Estrus characteristics, ovarian response to synchronization hormones, and fertility of crossbred dairy heifers managed under a semi-intensive system

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Abstract

This study aimed to characterize estrus sign/s which best predict the time of ovulation and determine ovarian responses to different synchronization protocols and conception rates to fixed-time artificial insemination in Boran*Holstein crossbred heifers. In the first part of the study, twenty-seven heifers were observed for estrus signs (from induced and natural) and the relationship of various estrus signs with ovulation time was evaluated by using ultrasonography. In the second part, 91 Boran*Holstein crossbred heifers were randomly grouped into three groups. Group one heifers (n=28) received 100μg gonadotropin-releasing hormone (GnRH) on day zero (D0), 500μg prostaglandin F2α (PGF2α) on day 7 and 100μg of GnRH on day 9. Group 2 heifers (n=32) were treated as group one but additionally received progesterone as controlled internal drug release (CIDR). Group three heifers (n=31) were treated as those in group 2 but without injection with GnRH on day 0. In all the 3 groups' insemination was made at 19h of the second GnRH. The results showed that irrespective of estrus source type (induced or natural), score for standing to be mounted, mounting other heifers, and non-receptive mount by other heifers showed a strong positive correlation with ovulation time (r=0.67, *P*<0.05). Standing estrus duration, and time elapsed from standing estrus to ovulation were shorter (*P*<0.05) in induced estrus. The conception rate was 39.3% in synchronized ovulation in the absence of CIDR. The conception rate in timed insemination was 56.3% when ovulation was synchronized by combining CIDR, PGF2α, and GnRH. In conclusion injection of GnRH on day zero together with treatment with CIDR improved ovulation rate, the number of

Ethiop. Vet. J., 2021, 25 (2), 115-131

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new corpus luteum, and conception rate. Heifers that ovulated within the first 24h after timed insemination had a higher (*P*<0.05) conception rate than heifers ovulated after 24h of timed insemination. Cycling heifers at day zero had a greater (*P*<0.05) conception rate than non-cycling.

Keywords: Conception rate; crossbred dairy heifers; estrus sign; ovarian response; synchronization.

Introduction

Estrus induction by PGF2α and subsequent insemination is used to speed up cattle genetic improvement in Ethiopia. The average conception rates after estrus induction and insemination were reported to be lower than 40% (Gizaw *et al*., 2016). Many factors influence the efficacy of prostaglandin-based estrus induction; among which are the stage of the estrus cycle at prostaglandin administration, estrus sign detection, and submission of animals in estrus for artificial insemination (AI). Previous studies (Bainesagne, 2015; Destalem, 2015) indicate that 24.2% to 50.9% of cows had milk progesterone concentration higher than 3ng/ml at insemination, which strongly indicated that insemination was done at the wrong time.

Protocols that allow for AI without the need for estrus detection are a better alternative to increase the number of animals inseminated at an appropriate time. These protocols need manipulation of follicular waves and ovulation before insemination. These protocols have been successfully applied in *Bos taurus* herds. However, where the environment is predominantly subtropical to tropical, and when *Bos taurus* breed was crossed to *Bos indicus*, a decrease in conception rates to ovulation synchronization and timed AI has been reported (Hiers *et al*., 2003). When dairy heifers of *Bos taurus* breed are subjected to Ovsynch protocol, a greater incidence of estrus before timed artificial insemination (TAI) was frequently observed (Rivera *et al*., 2004) and that leads to a low conception rate to TAI for dairy heifers. Suppression of premature estrus expression using Controlled Internal Drug Release (CIDR) improves conception rate to TAI in taurine dairy heifers.

Most of the data on ovulation synchronization and conception rate to timed AI was from sub-tropical countries like Brazil, Argentina, and the Southern United States. Similarly, the zebu cattle used for crossing to *Bos taurus* in these

countries were commonly the Nelore breed. Little is known on the subject in tropical African and non-Nelore zebu cattle crossed to *Bos taurus* cattle. The objectives of the present study were, therefore, to characterize estrus sign/s which best predict the time of ovulation, and determine ovarian responses to different synchronization protocols and conception rates to fix time of artificial insemination in crosses of *Bos indicus* X *Bos taurus*.

Materials and methods

Study area

This study was conducted in six dairy farms, three farms from Bishoftu, and three from Modjo towns. Both towns are located in the Eastern Shewa Zone of Oromia Regional State. Bishoftu is located at 47km southeast of Addis Ababa at 8°45′N latitude, 38°59′E longitude, and an elevation of 1885 meters above sea level. The mean annual rainfall of the town is 866 mm with a bimodal pattern. The mean annual minimum and maximum temperatures are 14^0C and 26^0C, respectively and relative humidity was 61.3%. Modjo town is located at 66Km southeast of Addis Ababa at 8°35'N latitude and 39°7'E longitude. The altitude is 1790 meters above sea level. The average annual rainfall, temperature, and mean relative humidity are 776mm, 19.4 ºC, and 59.9%, respectively (National Meteorology, 2010, unpublished data).

Experimental design and treatments protocols

This study has two experiments. Experiment one (Exp 1) involves 27 crossbred dairy heifers aged between 18 and 26 months and with a mean body condition score of 3.7± 0.2 (on 1-5 scale, Ferguson *et al.*, 1994). The heifers were given two doses of 500µg prostaglandin F2 alpha (PGF2α) (Synchromate®, cloprostenol sodium, Warburg, Germany) at 11 days intervals. After gathering data on estrus, the heifers were rested for one estrus cycle and crossed over for gathering data on natural estrus. In natural estrus, an observation started from day 16 of the previous estrus. Experiment 2 (Exp2) involved 91 crossbred dairy heifers randomly assigned to three groups. In group 1, 28 heifers received 100µg gonadotrophin-releasing hormone (GnRH) through IM injection (Gonadorelin diacetate tetrahydrate, Merial limited Duluth, USA) on day 0 (D0), 500µg PGF2α on day 7 and the second dose of GnRH (100µg) on day 9. The group was assigned as GnRH-PGF2α-GnRH (GPG). Group 2 (CIDR-GPG) heifers (n=32) received all treatments of Group 1 and were additionally treated with CIDR (CIDR 1380, EAZI BREED™, New Zealand) from day 0 to 7 and at

CIDR removal (D7) PGF2α was given. Group 3 (CIDR-P-G) heifers (n=31) had the same treatment as those in Group 2 but without the initial (day 0) GnRH treatment. In all groups, heifers were inseminated using frozen-thawed semen at 19h of the last GnRH injection.

Estrus detection

After 24h of the second PGF2α injection in experiment 1, heifers were visually observed every 3h for 30 minutes (6 am, 9 am, 12 am, 3 pm, 6 pm, 9 pm, 12 pm, and 3 am) for estrus sign for 120h in estrus induced group or starting from day 16 of the former estrus in spontaneous estrus. Observed estrus signs were scored using a method recommended by van Eerdenburg *et al.* (1996) with slight modifications in relation to signs associated with vaginal anatomy, which were not considered in the present study. Each time a symptom was observed, the assigned number of points was recorded (Table 1). If the sum of the points exceeded 50 during two consecutive observation periods, a heifer was considered to be in estrus. A heifer was said to be in standing estrus when it stood receptive on mounting by another heifer, with or without the other symptoms of estrus. Cessation of estrus was when heifers are no more receptive to mounting after previously being in standing estrus.

Table 1. Estrus scoring criteria. A heifer was considered to be in heat when the cumulative score was 50 at two observations (Modified from van Eerdenburg *et al.*, 1996).

Estrus sign	Points assigned
Flemen	3
Restlessness	5
Bellowing (repeated)	5
Sniffing vagina of another cow	10
Being mounted but not standing	10
Resting with chin on another cow	15
Mounting (or attempting) other cows	35
The mounting head side of another cow	45
Standing heat	100

Ovarian ultrasonography

Mindray ultrasound (DP.50vet, China) with a 7.5 MHz linear array rectal probe was used. In Experiment 1, starting from 24h of the second PGF2α in estrus induced group or day 16 of previously induced estrus in natural estrus group, ovarian structures were scanned twice a day (8:00 am; 8:00 pm) until heifers were in standing estrus. Beginning from first standing estrus, ovarian examinations were made at every 6h interval until heifers were off estrus. From off estrus to ovulation, ovarian examinations were made every 3h. In Experiment 2, ovaries were monitored on days 0, 2, 7, 8, and 9, and then at 24h, 36h, and 48h after day 9 to assess ovulatory outcomes and size of the ovulatory follicle. On ultrasonographic examination, the size of follicles, the location of the dominant follicle, and the corpus luteum were recorded. Ultrasonographic examinations were also performed on day 10 after AI to determine CL diameter. Ovulation was confirmed on the disappearance of a previously identified dominant follicle (≥8mm) and the presence of CL on the same site (modified from Ginther *et al.,* 1989).

Pregnancy determination

Conception was checked on day 32 of AI. On ultrasound, the presence of a fluid-filled uterine horn and a conceptus were used as positive indicators of conception (Fricke *et al.*, 1998).

Statistical analysis

Duration of estrus was calculated by subtracting the time initial estrus was detected from the time the last standing mount was detected. The interval from PGF2α injection to the onset of estrus was calculated as the interval from PGF2α to the first estrus detected. The estrous response was defined as the number of heifers displaying estrus after PGF2α injection divided by the total number of heifers treated. To evaluate the length of time intervals of each behavioral estrous sign to an ovulation time, analysis of variance (ANOVA) in STATA software was used. All heifers that ovulated between 10 pm and 6 am were not included in the ovulation time interval as determining ovulation every 3h within this time gap was interrupted in some heifers. Pearson correlation was used to evaluate the correlation between estrus signs and ovulation time, follicle size at standing estrus and ovulation, follicle size and ovulation time, ovulation from the same ovary at different estrus. $P < 0.05$ was considered to be significant. Conception rate was defined as the number of heifers

that became pregnant, divided by the number of heifers that were inseminated. All count measurements were indicated as mean ± SE (standard error of the mean). Five heifers that did not respond to PGF2α were excluded from estrus induced group.

Results

Estrus behavior characteristics and frequencies

The detailed descriptions of estrus signs manifested are listed in Table 2. There was no statistically significant difference among induced and natural estrus ($P>0.05$) on the frequency of various estrus signs. However, there was a tendency that standing to be mounted, attempt to mount other heifers, and non-receptive mount were frequently seen in heifers from the induced group. Irrespective of estrus type (induced or natural), sniffing ano-vaginal region and resting chin on other heifers were the most frequently observed estrus signs. Estrus signs were more frequently observed when more than two heifers were in estrus at the same time.

Table 2. The frequency and percentages of estrus sign observed (mean ±SE) in relation to estrus type and number of heifers in estrus

Estrus sign	Mean frequency (±SE) and (percent) of estrus signs		
	Induced estrus	**Natural estrus**	
	≥ two heifers were in estrus (synchronized)	*One heifer in estrus*	*≥ two heifers in estrus*
Sniffing	20.2±8.3, (92)[a]	15.6±3.6, (90)	16.3±2.7,(94)
Chin resting	22.6±10.5, (90)	18.5±5.2, (96)	21.2±10.6, (94)
Mounting others	9.9±4.2, (82)	6.7±2.6, (71)	7.9±3.2, (74)
Mount not received	8.2±1.5, (64)[a]	2.6±1.2, (63)	4.2±1.8, (69)
Standing estrus (mounts received)	7.5±2.8, (63.6)	4.7±1.5, (54)[a]	7.2±2.3, (59.1)
Bellowing	9.4±3.7, (58.9)	7.9±2.5, (50.9)	8.8±3.3, (54.9)

a= Cells with superscript **a** across the column were significantly different (*P*<0.05) from others

Estrus duration and time of ovulation

The details of durations of different estrus behaviors and intervals from different estrus signs to ovulation are listed in Table 3. The mean (± SE) estrus length was not affected by estrus type (induced and natural). The interval from estrus onset to ovulation was slightly shorter in induced estrus (30.7±0.6h)

than natural estrus (32.1±0.8h) although the difference was not statistically significant (*P*>0.05). Heifers that showed less than 24h estrus length in the induced group were also likely to have less than 24h estrus length when crossed over to the natural estrus manifestation group (r = 0.85; *P*<0.05). Standing estrus was started at an average of 7.1 ± 0.4h of onset of estrus in induced estrus and 8.6 ± 2.0h in natural estrus (Table 3). In all heifers that ovulated, ovulation occurred within the first 24h after the end of estrus. From the total of 32 ovulations observed, the large majority (60.4%) ovulated from the right ovaries (*P*<0.05). Heifers that had ovulations from right ovaries in estrus induced group were more likely to successively ovulate from the right ovaries (r= 0.6, *P*<0.05) when crossed over to ovulate from natural estrus. In the present study, it was found that standing to be mounted and mounting other heifers have a 100% correlation with ovulation rate (r=1) and interestingly the time of ovulation was quite consistent (range 16 -21h) and was with minimum variation between individual heifers with standing to be mounted.

Table 3. Duration of estrus, estrus onset to standing heat, and estrus signs to ovulation (hrs)

Estrus sign	Estrus type & interval to ovulation			
	Induced		Natural	
	Mean± SE	Range	Mean ±SE	Range
Duration of estrus	18.4±4.2	11.0-29.0	20.2 ±3.9	10.0 -30.0
Interval from onset to standing estrus	8.1 ±0 .6	6.0 -20.0	10.4±1.5	8.0-23.0
Standing estrus duration	7.1 ± 0.4[a]	5.0 – 12.0	10.6 ± 2.0[b]	3.0-16.0
Estrus onset to ovulation	30.7 ± 0.6	18.0-38.0	32.1±0.8	17.0-39.4
Estrus end to ovulation	12.3± 1.1	6.0-20.0	15.4±1.2	6.4-20.3
Standing estrus to ovulation	19.0±1.9[a]	12.0-21.0	25.0 ±4.0[b]	15.0-26.0
Mounting other heifers to ovulation	26.8±4.3[a]	16.0-35.4	30.3±2.1[b]	21.2-43.5
Non-receptive mount to ovulation	24.7±5.4	10.5-45.0	21.5±1.9	16.4-39.6
Sniffing (start) to ovulation	29.4±1.2	20.0-38.5	31.4±5.3	22.0-36.5
Chin resting (start) to ovulation	30.5±5.2	17.0-40.5	30.5±4.7	20.0-37.0

a, b cells with superscripts a,b within the row were different (*P*<0.05) from others

Follicle and corpus luteum size by types of estruses

The mean (±SE) diameter of the larger follicle at first standing estrus did not differ significantly (*P*>0.05) due to estrus type (8.64±1.32 in induced; 9.22± 0.73 in natural estrus). Similarly, the mean (±SE) diameter of the ovulatory follicle

didn't show a significant difference (*P*>0.05) between induced (16.15±0.53mm) and natural (17.43±1.53) ovulations. There was a strong positive correlation between follicle size at standing estrus and size at ovulation in which heifers with large follicles at standing estrus had larger ovulatory follicles at ovulation (r = 0.72, *P*<0.05). There was also a positive correlation between follicle size and ovulation time in which, heifers with larger follicles at standing estrus ovulated earlier than heifers with smaller follicles (r = 0.15, *P*>0.05). The diameter of CL did not significantly differ (*P*>0.05) between induced (17.5±1.6 mm) and natural estrus groups (17.7± 2.2mm).

Ovulation rate and ovulation time

The details of the ovulation rate to GnRH and time of ovulation were described in Table 4. Ovulation to day 0 GnRH injection was significantly higher (*P*<0.05) in heifers of the GPG group (28.6%) than the CIDR-GPG group (12.5%). Ovulation to day 9 GnRH injection was significantly higher (*P*<0.05) in the CIDR-GPG group than CIDR-P-G and GPG group. In the CIDR-GPG group, all heifers ovulated to day 0 GnRH injection ovulated also to day 9 GnRH injection. However, in the GPG group, 21.1% of heifers that ovulated to day 0 GnRH injection did not ovulate to day 9 GnRH injection.

The mean time (h) from PGF2 treatment to ovulation and from GnRH injection on day 9 to ovulation was not affected by treatment type (*P*>0.05) except in the CIDR-GPG group which had a significantly longer mean time (h) from PGF2α treatment to ovulation (*P*<0.05) than the other groups. When the distribution of time to ovulation after GnRH injection on day 9 was considered, more heifers (52.6%) from the GPG group ovulated within the first 24h than the remaining groups (Table 4). In about 73.7% of heifers from the GPG group, 78.6% from the CIDR-GPG group, and 83.3% from the CIDR-P-G group that ovulated to day 9 GnRH injection, the ovulations were from a follicle other than the large follicle that was present at the start date.

Diameter of follicles by treatment group

The mean size of the large follicle on day 0 did not differ (*P*>0.05) among the treatment groups (Table 4). Similarly, the mean size of the larger follicle at day 7 (48h of day 0 GnRH injection), the mean size of the large follicle of on day 9 GnRH injection, and the mean size of pre-ovulatory follicles that eventually ovulated did not statistically differ (*P*>0.05) among the treatment groups

(Table 4). However, when the data was combined with heifers ovulating from natural estrus (from experiment one), the mean diameter of the pre-ovulatory follicle that eventually ovulated was significantly greater ($P<0.05$) in natural ovulation (18.43±1.53) than hormone-treated groups.

Corpus luteum status by treatments

A large majority of heifers (60.4%) had visible CL on day 0 indicating that they were cyclic. The presence of a new CL on day 7 was greater ($P \leq 0.05$) for the CIDR-GPG group than the CIDR-P-G group. Ovulation to treatment with GnRH on day 0 resulted in a greater proportion of heifers with visible CL in the CIDR-GPG group than the CIDR-P-G group on day 7 (Table 4). The number of total CLs after PGF2α injection on day 7 was significantly greater ($P<0.05$) in heifers receiving GnRH on day 0 (CIDR-GPG) than those without GnRH treatment on day 0. Similarly, the proportion of heifers that have new CL on day 7 was significantly higher ($P<0.05$) in heifers that received GnRH on day 0.

Table 4. Ovarian characteristic and response to treatments

Ovarian status	Treatment group		
	GPG	**CIDR-GPG**	**CIDR-P-G**
PGF2*a* – *ovulation* interval (h)†	80.9± 2.7	99.5 ± 2.8	82.7 ±1.3
Ovulation due to D0 GnRH††	28.6%[a]	12.5%[b]	0%[c]
Ovulation due to D9 GnRH	67.9%[a]	87.5%[b]	77.4%[c]
D9 GnRH to ovulation interval(h) †	24.3±1.6	28.5±1.01	27.4±1.3
Ovulation in ≤ 24h of GnRH treatment (%)	52.6%	42.9%	45.8%
Ovulation in >24 of GnRH treatment (%)	47.4%	57.1%	54.2%
Follicle diameter at PGF2α	8.4±0.2	9.2±0.3	9.4±0.3
Follicle diameter 24h after PGF2α	10.0±1.2	10.2±0.6	11±1.4
Follicle diameter at D9 GnRH	11.8±1.7	12.7±2.5	12.5±0.7
Follicle diameter immediate to ovulation†	14.3±2.2	15.2±1.9	13.5±1.7
CL presence on d0 (%)	60.7%	65.6%	61.3%
New CL on D7(%) †††	35.7%[a]	47.5%[b]	16.1%[c]
Total CL on D7 (%)	82.1%[a]	62.5%[b]	51.6%[c]
D12 CL diameter†	16.3±2.4	17.6±1.9	16.5±2.7

a,b,c = within the row, cells with superscripts a,b,c differ *(P<0.05)* from each other, †= measurements were in mean ±SE, †† = heifers with follicle ≥ 10mm on D0 and CL on the same site 48h later,†††= heifers that had no CL on D0 but with new CL on D7

Conception rate

Among heifers inseminated, the pregnancy rate on day 32 was significantly less (*P*<0.05) in the GPG group than in CIDR-GPG and CIDR-P-G groups (Table 5). Similarly, pregnancy was significantly higher (*P*<0.05) in heifers that received GnRH at CIDR insertion than heifers without CIDR insert.

In all the 3 treatment protocols, heifers that ovulated within the first 24h after TAI had a higher pregnancy rate than heifers ovulated after 24h of insemination (Table 5). Cycling heifers at day 0 had a greater pregnancy rate compared to non-cycling heifers.

Table 5. Pregnancy rates in the three groups as affected by different factors

Factors considered	Pregnancy (%, N0)		
	GPG	**CIDR-GPG**	**CIDR-P-G**
Ovulation within 24h before TAI			
No of heifers (%)	4(21.1%)	7(25%)	5(20.8%)
No pregnant *(%)*	3(75%)	4(57.1%)	2(40%)
Ovulation within 24h after TAI			
No of heifers (%)	15(78.9%)	21(75%)	19(71.2%)
No pregnant *(%)*	8(53.3)	14(66.7%)	13(68.4%)
P. value	0.002	0.01	0.003
D0 luteal activity			
No cycling (% pregnant)	8 (44.4%)	12(57.1%)	10(52.6%)
No non-cycling (% pregnant)	3 (30.0%)	6 (54.5%)	5(41.7%)
P. value	0.014	0.07	0.001
Overall P/TAI	39.3% (11/28)	56.3(18/32)	48.4% (15/31)

Discussion

The slightly shorter estrus onset to ovulation in induced estrus compared to natural estrus was probably due to exogenous PGF2α which causes luteolysis and reduced progesterone concentration, which allows heifers to express estrus earlier than heifers under natural luteolysis. The estrus duration in both induced (18.4±4.2h) and natural estrus (20.2 ± 3.9h) groups were longer than of the previous report of Roelof *et al*. (2004) and Stevenson *et al*. (1998) who studied pure Holstein dairy heifers. Animal breed, management, and the environment may be among the factors that majorly contribute to the difference. The

disparity of results may also be due to differences in scoring estrus as Roelof *et al.* (2004) considered estrus when the sum of points during consecutive observation periods exceeded 100 as compared to 50 points considered in this study.

According to Yoshida and Nakao (2005), the average duration of standing estrus was 6.2 ± 3.9 h in Japan's dairy heifers which was slightly shorter than the average duration of standing estrus (7.1h in induced and 8.6h in natural estrus) of the present study. The same authors reported all the 10 heifers observed exhibited standing estrus. Heifers are considered to experience lesser stresses and therefore, should express strong estrus signs. However, in our study, only 63.5% from induced and 54.5% from the natural estrus group exhibited standing estrus behavior. Other authors (van Eerdenburg *et al.*, 1996; Le Blanc *et al.*, 1998; Lyimo *et al.*, 2000; van Eerdenburge *et al.*, 2002) reported that standing estrus behavior is not seen in many heifers and cows at different dairy farms. Some of the possible reasons for the lack of standing estrus behavior may be due to inherent breed effects and/or excessive animal handling for daily transrectal ultrasonic evaluation.

The present time interval from estrus onset to ovulation in induced (30.7 ± 1.6) and natural estrus (32 ±0.8hrs) was longer than the 27.7±2.4h and 26.1±1.2 h previously reported for induced and natural estrus groups, respectively (Pinheiro *et al.*, 1998). Similarly, Son *et al.* (2007) reported that ovulation occurred at 25.55±5.72h after the onset of estrus in dairy heifers. The differences may be possibly due to breed, and nutrition effects. Energy balance is known to affect reproduction in dairy cattle as reported by some authors (Wathes *et al.*, 2007; Van Hoeck *et al.*, 2014).

In the current study, the time interval between onset and end of most sexual estrus behaviors to ovulation showed large gap (range). The exceptions were standing to be mounted and mounting other heifers which showed lower variation and were easily detectable visually. Since all heifers that manifested mounting activities finally ovulated, signs related to mounting activities better-predicted ovulation time in this study. However, only 63.6% of heifers from induced and 54.5% from natural estrus groups were in standing estrus, which means when only standing estrus was considered heifers not in standing estrus could be missed. Lyimo *et al.* (2000) concluded that standing estrus, the standard symptom of estrus, was not the primary symptom for detecting estrus in cows as it was observed in only 53% of the cows.

Ovulatory follicle size is known to be affected by animal breed. In the present study, ovulation occurred at ovulatory follicle diameters range of 10mm to 19mm. The study of Gimenes *et al.* (2008) indicated that *B. indicus* cattle can ovulate from the follicle as small as 7mm in contrast to Holstein cattle which only ovulate from follicles with 10mm or more. Studies of Sartorelli *et al.* (2005) and Carvalho *et al.* (2008) indicate that ovulatory follicle diameter frequently ranges from 10 to 13mm in Bos *indicus*. In Holstein cattle, ovulation frequently occurs at a diameter of between 12 and 22mm (Savio *et al.*, 1988; Ginther *et al.*, 1989; Carvalho *et al.*, 2008). In one study (Alvarez *et al.*, 2000); ovulatory follicle was larger in Bos *indicus* than in *Bos taurus* cattle.

In the present study, the day 10 corpus luteum size of crossbred heifers ranged from 10mm to 22.6mm. The study of Carvalho *et al.* (2008) indicated that on day 10 after ovulation corpus luteum was smaller (15.3mm) in *B. indicus* cattle than in *B. taurus* heifers (18.4mm). Corpora lutea size can be as large as 24.1mm diameter for Holstein heifers (Sartori *et al.* (2004). In Nellore cattle (*B. indicus*) corpus luteum size range from 15.6mm diameter to 21.5mm diameter in the reports of Figueiredo *et al.* (1997) and Mollo *et al.* (2007).

Lima *et al.* (2010) compared the cost of timed AI and natural service using as inputs of reproductive results and economical information and, reported that the cost of pregnancy was less for timed AI than natural service. In the present study, Ovsynch protocol in absence of progestin (CIDR) resulted in a low conception rate to TAI (39.3%) in crossbred heifers. Similarly, previous studies (Pursley *et al.*, 1995; Schmitt *et al.*, 1996; Pursley *et al.*, 1997; Tenhagen *et al.*, 2005) indicated low pregnancy to TAI in Holstein heifers synchronized by Ovsynch protocol in absence of progestin. Tenhagen *et al.* (2005) proposed the limited success of Ovsynch protocol in heifers is suspected to be caused by the follicular dynamics of heifers that differs from that of lactating dairy cows.

The conception rate to TAI (56.3%) in the CIDR-GPG group in the present study was consistent with Colli *et al.* (2016) who reported 57.3% and 57.1% in crossbred (Angus *X* Nelore) heifers. However, in the work of Colli *et al.* (2016), the PGF2α was given twice, on day 0 and on the day of CIDR removal in the first group and two days prior to device removal in the second group heifers. Mendanha *et al.* (2012) reported lower conception to TAI (40.3% *and* 42.1%) in Girolando heifer when the PGF was given as in the work of Colli *et al.* (2016). The result indicates that animal breed and treatment protocol among the oth-

ers affect conception rate to TAI. Many other factors like sexual maturity of heifers, weight after weaning, and development of the reproductive tract may be the source of difference.

The lower pregnancy rate (48.4%) in CIDR-P-G heifers with a moderate ovulation rate (77.4%) may be due to the absence of treatment with GnRH on day 0 in this group. GnRH ovulates the dominant follicle (Pursley *et al.*, 1995) and prevents the formation of a persistent dominant follicle in heifers without CL at CIDR insert. Progestin treatment in the absence of a functional CL has been shown to result in the development of a persistent follicle resulting in poor pregnancy rates and oocytes ovulated from persistent follicles are known to be less fertile (Revah and Butler, 1996; Mihm *et al.*, 1999). Similarly, Bello *et al.* (2006) reported that synchronization response to Ovsynch was higher (87.9%) in cows that ovulated in response to the first GnRH of Ovsynch compared with those that did not (62.9%).

The variation in ovulation to treatment with GnRH on day 0 was probably due to the stage of follicular wave development at the time of GnRH injection and/ or differences in concentrations of gonadotropins, estrogen, inhibin, or progesterone at GnRH injection (Ireland *et al.*, 2000; Thatcher *et al.*, 2002). The work of Moreira *et al.* (2000) showed that initiation of Ovsynch on days 5 to 9 of the estrous cycle was a key to successful synchronization of ovulation.

Conclusions

Ovulation synchronization without CIDR insert leads to premature estrus manifestation and reduced fertility to timed insemination in crossbred heifers. Combining GnRH and CIDR at the start of ovulation synchronization increase follicle turnover, induce ovulation, and enhance new corpus luteum formation and increases fertility. Standing to be mounted and mounting other heifers are the best predictor for the time of ovulation, however, these behaviors were not seen in some heifers.

Acknowledgments

The authors also want to thank a technician that involved in estrus detection and the one involved in insemination work.

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