probably be necessary to delay the time of GnRH-2/TAI past 48 h. Recent reports from the Midwestern U.S. indicate that TAI at 66 h can markedly increase pregnancy rates in *Bos taurus* females (Schafer et al., 2004; Walker et al., 2005). However, given that only 60% of cows in the current studies formed a new follicular wave after GnRH-1, it will also be necessary to elucidate why GnRH-1 does not result in a higher number of ovulations and new follicular wave recruitment. While it is known that the stage of the cycle when the Ovsynch, CO-Synch and CO-Synch + CIDR protocols are initiated can affect the efficiency of synchronization of a new follicular wave, it is not likely that use of pre-synchronization procedures to improve this outcome can be economically-employed in commercial beef cattle enterprises in the southern U.S. Other options include the use of an estrogen to improve synchrony of follicular wave emergence in place of GnRH-1 (Martinez et al., 2002). However, given the fact that there are currently no commercially-available estrogens on the market in the U.S. and their use is not approved by the FDA, employing estrogens in synchronization of beef cattle is problematic at best. Another potential option is the use of hCG in place of GnRH-1 as a means of pharmacologically inducing a greater number of ovulations and thereby improving follicular wave synchrony; however, no data are available on this approach.

Finally, efforts to account for lower pregnancy rates in *Bos indicus* x *Bos taurus* influenced compared to straight *Bos taurus* cattle in relation to synchronization of ovulation and TAI often lead to conjecture about potential differences in overall fertility of straightbred Bos taurus and Bos indicus x Bos taurus crossbreds . Based upon the data presented herein, it should be clear that low pregnancy rates in these systems are accounted for mainly by failure to precisely control follicular growth and ovulation. Cumulative pregnancy rates after 30 and 90 d of breeding in our studies with both older (SMB) and newer (CO-Synch + CIDR) technologies consistently average greater than 75 and 90%, respectively, confirming that the cattle used for these experiments were highly fertile. Owing to the effects of hybrid vigor, the Brahman x Hereford F_1 (used extensively in our trials) is universally considered to be one of the most fertile of all commercial beef females in subtropical environments. Nonetheless, TAI pregnancy rates of 39% or less using the most advanced technology available are unacceptable and must be improved. In order for CO-Synch + CIDR and other similar, high input technologies to be economically-employed in the southern region of the U.S. and in other sub-tropical and tropical environments, it is likely that TAI pregnancy rates will have to consistently exceed 50%.

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