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## Efficacy of either a single or split treatment of PGF<sub>2α</sub> after a 14 day melengestrol acetate treatment to synchronize estrus and induce luteolysis in *Bos indicus* × *Bos taurus* heifers

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### Abstract

Two experiments evaluated a modified delivery of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) after a melengestrol acetate (MGA) treatment in Angus and *Bos indicus* × *Bos taurus* (BI) heifers. Experiment 1 was replicated three times with yearling BI heifers ( $n = 695$ ). Heifers received MGA (0.5 mg head<sup>-1</sup> day<sup>-1</sup>) for 14 days. In Replications 1 and 2, heifers received either 25 mg of PGF<sub>2α</sub> im 19 days after MGA (single) or 12.5 mg of PGF<sub>2α</sub> im 19 and 20 days after MGA (split). In Replication 3, heifers received the same treatments, with PGF<sub>2α</sub> initiated either 18 or 19 days after MGA. Estrus was detected for 72 h after PGF<sub>2α</sub>, with AI commencing 8–12 h after a detected estrus. Heifers not observed in estrus by 72 h were timed-AI concomitant with GnRH (100 μg im). Heifers from Replication 2 ( $n = 146$ ) had blood samples collected at the initial PGF<sub>2α</sub> and at timed-AI to determine corpus luteum (CL) regression by evaluating plasma progesterone concentrations. The interval from MGA withdrawal to PGF<sub>2α</sub> did not have a significant effect on any variable in Replication 3 and there were no treatment by replication effects for any variables, therefore data were pooled. Modifying the PGF<sub>2α</sub> treatment from a single treatment to two treatments on consecutive days increased ( $P < 0.05$ ) 72 h estrous response (43.2% versus 50.1%), timed-AI (23.9% versus 33.5%) and total-AI pregnancy rates (34.5% versus 42.5%), and CL regression (79.1% versus 92.5%), respectively. In Experiment 2, yearling Angus ( $n = 66$ ) and 2-year-old BI ( $n = 68$ ) heifers were synchronized as per Experiment 1 (with the initial PGF<sub>2α</sub> 19 days after MGA). Neither breed

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nor PGF<sub>2α</sub> treatment effected ( $P > 0.05$ ) 72 h estrous response, total-AI pregnancy rate, or CL regression rate. In conclusion, treating yearling BI heifers with split treatments of PGF<sub>2α</sub> (given on two consecutive days) improved estrous response and pregnancy rates by increasing PGF<sub>2α</sub>-induced luteolysis.

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*Keywords:* *Bos indicus*; Estrous synchronization; Prostaglandin F<sub>2α</sub>; Progestin

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## 1. Introduction

A limited amount of research data suggests that there are physiological and behavioral differences between *Bos indicus* and *Bos taurus* cattle that could affect reproductive function. *B. indicus* cattle exhibit an estrus that is shorter in duration and less intense [1–3] and a decreased release of LH in response to treatment with either GnRH [4] or estradiol [5] compared to *B. taurus* cattle. In addition, the estrous response following a single dose of PGF<sub>2α</sub> appears to be decreased in *B. indicus* [6,7] compared to *B. taurus* cattle [8,9]. However, administering half-doses of PGF<sub>2α</sub> 24 h apart improved estrous response in *B. indicus* cattle [10].

Feeding melengestrol acetate (MGA) for 14 days with a single treatment of PGF<sub>2α</sub> 17 days later (MGA/PGF<sub>2α</sub>) [11], followed by 5 days of estrous detection, is an effective method of synchronizing estrus in yearling *B. taurus* beef heifers. The synchrony of the estrus to the MGA/PGF<sub>2α</sub> protocol can be improved by extending the PGF<sub>2α</sub> treatment to 19 days after MGA was last fed [12]. Furthermore, timed-insemination of heifers not expressing estrus by the third day after PGF<sub>2α</sub> [13] reduced estrous detection from 5 to 3 days, without reducing synchronized pregnancy rates. Although the MGA/PGF<sub>2α</sub> system is an effective estrous synchronization system in *B. taurus* heifers, there are limited data regarding its efficacy in *B. indicus* heifers [14].

The objectives of the following experiments were to determine if two consecutive (split) treatments of PGF<sub>2α</sub> administered 24 h apart, initiated either 18 or 19 days after a 14-day MGA treatment, improved luteolysis, estrous response, and synchronized pregnancy rates compared to a single treatment of PGF<sub>2α</sub> in *B. taurus* and *B. indicus* × *B. taurus* beef heifers.

## 2. Materials and methods

### 2.1. Experiment 1

Experiment 1 consisted of three replications using *B. indicus* × *B. taurus* yearling heifers. Replications 1 ( $n = 139$ ) and 2 ( $n = 146$ ) were conducted at the Bar L Ranch, Marianna, FL in two consecutive years and the third replication was conducted at the Broseco Ranch, Omaha, TX ( $n = 410$ ) during the same year as Replication 2. Heifers were fed melengestrol acetate ( $0.5 \text{ mg head}^{-1} \text{ day}^{-1}$ ; MGA<sup>®</sup> Premix, Pharmacia Animal Health, Kalamazoo, MI) for 14 days in a total mixed ration in Replications 1 and 2, and in a high-protein range cube supplement in Replication 3. In Replications 1 and 2, heifers were

randomly assigned to one of two PGF<sub>2α</sub> treatments 19 days after MGA administration. Approximately half (Replication 1,  $n = 72$ ; Replication 2,  $n = 73$ ) of the heifers received 25 mg of PGF<sub>2α</sub> im (single; Lutalyse<sup>®</sup> Sterile Solution; Pharmacia Animal Health, Kalamazoo, MI) 19 days after MGA and the remaining heifers (Replication 1,  $n = 67$ ; Replication 2,  $n = 73$ ) received 12.5 mg of PGF<sub>2α</sub> im (split) on Days 19 and 20 after MGA. In Replication 3, heifers were randomly assigned to the same PGF<sub>2α</sub> treatments as previously described. To facilitate estrous detection in Replication 3, heifers were randomly and equally allocated to two groups and within each group, they received the initial PGF<sub>2α</sub> treatment on either Days 18 or 19 after MGA (single  $n = 209$ ; split  $n = 201$ ). Body condition score (BCS: 1–9 scale, 1 = emaciated, 5 = moderate, 9 = obese [15]) was recorded at PGF<sub>2α</sub> for all heifers in each replication. For all replications, heifers receiving the single PGF<sub>2α</sub> treatment were physically handled through working facilities each time heifers in the split treatment received PGF<sub>2α</sub>. Mean (least squares means  $\pm$  standard error; LSM  $\pm$  S.E.) BCS for Replications 1–3 were  $5.9 \pm 0.2$ ,  $5.7 \pm 0.4$ , and  $5.4 \pm 0.4$ , respectively. Within a replication, mean BCS (LSM  $\pm$  S.E.) for the split and single PGF<sub>2α</sub> treatments were  $5.9 \pm 0.2$  and  $5.9 \pm 0.1$  for Replication 1,  $5.7 \pm 0.4$  and  $5.7 \pm 0.4$  for Replication 2, and  $5.4 \pm 0.4$  and  $5.4 \pm 0.4$  for Replication 3, respectively.

Following the initial PGF<sub>2α</sub> treatment, behavioral estrus was observed for approximately 1 h at 07:00 and 17:00 for 72 h in all replications. Behavioral estrus was defined as a heifer standing to be mounted by another heifer and (or) other visual signs of estrus. To assist in estrous detection, heifers received Kamar detectors (Kamar<sup>®</sup> Marketing Group, Steamboat Springs, CO, USA) at the initial PGF<sub>2α</sub> treatment in Replications 1 and 2. If a heifer was not observed in estrus but a Kamar was one-quarter to completely activated (i.e., Kamar<sup>®</sup> was red in color), a heifer was deemed to have been in behavioral estrus. For all replications, heifers were artificially inseminated 8–12 h after the onset of behavioral estrus. Heifers not detected in estrus by 72 h after the initial PGF<sub>2α</sub> were timed inseminated (timed-AI) and received 100  $\mu$ g of GnRH im (Fertagyl<sup>®</sup>; Intervet, Boxmeer, The Netherlands). Bulls were placed with heifers approximately 7–10 days after timed-AI for all replications. Pregnancy to AI was determined approximately 50–60 days following timed-AI for all replications, using a real-time B mode ultrasound (Aloka 500, Corometrics Medical Systems, Wallingford, CT, USA) equipped with a 5.0 MHz rectal transducer.

Three-day estrous response was defined as the number of heifers that exhibited behavioral estrus during the 72 h after the initial PGF<sub>2α</sub> treatment, divided by the total number of heifers treated. Conception rate was defined as the number of heifers observed in estrus, inseminated, and became pregnant, divided by the total number of heifers inseminated after an observed estrus. Timed-AI pregnancy rate was defined as the number of heifers that became pregnant to timed-AI, divided by the total number of heifers receiving timed-AI. Total-AI pregnancy rate was defined as the number of heifers that became pregnant to either estrous detection with AI or timed-AI, divided by the total number of heifers treated.

Three-day estrous response, conception, timed-AI, and total-AI pregnancy rates were analyzed with the GENMOD procedures of SAS (SAS Institute Inc., Cary, NC, USA). Independent variables tested were treatment, replication, and treatment by replication. Body condition score was used as a covariate in each model. When significant ( $P < 0.05$ ),

BCS was categorized into BCS  $\leq 5.0$  and  $> 5.0$  and analyzed as an independent variable along with treatment, replication, and all two- and three-way interactions. For Replication 3, interval from MGA withdrawal to PGF<sub>2 $\alpha$</sub>  did not effect estrous response, conception rate, timed-AI pregnancy rate, and total-AI pregnancy rate; therefore, data were pooled. Across all three replications, there were no treatment by replication effects ( $P > 0.05$ ) for estrous response, conception rate, timed-AI pregnancy rate, and total-AI pregnancy rate, so replications were pooled. Mean interval from initial PGF<sub>2 $\alpha$</sub>  treatment to the mean onset of estrus was analyzed using GLM procedures of SAS. Additionally, the effect of PGF<sub>2 $\alpha$</sub>  treatment on the interval from the initial PGF<sub>2 $\alpha$</sub>  treatment to the onset of estrus was evaluated using the LIFETEST procedure of SAS. When added to the model as a covariate, interval from initial PGF<sub>2 $\alpha$</sub>  treatment to the onset of estrus affected ( $P < 0.05$ ) conception rate; therefore, it was tested as an independent variable along with treatment and replication using GENMOD and also by regression analysis using GLM. The AI technicians and sires were equally distributed across treatments and within each treatment, both were equally distributed whenever possible to heifers that were inseminated either after a detected estrus or timed-AI. The numbers of AI sires were seven, four and two, while the numbers of technicians were five, four and three for Replications 1, 2 and 3, respectively. Since the same AI sires and technicians were not represented across replications, they were not included in statistical model containing all three replications. However, within each replication, AI sires and technicians were tested as independent variables to determine their effect on conception and timed-AI pregnancy rates.

A second objective of Experiment 1 was to determine the effectiveness of the single and split PGF<sub>2 $\alpha$</sub>  treatments to induce luteolysis using heifers from Replication 2 ( $n = 146$ ). Blood samples were collected via coccygeal veinipuncture into an evacuated tube with an anticoagulant (EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at the initial PGF<sub>2 $\alpha$</sub>  (Day 33) from all heifers. An additional blood sample was collected at timed-AI (Day 36) from all heifers that had not expressed estrus within 72 h after the initial PGF<sub>2 $\alpha$</sub>  treatment. After collection, blood samples were immediately placed on ice until they were centrifuged ( $3000 \times g$  for 15 min). Plasma was separated and stored at  $-20^\circ\text{C}$  pending further analysis. Progesterone concentrations were determined by radioimmunoassay [16] using DPC kits (Diagnostic Products Corp., Los Angeles, CA, USA) in multiple assays with an intra- and interassay CV of 10 and 9%, respectively. Sensitivity of the assay was 0.01 ng/mL and 0.1 mL of plasma was assayed.

To evaluate the effectiveness of PGF<sub>2 $\alpha$</sub>  to initiate luteolysis, only heifers with a functional corpus luteum (CL), defined as progesterone concentration  $\geq 1.0$  ng/mL, at the initial PGF<sub>2 $\alpha$</sub>  were used in the final analysis. Heifers exhibiting estrus were deemed to have completed CL regression. Timed-AI CL regression was defined as any heifer with progesterone concentrations  $< 1.0$  ng/mL at timed-AI, excluding heifers that exhibited estrus. Total CL regression was defined as any heifer that displayed behavioral estrus within 72 h after PGF<sub>2 $\alpha$</sub>  or had progesterone concentrations  $< 1.0$  ng/mL at timed-AI. After retrospective analysis, it was determined that three heifers became pregnant to the timed-AI even though their progesterone concentrations were  $> 1.0$  ng/mL at timed-AI (mean progesterone concentration of 1.7 ng/mL). Therefore, the heifers were considered to have regressed their CL for both the timed-AI and total CL regression analysis and were included in the final analysis.

The effectiveness of PGF<sub>2α</sub> treatments to initiate luteolysis was analyzed using GENMOD, with the independent variable being treatment and the dependent variables being total and timed-AI CL regression. Progesterone concentrations (ng/mL) at the initial PGF<sub>2α</sub> were further divided into five categories; 1 to <3, ≥3 to <6, ≥6 to <9, ≥9 to <12, and ≥12 ng/mL in an attempt to equally distribute heifers into different progesterone concentration categories. Progesterone category data were analyzed using GENMOD, with the independent variable being treatment and the dependent variables being total and timed-AI CL regression. When progesterone category was significant ( $P < 0.05$ ) for an independent variable, mean comparisons between progesterone categories were made with Chi-square. The ability of PGF<sub>2α</sub> treatment to induce total CL regression across all progesterone concentrations categories were further analyzed using regression analysis.

## 2.2. Experiment 2

Experiment 2 was conducted at the University of Florida Beef Research Unit using yearling Angus ( $n = 66$ ) and 2-year-old *B. indicus* × *B. taurus* heifers ( $n = 68$ ) consisting of one-quarter to three-quarters Brahman breeding, with the remainder being Angus breeding. The same treatments used in Experiment 1 (single versus split PGF<sub>2α</sub>) were applied to Experiment 2, with the initial PGF<sub>2α</sub> given 19 days after MGA withdrawal. Within a breed group, heifers were equally distributed to treatments by BW and BCS (recorded on Day 33 of the experiment). Mean (LSM ± S.E.) BW and BCS were  $318 \pm 5.1$  kg and  $5.1 \pm 0.2$  for Angus heifers, and  $464 \pm 5.0$  kg and  $5.4 \pm 0.4$  for the *B. indicus* × *B. taurus* heifers, respectively. Throughout Experiment 2, heifers were maintained in their respective breed groups (Angus versus *B. indicus* × *B. taurus*) in adjacent pastures. Heifers were fitted with radiotelemetric estrous detection devices (HeatWatch<sup>®</sup>, DDx, Denver, CO, USA) [17] before the initial PGF<sub>2α</sub> treatment to determine the initiation, end, and duration of estrus, along with the total number of mounting events received during estrus. Blood samples were collected (same experimental days as described for Experiment 1), processed, and analyzed for progesterone to determine luteal regression as described in Experiment 1. The intra- and interassay CV were 9 and 11%, respectively. After the synchronized breeding, estrous detection and AI continued for another 25 days. Pregnancy to the synchronized breeding was determined using a real-time B mode ultrasound 64 days after the start of synchronized breeding.

Initiation of HeatWatch<sup>®</sup> estrus was defined as the first of three mounts (mounts >2 s) in a 4 h period and the end of estrus was defined as the time of the last mount, followed by at least 8 h of no mounting activity. Duration of estrus was calculated as the difference in time from the initial mount to the last mount. Mounting activity during estrus was defined as the number of mounts received during each consecutive 3 h period throughout the duration of estrus, divided by the total number of mounts received throughout the duration of estrus. Interval from initial PGF<sub>2α</sub> treatment to the onset of estrus was calculated by subtracting the date and mean time of the initial PGF<sub>2α</sub> treatment from the date and time of the initiation of estrus.

Estrous response, conception, timed-AI pregnancy, total-AI pregnancy, timed-AI CL regression, and total CL regression rates were defined and statistically analyzed as described in Experiment 1, with treatment, breed, and treatment by breed as the

independent variables. Heifer BCS was included as a covariate in all analyses. Twenty-eight AI sires were used in the synchronized breeding, with 8 AI sires in the Angus and 20 AI sires in the *B. indicus* × *B. taurus* heifers. Sire matings were preassigned to heifers before the synchronization treatment. The same AI sires were not represented across breed groups and within a breed group AI sires were not equally distributed across treatments; therefore, AI sire was not tested as an independent variable in any statistical model. One technician inseminated all but seven heifers during synchronized breeding and pregnancy rates were similar ( $P > 0.05$ ) between technicians.

Interval from initial PGF<sub>2α</sub> to the onset of estrus, duration of estrus, and total number of mounts received during estrus were analyzed using the GLM procedure of SAS with treatment, breed, and treatment by breed as the independent variables. Only heifers with a functional CL at the initial PGF<sub>2α</sub> (progesterone > 1 ng/mL) were included in the analysis. Two heifers in the single PGF<sub>2α</sub> treatment lost their HeatWatch<sup>®</sup> transmitters at the initiation of estrus and were removed from the behavioral estrus analysis. The effect of PGF<sub>2α</sub> treatment and breed on interval from initial PGF<sub>2α</sub> treatment to the onset of estrus was also evaluated using the LIFETEST procedure of SAS. Mounting activity during estrus was analyzed as a repeated measure using the MIXED procedure of SAS [18], with PGF<sub>2α</sub> treatment, breed, period, appropriate interactions, and heifer as a random variable. Period was defined as sequential 3 h periods from the initiation to the end of estrus. There were no significant PGF<sub>2α</sub> treatment effects; therefore, treatment was removed from the analysis. Data were subsequently analyzed with regression analysis and differences between curves were analyzed using homogeneity of regression. Slice mean comparisons [18] were used to examine differences among 3 h periods.

The effectiveness of PGF<sub>2α</sub> treatments to initiate luteolysis was analyzed using GENMOD with the independent variable being treatment, breed, and treatment by breed and the dependent variables being timed-AI and total CL regression, with progesterone concentration at the initial PGF<sub>2α</sub> used as a covariate. There were no ( $P > 0.05$ ) treatment, breed or treatment by breed effects on timed-AI and total CL regression. When used as a covariate in the model, progesterone concentration at the initial PGF<sub>2α</sub> influenced ( $P < 0.05$ ) total CL regression; therefore, progesterone concentrations were further divided into five categories (1 to <3, ≥3 to <6, ≥6 to <9, ≥9 to <12, and ≥12 ng/mL) as per Experiment 1 and analyzed with GENMOD.

### 3. Results

#### 3.1. Experiment 1

An increased ( $P < 0.05$ ) percentage of heifers expressed estrus during the 72 h following the split compared to the single PGF<sub>2α</sub> treatment (Table 1). Estrous response was affected by replication ( $P < 0.01$ ), but there was no ( $P > 0.05$ ) treatment by replication effect. The 72 h estrous estrus responses for Replications 1, 2, and 3 were 47.5 (66/139), 60.3 (88/146), and 41.5% (170/410), respectively. Replication 2 had a greater ( $P < 0.05$ ) estrous response than both Replications 1 and 3, which were similar ( $P > 0.05$ ) to each

Table 1

Estrous, conception, and pregnancy rates of *B. indicus* × *B. taurus* heifers in Experiment 1 synchronized with melengestrol acetate (MGA) for 14 days, with either a single or two consecutive split treatments of PGF<sub>2α</sub> 24 h apart initiated either 18 or 19 days after MGA<sup>a</sup>

Treatment	Seventy-two hour estrous response <sup>b</sup> , % (n)	Conception rate <sup>c</sup> , % (n)	Timed-AI pregnancy rate <sup>d</sup> , % (n)	Total-AI pregnancy rate <sup>e</sup> , % (n)
Single PGF <sub>2α</sub>	43.2 (354)	48.8 (153)	23.9 (201)	34.5 (354)
Replication 1	40.3 (72)	48.2 (29)	20.9 (43)	31.9 (72)
Replication 2	56.2 (73)	61.0 (41)	15.6 (32)	41.1 (73)
Replication 3	39.7 (209)	42.2 (83)	27.0 (126)	33.0 (209)
Split PGF <sub>2α</sub>	50.1 (341)	51.5 (171)	33.5 (170)	42.5 (341)
Replication 1	55.2 (67)	48.6 (37)	43.3 (30)	46.3 (67)
Replication 2	64.4 (73)	51.1 (47)	30.8 (26)	43.8 (73)
Replication 3	43.2 (201)	52.9 (87)	31.6 (114)	40.8 (201)
<i>P</i> -values <sup>f</sup>				
Treatment	<0.05	NS	<0.01	<0.05
Replication	<0.01	NS	NS	NS
Treatment × replication	NS	NS	MS	NS

<sup>a</sup> Heifers were fed MGA for 14 days and received either 25 mg of PGF<sub>2α</sub> im (single) or two consecutive 12.5 mg of PGF<sub>2α</sub> im 24 h apart (split). In Replications 1 and 2, PGF<sub>2α</sub> treatment was initiated 19 days after MGA and in Replication 3 PGF<sub>2α</sub> was initiated either 18 or 19 days after MGA. Heifers were AI 8–12 h after an observed estrus. Heifers not detected in estrus by 72 h after PGF<sub>2α</sub> were timed-AI and received 100 µg of GnRH.

<sup>b</sup> Percentage of heifers displaying estrus 72 h after PGF<sub>2α</sub> of the total treated.

<sup>c</sup> Percentage of heifers that exhibited estrus, received AI, and became pregnant.

<sup>d</sup> Percentage of heifers that received timed-AI and became pregnant.

<sup>e</sup> Percentage of heifers that became pregnant to either an observed estrus and AI or timed-AI.

<sup>f</sup> NS = not significant ( $P > 0.05$ ).

other. When BCS was included as a covariate in the main statistical model, BCS effected ( $P < 0.05$ ) 72 h estrous response. However, when BCS was tested as an independent variable in the main model, it was no longer significant. Mean BCS for Replication 1 (5.9) was greater ( $P < 0.05$ ) than Replications 1 (5.7) and 3 (5.4), which were also different ( $P < 0.05$ ) from each other. Within each replication, BCS were similar ( $P > 0.05$ ) between treatments.

Mean (LSM ± S.E.) intervals from initial PGF<sub>2α</sub> to the onset of estrus were similar ( $P > 0.05$ ) between treatments (single, 62.6 ± 1; split, 63.1 ± 0.9 h), with the majority of heifers exhibiting estrus between 48 and 72 h after the initial PGF<sub>2α</sub>. The distribution of estrus was similar ( $P > 0.05$ ) between treatments when analyzed with survival analysis (data not shown). There was no effect ( $P > 0.05$ ) of treatment (Table 1) or BCS on conception rate. However, when treatments were combined, interval from the initial PGF<sub>2α</sub> treatment to onset of estrus influenced ( $P < 0.05$ ) conception rate in a linear manner ( $y = 0.213 + 0.127x$ ;  $r^2 = 0.06$ ). Conception rates at 24, 36, and 48 h after PGF<sub>2α</sub> were similar ( $P > 0.05$ ) and were pooled for the statistical analysis. Conception rate for heifers expressing estrus within 48 h (25/78 = 32.1%) after PGF<sub>2α</sub> was decreased ( $P < 0.05$ ) compared to heifers expressing estrus 60 (39/80 = 48.8%) and 72 h (98/166 = 59.1%) after PGF<sub>2α</sub>, with the latter two similar ( $P > 0.05$ ) to each other.

Modifying the delivery of PGF<sub>2α</sub> from a single to two consecutive split treatments increased timed-AI pregnancy rate by 9.6% ( $P < 0.01$ ) and total-AI pregnancy rate by 8.0% ( $P < 0.05$ ; Table 1). Body condition had no effect ( $P > 0.05$ ) on pregnancy rates.

Within each replication there were no ( $P > 0.05$ ) technician effects on conception and timed-AI pregnancy rates (data not shown). Conception rates ranged from 38.5 to 80.0% across all three replications. For timed-AI pregnancy rate, there was a sire effect ( $P < 0.05$ ) in Replication 1, tended ( $P < 0.10$ ) to be a sire effect in Replication 2, and was no sire effect ( $P > 0.05$ ) in Replication 3. Timed-AI pregnancy rates for the four sires in Replication 1 were 5.6 (1/18), 27.8 (5/18), 31.8 (7/22), and 100% (1/1). Timed-AI pregnancy rates for the four sires in Replication 2 were 14.3 (2/14), 15.8 (3/19), 26.7 (4/15), 33.3 (2/6), 33.3 (2/6), 42.9 (3/7), 63.6 (7/11), and 100% (1/1). Timed-AI pregnancy rates for the two sires in Replication 3 were 12.5 (4/32), and 31.7% (66/208).

The effectiveness of PGF<sub>2α</sub> to induce luteolysis within 72 h following PGF<sub>2α</sub> was also affected by changing the delivery of PGF<sub>2α</sub> from a single to two consecutive split treatments 24 h apart. Timed-AI CL regression was increased ( $P < 0.05$ ) by 25.0%, whereas total CL regression was increased ( $P < 0.05$ ) by 13.4% for the split compared to the single PGF<sub>2α</sub> treatment (Fig. 1). Progesterone concentrations at PGF<sub>2α</sub> treatment did not effect ( $P > 0.05$ ) timed-AI CL regression but effected ( $P < 0.05$ ) total CL regression (Fig. 2) in a quadratic manner ( $y = 0.537 + 0.314x - 0.051x^2$ ;  $r^2 = 0.09$ ). Progesterone concentrations  $\geq 6$  to  $< 9$  ng/mL had greater ( $P < 0.05$ ) total CL regression than all other progesterone concentrations except, the  $\geq 3$  to  $< 6$  ng/mL category. Progesterone concentrations 1 to  $< 3$  ng/mL at PGF<sub>2α</sub> had decreased ( $P < 0.05$ ) total CL regression compared to progesterone concentrations from  $\geq 3$  to  $< 6$  ng/mL. Heifers with progesterone concentrations  $\geq 9$  ng/mL had similar ( $P > 0.05$ ) total CL regression rates compared to progesterone concentration of  $< 6$  ng/mL.

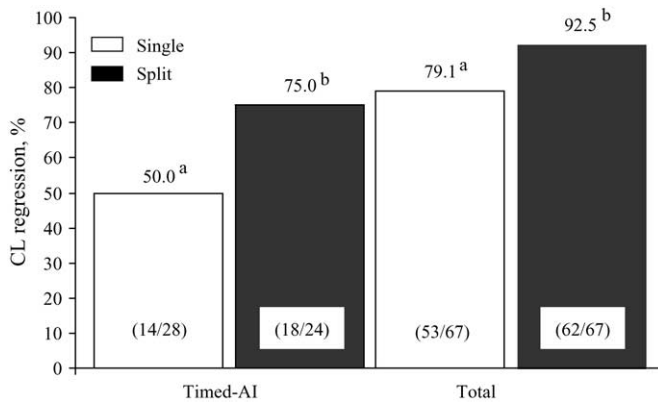


Fig. 1. Timed-AI and total corpus luteum (CL) regression of *Bos indicus* × *Bos taurus* heifers in Experiment 1 synchronized with melengestrol acetate (MGA) for 14 days, with either a single or two consecutive split treatments of PGF<sub>2α</sub> 24 h apart, initiated 19 days after MGA. Number in parenthesis indicates number of heifers within each group. Means without a common letter within a CL regression category differ ( $P < 0.05$ ).

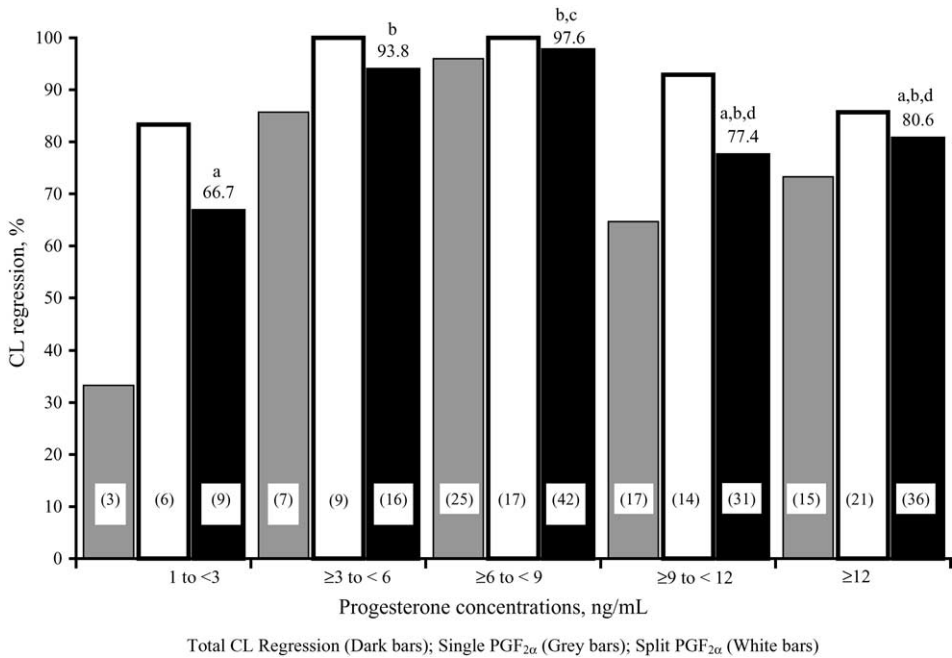


Fig. 2. Total corpus luteum (CL) regression at different progesterone concentrations in *Bos indicus* × *Bos taurus* heifers in Experiment 1 synchronized with melengestrol acetate (MGA) for 14 days, with either a single or two consecutive split treatments of PGF<sub>2α</sub> 24 h apart, initiated 19 days after MGA. Number in parenthesis indicates number of heifers within each group. Means between progesterone concentration categories without a common letter (a–d) differ ( $P < 0.05$ ). Treatment ( $P < 0.05$ ); progesterone concentration category ( $P < 0.05$ ); treatment by progesterone concentration category ( $P > 0.05$ ).

### 3.2. Experiment 2

The 2-year-old *B. indicus* × *B. taurus* heifers had greater ( $P < 0.05$ ) BW and BCS than the yearling Angus heifers, but mean BW and BCS were similar ( $P > 0.05$ ) between treatments. Estrous response, conception, timed-AI, and total-AI pregnancy rates for Experiment 2 are presented in Table 2. The 72 h estrous response was similar ( $P > 0.05$ ) between PGF<sub>2α</sub> treatments and there were no breed ( $P > 0.05$ ) or treatment by breed ( $P > 0.05$ ) effects for any variable tested. Modifying the delivery of PGF<sub>2α</sub> from a single to two consecutive split treatments tended ( $P = 0.07$ ) to increase conception rate. Additionally, conception rates were decreased ( $P < 0.01$ ) by 32.1% in *B. indicus* × *B. taurus* compared to Angus heifers. Both timed-AI and total-AI pregnancy rates were similar ( $P > 0.05$ ) between PGF<sub>2α</sub> treatments and there were no breed or treatment by breed effects ( $P > 0.05$ ). Body condition score had no effect ( $P > 0.05$ ) on 72 h estrous response, conception rate, timed-AI pregnancy rate, and total-AI pregnancy rate.

The mean interval from the initial PGF<sub>2α</sub> treatment to the onset of estrus was effected by treatment ( $P < 0.05$ ), breed ( $P < 0.05$ ), and treatment by breed ( $P < 0.01$ ; Table 3). The interval from PGF<sub>2α</sub> to the onset of estrus was similar ( $P > 0.05$ ) between PGF<sub>2α</sub> treatments for Angus heifers but *B. indicus* × *B. taurus* heifers receiving the split PGF<sub>2α</sub>

Table 2

Estrous response, conception, timed-AI, and pregnancy rates of yearling Angus and 2-year-old *B. indicus* × *B. taurus* (BI × BT) heifers in Experiment 2 synchronized with melengestrol acetate (MGA) for 14 days, with either a single or two consecutive split treatments of PGF<sub>2α</sub> 24 h apart 19 days after MGA<sup>a</sup>

Treatment	Seventy-two hour estrous response <sup>b</sup> , % (n)	Conception rate <sup>c</sup> , % (n)	Timed-AI pregnancy rate <sup>d</sup> , % (n)	Total-AI pregnancy rate <sup>e</sup> , % (n)
Single PGF <sub>2α</sub>	44.8 (67)	56.7 (30)	35.1 (37)	44.8 (67)
Angus	45.5 (33)	73.3 (15)	27.8 (18)	48.5 (33)
BI × BT	44.1 (34)	40.0 (15)	42.2 (19)	41.1 (24)
Split PGF <sub>2α</sub>	35.8 (67)	79.2 (24)	27.9 (43)	46.3 (67)
Angus	39.4 (33)	92.3 (13)	20.0 (20)	48.5 (33)
BI × BT	32.6 (24)	63.6 (11)	34.8 (23)	44.1 (34)
<i>P</i> -values <sup>f</sup>				
Treatment	NS	=0.07	NS	NS
Breed	NS	<0.01	NS	NS
Treatment × breed	NS	NS	NS	NS

<sup>a</sup> Heifers were fed MGA for 14 days and received either 25 mg of PGF<sub>2α</sub> im (single) or two consecutive 12.5 mg of PGF<sub>2α</sub> im 24 h apart (split). Heifers were AI 8–12 h after detected in estrus. Heifers not detected in estrus by 72 h after PGF<sub>2α</sub> received timed-AI and 100 μg of GnRH.

<sup>b</sup> Percentage of heifers displaying estrus 72 h after PGF<sub>2α</sub> of the total treated.

<sup>c</sup> Percentage of heifers that exhibited estrus, received AI, and became pregnant.

<sup>d</sup> Percentage of heifers that were timed-AI and became pregnant.

<sup>e</sup> Percentage of heifers that became pregnant to either an observed estrus and AI or timed-AI.

<sup>f</sup> NS = not significant ( $P > 0.05$ ).

treatment had a shorter ( $P < 0.01$ ) interval compared to heifers receiving single PGF<sub>2α</sub>. The interval from PGF<sub>2α</sub> to the onset of estrus was similar ( $P > 0.05$ ) between breeds when analyzed with survival analysis (data not shown). Interval from PGF<sub>2α</sub> to the onset of estrus had no affect ( $P > 0.05$ ) on conception rates either between or within treatments and breeds.

Duration of estrus was not affected ( $P > 0.05$ ) by PGF<sub>2α</sub> treatment but was decreased ( $P < 0.01$ ) in Angus compared to *B. indicus* × *B. taurus* heifers (Table 3). There was no treatment by breed effect ( $P > 0.05$ ) on the duration of estrus. The total number of mounts (Table 3) received during estrus was similar ( $P > 0.05$ ) between heifers receiving single and split PGF<sub>2α</sub> treatments and between breeds. However, the distribution of mounts received during estrus was different ( $P < 0.05$ ) between breeds (Fig. 3). Angus heifers received a greater percentage ( $P < 0.05$ ) of mounts during the first 6 h of estrus compared to *B. indicus* × *B. taurus* heifers; whereas, *B. indicus* × *B. taurus* heifers received a greater ( $P < 0.05$ ) percentage of mounts during the later stages of estrus, which included mounts recorded  $\geq 12$  h after the onset of estrus.

For heifers with a functional CL at the initial PGF<sub>2α</sub> treatment, progesterone concentrations were similar ( $P > 0.05$ ) between heifers receiving different PGF<sub>2α</sub> treatments ( $8.2 \pm 0.3$  ng/mL) but were greater ( $P < 0.01$ ) in *B. indicus* × *B. taurus* ( $9.1 \pm 0.4$  ng/mL) than Angus ( $7.1 \pm 0.5$  ng/mL) heifers. Timed-AI CL regression (single, 31/33 = 93.9%; split, 30/32 = 93.8%) and total CL regression (single, 57/59 = 96.6%; split,

Table 3

Intervals from PGF<sub>2α</sub> treatment to the onset of estrus, duration of estrus, and number of mounts received during estrus for yearling Angus and 2-year-old *B. indicus* × *B. taurus* (BI × BT) heifers in Experiment 2 synchronized with melengestrol acetate (MGA) for 14 days, with either a single or two consecutive split treatments of PGF<sub>2α</sub> 24 h apart, 19 days after MGA (Presented as least squares means ± standard error)<sup>a</sup>

Treatment <sup>b</sup>	N	Interval to estrus <sup>c</sup> (h:min)	Duration of estrus (h:min)	Total mounts <sup>d</sup>
Single PGF <sub>2α</sub>	25	62:32 ± 1:03	14:47 ± 0:51	52.1 ± 6.8
Angus	13	58:33 ± 1:28	12:55 ± 1:11	50.4 ± 9.9
BI × BT	12	66:33 ± 1:31	16:39 ± 1:14	54.4 ± 10.3
Split PGF <sub>2α</sub>	23	59:25 ± 1:05	14:06 ± 0:53	53.7 ± 7.1
Angus	12	60:12 ± 1:31	11:55 ± 1:14	50.0 ± 10.3
BI × BT	11	58:37 ± 1:35	16:08 ± 1:17	60.4 ± 10.8
<b>P-values<sup>e</sup></b>				
Treatment		<0.05	NS	NS
Breed		<0.05	<0.01	NS
Treatment × breed		<0.01	NS	NS

<sup>a</sup> Includes only heifers that had a functional corpus luteum (progesterone >1 ng/mL) at PGF<sub>2α</sub> and exhibited estrus by 72 h after PGF<sub>2α</sub>.

<sup>b</sup> Heifers were fed MGA for 14 days and received either 25 mg of PGF<sub>2α</sub> im (single) or two consecutive 12.5 mg of PGF<sub>2α</sub> im, 24 h apart (split). Heifers were AI 8–12 h after detected in estrus. Heifers not detected in estrus by 72 h after PGF<sub>2α</sub> were timed-AI and received 100 µg of GnRH.

<sup>c</sup> Mean interval from the initial PGF<sub>2α</sub> treatment to the onset of estrus.

<sup>d</sup> Total number of mounts received during the duration of estrus.

<sup>e</sup> NS = not significant (*P* > 0.05).

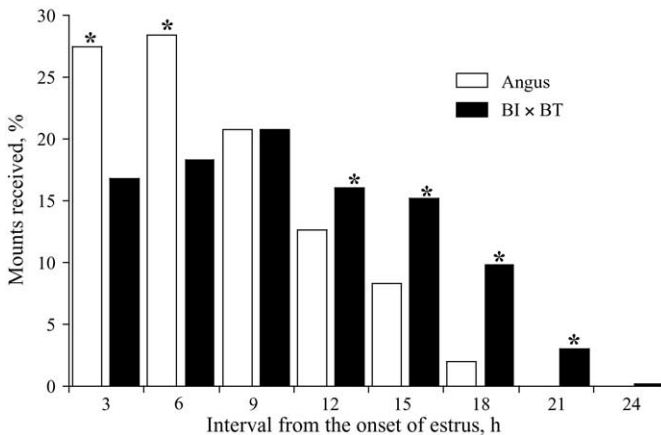


Fig. 3. Mounting activity during estrus as a percentage of mounts received during 3 h periods of estrus in Angus (white bars) and 2-year-old *Bos indicus* × *Bos taurus* (BI × BT; black bars) heifers in Experiment 2 synchronized with melengestrol acetate (MGA) for 14 days, with either a single or two consecutive split treatments of PGF<sub>2α</sub> 24 h apart, initiated 19 days after MGA. Breed (*P* < 0.05). Breed by period (*P* < 0.05). Periods denoted with an asterisk differ (*P* < 0.05) between breeds. Angus vs. *Bos indicus* × *Bos taurus* (*P* < 0.05).

51/53 = 96.2%) were similar ( $P > 0.05$ ) between PGF<sub>2α</sub> treatments. Timed-AI and total CL regression were similar ( $P > 0.05$ ) between Angus (23/25 = 92.0%; 45/47 = 95.7%) and *B. indicus* × *B. taurus* (38/40 = 95.0%; 63/65 = 97.0%) heifers, respectively. There were no ( $P > 0.05$ ) treatment by breed effects on timed and total CL regression. When progesterone concentration at PGF<sub>2α</sub> was included as a covariate in the model, it influenced ( $P < 0.05$ ) total CL regression. Total CL regression was similar ( $P > 0.05$ ) for all four progesterone categories from  $\geq 3$  to  $\geq 12$  ng/mL so data were pooled (data not shown). For heifers with progesterone concentration  $\geq 3$  ng/mL, total CL regression (57/59 = 96.6%), was greater ( $P < 0.05$ ) than progesterone concentrations  $< 3$  ng/mL (4/6 = 66.6%).

#### 4. Discussion

Modifying the delivery of PGF<sub>2α</sub> from a single to two consecutive split treatments, 24 h apart, increased 72 h estrous response by 6.9% in yearling *B. indicus* × *B. taurus* heifers in Experiment 1. The increase in 72 h estrous response was due to the increase in percentage of heifers that experienced luteolysis by 72 h in the split compared to the single PGF<sub>2α</sub> treatment. A similar improvement in estrous response was reported by Cornwell et al. [10] in *B. indicus* heifers receiving two consecutive split PGF<sub>2α</sub> treatments, 24 h apart, compared to a single PGF<sub>2α</sub> treatment. The 72 h estrous response for heifers receiving the split PGF<sub>2α</sub> treatment was similar to reports in yearling *B. taurus* heifers synchronized with a MGA/PGF<sub>2α</sub> protocol receiving a single PGF<sub>2α</sub> treatment 17 days after MGA [12,13,19], but slightly less than other reports in *B. taurus* heifers receiving PGF<sub>2α</sub> either 17 [20] or 19 days [12] after MGA. Conversely, the 72 h estrous response for heifers receiving the split PGF<sub>2α</sub> treatment was considerably less than *B. indicus* × *B. taurus* heifers synchronized with MGA/PGF<sub>2α</sub> receiving a single PGF<sub>2α</sub> treatment 17 days after MGA (30/49 = 61%) [14]. In contrast to Experiment 1, the 72 h estrous response was similar between the split and single PGF<sub>2α</sub> treatments for the 2-year-old *B. indicus* × *B. taurus* heifers in Experiment 2. Furthermore, the 72 h estrous responses were similar between Angus and 2-year-old *B. indicus* × *B. taurus* heifers, regardless of treatment. Unlike Experiment 1, luteolysis was not enhanced by the split PGF<sub>2α</sub> treatment in Experiment 2 as total CL regression was  $>96\%$  for both the split and single PGF<sub>2α</sub> treatments.

The decreased luteolysis for the single PGF<sub>2α</sub> treatment in Experiment 1 compared to Experiment 2 suggests an effect of animal age on the efficacy of PGF<sub>2α</sub> to induce luteolysis in *B. indicus* × *B. taurus* cattle. The exact reason(s) for the difference are unclear. When *B. indicus* × *B. taurus* heifers in Experiments 1 and 2 that had progesterone  $\geq 9$  mg/mL at the initial PGF<sub>2α</sub> and received single PGF<sub>2α</sub> treatment are compared, 2-year-old heifers were more responsive to PGF<sub>2α</sub> than *B. indicus* × *B. taurus* yearling heifers, suggesting a possible difference in CL responsiveness between the two age groups. Additionally, the uterus of the 2-year-old *B. indicus* × *B. taurus* heifers could be more physiologically mature and therefore more responsive to PGF<sub>2α</sub> than in yearling *B. indicus* × *B. taurus* heifers. It has been well documented that *B. indicus* heifers [21] attain puberty at older ages than *B. taurus* breeds [22]. Whether the uterus of a *B. indicus* × *B. taurus* heifer completing her pubertal or second estrous cycle is still immature and less responsive to PGF<sub>2α</sub> than the uterus of a *B. indicus* × *B. taurus* heifer that has completed several estrous

cycles is unclear. Because the yearling Angus heifers in Experiment 2 that received only a single PGF<sub>2α</sub> treatment had nearly a 16% greater CL regression than similarly treated yearling *B. indicus* × *B. taurus* in Experiment 1 suggest that events associated with luteolysis [23] could be compromised in yearling *B. indicus* × *B. taurus* heifers. Additional research will be required to confirm this.

The reason(s) for the decreased 72 h estrous response for both the *B. indicus* × *B. taurus* and *B. taurus* heifers in the present experiment compared to *B. taurus* heifers synchronized with a similar MGA/PGF<sub>2α</sub> system are unclear. Numerous factors, including environmental, frequency of estrous detection, inability to detect estrus, follicle development, and possibly handling stress could all contributed to the compromised 72 h estrous response in the present experiments. However, it should be noted that if the timed-AI would not have been conducted in the present experiments and estrous detection had continued for another 48–96 h, estrous response would have surely been greater. This was particularly true in Experiment 2, where CL regression was nearly 100% across both breeds and treatments.

The inability to detect behavioral estrus may partially explain the diminished 72 h estrous response in Experiment 1. Behavioral estrus is less intense in *B. indicus* × *B. taurus* than *B. taurus* cows [24], combined with the common occurrence of silent estrus in *B. indicus* cattle [25,26] are two reasons that estrus is difficult to detect in *B. indicus* cattle. Inadequate estrous detection was probably not the primary reason for the decreased estrous response in Experiment 2, since heifers were under constant observation of HeatWatch<sup>®</sup>, which has been shown to improve estrous detection efficiency [14,27]. In contrast, Stevenson et al. [14], using a group of cycling *B. indicus* × *B. taurus* heifers synchronized with MGA/PGF<sub>2α</sub> and monitored with HeatWatch<sup>®</sup>, reported a 72 h estrous response that was 20% greater than the present experiments. Whether environmental differences between the subtropical climates of Texas and Florida in the present study compared to the temperate climate of Kansas [14] could impair the expression estrus is unclear. Additionally, Galina et al. [24] and Orihuela et al. [6] suggest that estrus in *B. indicus* cattle may be more covertly expressed behaviors, e.g. head butting and (or) other secondary estrous activities, besides mounting. If estrous expression is more overt and there is an increase incidence of silent estrus in *B. indicus* cattle, even the use of HeatWatch<sup>®</sup> would not increase estrous detection efficiency. Although stage of follicle development at PGF<sub>2α</sub> was not evaluated in the present experiments, numerous studies have reported that follicular wave status and dominant follicle size at a PGF<sub>2α</sub>-induced luteolysis can influence the interval from PGF<sub>2α</sub> to the onset of estrus [28,29]. *B. indicus* cattle have also been reported to have an increased occurrence of three follicular waves [30], which could affect the interval from PGF<sub>2α</sub> to the onset of estrus and the synchrony of that estrus. Frequent handling of cattle, which has been shown to have a negative affect on the expression of estrus in cattle of *B. indicus* breeding [31,32], could have also had a negative effect on the expression of estrus in the present experiments.

Because HeatWatch<sup>®</sup> was used to monitor the estrous activity in Experiment 2, it was of interest to determine if breed or PGF<sub>2α</sub> treatment influenced characteristics associated with estrus. Duration of estrus was not affected by PGF<sub>2α</sub> treatment, but was affected by breed. Angus heifers had a shorter estrus than *B. indicus* × *B. taurus* heifers. In contrast, Rae et al. [3] reported no breed effect on duration of estrus between Angus and *B. indicus* × *B. taurus* heifers synchronized with SyncroMate B with estrus detected with HeatWatch<sup>®</sup>. Both the

Rae et al. [3] study and Experiment 2 were conducted at the same location, under similar management, using similar HeatWatch<sup>®</sup> parameters evaluating estrus, and with the Angus and *B. indicus* × *B. taurus* maintained in separate pastures, similar to the present study. The absence of a breed effect in the Rae et al. [3] study could be due to type of synchronization treatment or environmental differences between years.

The total number of mounts received during estrus was not affected by breed or PGF<sub>2α</sub> treatment. The total number of mounts received for the *B. indicus* × *B. taurus* heifers were similar to observations by Stevenson et al. [14], but considerably greater than other reports in synchronized *B. indicus* × *B. taurus* heifers [3,32]. Although breed had no effect on the total numbers of mounts received in Experiment 2, breed affected the distribution of mounts received during estrus. Angus heifers had an increased percentage of total mounts received during the early stages of estrus (0–6 h) than *B. indicus* × *B. taurus* heifers, which had the greatest percentage of total mounts received during the later stages of estrus (12–24 h). The mount distribution pattern for the Angus heifers in the present study was similar to observations in commingled and synchronized non-lactating Angus, Brahman, and Senepol cows [33], although, there was no breed effect on mount distribution in the latter study. As previously mentioned, the Angus and *B. indicus* × *B. taurus* heifers in the present were maintained in separate groups but exposed to similar environmental and management conditions. It appears that the yearling Angus heifers had a more intense estrus of shorter duration compared to a less intense estrus of longer duration for the *B. indicus* × *B. taurus* 2-year-old heifers. The differences in duration of estrus and distribution of mounting activity between the Angus and *B. indicus* × *B. taurus* heifers could be associated with different social hierarchical relationships within each breed [33,34].

Prostaglandin F<sub>2α</sub> treatment had no effect on conception rates in Experiment 1; however, conception rates tended to be greater for the split than the single PGF<sub>2α</sub> treatment in Experiment 2. Breed also had a significant affect on conception rates in Experiment 2, with 32% more Angus than *B. indicus* × *B. taurus* heifers conceiving. Mean conception rates for the *B. indicus* × *B. taurus* heifers were similar between Experiments 1 and 2, but still less than what is reported for *B. taurus* synchronized with the MGA/PGF<sub>2α</sub> system and inseminated after an observed estrus [12,13,19]. That conception rates in Experiment 1 were below average does not appear to be due to an AI sire/technician effect, since conception rates for the different AI sires and technicians were similar within each of the three replications. Differences in conception rates may be due to age at puberty. Cattle of *B. indicus* breeding [21] attain puberty at older ages than *B. taurus* breeds [22]; reaching puberty later may have decreased the number of estrous cycles heifers had before synchronization and AI. This is important, since fertility appears to increase as the number of estrous cycles after the pubertal estrus increases [34,35]. However, this logic does not apply for the compromised conception rates for the 2-year-old *B. taurus* × *B. indicus* heifers in Experiment 2, since heifers should have had several estrous cycles before synchronization and AI. Because of the limited numbers of heifers inseminated, conception rate data in Experiment 2 should not be over interpreted.

In Experiment 1, conception rates increased in a linear manner as the interval from PGF<sub>2α</sub> to the onset of estrus increased. A similar trend was not observed in Experiment 2, which may be due to a small number of heifers ( $n = 5$ ) exhibiting estrus by 48 h after PGF<sub>2α</sub> administration in Experiment 2. Others have reported decreased conception rates in *B.*

*taurus* heifers that exhibited estrus <48 h after PGF<sub>2α</sub> and compared to heifers expressing estrus >48 h following PGF<sub>2α</sub> [13]. However, it contrasts with a report in dairy heifers where conception rates remained the same as the interval from PGF<sub>2α</sub> to the onset of estrus increased [36]. The reduced conception rates observed in Experiment 1 could have also been due to differences in duration of dominance of the ovulatory follicle. Follicles with longer durations of dominance have decreased fertility when inseminated after an observed estrus [37–39]. Heifers in estrus early after PGF<sub>2α</sub> may have ovulated an oocyte from a follicle that had been dominant for an extended period, thus reducing their fertility. Heifers with extended intervals to estrus (60 and 72 h) probably represented heifers with a dominant follicle in a growing phase at luteolysis, which may have resulted in a decreased duration of dominance and increased fertility. Differences in conception rates may also be due to differences in follicular wave status of heifers that could be driving the difference in follicle dominance. In cattle with three follicular waves, the emergence of the third follicular wave occurs on approximately Day 16 of the estrous cycle [28,40]. In the present study, PGF<sub>2α</sub> was administered on approximately Days 12–17 of the estrous cycle. Heifers with three follicular waves might have initiated development of the third wave around luteolysis, resulting in an extended interval from PGF<sub>2α</sub> to the onset of estrus. In dairy cattle, fertility was higher after ovulation of a third-wave dominant follicle compared to ovulation of a second-wave dominant follicle [39].

Timed-AI pregnancy rate was 9% greater for the split than the single PGF<sub>2α</sub> treatment in Experiment 1, which was attributed to the improved luteolysis for the split PGF<sub>2α</sub> treatment. In contrast, timed-AI pregnancy rates and CL regression rates were similar between PGF<sub>2α</sub> treatments and breeds in Experiment 2. The timed-AI pregnancy rate of the split PGF<sub>2α</sub> treatment was similar to a timed-AI pregnancy rate in *B. taurus* heifers synchronized with the MGA/PGF<sub>2α</sub> system receiving a single PGF<sub>2α</sub> treatment [13]. The enhanced luteolytic effect of the split PGF<sub>2α</sub> treatment may have improved timed-AI pregnancy rates by removing the negative effect of progesterone and increasing the proportion of dominant follicles ovulating at timed-AI in response to GnRH. In contrast, heifers receiving a single PGF<sub>2α</sub> treatment likely had smaller ovulatory follicles at GnRH, due to longer progesterone exposure and a decreased growth rate, which reduced their ability to ovulate in response to GnRH [41]. Taponen et al. [42] reported that inducing ovulation of small follicles with GnRH resulted in a CL with a shortened life span and reduced fertility.

Because the split PGF<sub>2α</sub> treatment enhanced luteolysis, 72 h estrous response, and timed-AI pregnancy rates, total-AI pregnancy rate in Experiment 1 was increased by 8% compared to the single PGF<sub>2α</sub> treatment. In contrast, total-AI pregnancy rates were similar between PGF<sub>2α</sub> treatments and breeds in Experiment 2. Although the split PGF<sub>2α</sub> treatment improved the total-AI pregnancy rate of the *B. indicus* × *B. taurus* heifers in Experiment 1, the pregnancy rate was still less than reports in *B. taurus* cattle synchronized with MGA/PGF<sub>2α</sub> receiving a single PGF<sub>2α</sub> treatment, including a combination of estrous detection and timed-AI (59%) [43] or 5 days of estrous detection and AI, with pregnancy rates ranging from 47% [20] to 57% [11,43]. The decreased percentage of heifers detected in estrus, coupled with the slightly decreased conception rates for the *B. indicus* × *B. taurus* heifers in the present experiments, are probably the primary reasons for the decreased total-AI pregnancy rates.

In Experiment 1, modifying the delivery of PGF<sub>2α</sub> from a single to two consecutive split PGF<sub>2α</sub> treatments increased timed-AI CL regression by >25% and total CL regression by 13%. Using estrous response as an indirect indicator of luteolysis, several studies suggest that luteolysis is reduced in *B. indicus* cattle following a single PGF<sub>2α</sub> treatment [6,7]; however, few studies have analyzed blood progesterone concentrations to evaluate luteolysis. Pinheiro et al. [2] reported that 50% ( $n = 13/26$ ) of Nelore (*B. indicus*) cows with progesterone concentrations >1.0 ng/mL at PGF<sub>2α</sub> failed to either express estrus or have progesterone concentrations <1.0 ng/mL by 48 h following a single PGF<sub>2α</sub> treatment. Unlike Experiment 1, timed-AI and total CL regression were similar between PGF<sub>2α</sub> treatments in Experiment 2, suggesting that older *B. indicus* × *B. taurus* cattle may not benefit from two consecutive split PGF<sub>2α</sub> treatments like the yearling *B. indicus* × *B. taurus* heifers. This is supported by a recent report in mature, non-lactating *B. indicus* × *B. taurus* cows, where two consecutive split PGF<sub>2α</sub> treatments administered 7 and 8 days after a GnRH treatment did not improve pregnancy rates to a timed-AI compared to a single PGF<sub>2α</sub> treatment on Day 7 [44].

During a normal PGF<sub>2α</sub>-induced luteolysis, progesterone concentrations are <0.5 ng/mL within 24 h after a single 30 mg PGF<sub>2α</sub> treatment [45]. Cornwell et al. [10] reported that progesterone concentrations initially declined for 12 h after PGF<sub>2α</sub>, but rebounded to near pretreatment concentrations by 48 h in Brahman heifers failing to express estrus after a single PGF<sub>2α</sub> treatment. Chenault et al. [46] also reported an initial decrease in progesterone concentrations in dairy cattle not exhibiting estrus, and progesterone concentrations never declined to <1.0 ng/mL and luteolysis did not occur. Similar progesterone profiles have been reported in water buffalo that failed to ovulate following a single PGF<sub>2α</sub> treatment [47]. Administering two consecutive split PGF<sub>2α</sub> treatments likely improved luteolysis by two mechanisms. First, the split PGF<sub>2α</sub> treatments prevented the “rebounding” of progesterone concentrations and resulted in luteolysis [10]. Second, two consecutive PGF<sub>2α</sub> treatments may act to mimic the episodic release of PGF<sub>2α</sub> required to induce a spontaneous luteolysis [23] and thereby enhancing luteolysis in yearling *B. indicus* × *B. taurus* heifers.

Progesterone concentrations at PGF<sub>2α</sub> had an effect on total CL regression in both experiments. Heifers with progesterone between 1 and <3 ng/mL at PGF<sub>2α</sub> may represent a group of heifers that expressed estrus shortly before PGF<sub>2α</sub> and were in early diestrus (Days 5–8) when PGF<sub>2α</sub> was administered resulting in a CL that was less responsive to PGF<sub>2α</sub> [9,48,49]. This corresponds to similar findings in water buffalo cows where low progesterone concentrations at PGF<sub>2α</sub> resulted in a compromised luteolysis and lack of ovulation [47]. The decreased luteolysis observed when progesterone concentrations were >12 ng/mL in the yearling heifers in Experiment 1 is less clear. Progesterone concentrations of this magnitude usually indicate a robust CL typical of a late-luteal stage CL that is very responsive to PGF<sub>2α</sub> [8,9], which was observed in the Angus and 2-year-old *B. indicus* × *B. taurus* heifers in Experiment 2.

In summary, administering two consecutive split PGF<sub>2α</sub> treatments in yearling *B. indicus* × *B. taurus* heifers increased estrous response, timed-AI, and total-AI pregnancy rates due to an enhanced luteolysis compared to a single PGF<sub>2α</sub> treatment in Experiment 1. Furthermore, as the interval from PGF<sub>2α</sub> to the onset of estrus decreased, there was a significant decrease in conception rates. In Experiment 2, estrous response, timed-AI, and

total-AI synchronized pregnancy rates were similar between PGF<sub>2α</sub> treatments in the 2-year-old *B. indicus* × *B. taurus* and yearling Angus heifers. However, luteolysis was not improved by administering two consecutive split PGF<sub>2α</sub> treatments in Experiment 2. Estrous response, conception, and total-AI AI pregnancy rates for the *B. indicus* × *B. taurus* heifers in the present experiments were considerably decreased compared to reports in *B. taurus* heifers synchronized with similar MGA/PGF<sub>2α</sub> systems. In Experiment 2, breed influenced the interval from PGF<sub>2α</sub> to the onset of estrus, duration of estrus, and distribution of mounts received during estrus. In both experiments, the ability of PGF<sub>2α</sub> to induce luteolysis, regardless of treatment, was significantly influenced by progesterone concentrations at the time of PGF<sub>2α</sub> treatment.

## 5. Implications

Modifying the administration of PGF<sub>2α</sub> from a single (25 mg) to two consecutive split (12.5 mg) treatments 24 h apart initiated either 18 or 19 days after a 14 day MGA treatment increased estrous response, timed-AI and total-AI AI pregnancy rates in yearling *B. indicus* × *B. taurus* heifers by improving luteolysis. Similar improvements were not obtained in 2-year-old *B. indicus* × *B. taurus* or yearling Angus heifers. Therefore, adding a second PGF<sub>2α</sub> treatment to the MGA/PGF<sub>2α</sub> synchronization system is a low-cost management tool that producers can use to improve its effectiveness in yearling *B. indicus* × *B. taurus* heifers.

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## References

- [1] Plasse D, Warnick AC, Koger M. Reproductive behavior of *Bos indicus* females in a subtropical environment. IV. Length of estrous cycle, duration of estrus, time of ovulation, fertilization and embryo survival in grade Brahman heifers. *J Anim Sci* 1970;30:63–72.
- [2] Pinheiro OL, Barros CM, Figueiredo RA, do Valle ER, Encarnação RO, Padovani CR. Estrous behavior and the estrus-to-ovulation interval in Nelore cattle (*Bos indicus*) with natural estrus or estrus induced with prostaglandin F<sub>2α</sub> or norgestomet and estradiol valerate. *Theriogenology* 1998;49:667–81.
- [3] Rae DO, Chenoweth PJ, Giangreco MA, Dixon PW, Bennett FL. Assessment of estrus detection by visual observation and electronic detection methods and characterization of factors associated with estrus and pregnancy in beef heifers. *Theriogenology* 1999;51:1121–32.

- [4] Griffin JL, Randel RD. Reproductive studies of Brahman cattle. II. Luteinizing hormone patterns in ovariectomized Brahman and Hereford cows before and after injection of gonadotropin releasing hormone. *Theriogenology* 1978;9:437–46.
- [5] Rhodes III RC, Randel RD. Reproductive studies in Brahman cattle. I. Behavioral effect of various dose levels of estradiol-17B upon ovariectomized Brahman, Brahman X Hereford and Hereford cows. *Theriogenology* 1978;9:429–35.
- [6] Orihuela A, Galina C, Escobar J, Riquelme E. Estrous behavior following Prostaglandin  $F_{2\alpha}$  injection in Zebu cattle under continuous observation. *Theriogenology* 1983;19:795–809.
- [7] Landivar C, Galina CS, Duchateau A, Navarro-Fierro R. Fertility trial in Zebu cattle after a natural or controlled estrus with prostaglandin  $F_{2\alpha}$ , comparing natural mating with artificial insemination. *Theriogenology* 1985;23:421–9.
- [8] Tanabe TY, Hann RC. Synchronized estrus and subsequent conception in dairy heifers treated with prostaglandin  $F_{2\alpha}$ . I. Influence of stage of cycle at treatment. *J Anim Sci* 1984;58:805–11.
- [9] Watts TL, Fuquay JW. Response and fertility of dairy heifers following injection with prostaglandin  $F_{2\alpha}$  during early, middle, or late diestrus. *Theriogenology* 1985;23:655–61.
- [10] Cornwell DG, Hentges JF, Fields MJ. Lutalyse as a synchronizer of estrus in Brahman heifers. *J Anim Sci* 1985;61:416 (abstract).
- [11] Brown LN, Odde KG, King ME, LeFever DG, Neubauer CJ. Comparison of melengestrol acetate-prostaglandin  $F_{2\alpha}$  to Syncro-mate B for estrus synchronization in beef heifers. *Theriogenology* 1988;30:1–12.
- [12] Lamb GC, Nix DW, Stevenson JS, Corah LR. Prolonging the MGA-Prostaglandin  $F_{2\alpha}$  interval from 17 to 19 days in an estrus synchronization system for heifers. *Theriogenology* 2000;53:691–8.
- [13] Larson RL, Corah LR, Peters CW. Synchronization of estrus in yearling beef heifers with the melengestrol acetate/prostaglandin  $F_{2\alpha}$  system: efficiency of timed insemination 72 h after prostaglandin treatment. *Theriogenology* 1996;45:851–63.
- [14] Stevenson JS, Smith MW, Jaeger JR, Corah LR, LeFever DG. Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beef heifers. *J Anim Sci* 1996;74:729–35.
- [15] Richards MW, Spitzer JC, Warner MB. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. *J Anim Sci* 1986;62:300–6.
- [16] Seals RC, Lemaster JW, Hopkins FM, Schrick FN. Effects of elevated concentrations of prostaglandin  $F_{2\alpha}$  on pregnancy rates in progesterone supplemented cattle. *Prostaglandins* 1998;56:377–89.
- [17] Dransfield MBG, Nebel RL, Pearson RE, Warnick LD. Timing of insemination for dairy cows identified in estrus by radiotelemetric estrus detection system. *J Dairy Sci* 1998;81:1874–82.
- [18] Littell RC, Milliken GA, Stroup WW, Wolfinger RD. SAS for mixed models. Cary, NC: SAS Institute Inc.; 1999.
- [19] Jaeger JR, Whittier JC, Corah LR, Meiske JC, Olson KC, Patterson DJ. Reproductive response of yearling beef heifers to a melengestrol acetate-prostaglandin  $F_{2\alpha}$  estrus synchronization system. *J Anim Sci* 1992;70:2622–7.
- [20] Funston RN, Ansotegui RP, Lipsey RJ, Geary TW. Synchronization of estrus in beef heifers using either melengestrol acetate (MGA)/prostaglandin or MGA/Select Synch. *Theriogenology* 2002;57:1485–91.
- [21] Plasse D, Warnick AC, Koger M. Reproductive behavior of *Bos indicus* females in a subtropical environment. I. Puberty and ovulation frequency in Brahman and Brahman  $\times$  British heifers. *J Anim Sci* 1968;27:94–100.
- [22] Patterson DJ, Corah LR, Brethour JR, Spire MF, Higgins JJ, Kiracofe GH, et al. Evaluation of reproductive traits in *Bos taurus* and *Bos indicus* crossbred heifers: effects of postweaning energy manipulation. *J Anim Sci* 1991;69:2349–61.
- [23] McCracken JA, Custer EE, Lamsa JC. Luteolysis: a neuroendocrine-mediated event. *Physiol Rev* 1999;79:263–324.
- [24] Galina CS, Calderón A, McCloskey M. Detection of signs of estrus in the Charolais cow and its Brahman cross under continuous observation. *Theriogenology* 1982;17:485–98.
- [25] Dawuda PM, Eduvie LO, Esievo KAN, Molokwu ECI. Silent oestrus manifestation in Nigerian Bunaji Zebu cows. *Anim Reprod Sci* 1989;21:79–85.

- [26] Lamothe-Zavaleta C, Fredriksson G, Kindahl H. Reproductive performance of Zebu cattle in Mexico. 1. Sexual behavior and seasonal influence on estrous cyclicity. *Theriogenology* 1991;36:887–96.
- [27] White FJ, Wettemann RP, Looper ML, Prado TM, Morgan GL. Seasonal effects on estrous behavior and time of ovulation in nonlactating beef cows. *J Anim Sci* 2002;80:3053–9.
- [28] Sirois J, Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. *Biol Reprod* 1988;39:308–17.
- [29] Kastelic JP, Knopf L, Ginther OJ. Effect of day of prostaglandin  $F_{2\alpha}$  treatment on selection and development of the ovulatory follicle in heifers. *Anim Reprod Sci* 1990;23:169–80.
- [30] Viana JHM, Ferreira A, Fierreira W, Camargo L. Follicular Dynamics in Zebu cattle. *Pesq Agropec Bras* 2000;35:2501–9.
- [31] Vaca LA, Galina CS, Fernandez-Baca S, Escobar RJ, Ramirez B. Oestrous cycles, oestrus and ovulation of the zebu in the Mexican tropics. *Vet Rec* 1985;117:434–7.
- [32] Lemaster JW, Yelich JV, Kempfer JR, Schrick FN. Ovulation and estrus characteristics in crossbred Brahman heifers treated with an intravaginal progesterone-releasing insert in combination with prostaglandin  $F_{2\alpha}$  and estradiol benzoate. *J Anim Sci* 1999;77:1860–8.
- [33] Landaeta-Hernández AJ, Yelich JV, Lemaster JW, Fields MJ, Tran T, Chase Jr CC, et al. Environmental, genetic and social factors affecting the expression of estrus in beef cows. *Theriogenology* 2002;57:1357–70.
- [34] Galina CS, Orihuela A, Rubio I. Behavioural trends affecting oestrus detection in Zebu cattle. *Anim Reprod Sci* 1996;42:465–70.
- [35] Byerley DJ, Staigmiller RB, Berardinelli JG, Short RE. Pregnancy rates of beef heifers bred either on puberal or third estrus. *J Anim Sci* 1987;65:645–50.
- [36] Hernandez CJ, Almeraya AP, Arista AS, Tamayo VL. Estrus induction with prostaglandin  $F_{2\alpha}$ . Effect of interval between the treatment and the onset of estrus on conception rate in Holstein heifers. *Vet Mexico* 1994;25:19–22.
- [37] Mihm M, Basguisi A, Boland MP, Roche JF. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. *J Reprod Fertil* 1994;102:123–30.
- [38] Austin EJ, Mihm M, Ryan MP, Williams DH, Roche JF. Effect of duration of dominance of the ovulatory follicle on onset of estrus and fertility in heifers. *J Anim Sci* 1999;77:2219–26.
- [39] Townson DH, Tsang PC, Butler WR, Frajblat M, Griel Jr LC, Johnson CJ, et al. Relationship of fertility to ovarian follicular waves before breeding in dairy cows. *J Anim Sci* 2002;80:1053–8.
- [40] Savio JD, Keenan L, Boland MP, Roche JF. Pattern of growth of dominant follicles during the oestrous cycle of heifers. *J Reprod Fertil* 1988;83:663–71.
- [41] Sartori R, Fricke PM, Ferreira JC, Ginther OJ, Wiltbank MC. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. *Biol Reprod* 2001;65:1403–9.
- [42] Taponen J, Katila T, Rodríguez-Martínez H. Induction of ovulation with gonadotropin-releasing hormone during proestrus in cattle: influence on subsequent follicular growth and luteal function. *Anim Reprod Sci* 1999;55:91–105.
- [43] Salverson RR, DeJarnette JM, Marshall CE, Wallace RA. Synchronization of estrus in virgin beef heifers using melengestrol acetate and  $PGF_{2\alpha}$ : an efficacy comparison of cloprostenol and dinoprost tromethamine. *Theriogenology* 2002;57:853–8.
- [44] Hiers EA, Barthle CR, Dahms MKV, Portillo GE, Bridges GA, Rae DO, et al. Synchronization of *Bos indicus* × *Bos taurus* cows for timed artificial insemination using gonadotropin-releasing hormone plus prostaglandin  $F_{2\alpha}$  in combination with melengestrol acetate. *J Anim Sci* 2003;81:830–5.
- [45] Thatcher WW, Chenault JR. Reproductive physiological responses of cattle to exogenous prostaglandin  $F_{2\alpha}$ . *J Dairy Sci* 1976;59:1366–75.
- [46] Chenault JR, Thatcher WW, Kalra PS, Abrams RM, Wilcox CJ. Plasmal progestins, estradiol, and luteinizing hormone following prostaglandin  $F_{2\alpha}$  injection. *J Dairy Sci* 1976;59:1342–6.
- [47] Brito LFC, Satrapa R, Marson EP, Kastelic JP. Efficacy of  $PGF_{2\alpha}$  to synchronize estrus in water buffalo cows (*Bubalus bubalis*) is dependent upon plasma progesterone concentration, corpus luteum size and ovarian follicular status before treatment. *Anim Reprod Sci* 2002;73:23–35.
- [48] Lauderdale JW. Effects of  $PGF_{2\alpha}$  on pregnancy and estrous cycle of cattle. *J Anim Sci* 1972;35:246 (abstract).
- [49] Henricks DM, Long JT, Hill JR. The effect of prostaglandin  $F_{2\alpha}$  during various stages of the oestrous cycle of beef heifers. *J Reprod Fertil* 1974;41:113–20.