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Pregnancy rates to fixed-time AI in *Bos indicus*-influenced beef cows using PGF2α with (Bee Synch I) or without (Bee Synch II) GnRH at the onset of the 5-day CO-Synch + CIDR protocol

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ABSTRACT

Objectives were to 1) characterize fixed-time AI (FTAI) pregnancy rates using the 5-Day CO-Synch + CIDR protocol in mature, suckled *Bos indicus*-influenced beef cows, 2) compare FTAI pregnancy rates in the latter to a modified version (5-Day Bee Synch + CIDR; Bee Synch I) that included treatment with prostaglandin F2α (PGF2α) at CIDR insertion on Day 0, and 3) test the hypothesis that elimination of both GnRH-1 at the onset of synchronization and the double dose of PGF on Day 5 (Bee Synch II) would not reduce FTAI pregnancy rates compared to Bee Synch I. For Experiment 1-trial 1, Brahman x Hereford (F-1) cows (n = 168) at least 40 d postpartum (PP; r = 40–92 d) at the time of CIDR insertion were administered the 5-Day CO-Synch + CIDR protocol with FTAI at 72 h after CIDR removal. Pregnancy rates to FTAI averaged 34.9 ± 1.9%. In Experiment 1-trial 2, fall- and spring-breeding Brahman x Hereford (F-1) beef cows (n = 269) were stratified by days PP and assigned randomly to receive either the 5-Day CO-Synch + CIDR (n = 136) or Bee Synch I (n = 133) protocol, with FTAI at 66 h after CIDR removal. Pregnancy rate to FTAI was greater (P < 0.05) in Bee Synch I (52.6 ± 0.9%) than in the 5-Day CO-Synch + CIDR procedure (40.4 ± 5.7%). For Experiment 2, 422 mature Braford, Brangus, Nelore x Brahman, and Brahman crossbred cows (*Bos indicus* proportion unknown) at 4 locations were treated with Bee Synch I, with FTAI at 66 h. Overall FTAI pregnancy rate averaged 51.7 ± 2.1%. Finally, from 2013 through spring 2018, we used a switchback design using fall- and spring-breeding herds to compare Bee Synch I (402 observations) to Bee Synch II (393 observations). Overall frequency of detected estrus at 66 h using ESTROTECT™ breeding indicator patches was 57.2 ± 2.4%, conception rates of those detected in estrus was 64.4 ± 3.5%, and FTAI pregnancy rates averaged 52.3 ± 2.4%, none of which differed between treatments. Moreover, pregnancy rates to FTAI in both treatments did not differ in cows synchronized between 40 and 80 d PP but increased after 80 d PP (P < 0.05). Bee Synch II, which eliminates GnRH-1 and the double dose of PGF2α on Day 5, results in FTAI pregnancy rates essentially identical to Bee Synch I but reduces synchronization costs and avoids the need for off-label (double dose PGF2α) drug use.

1. Introduction

Since the mid 1980's, we have reported on the efficacy of numerous protocols for synchronization of ovulation and fixed-time AI (FTAI) in *Bos indicus*-influenced beef cattle. Results have been reported for Syncro-Mate-B, Ovsynch, 7-Day Co-Synch + CIDR, and 7-Day Co-Synch + CIDR with pre-synchronization, among others [1–8]. All have yielded inconsistent and disappointingly low pregnancy rates (33–45%), including the latter 7-day protocols that have been reported to result in FTAI pregnancy rates of 55–60% in *Bos taurus* females [9].

Following the initial reporting by Bridges et al. [10] of the 5-Day CO-Synch + CIDR procedure in *Bos taurus* cattle, our group began to test the suitability of a similar version [11] in *Bos indicus*-influenced females. The latter involved application of a single 50-mg dose of PGF2α at CIDR removal and had been shown to result in FTAI pregnancy rates in *Bos taurus* females not different from those

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observed when two 25-mg injections were administered 8 h apart. Unfortunately, advantages reported for the 5-day procedure compared to the 7-day in *Bos taurus* [10] were not obvious in trials involving *Bos indicus*-influenced cattle [12, 13, current report]. Thus, a modified version of the 5-Day CO-Synch + CIDR protocol, termed 5-day Bee Synch + CIDR (Bee Synch), was developed. The protocol employed treatment with PGF2α at CIDR insertion (Day 0), a double dose of PGF2α (50 mg) administered in one injection on Day 5, and FTAI with GnRH at 66 h after CIDR removal. In preliminary reports, FTAI pregnancy rates consistently averaged ≥50% [12,13].

The underlying basis for development of Bee Synch (now termed Bee Synch I) was the hypothesis that *Bos indicus*-influenced beef females are more sensitive to progesterone negative feedback than *Bos taurus* cows. If so, circulating concentrations of progesterone during both 7- and 5-Day CO-Synch + CIDR synchronization in females with mature corpora lutea (CL) could reduce maturational rate of the synchronized dominant follicle. Indirect evidence for this was provided in our earlier report comparing ovulatory follicle sizes in *Bos indicus*-influenced cows synchronized with either the 7-Day Select Synch or 7-Day CO-Synch + CIDR protocol [5]. In that study, cows allowed to ovulate naturally following CIDR removal (Select Synch) exhibited ovulatory follicle sizes of ≥13 mm, whereas those induced to ovulate with an injection of GnRH at 66 h after CIDR removal had mean ovulatory follicle sizes of ~11.6 mm.

In addition to addressing the issue of presence of a mature CL during the synchronization period, recent reports using *Bos taurus* heifers [14] have questioned the value of GnRH treatment (GnRH-1) in a 5-Day CIDR-based protocol. The basis for this question lies in observations that a very low percentage of *Bos taurus* heifers ovulate in response to a random GnRH treatment and, as a result, does not optimize synchrony of a new follicular wave [14]. Since ovulation rate to GnRH-1 has also been shown to be highly variable (and often quite low) in *Bos indicus*-influenced mature cows [4,5], we questioned whether GnRH-1 is necessary in the Bee Synch I protocol.

Objectives of studies reported here were to 1) characterize pregnancy rate to FTAI using the standard 5-Day CO-Synch + CIDR protocol in mature, suckled *Bos indicus*-influenced beef cows, 2) compare FTAI pregnancy rates in the latter to a modified version (Bee Synch I) that includes treatment with PGF2α at CIDR insertion on Day 0, and 3) test the hypothesis that elimination of both GnRH-1 at the onset of synchronization and the double dose of PGF2α on Day 5 (Bee Synch II) would not reduce FTAI pregnancy rates compared to Bee Synch I. Details associated with follicular and luteal dynamics resulting from the inclusion or omission of GnRH-1 with 5-day protocols in *Bos indicus*-influenced females have been reported recently by our group elsewhere [15].

2. Materials and methods

All animal-related procedures in this study were approved by the Agricultural Animal Care and Use Committee, Texas A&M AgriLife Research, Texas A&M University System.

2.1. Experiment 1. use of the standard 5-day CO-Synch + CIDR in braford (F-1) mature cows and comparison to Bee Synch I

2.1.1. Trial 1

For Trial 1, fall- and spring-calving, pluriparous Braford (F-1) cows (n = 168) at the Texas A&M AgriLife Research Station-Beeville were used. Cows were maintained on improved pastures consisting of mixed grasses, including Coastal bermudagrass, Kleingrass and other native species, with energy and protein supplementation as required to maintain a minimum body condition score (BCS) of 5 (1–9 scale). To be included in trials, cows were required to have a minimum body condition score (BCS) of 5 (1–9 scale) [16], be suckling a calf, and be a minimum of 40 d postpartum (PP; range = 40–92) at onset of treatment. A few cows that did not meet minimum BCS requirements or whose calf died, were often included in the synchronization and FTAI process but only those that lost calves but met BCS requirements were included in data analysis.

The 5-Day CO-Synch + CIDR protocol employed in these studies [11] included insertion of a CIDR (Zoetis Animal Health, New York, NY) on Day 0 and intramuscular injection of GnRH (100 μg; Factrel; Zoetis Animal Health). On Day 5, the CIDR was removed and cows received a single intramuscular injection (50 mg) of $PGF_{2\alpha}$ (Lutalyse; Zoetis Animal Health, New York, NY). All females were inseminated by one of two technicians beginning at 72 h following CIDR removal using semen from a single Angus sire and received an intramuscular injection of GnRH (100 μg; Factrel, Zoetis Animal Health, New York, NY). Fig. 1 provides a timeline of the standard 5-Day CO-Synch + CIDR procedure with a double dose (50 mg) of PGF2α administered at CIDR removal as reported by Bridges et al. [11] in *Bos taurus* beef females. Technicians were stratified equally across all inseminations. Pregnancy to FTAI was determined at 32 d post-insemination using transrectal ultrasonography. Clean-up bulls (Angus) were placed with all cows at a ratio of approximately 1:40 beginning 10 days after FTAI and remained for 75 d. All bulls were required to pass a standard breeding soundness examination before use. Final pregnancy determinations were made by palpation per rectum at least 45 d following the end of bull exposure.

2.1.2. Trial 2

In Trial 2, Braford (F-1) fall and spring-calving cows (n = 269) at the same location as in Trial 1 were used to compare the 5-Day CO-Synch + CIDR protocol [11] as described in Trial 1 FTAI (Fig. 2, top panel) to a modified version (5-Day Bee Synch + CIDR; Bee Synch I) that included a single injection of PGF2α (25 mg) at the time of CIDR insertion on Day 0, CIDR removal and 50 mg PGF2α i.m. on Day 5 (Fig. 2, middle panel). We used FTAI at 66 h instead of 72 h for both treatment groups. This modification in timing was made because we observed in previous trials (Williams et al., unpublished) that up to 50% of cows receiving the 5-Day CO-Synch + CIDR treatment in *Bos indicus*-influenced mature cows were in estrus at 48–54 h. Cows were treated intramuscularly with 100 μg GnRH at insemination. A timeline showing all procedures employed for 5-Day CO-Synch + CIDR and Bee Synch I as employed in these experiments is presented in the top two panels of Fig. 2. Within each of the two fall-calving replicates and the single spring-calving replicate, cows were stratified by days PP and BCS before random assignment to treatment. As in Trial 1, cows were required to have a minimum BCS of 5 and be at least 40 d PP at treatment onset.

Females in both groups were inseminated in approximately equal numbers by the same two technicians as in Trial 1 using semen from a single Angus sire. Pregnancy detection was performed on Day 32 using transrectal ultrasonography. Clean-up bulls were employed as described previously and final pregnancy determinations were made by palpation per rectum at least 45 d following the end of bull exposure.

2.2. Experiment 2. location trials using Bee Synch I in braford (F-1), brangus, nelore crossbred and brahman crossbred mature cows

Based on the results observed in Experiment 1 above, we replicated Bee Synch I trials at various Texas A&M AgriLife Research locations (years 2012–2013), including Braford cows from the

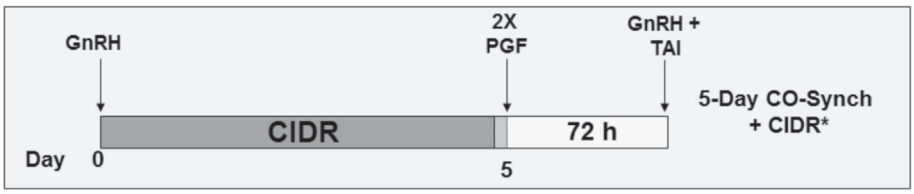

Fig. 1. Timeline of treatments and events associated with the 5-Day CO-Synch + CIDR protocol for synchronization of ovulation in which the double dose of PGF2α was administered at CIDR removal [10].

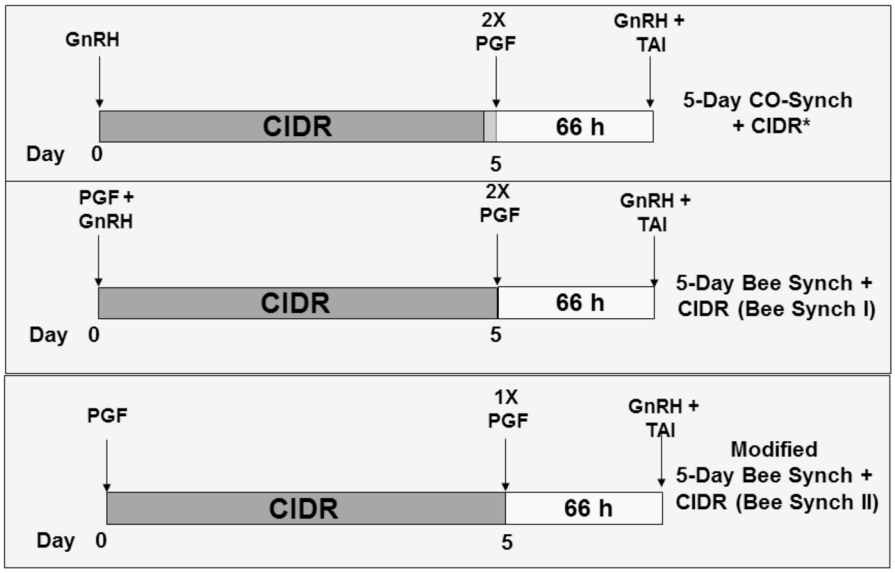

Fig. 2. Comparison of timelines for the standard 5-Day CO-Synch + CIDR protocol with FTAI at 66 h to Bee Synch I and II. **Top panel**: 5-Day CO-Synch + CIDR with FTAI at 66 h; **Middle panel**: Modified 5-Day CO-Synch + CIDR protocol (5-Day Bee Synch + CIDR I; Bee Synch I) in which females are treated with a standard dose of PGF on Day 0 with FTAI at 66 h; **Bottom panel**: Modified version of Bee Synch I (Bee Synch II) in which treatment with GnRH on Day 0 is omitted and a standard dose of PGF is administered on Day 5 with FTAI at 66 h.

spring- (n = 119) and fall-calving (68) herds in Beeville, TX, Brangus and unspecified Brahman crossbred (n = 40) in College Station, TX, and Nellore x Brangus crossbred cow (n = 81) in McGregor, TX. Finally, an additional group of 114 Brangus cows at the Buck Island Ranch in Lake Placid, Fla were included which provided a total of 422 cows.

Specifications for BCS and days postpartum were the same as for all previous trials as outlined above. The Bee Synch I treatment was employed as described earlier, including FTAI at 66 h. More than one AI bull was used at each location and were stratified equally across treatment groups. Clean-up bull information was available only for the Beeville location.

2.3. Experiment 3. effects of eliminating GnRH on day 0: Bee Synch I vs Bee Synch II for FTAI in suckled braford and brangus cows

During years 2013, 2015, 2016, 2017 and 2018, we compared FTAI pregnancy rates in mature suckled cows treated with either Bee Synch I or a modified version of Bee Synch I, Bee Synch II (Fig. 2, bottom panel). The treatment protocol for Bee Synch II eliminated GnRH treatment on Day 0 and employed a standard dose (25 mg) of PGF2α on Day 5 at CIDR removal.

For this study, minimum days PP was set at 50 d. Beginning the first year of the study, cows available for use from fall- and spring-breeding herds were stratified by d PP and BCS and assigned randomly to receive either Bee Synch I or II. Cows were inseminated by one of two technicians and pregnancy to FTAI was determined by transrectal ultrasonography between 32 and 40 d PP. For each subsequent year thereafter, the synchronization treatment for each female within each herd was either 1) switched to the opposite treatment she had been assigned the previous year, or 2) stratified by d PP and BCS and assigned randomly to treatment as a newly available female suckling a calf. Thereafter, those remaining in the herd entered the switchback for subsequent years.

Beginning in 2016 and continuing through 2018, ESTROTECT™ heat detection patches (Spring Valley, WI) were utilized to estimate incidence of behavioral estrus. Patches were placed on the tail heads at the time of CIDR removal on Day 5 and the number of fully-activated patches recorded at the time of AI. Clean-up bulls (either Angus for Braford cows or Hereford for Brangus cows) were

placed with cows 7–10 d after FTAI. Pregnancy rate to FTAI was determined by transrectal ultrasonography at 32–34 d after AI and final pregnancy determinations were made by palpation per rectum at least 45 d following the end of bull exposure. All bulls were required to pass a standard breeding soundness examination before use. In the Fall of 2018 and spring of 2019, only Bee Synch II was employed for synchronization and FTAI in order to add additional numbers of observations for verifying efficacy of this treatment.

2.4. Statistical analyses

Descriptive data (d PP, BCS) are presented as means (±SEM) and ranges throughout. For examining treatment effects on FTAI pregnancy and breeding season pregnancy rates in Exp 1, Trial 2, the Proc Mixed procedure of the Statistical Analysis System (SAS version 9.3 for Windows; SAS Institute, Cary, NC, USA) was used. The model included treatment, AI sire, AI technician and appropriate interactions. Only significant main and interaction terms were included in the final model. For Exp. 2, the main effect of location (For Beeville represented by 2 different herds) was examined using Proc Mixed, with AI technician and AI sire also included in the model. In Exp. 3, 'herd within year' served as the experimental unit since a switchback design was employed with a significant proportion of cows used repeatedly over multiple years. Thus, in any given year, 'herd' was represented by cows in the spring- or fall-breeding herd that had been included previously or new cows entering the study for the first time. The latter varied from 10 to 57%, depending on year. Therefore, data were analyzed two ways: 1) using the Wilcoxon Signed-Rank test for ordinal data and 2) Proc mixed with herd within year as the experimental unit. Sire and AI technician served as other sources of variation in the latter analysis.

3. Results

3.1. Experiment 1

Fall-calving females (n = 68) utilized for Trial 1 calved from August 25 through October 21 and averaged 67.2 ± 3.5 d PP (range = 40–92 d) at onset of synchronization treatments. Cows (n = 100) utilized from the spring-calving herd calved from January 4 through February 3 and averaged 71 ± 5.7 d PP (range 45–100 d) at treatment onset. Mean BCS for both groups was 5.6 ± 0.2. For the fall-and spring-calving cows used in Trial 2, mean (±SEM) days PP and BCS were 66.8 ± 3.1 d (range 42–89) and 5.8 ± 0.2 (range = 5–6.5), and 74 ± 4.1 d (range = 47–96) and 5.5 ± 0.3 (range = 5–7), respectively.

Table 1 presents FTAI pregnancy rates in mature females treated with 5-Day CO-Synch + CIDR (Trial 1) and Bee Synch I (Trial 2). In trial 1, mean overall FTAI pregnancy rates for the standard 5-Day CO-Synch + CIDR treatment were less than 35%. For Trial 2, the 5-Day CO-Synch + CIDR protocol averaged 40.4% compared to 52.6% for Bee Synch I ($P < 0.05$). The only other trend observed in the final model was a tendency for AI technician to affect FTAI pregnancy rate in Trial 2, where technician 1 had a lower ($P < 0.10$) pregnancy rate than technician 2 (47 vs 51%).

Final pregnancy rates for spring and fall -bred cows in Trial 1 were 95.8 and 94.6%, respectively. For Trial 2 involving comparison of 5-Day CO-Synch + CIDR to Bee Synch I in the fall and spring-calving herds, final pregnancy rates were 88.1 and 95.8%, respectively, and did not differ due to synchronization treatment.

3.2. Experiment 2

Mean (±SEM) d PP and BCS at the Beeville location for fall-calving cows were 68.1 ± 3.5 d PP (range 40–88 d) and 5.5 ± 0.3. Spring-calving cows averaged 70 ± 4.2 d PP (range = 40–91) with a mean (±SEM) BCS of 5.4 ± 0.5. Minimum BCS requirements were also targeted and met at all other Texas locations (e.g., no cattle < BCS 5). However, quantitative means and ranges for days PP at the latter locations were not available, although minimum days PP were not less than 40 d. At the Florida location, approximately 27% of the 114 cows contributing to the data set had a BCS <5 but the overall mean was 5 ± 0.1 (range = 3.5–7). Days PP were not available; however, records indicated that no cows were less than 40 d PP and the majority exceeded 60 d. A second group of 153 cows intended for inclusion were eliminated completely from consideration due to >45% of cows having a BCS <5.

Fixed-time AI pregnancy rates are shown in Table 2 and ranged

Table 2
Fixed-time AI (FTAI) pregnancy rates in suckled Braford (F-1), Brangus, and Nelore x Brangus beef cows (Exp. 2) following synchronization of ovulation with the 5-Day Bee Synch + CIDR (Bee Synch I) protocol at 4 locations and 2 different herds at Beeville.

Location	No. Cows	FTAI Pregnancy Rate, %
Spring 2011, Beeville	119	52.1
Spring 2012, College Station	40	55.0
Spring 2012, McGregor	81	59.3
Fall 2012, Beeville	68	52.2
Spring 2012, Lake Placid, FL	114	40.0
Total	422	51.7 ± 2.1[a]

[a] Mean ± SEM.

Table 1
Fixed-Time AI (FTAI) pregnancy rates in suckled Braford (F-1) cows following synchronization of ovulation using the standard 5-Day CO-Synch + CIDR protocol (Trial 1) and comparison (Trial 2) to 5-Day Bee Synch + CIDR (Bee Synch I) in Exp. 1.

Trial	Season	Treatment	No. Cows	FTAI Pregnancy Rate, %
1	Spring	5-Day CO-Synch + CIDR	68	36.8
1	Fall	5-Day CO-Synch + CIDR	100	33.0
Combined		5-Day CO- Synch + CIDR	168	34.9 ± 1.9
2	Fall	5-Day CO-Synch + CIDR	70	35.7
		Bee Synch I	69	52.1
2	Spring	5-Day CO-Synch + CIDR	66	45.4
		Bee Synch I	64	53.1
Combined		**5-Day CO-Synch + CIDR**	**136**	**40.4 ± 5.7**
		Bee Synch I	**133**	**52.6 ± 0.9**[a]

[a] $P < 0.05$; Combined values are mean ± SEM

from 40% (Florida location) to 59.3% (McGregor, TX), with all locations consistently greater than 50% except at the Florida location. Final pregnancy rates following removal of clean-up bulls at Beeville averaged 94% but data were not available at the other locations.

3.3. Experiment 3

The comparison of FTAI pregnancy rates between Bee Synch I and II can be seen in Table 3. Individual year within season pregnancy rates ranged from 44.8% to 67.3% but did not differ overall due to treatment based on both the Wilcoxon test and mixed model analysis. Although numerical differences were observed for AI sires, numbers of observations per bull were inadequate to detect statistical differences. Both treatments had a combined FTAI pregnancy rate averaging approximately 52% over a 5-year period in which the two treatments were compared (Table 3A). In addition to FTAI pregnancy rates, frequencies of estrus (based on full activation of ESTROTECT™ patches at the 66-h FTAI) and conception rates of cows detected in estrus were also obtained for years 2016, 2017, and 2018. Frequency of estrus in both treatment groups generally paralleled pregnancy rates. The Pearson correlation coefficient (R) between tail patch activation and FTAI pregnancy rate was 0.6486 ($P = 0.059$). Conception rates of cows detected in estrus based on tail patch activation averaged 60% or greater overall (Table 3 A). In 2018 (Fall) and 2019, cows were synchronized with Bee Synch II only and resulted in frequencies of estrus, conception rates, and FTAI pregnancy rates similar to those observed during the previous 5 years (Table 3B).

Table 4 summarizes the effect of days postpartum at CIDR insertion, regardless of treatment, on tail patch activation, conception rate of females with activated patches, and FTAI pregnancy rates using data from Table 3A. Data were stratified arbitrarily within 3 postpartum ranges: 40–65, 66–81, and ≥81 d. There was a trend for FTAI pregnancy rates to increase with increasing d PP, with pregnancy rates greater ($P < 0.05$) in cows in which synchronization began ≥ 81 d PP compared to those began at 40–65 d.

4. Discussion

Based in part on our earlier preliminary reports [12,13,17], a protocol referred to in the Beef Sire Directories as PG-5-Day–CO–Synch + CIDR [15], became the only nationally recommended approach for synchronization of ovulation for FTAI of *Bos-indicus*-influenced cows. The difference between the latter method and Bee Synch I as summarized herein was the recommendation to inject two standard doses of PGF2α 8 h apart on Day 5, whereas Bee Synch I utilizes a single double dose (50 mg) of PGF2α on Day 5. The current report formalizes the results of multi-year trials involving the original 5-day Bee Synch + CIDR protocol (Bee Synch I) with the double dose of PGF2α administered at CIDR removal and a modified version, Bee Synch II.

Early studies at this location involving the 5-Day CO-Synch + CIDR procedure in *Bos indicus*-influenced cattle (mainly Braford) did not yield satisfactory results and appeared to offer no improvement in FTAI pregnancy rates compared to the 7-Day CO-Synch + CIDR, with pregnancy rates still generally in the 35–45% range [12, current report]. Studies reported here addressed the hypothesis that eliminating functional CL from all cycling cows at the start of the 5-day procedure (Bee Synch I) would benefit the rate of maturation of the synchronized, dominant follicle and improve FTAI pregnancy rates by lowering mean circulating progesterone in a predominant proportion of cows during the synchronization period. Based on numerous preliminary reports [12,13,17] and the current formalized summary, this hypothesis appears to have proven correct as FTAI pregnancy rates using Bee Synch I have averaged around 52% over a 7-year period at various locations and when used repeatedly within the herd at Beeville. These results have been achieved in Braford, Brangus, and other non-specific Nelore x *Bos taurus* and Brahman x *Bos taurus* crossbreds. Collectively, we believe that our data provide convincing evidence that elimination of functional CL at the onset of the 5-day protocol is beneficial for increasing risk of FTAI pregnancy in *Bos indicus*-influenced beef cows. These results do not necessarily apply to pure-bred *Bos indicus* cattle (e.g., Brahman; Nelore) because none of the trials we have conducted have included them.

Table 3
Estrual (**Estr**) responses (females with activated patches at 66-h FTAI), conception rates (**CR**), and FTAI pregnancy rates (**Preg**) in suckled Braford (F-1) and Brangus cows following synchronization of ovulation using either 5-Day Bee Synch + CIDR (Bee Synch I) or a modified version of Bee Synch I (Bee Synch II). Estrus detection patches were not used before the year 2016. **A:** Fixed-time AI pregnancy rates only (2013–2015); estrus, conception rates and pregnancy rates (2016–2018); **B**: Estrus, conception rates and FTAI pregnancy rates for Bee Synch II only (2018–2019).

				Bee Synch I				Bee Synch II		
	Year	Season	No.*	Estr, %	CR %	Preg, %	No.*	Estr, %	CR. %	Preg, %
A	**2013**	Spring	47	–	–	46.8	49	–	–	44.9
	2013	Fall	33	–	–	48.5	32	–	–	59.4
	2015	Spring	75	–	–	52	69	–	–	47.8
Subtotals/means (±SEM)			**155**	**-**	**-**	**49.1 ±1.5**	**150**	**-**	**-**	**50.7 ±4.4**
	2016	Fall	60	60	63.8	50.0	54	57.4	48.3	48.1
	2017	Spring	51	43.1	59.1	51.0	51	54.9	67.3	50.0
	2017	Fall	81	53.1	65.1	53.7	84	57.1	64.5	53.0
	2018	Spring	55	65.4	86	67.3	54	66.6	61.1	59.3
Subtotals/means (±SEM)			**247**	**55.4 ±4.8**	**68.5 ±5.9**	**55.5 ±2.6**	**243**	**59 ±2.6**	**60.3 ±4.2**	**52.6 ±2.2**
Grand total/means (±SEM)			**402**	**-**	**-**	**52.8 ±2.6**	**393**	**-**	**-**	**51.8 ±2.2**
B				**Year**	**Season**				**Bee Synch II**	
				2018	Fall	92		59.8	72.7	51.1
				2019	Spring	59		57.7	53.3	50.8
				Total/means		**151**		**58.8 ±1.05**	**63 ±9.7**	**51 ±0.15**

* For 3A, No. represents the number of available females in fall- and spring-calving herds each year, with herd as the experimental unit, in a switchback design. Estrus based on full activation of ESTROTECT estrus detection patches in years 2016–2019. Means (±SEM) did not differ ($P > 0.10$).

Table 4
Effect of days postpartum at initiation of synchronization treatments (Bee Synch I and II combined) on percentage of Braford and Brangus cows detected in estrus (cows with fully activated ESTROTECT™ patches at the 66-h FTAI), conception rate of cows with activated patches, and total FTAI pregnancy rates.

Data Source (yr)[a]				
Days Postpartum	2016–2018		2013–2018	
	No.	Estrus, %	No.	FTAI pregnancy,%
40–65	202	56.9[a]	437	48.0[a]
66–81	266	59.1[a,b]	368	53.5[a,b]
≥81	64	65.1[b]	82	61.2[b]

[a,b]Means within columns with different superscripts differ $P < 0.05$.
[a] Computed from Table 3A.

Moreover, we have no data to indicate the proportion of *Bos indicus* influence required for either Bee Synch I or Bee Synch II to provide benefit for FTAI pregnancy rates compared to the standard 5-day protocol. Moreover, although we have provided anecdotal reports on the efficacy of Bee Synch I and II for use in *Bos indicus*-influenced heifers, we do not have adequate data at this time to provide accurate estimates of FTAI pregnancy rates. Based on preliminary observations, we do recommend that the timing of FTAI in the latter be shortened from 66 to 54–60 h because a very high proportion of *Bos indicus*-influenced heifers treated with Bee Synch I or II have been observed in estrus as early as 36–42 h following CIDR removal. Thus, waiting until 66 h has the potential of reducing pregnancy rates (Williams et al., unpublished) and these observations concur with those reported by Kasimanickam et al. [18] when the standard 5-day protocol was employed in *Bos taurus* heifers. Based on our preliminary observations involving relatively small numbers of animals (Williams and Stanko, unpublished), we believe that FTAI pregnancy rates in *Bos indicus*-influenced heifers that are confirmed pubertal, when inseminated at 54–60 h, may be similar to those we have reported for mature cows inseminated at 66 h.

In Experiment 3, we tested the hypothesis that GnRH-1 is not essential for optimizing FTAI pregnancy rates in mature cows treated with a Bee Synch protocol. Using GnRH at the initiation of synchronization is based on the premise that ovulation of a dominant follicle is required to optimize synchrony of new follicular wave emergence [4,19,20]. Cruppe et al. [14] reported that, due to the low ovulation rate after GnRH treatment in randomly cycling *Bos taurus* heifers, its use at the start of the standard 5-day CO-Synch + CIDR protocol (GnRH-1) for synchronizing new follicular wave emergence has little value in a practical sense because FTAI pregnancy rates were similar with or without GnRH-1. Moreover, in a direct comparison of 5- and 7-day CO-Synch + CIDR programs in *Bos taurus* primiparous beef cows, failure to respond to GnRH-1 proved detrimental to estradiol and progesterone concentrations in 7-day programs but it had no effect in 5-day programs [21]. Similarly, it has been our experience that mature *Bos indicus*-influenced cows also exhibit generally low and highly variable ovulation rates following random treatment with GnRH [4,5]. This implied that omission of GnRH-1 from the Bee Synch I protocol in mature *Bos indicus*-influenced females would not be detrimental to FTAI pregnancy rates. We tested this hypothesis using a switchback design implemented from 2013 to 2019 at Texas A&M AgriLife Research Station-Beeville. As can be noted from the data, no significant differences in FTAI pregnancy rates could be detected between Bee Synch I and II, both averaging approximately 52%. In this scenario, because GnRH-1 is eliminated on Day 0 in Bee Synch II and no new CL are induced, we also eliminated the double dose of PGF2α on Day 5 at the time of CIDR removal, using only the standard dose.

Recently, we reported a comparison of follicular and luteal dynamics in *Bos indicus*-influenced beef cows comparing truncated versions (ie., no FTAI or GnRH-2) of Bee Synch I and II [15]. Results indicated greater synchronization of new follicular wave emergence and a reduced incidence of early ovulations with use of Bee Synch I compared to Bee Synch II. However, despite these observations, GnRH-1 did not enhance the synchronized development of a dominant follicle at 66 h after CIDR removal, the scheduled time of FTAI, compared to Bee Synch II. Those findings, coupled with the current field trial results, substantiate the argument that there is no advantage for selecting Bee Synch I over Bee Synch II. Thus, GnRH-1 and the double dose of PGF2α on Day 5 can be eliminated without a reduction in fertility. This results in an overall drug cost savings without compromising FTAI pregnancy rates.

In 2016, we began using ESTROTECT™ estrus detection patches during Bee Synch I and II comparison trials. As shown in these direct comparisons, 55.4% of Bee Synch I and 59% of Bee Synch II-treated females, respectively, were determined to have expressed estrus by the time of 66-h FTAI in both treatment groups (Table 3 A). In some cases, > 70% have been determined to have expressed estrus by 66 h after CIDR removal using Bee Synch II (Table 3B). Conception rates of cows with activated patches averaged 60.3% (Bee Synch II) and 68.5% (Bee Synch I), respectively, in years 2016–2018 and did not differ ($P > 0.10$). During those years, FTAI pregnancy rates averaged approximately 53% (Bee Synch I) and 56% (Bee Synch II), respectively ($P > 0.10$). Thus, approximately 75% of FTAI pregnancies in these comparisons were generated in cows with activated tail patches at FTAI, with an additional 25% generated in females without activated patches. As noted earlier, FTAI pregnancy rates were generally correlated with the proportion of females with activated patches. However, FTAI pregnancy rates tended to remain within the 52–55% range even during years in which proportions of cows detected in estrus was ≥70%. Thus, a significant proportion of females may have been in estrus too early or too late for optimal fertility at 66 h and other AI options may need to be explored to capture these potential losses.

Finally, we examined the effects of d PP at onset of synchronization on FTAI pregnancy rates, irrespective of synchronization treatment. In earlier trials (2013–2015), minimum d PP was set at 40 d. In later trials, this was adjusted slightly to 50 d. Anecdotally, this latter adjustment was associated with greater FTAI pregnancy rates during those years. As summarized in Table 4, FTAI pregnancy rates were greater in cows in both treatment groups if they were ≥81 days postpartum at onset of treatment compared to those ranging from 40 to 65 days. However, there was no difference in pregnancy rates of females between 66 and 81 days at onset of treatment compared to those ≥81 days.

Managerial decisions must always be considered when deciding which cows to include in a synchronization/FTAI operation. Depending on specific operational goals and other factors, increases in FTAI pregnancy rates in cows associated with longer postpartum intervals at treatment onset may be offset by disadvantages created

due to the overall delay in initiation of breeding. In addition, many other factors determine success of synchronization protocols, regardless of procedure employed. These may include appropriateness of certain AI sires for use in FTAI, BCS of females, technical expertise of personnel, and unknown factors that are often difficult to identify. These variances often frustrate cattlemen and efforts to educate potential users of synchronization protocols must always include alerts to these pitfalls. In the end, cattlemen should be encouraged to consider the broader advantages provided by procedures with the potential to yield FTAI pregnancy rates over the long term that average $\geq$ 50%, despite the sporadic occurrence of pregnancy rates well below 50% within certain subgroups. Results reported herein indicate that *Bos indicus*-influenced females that are adapted to subtropical climates and that represent 30% or more of all beef females in the US [22], can be managed successfully for FTAI using Bee Synch II. The latter involves the use of commercially available, FDA-approved pharmaceuticals only and does not require off-label use of any approved or unapproved (i.e., estrogens) pharmaceutical products. Utilizing this procedure has the potential to contribute to large increases in growth performance and beef quality characteristics of offspring in areas where sub-tropically adapted females are strategically employed and necessary for successful beef production.

In summary, treatment of mature *Bos indicus*-influenced beef females with PGF2α on Day 0 of a 5-Day CO-Synch + CIDR protocol, with (Bee Synch I) or without (Bee Synch II) treatment with GnRH (GnRH-I) at the time of CIDR insertion, results in FTAI pregnancy rates that are equivalent and generally exceed 50%. Thus, the inclusion of GnRH-1 appears unnecessary, making Bee Synch II preferable because of reduced pharmaceutical costs and avoidance of off-label (double dose) application of PGF2α on Day 5.

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