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Estrus Performance of Boran and Boran × Holstein Friesian Crossbred Cattle Synchronized with a Protocol based on Estradiol Benzoate or Gonadotrophin-Releasing Hormone

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ABSTRACT

The objectives of this study were to determine estrus behavior, interval to estrus, duration of estrus and time of ovulation of Boran, and Boran × Holstein Friesian (HF) breeds. A total of 113 cows and heifers from the two breeds were synchronized with two estrus synchronization protocols: 1) estradiol benzoate (EB) + controlled internal drug release (CIDR) + prostaglandin ($PGF_{2\alpha}$); and 2) gonadotrophin (GnRH) + CIDR + $PGF_{2\alpha}$. The results (mean ± SE) showed that the Boran breed had significantly longer interval to estrus (70.67 ± 5.9 versus 54.58 ± 4 h), shorter duration of estrus (8.65 ± 0.83 versus 12.1 ± 0.7 h) and lower behavioral score (mean ± SD; 883 ± 639 versus 3,399 ± 957) compared to Boran × HF crossbred cattle. Animals treated with EB + CIDR + $PGF_{2\alpha}$ had a significantly shorter interval to estrus (46.75 ± 4 versus 78.5 ± 4 h), higher behavioral score (3,058 ± 1,223 versus 2,663 ± 991) and longer duration of estrus (11.31 ± 0.71 versus 9.4 ± 0.75 h) compared to animals treated with GnRH + CIDR + $PGF_{2\alpha}$. The interval from estrus to ovulation (26 ± 2.5 h) was not different between breeds and between synchronization methods. The Boran (*Bos indicus*) breed tended to have a longer interval to estrus, shorter duration of estrus and an estrus behavior score lower than Boran × HF crossbred cattle treated and managed under similar conditions. The protocol using EB + CIDR + $PGF_{2\alpha}$ was more effective than GnRH + CIDR + $PGF_{2\alpha}$ in terms of enhancing estrus behavior and creating tight synchrony.

Keywords: estrus behavior, interval to estrus, estrus duration, synchronization, Boran cattle

INTRODUCTION

In order to improve production in crossbred cattle operations, it is necessary to achieve a 12-month calving interval. To achieve this goal, cows must cycle and become pregnant within an average of 85 d postpartum. However, a long postpartum anestrous period is a very common problem in cows reared in a tropical environment. Inaccurate detection of estrus is the single most important problem limiting successful reproductive performance and use of artificial insemination (Senger, 1994; Baruselli *et al*., 2004). Behavioral differences between *Bos indicus* and *Bos taurus* cattle might lead to inaccuracies or inconsistencies in human observation and

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interpretations (Chenoweth, 1994). Although *Bos indicus* and *Bos taurus* breeds share a common ancestor, there are differences in various aspects of their reproductive physiology and behavior (Baruselli *et al.*, 2006). These may be associated with different natural and human selection pressures, compounded by strong genotype-environment interactions.

Previous results indicate that hormonal treatments that stimulate gonadotrophin secretion, follicular development and ovulation, such as GnRH, gonadotrophin, prostaglandin and progestagen implants, have been tested in temperate and Zebu cattle (Niasari Naslaji *et al.*, 1996; Pinheiro *et al.*, 1998; Mattoni and Ouedraogo, 2000). However, the results were not very promising, particularly with respect to Zebu and related crossbred cattle. Recently, Baruselli *et al.* (2006) in his review suggested that treatments using progesterone-releasing devices and GnRH/EB may improve reproductive performance in *Bos indicus* cows due to their beneficial effect on LH pulse frequency, follicular growth and ovulation. Furthermore, Solano *et al.* (2004) suggested that estrogen applied to a small number of animals is necessary to prime estrus synchronization and increase the number of cows showing mounting behavior.

The objective of the present study was to determine the estrus behavior and score, interval to estrus, duration of estrus and time of ovulation of Boran and Boran × HF crossbred cattle, and the effect of EB or GnRH in combination with CIDR and $PGF_{2\alpha}$ on these parameters.

MATERIALS AND METHODS

Site and animal description

The experiment was conducted at the Holetta Agricultural Research Center dairy farm in the central highland of Ethiopia. Sixty six cows (21 Boran and 45 Boran × HF) with a postpartum period of more than 50 d and 47 heifers (18 Boran and 29 Boran × HF crosses) each with a body condition score of 4 and above (on a 1˘9 scale) were synchronized with two estrus synchronization protocols.

The experiment consisted of two breeds and two types of estrus synchronization protocols to animals grouped into two parity groups (cows and heifers). Animals were assigned randomly (within breed and parity) to one of the two treatments: treatment 1; EB + CIDR + $PGF_{2\alpha}$ (n=58) and treatment 2; GnRH + CIDR + $PGF_{2\alpha}$ (n=55). Cows and heifers allocated to treatment 1 were injected intramuscularly with 2 mg of EB (Ciderol, Genetics, Australia) and a CIDR (1.9 g of progesterone; Pfizer Animal Health, New Zealand) implant was inserted into the vagina. Seven days later, the CIDR was removed and each cow/heifer was injected intramuscularly with 2 mL of $PGF_{2\alpha}$ (Estrumet; Intervet). Cows/heifers allocated to treatment 2 were treated similarly except that GnRH (2 mL; Busereline acetate-Receptal; Intervet) was used in place of EB (Figure 1). Following $PGF_{2\alpha}$ injection or CIDR

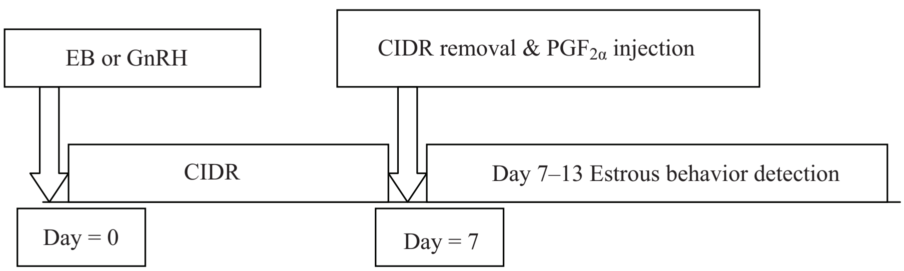

Figure 1 Schematic presentation of estrus synchronization protocols used in both treatments.

withdrawal, animals were observed for behavioral estrus for 24 h per day over 5˘7 d.

All animals were maintained on grazing pasture during the daytime and housed inside on a concrete floor at nighttime. Concentrate feed composed of wheat bran (59%), noug seed cake *(Guizotia abyssinica*; 40%) and salt (1%) was fed to the lactating cows and heifers.

Detection of estrus behavior

After completion of the synchronization treatment, animals were observed for estrus behavior by trained fulltime (24 h) observers for 5˘7 d and estrous behavior was recorded with an assigned value (Table 1; van Eerdenburg *et al*., 2002). Each time a behavior sign was observed, the assigned number of points was given except for vaginal mucous discharge and swollen vulva, which were recorded only once. During the 7 d after $PGF_{2\alpha}$ injection, treated animals were maintained in a holding yard with a teaser bull to increase the accuracy of observations. Estrus was defined as a period of sexual receptivity where two or more mounts were received, preceded and followed by a 4 h period of no activity (Orihuela *et al*., 1983). Onset of estrus was determined when the first of two mounts was received within the 4 h period. The end of estrus was the last mount received, with a mount 4 h before, and no mounts received during the next 12 h (White *et al*., 2002).

Ultrasound examination of ovarian structure

Starting 16 h after the onset of estrus, transrectal ultrasonography (Beal *et al.,* 1992) was applied every 4 h until the dominant follicle had disappeared from the ovary. The time of ovulation was determined as 2 h preceding the time that the dominant follicle disappeared from the ovary. The follicular diameter was measured at the time of CIDR removal, at the onset of estrus and 12 h after the onset of estrus using the shortest length of the largest follicle.

Statistical analysis

Frequency distribution was used to determine the estrus expression rate, estrous behavior signs and the distribution of the number of animals (from the total animals in estrus) by the interval to estrus group for the effect of breed, synchronization method and parity. The mean, median and standard deviation were used to describe the estrous behavioral score (total score during the estrus period) for breed, synchronization method and parity groups (cows and heifers).

Data on the interval to estrus, duration of estrus and ovulation time were analyzed using a general linear model. Least square means were used to compare the differences between breeds, synchronization methods and parities. Relationships of the estrous behavioral score with the diameter of largest follicle (DLF), duration of

Table 1 Scoring scale used for observed symptoms of estrus behavior.

Estrus behavior characteristics	Score assigned (point)
Mucous vaginal discharge	3
Restlessness	5
Sniffing the vagina of another cow	10
Chin resting	15
Mounted but not standing	10
Mounting (or attempt) another cow	35
Mounting head side of another cow (butting)	45
Standing heat	100

Source: van Eerdenburg *et al*. (2002).

estrus and interval to estrus were determined using Pearson s correlation coefficient and a t-test was used to evaluate the level of significance of the relationship at the level $P < 0.05$ (van Eerdenburg *et al.*, 2002).

RESULTS

Estrus behavior characteristics

The estrus expression rates of Boran, and Boran × HF crossbred cows and heifers synchronized with two hormonal treatment programs are summarized in Table 2. The proportion of females that responded to synchronization treatment was 48.6 and 54.8% for cows and 39.1 and 33.3% for heifers using EB + CIDR + $PGF_{2\alpha}$ and GnRH + CIDR + $PGF_{2\alpha}$, respectively. The estrus expression rate was 33.3 and 60% for Boran, and Boran × HF crossbred cows ($P > 0.05$) and 61.1 and 20.7% for Boran, and Boran × HF crossbred heifers ($P < 0.005$), respectively. The difference between crossbred cows and heifers was significant ($P < 0.05$) while the difference between Boran cows and heifers was not significant.

The most frequently and consistently observed estrus behavior in both breeds and treatments exhibiting estrus was standing to be mounted by a herd-mate, vaginal mucous discharge and a swollen vulva. Other secondary signs included an attempt to mount another animal, bellowing, mounted but not standing, restlessness, chin resting and sniffing the vagina of another cow.

Vaginal mucous discharge, mounting other animals, sniffing the vagina of another cow and chin resting were observed more in Boran × HF crosses compared with Boran females. However, the other behavioral signs were equally manifested by both breeds (Figure 2).

Mounted but not standing and restlessness were more observed in females treated with EB + CIDR + $PGF_{2\alpha}$ compared with females treated with GnRH + CIDR + $PGF_{2\alpha}$, whereas a swollen vulva was observed more frequently in females treated with GnRH + CIDR + $PGF_{2\alpha}$, while the remaining estrous behavioral signs were equally observed in both treatments (Figure 3).

Differences in estrous behavioral signs between cows and heifers are presented in Figure 4. A swollen vulva, mounting other cows, bellowing, restlessness and sniffing the vagina of another cow were more observed by cows in estrus compared to heifers; however there was no difference with respect to the behavioral types of standing to be mounted, mounted but no standing, vaginal mucous discharges and chin resting.

The distribution of interval to estrus following treatment was not different between

Table 2 Estrus expression rate (EER) of Boran, and Boran × HF crossbred dairy cows and heifers in response to EB + CIDR + $PGF_{2\alpha}$ or GnRH + CIDR + $PGF_{2\alpha}$.

	Cows		Heifers	
	No.	EER (%)	No.	EER (%)
All	66	51.5 (34)	47	36.2 (17)
Breed group				
Boran (*Bos indicus*)	21	33.3 (7)^a	18	61.1 (11)^a
Boran × HF	45	60.0 (27)^a	29	20.7 (6)^b
Treatment				
EB + CIDR + $PGF_{2\alpha}$	35	48.6 (17) ^{a}	23	39.1 (9)^a
GnRH+CIDR+$PGF_{2\alpha}$	31	54.8 (17)^a	24	33.3 (8)^a

Within a column, in a group, mean values followed by the same letter are not significantly different ($P < 0.05$). No. = number of animals treated; number of animals in estrus in parenthesis.

Boran, and Boran × HF crosses. The majority of animals (45% of Boran × HF crossbred and 38.9% of Boran females) were in estrus within 48 h after $PGF_{2\alpha}$ injection (Figure 5).

The percentage distribution of animals (in estrus) across the interval to estrus was different between the two synchronization methods (Figure 6). The majority of females that were treated with and responded to EB+CIDR+$PGF_{2\alpha}$ (69%) were in estrus within 48 h after $PGF_{2\alpha}$, injection whereas only 16% of the animals that were treated with and responded to GnRH + CIDR + $PGF_{2\alpha}$ showed estrus during this period. There was no difference between the percentage distribution of cows and heifers in the interval to estrus. The majority of responding cows and heifers were in estrus within 48 h after $PGF_{2\alpha}$ injection (Figure 7).

The mean, median and standard deviation of the estrus behavioral scores for breed synchronization method and parity are presented in Table 3. The mean estrus behavioral score during the observation period was higher (3,398 ± 957 points) for the Boran × HF breed and lower (1,883 ± 639 points) for the Boran breed. Animals treated

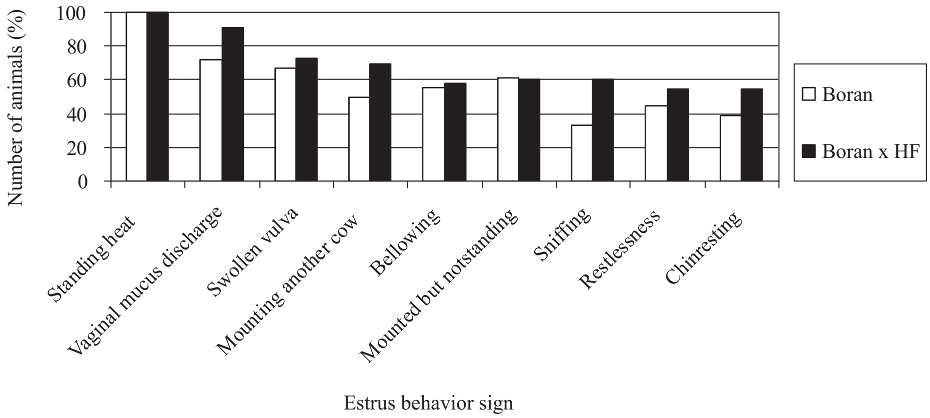

Figure 2 Percentage of Boran (n=18) and Boran × HF (n=33) females showing different signs of estrus behavior after treatment.

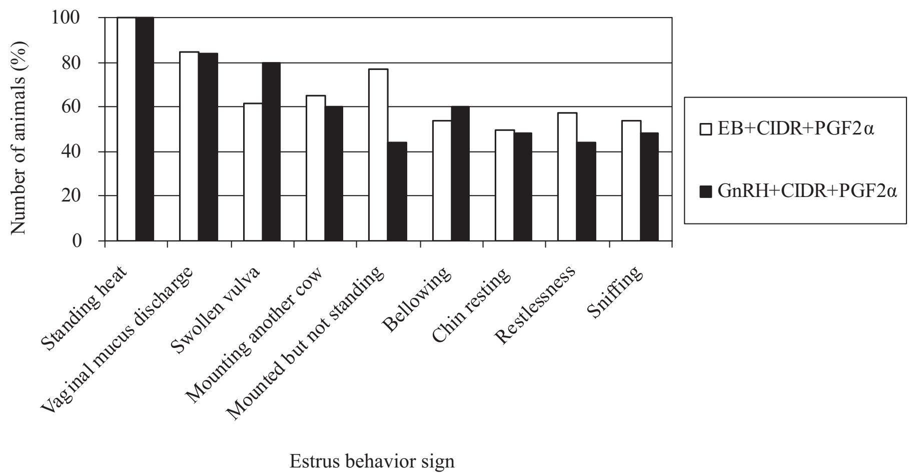

Figure 3 Percentage of animals showing different signs of estrus behavior in EB + CIDR + $PGF_{2\alpha}$ (n=26) and GnRH + CIDR + $PGF_{2\alpha}$ (n=25) treatments.

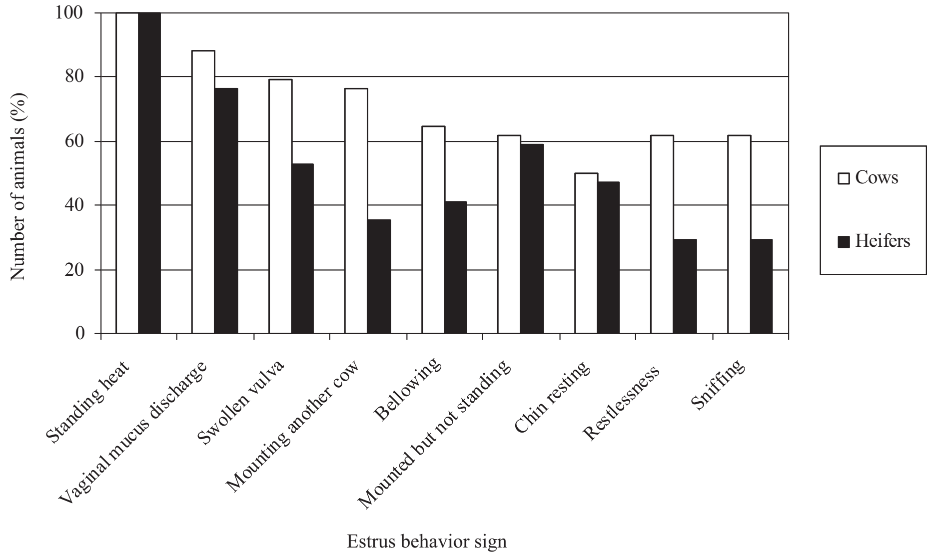

Figure 4 Distribution of number of cows (n=34) and heifers (n=17; in estrus) showing different signs of estrus behavior.

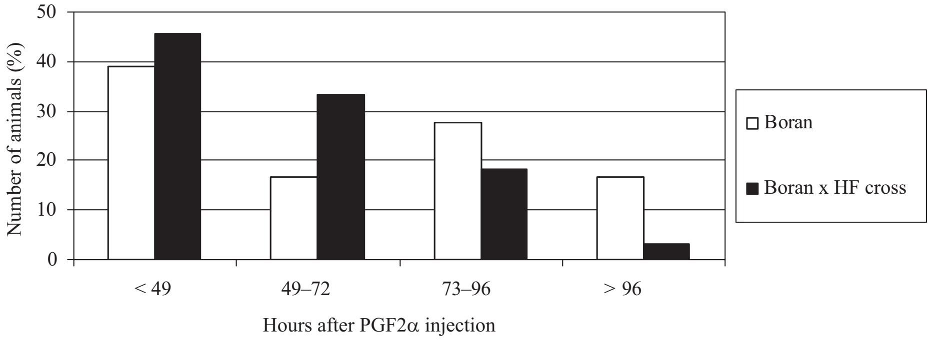

Figure 5 Distribution of number of animals across interval to estrus group after $PGF_{2\alpha}$ injection for Boran and Boran × HF crossbred animals.

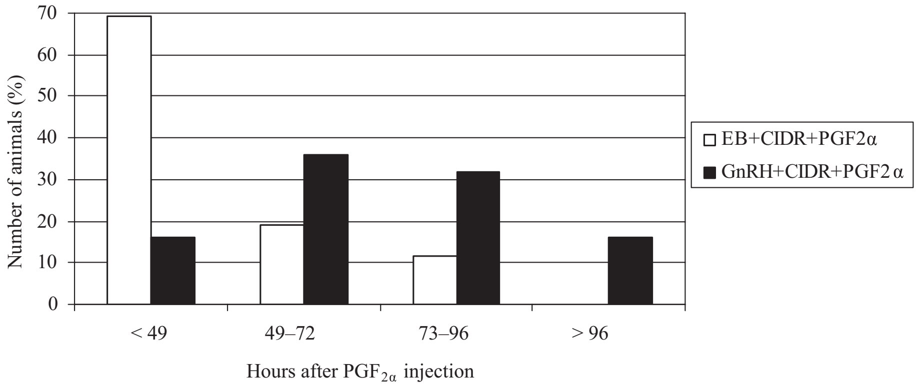

Figure 6 Distribution of number of animals across interval to estrus after $PGF_{2\alpha}$ injection for EB + CIDR + $PGF_{2\alpha}$ and GnRH + CIDR + $PGF_{2\alpha}$ treatments.

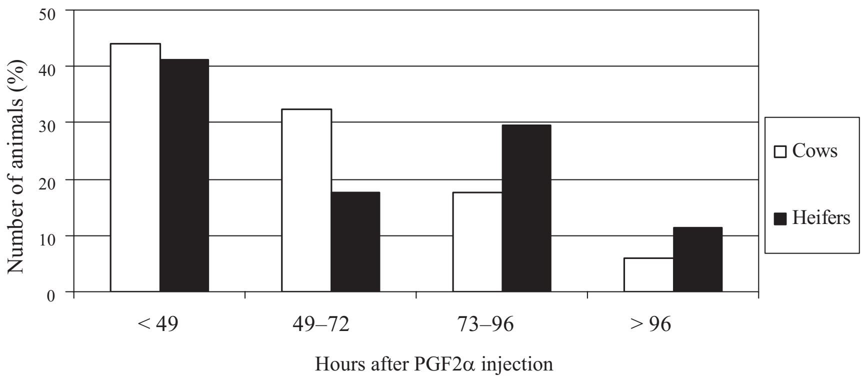

Figure 7 Distribution of number of cows and heifers across interval to estrus after $PGF_{2\alpha}$ injection.

Table 3 Results of estrus behavior score for breed, synchronization method and parity.

	Mean	Median	Minimum	Maximum	SD
Breed group					
Boran	1,883	1,765.5	1,008	3,188	639
Boran × HF	3,399	3,223.0	1,600	5,778	957
Synchronization method					
EB + CIDR + $PGF_{2\alpha}$	3,058	3,073.0	1,203	5,778	1,224
GnRH + CIDR + $PGF_{2\alpha}$	2,663	2,658.0	1,008	4,633	991
Parity group					
Cows	3,244	3,200.5	1,478	5,778	936
Heifers	2,104	1,843.0	1,008	5,588	1,101

with the EB + CIDR + $PGF_{2\alpha}$ protocol had higher behavioral scores compared to animals treated with the GnRH + CIDR + $PGF_{2\alpha}$ protocol (3,058 ± 1,224 versus 2,663 ± 991 points), while the mean behavioral score was higher for cows compared to heifers (3,244 ± 936 versus 2,104 ± 1101 points). There was a positive correlation for the estrus behavioral score with DLF (r = 0.36; $P > 0.05$) and for the estrus behavioral score with the duration of estrus (r = 0.70; $P < 0.001$) and a negative correlation between the estrous behavioral score and the interval to estrus (r = -0.33; $P < 0.05$).

The results on the mean interval to estrus and the duration of estrus are presented in Table 4. The interval from $PGF_{2\alpha}$ to estrus was significantly affected by breed, synchronization method and interaction between breed × synchronization method, while the effect of parity was not significant. The interval to estrus was significantly longer (70.67 ± 5.9 h; $P < 0.001$) for the pure Boran breed compared with the Boran × HF crosses (54.58 ± 4.0 h). The mean interval to estrus was significantly longer (78.5 ± 4 h; $P < 0.001$) for GnRH + CIDR + $PGF_{2\alpha}$ treated animals compared to the mean interval of 46.75 ± 4 h for females treated with EB + CIDR + $PGF_{2\alpha}$. There was no significant difference between cows and heifers on the interval to estrus (Table 4).

The duration of estrus was significantly affected by breed while the effects of synchronization method and parity were not significant (Table 4). The mean duration of estrus was significantly longer for Boran × HF crosses (12.1 ± 0.7 h; $P < 0.05$) compared with the Boran breed (8.65 ± 0.83 h). The mean duration of estrus

Table 4 Least square means interval to estrus and duration of estrus.

	No.	Interval to estrus (h after $PGF_{2\alpha}$)	Duration of estrus (h)
Breed group		**	*
Boran	18	70.67 ± 5.9^a	8.65 ± 0.83^b
Boran × HF	33	54.58 ± 4.0^b	12.1 ± 0.7^a
Synchronization method		***	NS
EB + CIDR + $PGF_{2\alpha}$	26	46.75 ± 4^b	11.31 ± 0.71^a
GnRH + CIDR + $PGF_{2\alpha}$	25	78.5 ± 4^a	9.4 ± 0.75^a
Parity		NS	NS
Cow	34	61.31 ± 4^a	11.3 ± 0.68^a
Heifers	17	63.9 ± 5^a	9.3 ± 0.85^a

Within a column, in a group, mean values followed by different letters are significantly different; *** = ($P < 0.0001$); ** = ($P < 0.001$); * = ($P < 0.05$); NS = Not significant ($P > 0.05$); No. = number of records.

was longer for animals treated with the EB + CIDR + $PGF_{2\alpha}$ protocol compared with animals treated with GnRH + CIDR + $PGF_{2\alpha}$ (11.3 ± 0.71 versus 9.4 ± 0.75 h). The mean interval from estrus to ovulation was estimated to be 26 ± 2.5 h and was not affected by breed, synchronization method and parity.

DISCUSSION

The overall percentages of Boran, and Boran × HF crossbred females that expressed estrus after treatment with EB + CIDR + $PGF_{2\alpha}$ or GnRH + CIDR + $PGF_{2\alpha}$ in the present study (45%) was lower than the previous result of 62% reported for Boran, and Boran × HF crossbred cattle in Ethiopia (Tegegne *et al*., 1989) and similar to the estrus expression rate of 46.4% reported by Landivar *et al*. (1985) for IndoBrazil and Gyr cows treated with $PGF_{2\alpha}$. The lower estrus response in the present study compared with the previous result could have been related to the presence of cows/heifers with inactive ovaries at the start of the experiment. When the percentage of cows cycling at the start of the breeding was less than 50%, Stevenson *et al*. (2000) also observed a substantial decrease in estrous rates of *Bos taurus* cows synchronized with the Select-Synch protocol.

The poor estrus expression of heifers compared to cows could be attributed to the greater nutritional stress being imposed on younger animals due to their requirements for growth (Elhag, 2003) and poor feed availability during the dry season was also a contributing factor, since this experiment was conducted in the middle of the dry season when animals had a poor nutrition supply and were in poor body condition. Voh *et al*. (1987) reported a seasonal effect on the estrus response between the wet and dry seasons (a longer duration of standing estrus in the wet season than in the dry season) in Zebu cows treated with $PGF_{2\alpha}$.

The lower manifestation of estrous behavior signs in the Boran breed compared with the Boran × HF crossbred animals was in agreement with the poor estrus expression of the *Bos indicus* breed in tropical environments (Galina and Arthur, 1990; Galina *et al*., 1996; Bó *et al*., 2003). The slightly higher estrous behavior signs and higher behavioral score observed in animals treated with EB + CIDR + $PGF_{2\alpha}$ compared with GnRH + CIDR + $PGF_{2\alpha}$ is not clear and could have been related to the direct effect of estradiol on behavior expression. In a study on ewes, Caraty *et al*. (2002) reported that at least two mechanisms are necessary for the expression of sexual behavior around the time of estrus/ovulation, with one involving direct estradiol and the other involving estradiol-induced GnRH secretion. In another study, for example, hypogonadal mice that did not have detectable GnRH in the forebrain, displayed normal standing behavior after estradiol and progesterone treatment (Ward and Charlton, 1981) indicating the role of estradiol in estrus behavior without the involvement of GnRH. Solano *et al.* (2004) suggest that estrogen applied to a small number of animals is necessary to prime estrus synchronization increasing the number of cows showing mounting behavior.

In the present study, it was shown that the number of points that a cow scored on the behavior scale was strongly correlated with the duration of estrus (r = 0.70; $P < 0.001$) and significantly and negatively correlated with the interval to estrus; however, the relationship between estrous behavioral score and DLF was weak. The latter result was in agreement with the nonsignificant correlation coefficient (r = 0.01) between preovulatory follicle size and estrus behavioral score (van Eerdenburg *et al*., 2002).

The interval to estrus obtained for Boran breed animals in the present study was similar with the interval of 70 ± 2.9 h reported for the *Bos indicus* breed (Saldarriaga *et al*., 2007). Solano *et al*. (2000) reported that cows treated with norgestomet and estrogen displayed mounting activity at 57.6 ± 0.40 h after implant withdrawal, which is in agreement with what was observed in the Boran, and Boran × HF crossbred animals. In the same study, animals treated with norgestomet alone had a higher average interval (132 ± 85 h). The shorter interval to estrus obtained in animals treated with EB + CIDR + $PGF_{2\alpha}$ compared with GnRH + CIDR + $PGF_{2\alpha}$ indicated the better synchronizing ability of EB compared to GnRH. A similar longer interval of 66 ± 3 h for dairy cattle treated with GnRH + CIDR + $PGF_{2\alpha}$ was reported by Richardson *et al*. (2002). The synchrony of estrus within 48 h after $PGF_{2\alpha}$ injection that was observed for the majority of Boran or Boran × HF crossbred animals treated with EB + CIDR + $PGF_{2\alpha}$ may make this treatment compatible with timed insemination at 54˘66 h after $PGF_{2\alpha}$ injection.

The significant and longer duration of estrus for Boran × HF crossbred animals compared with the Boran (*Bos indicus*) breed is in agreement with the longer period of sexual receptivity reported for White Fulani crossbreds mated with an HF bull as compared with White Fulani heifers (Johnson and Oni, 1986). In agreement with the present study, a significant breed effect on the duration of estrus (longer for crossbred cows) and estrus behavioral score was also reported for Angus, Brahman, and Angus × Brahman cows (Rae *et al*., 1999). Similarly, the average duration of estrus in *Bos indicus* cattle was reported to be about 10 h, with variations between 1.3 and 20 h (Galina and Arthur, 1990; Pinheiro *et al*., 1998).

The interval from the onset of estrus to ovulation obtained in the present study was similar to the mean interval from the onset of estrus to ovulation of 27.1 ± 3.3 h reported in Nellore cows (Mizuta, 2003). In addition other reports have shown an interval of about 25˘29 h (Cavalieri *et al*., 1997; Pinheiro *et al*., 1998). However, earlier, Randel (1976) reported a lower interval of 19 h between the onset of estrus to ovulation in Brahman heifers. The reason for this difference is unclear. However, there may have been a difference in the accuracy of determining the time of ovulation, as the latter studies used real-time ultrasonography while the earlier study used rectal palpation. Other factors such as rain, strong wind, movement of animals from one pasture to another or keeping animals in a corral also tended to suppress mounting and estrus related activities (Galina *et al*., 1996). Dobson and Smith (2000) suggested that exposure to environmental, management or animal-derived stresses affects the frequency and amplitude of GnRH and LH-pulses and delays the preovulatory LH-surge with a subsequent decrease in estrus expression and incidence of normal ovulation.

CONCLUSION

The results from the present study indicated that the Boran (*Bos indicus*) breed tended to have a longer interval to estrus, shorter duration of estrus and lower estrus behavior scores compared with Boran × HF crosses under similar treatment and management conditions, indicating the relatively poor response of this breed to hormonal interventions.

Estrogen administered with CIDR implants in the protocol involving EB + CIDR + $PGF_{2\alpha}$ treatment resulted in a shorter interval to estrus, longer duration of estrus and higher behavioral score compared with GnRH + CIDR + $PGF_{2\alpha}$. The higher estrus behavioral score observed in the EB + CIDR + PGF_2_estrous synchronization protocol compared with the GnRH + CIDR + $PGF_{2\alpha}$ protocol was the result of both a shorter interval to estrus and a longer duration of estrus.

The use of EB with CIDR and $PGF_{2\alpha}$ was more effective than GnRH in terms of enhancing estrus behavior and creating tight synchrony.

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