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Pregnancy Rate of Cross Breed Dairy Heifers Subjected to Ovulation Synchronization and Fixed Time Artificial Insemination

[1]Tilaye Demissie, [1]Tefera Yilma, [2]Tamrat Degefa, [3]Adise Ambilo, [3]Tsigereda Tadesse and [1]Alemayehu Lema
[1]*College of Veterinary Medicine and Agriculture, Addis Ababa University, PO. Box 34, Bishoftu, Ethiopia*
[2]*Debre-Zeit Agricultural Research Center, PO. Box 32, Bishoftu, Ethiopia*
[3]*College of Veterinary Medicine, Wolaita Sodo University, PO. Box 138, Wolaita, Ethiopia*

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Corresponding Author:
Tilaye Demissie
College of Veterinary Medicine and Agriculture, Addis Ababa University, PO. Box 34, Bishoftu, Ethiopia

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Abstract: Cattle breed, climate in which cattle reared and management result in differences in ovulation time and responses to reproductive hormones. Most of the data on ovulation synchronization and conception rate to timed AI in cattle of *Bos indicus* and their cross to *Bos taurus* were from humid tropical countries. Little has been known from tropical hot African countries. The study aimed to evaluate the effect on pregnancy rate when progesterone is added to or when initial GnRH is removed from the usual ovulation synchronization that uses GnRH- PGF2α-GnRH combination. To achieve the objective, cross breed dairy heifers (n = 91) were randomly grouped into 3 groups. Group 1, heifers received 100 µg GnRH on day of start (D0), 500 µg PGF2α on day 7 and 100µg of GnRH on day 9. Group 2 heifers were treated as group1 but heifers received progesterone as Controlled Internal Drug Release (CIDR). Group 3 heifers were treated as in group 2 but without D0 GnRH. All inseminations were made at 19h of the 2nd GnRH. Transrectal ultrasonography was used to assess ovarian changes and pregnancy. Group with D0 GnRH had significantly higher ($p<0.05$) ovulation 48h after D0 GnRH (28.6% in Ovsynch and 12.5% in CIDR+Ovsynch) and significantly higher new corpus luteum at D7. Pregnancy rate was significantly higher ($p<0.05$) in group with CIDR Ovsynch (56.3%) than without CIDR (39.3%). The mean diameter of larger follicle at first standing, the mean diameter of ovulatory follicle and that of corpus luteum were not affected ($p>0.05$) treatment protocol. In conclusion, in *Bos indicus X Bos taurus* reared in hot dry climate both CIDR insert and D0 GnRH improved pregnancy rate to timed artificial insemination and recommended.

INTRODUCTION

Estrus induction and artificially insemination is used to speed up cattle genetic improvement in Ethiopia. The average conception rate to artificial insemination was 27%[1]. Many factors influence conception rate to artificial insemination; among which are estrus sign detection and submission of animals in estrus for AI, cattle breed and the climate. Previous studies from Ethiopia[2, 3] indicate that 24.2-50.9% of cows had milk progesterone concentration >3 ng mL^{-1} at insemination which strongly indicated insemination was done at wrong time. Protocols that allow for AI without the need for estrus detection may be better alternative to increase the number of animals inseminated at appropriate time. These protocols need manipulation of follicular waves and ovulation before insemination. In fact, these protocols have been successfully applied in *Bos taurus* cattle reared in temperate climate. In areas where the environment is predominantly subtropical to tropical and when *Bos taurus* breed were crossed to *Bos indicus*, a decrease in conception rates to ovulation synchronization and timed AI have been reported[4]. When dairy heifers of *Bos tourus* breed are subjected to Ovsynch protocol, a greater incidence of estrus before TAI was frequently observed[4] and that leads to low conception rate to TAI. Progesterone suppress premature estrus and improve conception rate to TAI in taurine dairy heifers. Most of the data on ovulation synchronization and conception rate to timed AI in cattle of *Bos indicus* and their cross to *Bos taurus* were from sub-tropical countries. Little has been known from tropical hot African countries. The objective of the present study was to evaluate the difference in conception rate after omission of initial GnRH or addition of CIDR to commonly used ovsynch protocol that use GnRH-PGf2α-GnRH combination.

MATERIALS AND METHODS

Study area: This study was conducted in two dairy farms located in Bishoftu and Modjo towns. Bishoftu is located at 8°45 N latitude, 38°59 E longitude and at elevation of 1885 m above sea level. The monthly average low temperature ranges from 10-13°C and monthly average high temperature ranges from 23-26°C and the relative humidity was 61.3%. The mean annual rainfall of the town is 866 mm. Modjo town is located at 8°35'N latitude and 39°7'E longitude. The altitude is at 1777 meters above sea level. The monthly average low temperature ranges from 12-16°C and monthly average high temperature ranges from 25-30°C. The annual average rainfall is 776 mm and relative humidity is 59.9%.

Experimental design and treatments protocols: In this experiment, dairy heifers (n = 91) were randomly assigned to three groups. Group 1, heifers (n = 28) received 100 µg GnRH IM injection (Gonadorelin diacetate tetrahydrate, Merial limited Duluth, USA) on starting day (D0). On seventh day (D7) all heifers received 500 µg PGF2α IM. On ninth day (D9) heifers received a second dose 100 µg GnRH and the group was assigned as Ovsynch. Group 2 heifers (n = 32) were treated as in Ovsynch (group1) but on D0 heifers received CIDR (CIDR 1380, EAZI BREED™, New Zealand). The CIDR was retained for 7 days and at CIDR removal (D7), PGF2α was given. The group was assigned as CIDR+ Ovsynch. Group3 heifers (n = 31) were treated as in CIDR+Ovsynch but without the initial (D0) GnRH treatment and the group was assigned as CIDR+Ovsynch-D0 GnRH. In all the three groups, heifers were inseminated at 19h of second GnRH, using frozen-thawed semen of the same batch and the same bull.

Ovarian ultrasonography: Mindray ultrasound (DP.50vet, China) with 7.5 MHz linear array rectal probe was used. In all heifers ovaries were monitored on D0, D2, D7, D8, D9 and then at 24h, 36h and 48h after D9 to assess ovulatory outcomes and size of ovulatory follicle. On ultrasongraphic examination, the size of follicles, the location of the dominant follicle and corpus luteum were recorded. Ultrasonographic examinations were also performed on D10 of AI to determine CL diameter. Ovulation was confirmed on disappearance of a previously identified dominant follicle ≥8 mm and presence of CL on the same site[5]. Response to first GnRH injection was assessed by the development of a new CL on D7 regardless of their initial CL.

Pregnancy determination: Conception was checked on D32. On ultrasound, the presence of fluid-filled uterine horn and presence of a conceptus were used as positive indicators of conception[6].

Statistical analysis: Time of ovulation was calculated by subtracting the time initially dominant follicle was detected from the time its disappearance was detected. The time intervals between hormone administrations to ovulation were analyzed by Analysis of Variance (ANOVA), using STATA Software. Pearson correlation was used to evaluate the correlation between follicle size at first measure and size at ovulation, follicle size and ovulation time, ovulation from the same ovary at different estrus. $p<0.05$ was considered to be significant. Pregnancy 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).

RESULTS

Ovulation rate and ovulation time: The details of the ovulation rate to GnRH and time of ovulation was described in Table 1. Ovulation to D0 GnRH was

Table 1: Ovarian characteristic and response to treatments

	Treatment group		
Ovarian status	Ovsynch	CIDR+Ovsynch	CIDR+Ovsuch-D0 GnRH
PGF2α to ovulation (h)	80.9±2.7	99.5±2.8	82.7±1.3
Ovulation to D0 GnRH†	28.6%[a]	12.5%[b]	NA
Ovulation 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≤24h of GnRH (%)	52.6%	42.9%	45.8%
Ovulation >24 of GnRH (%)	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 & a,b,c] = within the row, cells with superscripts a, b or a,b, c differ ($p<0.05$) from each other; † = heifers with follicle ≥1 0mm on D0 and CL on the same site 48h later, †† = heifers with either follicle ≥8 mm on D7(at PGF2α) or follicle ≥10 mm on D9 and CL on the same site after D9; ††† = heifers that had no CL on D0 but with new CL on D7; NA = not applicable; * = measurements were in mean ±SE (standard error of the mean)

Table 2: Conception rates by factors considered

	Conception rate (N0, %)		
Factors considered	Ovsynch	CIDR+Ovsynch	CIDR+Ovsynch-D0 GnRH
Ovulation Within 24h of 2nd GnRH			
N0 of heifers (%)	4(21.1%)	7(25%)	5(20.8%)
N0 conceived (%)	3(75%)	4(57.1%)	2(40%)
Ovulation beyond 24h of 2nd GnRH			
N0 of heifers (%)	15(78.9%)	21(75%)	19(71.2%)
N0 conceived (%)	8(53.3)	14(66.7%)	13(68.4%)
p-values	0.002	0.01	0.003
D0 luteal activity			
N0 cycling (% conceived)	8 (44.4%)[a]	12(57.1%)	10(52.6%)
N0 non-cycling (% conceived)	3 (30.0%)	6 (54.5%)	5(41.7%)
p-values	0.014	0.07	0.001
Overall P/TAI (%)	39.3%[a]	56.3%	48.4%

a = cell with superscript a in the row was statistically lower ($p<0.05$) than others

significantly higher ($p<0.05$) in heifers of Ovsynch group (28.6%) than CIDR+Ovsynch (12.5%). Ovulation to D9 GnRH was significantly higher ($p<0.05$) in CIDR+Ovsynch group, than CIDR+Ovsynch-D0 GnRH and Ovsynch group in the respective order mention. In CIDR+Ovsynch group, all heifers ovulated to D0 GnRH were ovulated to D9 GnRH. However, in Ovsynch group 21.1% heifers that ovulated to D0 GnRH did not ovulate to D9 GnRH.

The mean time (h) from PGF2 to ovulation and the mean time from D9 GnRH to ovulation was not affected by treatment type ($p>0.05$) except in CIDR+Ovsynch group in which mean time (h) from PGF2 to ovulation was significantly longer ($p<0.05$). When the distribution of time to ovulation after D9 GnRH was considered, more heifers (52.6%) from the Ovsynch group ovulated within the immediate 24h post D9 GnRH than the remaining groups (Table 1). Of heifers ovulating to D9 GnRH, 73.7% from Ovsynch, 78.6 from CIDR+Ovsynch and 83.3% CID+Ovsynch-D0 GnRH ovulated from a follicle other than the large follicle present at day of start.

Diameter of follicles by treatment group: The mean large follicle on D0 did not differ ($p>0.05$) among the group (Table 1). Similarly, the mean size of the larger follicle at 48h of D0 GnRH; the mean size of D9 larger follicles and the mean size of preovulatory follicles that eventually ovulated did not statistically differ ($p>0.05$) among the treatment group (Table 1).

Corpus luteum status by treatments: Large majority of heifers (60.4%) were cyclic and had visible CL on D0. The presence of a new CL on D7 were greater ($p\leq0.05$) for CIDR+Ovsynch than for CIDR+Ovsynch-D0GnRH heifers (Table 1). Similarly, the numbers of total CL at PGF2α injection (D7) were significantly affected ($p<0.05$) by treatment type.

Pregnancy rate: Among heifers inseminated, the D32 pregnancy rate was significantly less ($p<0.05$) in Ovsynch group than CIDR+Ovsync and CIDR+Ovsynch-D0 GnRH group (Table 2). Similarly, pregnancy rate 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 immediate 24h of 2nd GnRH had higher conception than heifers ovulated after 24h of 2nd GnRH (Table 2). Cycling heifers at D0 had a greater conception rate compared to non-cycling heifers.

DISCUSSION

Ovulatory follicle size is known to be affected by animal breed. In present study we used *B. indicus* X *Bos taurus* heifers and found that ovulation occurred at ovulatory follicle diameters range of 10-19 mm. Gimenes *et al*.[7]. indicated *B. indicus* cattle can ovulate from follicle as small as 7 mm. Studies of Sartorelli *et al*.[8] and Carvalho *et al*.[9] indicate ovulatory follicle diameter frequently range from 10-13 mm in *Bos indicus*. In Holstein cattle, ovulation frequently occurs at diameter of between 12 and 22 mm[5, 9]. In one study[10] ovulatory follicle was larger in *B. indicus* than in *Bos taurus*.

In present study, the D10 corpus luteum size range from 10-22.6 mm. The study of Carvalho *et al*.[9] indicates that corpus luteum was smaller (15.3 mm) in *B. indicus* cattle than in *B. taurus* heifers (18.4 mm). Corpora lutea size can be as large as 24.1 mm diameter for Holstein heifers[11]. In Nellore cattle (*B. indicus*) corpus luteum size range from 15.6 mm diameter to 21.5 mm diameter[12, 13].

In present study, Ovsynch protocol in absence of progestin (CIDR) resulted in low pregnancy rate to TAI (39.3%) in crossbred heifers. Similarly, previous studies[14, 15] indicated low pregnancy to TAI in Holstein heifers synchronized by Ovsynch protocol in absence of progestin. Tenhagen *et al*.[15] 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.

Pregnancy rate to TAI (56.3%) in CIDR+Ovsynch group in present study was consistent with Colli *et al*.[16] who reported pregnancy rate of 57.3% from Angus (*Bos taurus*) and 57.1% from Nelore (*Bos indicus*) heifers. However, in work of Colli *et al*.[16] the PGF2α was given twice, on day 0 and on the days of device removal in the first group and two days prior to device removal in the second group heifers. The result indicates that treatment protocol and animal breed among the others affect conception rate to TAI.

The lower pregnancy rate (48.4%) in CIDR+Ovsynch-D0 GnRH heifers with moderate ovulation rate (77.4%) may be due absence of D0 GnRH in this group. GnRH ovulate the dominant follicle[14] and prevent 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[17, 18]. Similarly, Bello *et al*.[19] reported that synchronization response to Ovsynch was higher (87.9%) in cows that ovulated in response to first GnRH of Ovsynch compared with those that did not (62.9%).

The variation in ovulation to D0 GnRH was probably be due 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[20, 21]. The work of Moreira *et al*.[22] showed that initiation of Ovsynch on day 5-9 of the estrous cycle was a key to successful synchronization of ovulation.

CONCLUSION

Combining GnRH and CIDR at the start of ovulation synchronization increase follicle turnover, induce ovulation and enhance new corpus luteum formation and increases fertility in crossbred heifers used in the study.

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