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Evaluation of single shot prostaglandin F2α analog (dinoprost tromethamine) for estrus synchronization in cattle with corpus luteum

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Abstract

An experiment was conducted from June 2022 to April 2023 at Debrezeit Agricultural Research Center to evaluate the effectiveness of single shot prostaglandin based estrus synchronization protocol in Boran, Boran*Holstein-Friesian crosses, and Holstein-Friesian cattle upon manual detection of mature corpus luteum (CL). A total of 120 animals (80 cows and 40 heifers) with detected CL from different age, body condition (BC), parity, days in milk, and lactation status were included in the experiment. A single injection of 5 ml LUTALYSE® Injection (dinoprost tromethamine, a prostaglandin F2α analog) was used to synchronize the animals. The observed estrus response and conception rate were 83.3% and 73.0%, respectively. Among the predictor factors considered, BC score significantly influenced both estrus response and conception rates, while days in milk (DIM) had significant effect on conception (p <0.05). Estrus response and pregnancy rates were higher in animals with better BC, and cattle with >60 DIM had significantly higher conception rate compared to those with less DIM. Both estrus response and pregnancy rates were not significantly different between cows and heifers, as parity had no significant association (p >0.05) with both outcomes. The result showed that manual per rectal detection of CL and injection of a prostaglandin analog were effective in inducing heat. Therefore, synchronization with a single injection of LUTALYSE in heifers and cows after manual detection of mature CL could minimize drug cost and can achieve optimal results.

Keywords: Boran*Holstein crosses; Conception rate; Corpus luteum; Prostaglandin; Synchronization.

Introduction

Ethiopia owns enormous livestock in Africa, having 70 million cattle, 42.9 million sheep, 52.5 million goats, 8.1 million camels and 57 million chickens in 2020. Ethiopia's livestock consists almost entirely of indigenous breeds. Recent estimates indicate that 97.4%, 2.3%, and 0.3% of cattle are native, hybrid, and exotic, respectively (CSA, 2020). Despite their large numbers, indigenous cows are not selected for milk production, so their productivity is very low, with an average milk production of about 1-2 liters per day and a short lactation period of about 7 months (Tegegne *et al*., 2010).

Annual milk production in the Ethiopia is very low, estimated at about 4 billion liters (CSA, 2020). On the other hand, the current demand for milk is increasing rapidly due to various factors such as increasing urbanization, population growth and improving milk consumption habits. However, per capita annual consumption of milk is still lower than 20 liters (Tegegne, 2018). Based on FAO recommendations, Ethiopia's human population now needs about 22 billion liters of milk per year, indicating a supply gap of about 18 billion liters per year (Tegegne, 2018). This huge gap between demand and supply has to be met by increasing milk production largely from cows, and this should be accompanied by an increase in the number of crossbred cows (Tadesse, 2002). Cattle reproductive biotechnologies such as estrus synchronization (ES) and artificial insemination (AI) could be used to fast produce crossbred cows (Tegegne *et al*., 2016).

The inability to timely and accurately detect heat in dairy herds is a major factor limiting reproductive efficiency (Mekonnin *et al*., 2016). Fertility is an important factor in dairy herd production and profitability (Dogruer *et al*., 2010). A calving interval of 12-13 months is generally considered to be economically optimal (Rajamahendran *et al*., 2001), but is often difficult to achieve in Ethiopia. To reach this goal, cows must begin their cycle and become pregnant within an average of 85 days postpartum. In addition, efficient and accurate heat detection, proper semen handling and timely insemination in relation to ovulation are also important factors (Million *et al*., 2011).

Estrus synchronization is one of the reproductive management tools that induce estrus in groups of females to breed relatively in a confined period of time. This program improves reproductive efficiency by shortening the length of breeding, and calving intervals and increasing calf uniformity (Gupta *et al*., 2009). Products such as prostaglandin (PG) F2α, PGF2α analog (dinoprost tromethamine), melengesterone acetate (MGA), controlled internal drug release (CIDR), and gonadotropin-releasing hormone (GnRH) are used to synchronize estrus and ovulation. Prostaglandin-based protocols depend on the presence of a functional CL aged a minimum of 5 days, especially during the diestrus phase of the estrous cycle (day 7 to 17 of the cycle) (Oaxaca *et al*., 2009).

The natural prostaglandins (PG) produced in endometrium of uterus induce functional or structural regression of the corpus luteum (CL). Regression of the CL results in the removal of progesterone's negative feedback control on the secretion of GnRH, follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2), leading to the growth and development of follicles. Luteolysis leads to increased E2 concentrations (Galina and Orihuela, 2007; Sprott and Carpenter, 2007; Stocco, 2007; Araujo *et al*., 2009; Ginther *et al*., 2011).

Ethiopia has undertaken mass synchronization of cattle in various regions in recent years to improve planned breeding programs. Still various factors have led to a decline in estrus and conception rates of synchronized cattle. It was therefore hypothesized that effective detection of CL and identification of factors affecting estrus response and conception would help to minimize drug cost and improve the efficiency of single-shot PGF2α based synchronization protocol. Therefore, the current study was designed to evaluate the effectiveness of single injection of PGF2α analog (dinoprost tromethamine), following manual CL detection, in estrus synchronization and pregnancy in dairy cows and to identify cow factors associated with estrus response.

Materials and methods

Study area

The experiment was conducted from June 2022 to April 2023 at Debrezeit Agricultural Research Center (DZARC) of Ethiopian Institute of Agricultural Research (EIAR) in Bishofu town. Bishoftu is located about 45 km east of Addis Ababa, the capital city of Ethiopia. The town is located at a geographic coordinate of 8°46′ latitude N, and 38°59′ longitude E at an altitude of 1920 masl (Fig. 1) (Addis *et al*., 2015).

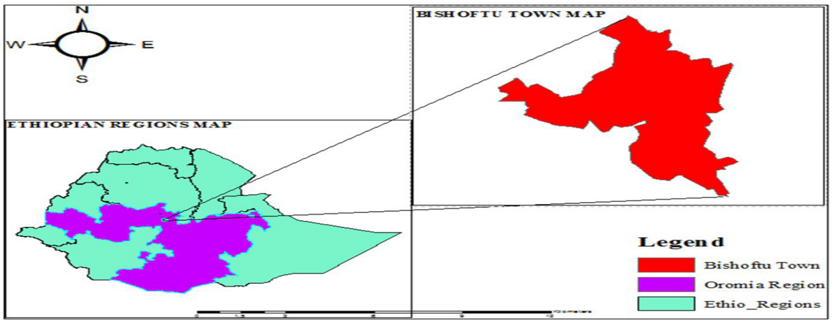

Figure 1. Map of the study location (Arc GIS).

Study animals

The study was conducted on 120 dairy cattle (80 cows and 40 heifers) at the Debre zeit agricultural research center (DZARC). Prior to the start of the experiment, reproductive organs of animals were palpated per rectum to confirm the reproductive stage, presence of CL and whether they were free from any obvious reproductive tract abnormalities. Animals having CL on palpation were selected for the experiment.

The experimental animals aged between 2 and 10 years with a parity of 0 to 5. The body conditions (BC) of the animals ranged between 2.5 to 3.5, on a 5-point scale assessment (Wildman *et al.*, 1982). On a scale of 1 through 5, condition score 1 indicates severe under condition, 3 optimal and 5 indicates severe over condition. Boran, Boran*Holstein-Friesian crosses, and Holstein-Friesian cattle were included in the experiment. Regarding parity, the experimental animals were grouped into heifers, parity 1 and 2, and ≥3. The minimum days in milk of the experimental animals was 54 days. The animals were managed intensively and fed on straw, hay, concentrates and alfalfa.

Study design

The experiment was carried out on 120 dairy cattle to evaluate estrus response and subsequent pregnancy rate for a single injection of LUTALYSE® Injection. The reproductive organs of the animals were examined manually per rectum for any gross abnormalities and for presence of mature CL. Animals with apparently normal reproductive organs and mature CL at the time of examination were included in the study and received a single shot of dinoprost tromethamine (PGF2α analog) on the day of examination and monitored for 5 days thereafter for signs of heat. Animals that showed heat during this period were inseminated artificially.

PGF2α treatment, estrus detection and insemination

Animals which were found to have normal reproductive organs and mature CL received single injection of 5ml LUTALYSE® Injection (dinoprost tromethamine injection, Zoetis Inc., Spain) intramuscularly (IM). Estrus was detected with visual observation and the help of ESTROTECT breeding indicator. ESTROTECT were applied halfway between the hip and tail head perpendicular to the spine. In the event of estrus, because the animal allows itself to be mounted, the surface of the indicator, with each mount, will gradually change its color (to deep green in our case) indicating a true standing heat. Visual observations were made twice daily, once in the early morning (6am) and once in the afternoon (5pm). All observations made lasted approximately for 1 hour for up to 5 days. The onset of behavioral signs of estrus such as standing to be mounted, mounting others, ruffled hair on tail head or rump, pink vulvar mucosa, vaginal mucus discharge, frequent urination, anogenital sniffing, restlessness, walking in circles, and bellowing were recorded along with color change in the breeding indicator. Animals that showed behavioral estrus, within 5 days of the injection, were inseminated in the AM-PM principle (Fig. 2) i.e., animals that showed estrus in the morning were inseminated in the afternoon and those which showed estrus in the afternoon were inseminated in the next morning. Holstein-Friesian semen purchased from the National Artificial Insemination Center (NAIC), Kality, Ethiopia, was used for insemination.

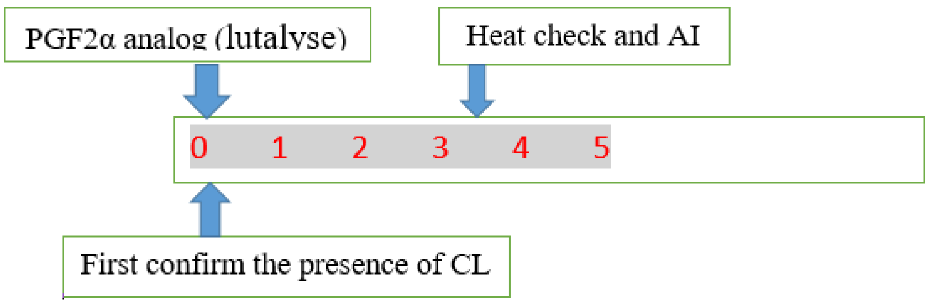

Figure 2. Synchronization and artificial insemination protocol.

Pregnancy diagnosis

History of return to estrus of inseminated animals within the normal period of interestrus was obtained from the farm attendants. The experimental animals that did not return to estrus were checked manually for pregnancy at 60th day of insemination. Pregnancy was confirmed when there was asymmetry of uterine horn, manual detection of CL, and fluid filled uterus was grasped in the palm (Purohit, 2010).

Age of the animal, breed, parity, body condition score (BCS), days postpartum, lactation status, the estrus response of the animal to hormonal treatment, date and time of estrus detection, insemination, and pregnancy were recorded.

Data analysis

All data collected from study animals were coded, entered and filtered in Microsoft excel spreadsheet and analyzed in STATA (version 17). Descriptive statistics were employed to calculate the percentage of estrus response and pregnancy rate. The effect of age, breed, BCS, parity, days postpartum, and lactation status on the rate of estrus and conception was analyzed using Chi-square test. Factors that were significant in the X^2 were considered for further logistic regression to estimate the strength of the association. The variation between groups was considered significant when the *p* value was less than 0.05.

Results

Estrus response and conception rate

Out of the 120 cattle injected with a single dose of 5ml PGF2α analog (dinoprost tromethamine) during the experiment 100 (83.33%) animals showed estrus (Table 1), and of these 100 animals, which showed estrus and were inseminated in AM-PM principle 73 (73%) became pregnant (Table 3).

Association of estrus response with factors

The multivariable logistic regression demonstrated that estrus response was significantly influenced by BCS ($p<0.05$), whereas all the other factors considered in the study, namely days in milk, parity, and lactation status of the animals did not significantly affect estrus response ($p>0.05$) (Table 2).

Table 1. Chi-square analysis for estrus response to lutalyse (PGF2α analog) injection in the experiment.

Factors	Category	No. injected	No. in Estrus	Response rate (%)	X^2	*p* value
Days in milk	≤ 60 days	21	12	57.14%	12.68	0.000
>60 days	59	54	91.52%			
Lactation status	Non lactating	73	65	89.04%	4.37	0.0037
	Lactating	47	35	74.46%		
BCS	2.5	30	12	40%	54.27	0.000
	3	57	55	96.49%		
	3.5	33	33	100%		
Parity	Zero (Heifer)	40	34	85%	8.51	0.014
	One & two	39	37	94.87%		
	≥ 3	41	29	70.73%		
Breed	Boran	36	29	80.55%	1.85	0.396
	Boran*HF	58	51	87.93%		
	HF	26	20	76.92%		
Total		120	100	83.33%		

HF: Holstein-Friesian

The odds of estrus response in animals having BCS of 3 was 40.7 times more likely than cattle with 2.5 BCS, keeping other variables constant. The detail multivariable output is presented in Table 2.

Table 2. Multivariable binary logistic regression for association of lutalyse injection with estrus response.

Factor	Category	Odds ratio	95% CI	*p* value
BCS	2.5	Ref		
	3	40.68	3.77, 439.55	0.002
	3.5	1		
Lactation status	Lactating	Ref		
	Non lactating	0.66	0.077, 5.63	0.704
Days in milk	≤60 days	Ref		
	>60 days	1.599	0.257, 9.97	0.615
Parity	Heifers	Ref		
	One & two	1.43	0.146, 13.92	0.761
	≥3	1		
Cons		.691	0.082, 5.812	0.734

BCS: Body condition score; Ref: Reference; SE: Standard error; CI: Confidence interval

Association of pregnancy with factors

Pregnancy was significantly different among animals with different body conditions i.e. animals with 3.5, 3, and 2.5 BCS had 93.94%, 72.73%, and 16.67% pregnancy rate, respectively (p <0.05) (Table 3). Days in milk as a predictor factor also statistically significantly influenced pregnancy (p <0.05). Pregnancy rate, however, was not affected by lactation status, parity, and breed of the animals (p >0.05) (Table 4).

Table 3. Univariable factor wise effects Chi-square on conception rate of lutalyse.

Factors	Category	No. inseminated	No. conceived	Conception rate (%)	X^2	*p* value
Days in milk	≤60 days	12	4	33.33%	16.12	0.000
	> 60 days	59	47	79.66%		
Lactation status	Non lactating	65	50	76.92%	1.45	0.229
	Lactating	35	23	65.71%		
BCS	2.5	12	2	16.67%	26.66	0.000
	3	55	40	72.73%		
	3.5	33	31	93.94%		
Parity	0 (Heifers)	34	22	64.71%	2.42	0.299
	One and two	37	30	81.08%		
	≥3	29	21	72.41%		
Breed	Boran	29	18	62.07%	2.56	0.278
	Cross (Boran*HF)	51	40	78.43%		
	HF	20	15	75%		
Total		100	73	73%		

BCS: Body condition score; HF: Holstein-Friesian

The odds of conception rate in cows with greater than 60 days postpartum was 9.4 times more likely than cows with less than 60 days in milk, keeping BCS, lactation status, and parity constant. Cattle with 3.5 BCS were 32.3 times more likely to conceive than cattle with 2.5 BCS, keeping other predictors constant. Results of multivariable analysis of association between conception rate and predictors are presented in Table 4.

Table 4. Multivariable factor logistic regression on conception rate.

Factor	Category	Odds ratio	95% CI	*p* value
BCS	2.5	Ref		
	3	9.43	1.33, 66.99	0.025
	3.5	32.30	2.13, 488.73	0.012
Days in milk	≤60 days	Ref		
	>60 days	9.4	1.92, 46.26	0.006
Cons		.079	0.01, 0.68	0.021

BCS: Body condition score; Ref: Reference; SE: Standard error; CI: Confidence interval

Discussion

The experiment revealed that estrus was induced in 83.3% of the animals with a single injection of PGF2α analog (dinoprost tromethamine). Using the same protocol with the current study, a similar (84.2%) estrus response rate was reported in Tigray region, Ethiopia (Gugssa, 2015). Our result was also within the range of 75 to 90% estrus response rate recorded within 2 to 5 days of hormonal injection by Murugavel *et al.* (2003). However, the estrus response rate observed in our study was lower compared to results reported by Tegegne *et al.* (2012) from Hawassa-Dale (97.7%) and Adigrat-Mekelle (100%) milksheds, Gebrehiwot *et al.* (2015) from Wukro area (91.67%) in Tigray, and slightly lower compared to 89.3% response rate reported from Bahir Dar milkshed (Kebede *et al.*, 2013). The variations might be due to differences in the animals involved in the studies (body condition, parity, age, breed, days postpartum) and their management, and even differences in agro-ecological conditions of the study areas.

Estrus response in the present study was influenced by BCS of the animals. Cows and heifers with optimal BCS (3) showed greater response than those with sub-optimal body condition (2.5 BCS). The negative effect of low BCS on estrus response may be related to reduced functional competence of follicles produced during a period of negative energy balance (Britt, 1991; Beam and Butler, 1999). Acute nutritional deprivation leading to a negative energy balance (poor conditions) in heifers and cows has been noted to have immediate adverse effect on follicular growth and ovulation (Roche *et al.*, 2000). Energy was also reported as the most important limiting nutrient in establishing estrous cycles in the postpartum cow (Hill *et al.*, 2014). Negative energy balance in dairy cattle influenced follicular growth and size of ovulating follicles (Armstrong *et al*., 2001; Wiltbank *et al*., 2002; Diskin *et al*., 2003).

The conception rate of 73% recorded in this study was higher than the national rate of pregnancy (27%) reported by Tegegne *et al.* (2012), and the preliminary results of mass synchronization in SNNPR (63%) and Tigray (62%) regions (Tegegne *et al*., 2010). Gebrehiwot *et al.* (2015) also reported a lower conception rate of 32.17% in Wukro area from Tigray. This difference might be due to careful selection of the animals on the basis of presence of CL in the present study, close follow-up of the animals, proper heat detection,and insemination at the right time.

Conception rate in our study was affected by body condition of the animals. Animals with better body condition had higher conception rate. This finding follows previous works of several researchers (Barros *et al*., 2000; Pancarci *et al*., 2002; Patton *et al*., 2007; Ciptadi *et al*., 2012; Tazangi and Mirzaei, 2015) who reported influence of body condition on conception rate where animals with higher body conditions had higher conception rates than animals with lower BCS. The low pregnancy rate in animals with low BCS may reflect reduced functional competence of the ovulated follicle because of its development during negative energy balance, or it may be a result of subtle changes in steroid hormone secretions that regulate gene expression and the secretion of proteins by the endometrium, thereby affecting implantation or pregnancy recognition (Beam and Butler, 1999; Roche *et al*., 2009).

Cows with longer days in milk (>60 days) during hormonal treatment had higher pregnancy rate than cows with less DIM. This is supported by different scholars who concluded that conception increased as the voluntary waiting period increased, peaking at about 80 days postpartum (Tenhagen *et al*., 2003; Stevenson, 2004).

Conclusions

Based on the finding, manual detection of CL and injection of prostaglandin analog is effective in inducing estrus in different breeds of bovine species. The present study revealed that estrus response to a single injection of lutalyse (dinoprost tromethamine*)* was significantly affected by body condition of the animals. The pregnancy rate was also significantly affected by body condition and days postpartum of the animals at the time of hormonal injection. Our study suggested that a single injection of lutalyse (dinoprost tromethamine), following manual detection of CL, could be used for a cost-effective synchronization of estrus in dairy cows and heifers in Ethiopia. More controlled studies are recommended to support the findings of our study.

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