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On Farm Trial of Prostaglandin Based Estrus Synchronization Protocols in Selected Milk-shed Areas of Amhara Region, Ethiopia

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Abstract. The present study was conducted from 2019 to 2020 in the Debre Birhan area of the Amhara region, Ethiopia, aiming to evaluate the effect of single-dose prostaglandin hormone in dairy cows and heifers at the smallholder farmer level. A total of 458 dairy cows and heifers were treated with 2ml of Synchromate® hormone, and after 77.82±2.74 hours, 286 of which (62.4%) were reported to have manifested estrus signs. Insemination was performed to 215 animals, 71 animals were not inseminated (the time for AI was passed when checked by rectal palpation) because of later reports by farmers after the cessation of estrus periods. Of the 215 animals that were inseminated, 82 (38.1%) conceived, and from the 82 animals that conceived, 79 (96.3%) gave birth. Estrus response and conception rate have not shown a significant difference between parity and body condition scores. However, treatment to estrus interval has been found to be significantly ($p<0.05$) influenced by parity, cows had shorter intervals than the heifers. This study indicated that there were problems in the detection and reporting of estrus response by the smallholder farmers, hence, requiring continuous training on dairy cow management.

Keywords: dairy cows and heifers, estrus response, insemination, prostaglandin, PGF2α

Introduction

Ethiopia has about 60 million heads of cattle populations. From this, about 12 million are cows (98 percent are indigenous breeds and the rest 2 percent are cross and pure breeds) (CSA, 2019). Despite their large number, indigenous cows are not selected for milk production and hence, their productivity is very low (Azage et al., 2010), with average milk production of about 1-2 liters/day and lactation length of about 7 months. In addition, indigenous cows are found in rural areas with low or no supplementary feed apart from grazing on natural pasture and animal health services and housing conditions are also very poor (Azage, 2018). As a result, the national annual production of cow milk is very low (about 4 billion liters) and much of this is even not available in the market, it is rather used for household consumption in the form of butter and other milk products (CSA, 2019).

On the other hand, the current demand for milk is increasing very rapidly due to a multitude of factors including the growing urbanization, increased population number and improvements in milk consumption habits. However, the national annual per capita consumption for cow milk is still lower (20 liters) as compared to neighboring countries (120 liters for Kenya and 180 liters for Sudan), the African average (40 liters), the world's average (105 liters) and the Food and Agricultural Organization's (FAO) recommendation (200 liters) (AGP, 2013; Azage, 2018). Based on the FAO's recommendation, the current 110 million people in Ethiopia require about 22 billion liters of milk per year, which indicates annual supply gap of about 18 billion liters (Azage, 2018). This huge gap between demand and supply has to be met by increasing milk production from cows, which is associated with increasing the number of crossbred cows (Tadesse, 2002).

However, crossbred cows are very costly to buy (Tegegne et al., 2016) and not easily affordable for smallholder farmers. Therefore, increasing the number of crossbred cows at the smallholder level requires the use of cattle reproductive biotechnologies like Artificial Insemination (AI) technology. However, AI technology alone had been tried in Ethiopia for half a century and it was practiced based on the natural estrous cycle of individual cows which is slow and inefficient to increase the number of crossbred dairy cows in the short term. As a result, estrous synchronization (ES) using prostaglandin hormone was triggered as supportive reproductive biotechnology for a faster increase in the number of crossbred dairy cows and it is currently under evaluation in most parts of Ethiopia.

ES is either natural (using bulls) or artificial insemination of many cows in a predetermined period after hormonal administration. Hormonal ES as a reproductive management tool is superior than the natural synchronization in terms of induction of estrus in a group of cows and heifers so that they can be bred relatively in around the same time (Rick and Gene, 2013). It improves reproductive efficiency by reducing the length of breeding and calving intervals by regressing the corpus luteum of cows/heifers before the time of natural lutteolysis, increase calf uniformity and enhance the possibilities for utilizing AI (Lamb, 2010). Due to the reductions in breeding and calving intervals and enhancement of utilizing AI, ES can be used to speed-up the dairy cattle breed improvent (gaining more numbers of crossbred dairy animals in few years than following the natural method) strategy. In addition, the use of prostaglandin or its analogue (PGF2α) in a synchronization protocol shortens the estrous cycle and estrus is observed within 2 to 7 days post injecting the hormone (Gupta et al., 2008).

Prostaglandin-based ES technique had been evaluated in the past in four regions of Ethiopia, Oromia, Amhara, Tigray and Southern Nations, Nationalities and Peoples regions. Yeshimebet et al. (2017) in Debre Birhan Area of Amhara region used 5 ml PGF2α hormone and reported 87% estrus respons in cows and heifers. In addition, Destalem (2015) in central Tigray, Bainesagn (2015) in West Shoa zone of Oromia region, Tewodros et al. (2015) in Fogera district of Amhara region and Abiyot and Eyob (2019) in Sodo zuria district of Southern Nations, Nationalities and Peoples' regions also reported estrous response of 84.9%, 72.3%, 98.9% and 91.9%, respectively. However, most of these previous works on prostaglandin-based estrous synchronization techniques focused on dairy cows that are found in urban and peri-urban areas under improved management system, but only fewer works have been conducted for wider evaluation of the protocol at the smallholder farmer level. Therefore, the objective of this study was to evaluate prostaglandin-based estrus synchronization protocol in Debre Birhan area, one of the selected milk-shed areas of Amhara Region, Ethiopia.

Materials and Methods

Description of The Study Area

The study was conducted from October 2019 to December 2020 in BasonaWorana and Angolelanatera districts (Debre Birhan area) in Amhara region, Ethiopia. The study areas are found in the central highlands of Ethiopia at a road distance of 110 to 130 kilometers from Ababa, the capital city of the nation. Geographically, the area lies between 09^0 35'45" North latitude and $39^0$29'40" East longitude with an average elevation range of 2800 meters above sea level and with a mean annual temperature of about 19.9°C. The average annual rain falls of the area ranges from 897.8 to 1149mm and it is characterized by bimodal pattern with cold, harsh climate that occasionally has frost, particularly between November and January.

Study Animals and Method of Data Collection

A total of 458 non-pregnant, apparently healthy dairy cows and heifers with functional corpus luteum (CL), body condition score of 3.0-4.0, age between 38 years old (84, 129, 97, 104, 31, and 13 heifers and cows with 3, 4, 5, 6, 7 and 8 years old), one to five parities (84, 141, 116, 78, 33 and 6 heifersand cows with parities 1, 2, 3, 4 and 5, respectively) were selected purposively to be considered for this study and synchronized based on the following diagram.

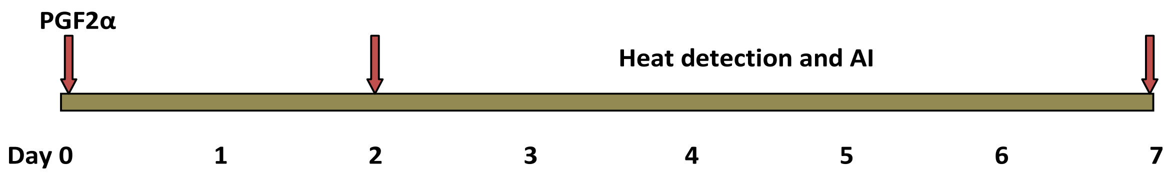

Figure 1. Schematic representation of Single PGF2α injection estrus synchronization protocol. Dairy cows/heifers (n=458) with mature corpus leutium were administeres single injection of 2 ml PGF2α (Synchromate, Germany) at day 0, heat detected from days 2 to 7 and AI conducted 8-12 hrs after standing heat.

The body condition of cows ranged from 1 to 5 according to Ibrahim (2015) while the age and parities of cows were recorded based on the history from the owners. The experimental animals were managed under extensive system at the smallholder farmers and feed mainly on grazing natural pasture (managed in the home from the time of hormonal injection to AI) and crop byproducts with no or little salt, and commercial concentrate supplement. Trans-rectal palpation examination was used to confirm the normality of the reproductive tract, the presence or absence of pregnancy, and presence or absence of mature corpusleutum. As per the results of the rectal examination, 2ml prostaglandin (Synchromate®, Germany) was administered intramuscularly to those cows possessing the above requirements. The hormone synchromate was selected based on its cost and availability in the market. All hormone-injected cows were left for continuous observation by the farmers themselves for detecting the manifestations of heat (estrous) signs which include frequent mounting of other cows/heifers, standing to be mounted by other cows/heifers, frequent (overnight) bellowing, swollen and congested vulva, mucosal vaginal discharge, restlessness, nervousness, lack of willingness to be milked and decrease in milk production. Then, cows reported by farmers for the manifestation of heat signs were assessed by the inseminator for true signs of estrus (the proper time for AI time was cheked by rectal palpation), treatment to estrus interval recorded and inseminated based on the standered protocoal (8-12 hrs after standing heat) using uniform dose(300 million spermatozoa/0.25 ml straw) of frozen-thawed Holstein Friesian semen which was sourced from the National Animal Genetic Improvement Institute, Kality, Ethiopia. Confirmation for conception (pregnancy) was conducted three months post insemination using rectal palpation, and calf crop data were recorded after nine months of insemination.

Methods of Data Analysis

All data collected from study animals were coded with appropriate variables, recorded in Microsoft Excel 2010, and summarized using descriptive statistics of the statistical package for social science (SPSS) software version 20. Estrus response rate and conception rate were calculated according to Khatun *et al*. (2014). The effects of parity and body condition score (BCS) on the rate of estrus and conception were analyzed using Chi-square while data on the treatment to estrus interval were analyzed using an independent sample t-test. The level of significance was held at $p < 0.05$ to show statistically significant differences between variables.

Results and Discussion

The current study was conducted in two years of 2019 and 2020, during which 458 cows and heifers were injected using a single dose of prostaglandin hormone (2ml Synchromate). After injection, 286 showed estrous signs, 215 was inseminated, 82 was conceived and, from the 82 animals that conceived, 79 gave birth as summarized in Table 1.

Table 1. Summary of heat response and conception rate for single-dose PGF2α injection of cattle in Debre birhan area

Years	No. injected	No. in heat	No. inseminated	No. conceived (%)	Birth (%) compared to AI	Birth (%) compared to conception
2018/19	63	43 (68.3)	34	16 (47.1)	15 (44.1)	15 (93.8)
2019/20	395	243 (61.5)	181	66 (36.5)	64 (35.4)	64 (97.0)
Total	458	286 (62.4)	215	82 (38.1)	79 (36.7)	79 (96.3)

Table 2. Estrus response rate across different factors

Factor	Category	No.	No. Responded (%)	X^2	P-value
BCS					
	3.0	302	184 (61.0)		
	>3.0	156	102 (65.4)	0.872	0.362
Parity					
	0	84	59 (70.2)	2.664	0.107
	≥1	374	227 (60.7)		
Total		458	286 (62.4)		

Table 3. Treatment to estrus interval across different factors

Factor	Category	No.	Mean± SEM *(hrs)*	P-value
BCS				
	3.0	184	76.20±2.92	
	>3.0	102	80.81±4.60	0.058
Parity				
	0	59	79.78±3.65	
	≥1	227	77.31±3.33	0.000
Total		286	77.82±2.74	

Estrus Response Rate

The overall estrus response rate for the current study was 62.4%. When risk factors (factors that can be considered to influence estrus response) are considered, the estrus response rate animals with body condition score of 3.0 had lower estrus response rate than animals with body condition scores above 3.0. When parity is considered, heifers (parity 0) had higher estrus response rate than cows (parity 1 and above). However, there is no statistical significance between the body condition scores and parities as indicated in (Table 2).

Time Interval from Treatment to Estrus Using Single-Dose PGF2α Injection

The overall mean (±SEM) for treatment to estrus interval was 77.82± 274hrs. The effect of body condition score was not statistically significant (*P>0.05*) while the effect of parity on treatment to estrus interval was significant (*P<0.05*) (Table 3).

Conception Rate across Different Factors

The overall conception rate was 38.1%. Animals with body condition score of 3.0 had a higher conception rate than animals with body condition scores above 3.0, and heifers (parity 0) had higher conception rate than cows (parity 1 and above), but the association for both factors was not significant (P>0.05) as indicated in (Table 4).

As shown from Table 2 above, the overall estrus response for the current study was 62.4%. Although it is not statistically significant (P>0.05), the response in heifers (parity 0) (70.2%) is higher than that in cows (parity) (60.7%). It is probably due to reduced exposure of heifers (nulliparous) for infectious agents that affect the reproductive tract, unlike cows which have increased chance of being infected in their successive parities.

Table 4. Rate of conception across different factors

Factor	Category	No. inseminated	No. conceived (%)	X^2	P-value
BCS					
	3.0	138	56 (40.6)		
	>3.0	77	26 (33.8)	0.972	0.380
Parity					
	0	33	14 (42.4)	0.303	0.679
	≥1	182	68 (37.4)		
Total		215	82 (38.1)		

This result is not consistent with most of the studies conducted in Ethiopia. Yeshimebet et al. (2017) reported that 87% of cows and heifers injected using single dose of 5 ml PGF2α hormone showed estrous. Similarly, Kebede et al. (2013) who administered 5 ml of PGF2α to local cows and heifers in a study conducted in three districts of Bahir Dar milk shed reported high estrus response (89.3%). Destalem (2015) in central Tigray, Bainesagn (2015) in West Shoa zone of Oromia region, Tewodros et al. (2015) in Fogera district and Abiyot and Eyob, (2019) in Sodo zuria district of Wolaita zone also reported overall estrous response of 84.9%, 72.3%, 98.9% and 91.9%, respectively, which are also higher than the results recorded from this study.

The average treatment to estrus interval in the current study is 77.82+ 2.74hrs. Although the result is not statistically significant (*P>0.05*), the interval is shorter in animals with body condition score 3.0 than animals with body condition score >3.0. It may be due to differences in the stage of estrous cycle or luteal phases (animals in late luteal phase usually come to heat earlier than animals in early and mid-luteal phases) during hormonal injection. The results from a study by Adebebay et al. (2013) in Bahirdar milk shed indicated that the average numbers of hours to estrus interval in cows post injection was 51 hrs, which is shorter than the result from this study. Similarly, from his work in Siltie zone, Hamid (2012) indicated that the interval from treatment to estrus was about 57.9 hrs. On the other hand, Azage et al. (2012) in Dale milk shed, Ejigayehu (2017) in Debre Zeit, and Bekana et al., (2005) in Fogera heifers recorded treatment to estrus response interval of 13-154 hrs, 68-74hrs and 82hrs, respectively, which are consistent with this study.

The average conception rate recorded by this study was 38.1%. This result is comparable with the results from Destalem (2015) in Central Zone of Tigray, who indicated conception rate of 37.95%., and higher than the results from Adebebay et al. (2013) in Bahirdar milk shed, Girmay et al. (2015) in Wukro kilte Awulaelo district of Tigray region, Tegegn and Zelalem (2017) in Mizan Aman area of the South West Ethiopia and Tewodros et al. (2015) in Fogera district of Amhara region, which indicated the average conception rates of about 13.2%, 32.17%, 24.69% and 31.29%, respectively. However, it is lower than the findings of Azage et al. (2013) and Azage et al. (2012) in Adigrat-Mekelle milk shed, Yeshimebet et al., (2017) in Debre Birhan area of Amhara region, Hamid (2012) in Siltie zone of Southern Ethiopia, and Debir (2016) in Sidama Zone of Southern Ethiopia, which indicated the average conception rates of about 60%, 57.7%, 50.9%, 48.1% and 58.4%, respectively. On the other hand, the current result is higher than the result from Desalegn (2009) who reported that the average national conception rate for artificially inseminated dairy cows in Ethiopia is 27.1%.

The variations in estrus response, treatment to estrous interval and average conception rate in the present study could possibly be associated with either the management factors (husbandry, feeding and estrus detection by smallholder dairy farmers), time of artificial insemination, and semen handling by artificial insemination technicians, or cow factors such as age, body condition score, post-parturient problems, disease events, milk yield and genetics as indicated by Tegegne et al. (2010). When the heat detection and reporting to technicians is considered in the Ethiopia condition, it has been performed by dairy cattle producers during the time of observing signs of heat like mounting on other animals, bellowing, swelling, redness and mucus discharge of the vulva, restlessness, and nervousness (Nuraddis et al., 2015). This is indicated by Woldu et al. (2011) that small holder farmers are engaged in various farm activities and is very difficult for them to detect proper time of heat.

The dairy producer could detect the heat time but it might not match with the appropriate time of insemination. Cows and heifers used for this study had lower exotic blood level and they were in the hands of small holder farmers. Therefore, the difference in results between this study and other works could also be due to difficulties of detecting estrous in the cows of lower exotic blood level (indigenous cows) because of the low intensity and short duration of estrous signs as compared to the cross-breed cows (Bo et al., 2003; Galina and Orihuela, 2007).

One of the reasons for weak signs of estrus in cows of lower exotic blood level is the presence of small follicular diameter which directly affects quantity of estrogen that is synthesized by the cells of the *theca* interna (Bo et al., 2003; Bridges and Fortune, 2007). These reasons lead to the heat period of the cows and heifers missed or passed before the AI service have been delivered or inappropriate time of insemination that cause failure to conception or pregnancy.

These conditions were also evidenced by this study that out of 286 cows and heifers that were reported by the smallholder farmers for showing estrus signs, only 215 were inseminated, while the rest 71 cows were not inseminated because of later reports after cessation of estrus periods.

Conclusions

Estrous synchronization can be a useful supportive reproductive technology for planned insemination of large number of dairy cows at the smallholder farmer level. However, a lot needs to be done, particularly regarding the management of dairy cows and heifers. As such, the problems in heat detection and reporting must be solved through continuous training so that the number of cows and heifers with missed and passed heat responses could be reduced while more cows and heifers could be inseminated timely, hence, producing more calves. Moreover, the requirements of artificial insemination of cows and heifers at detected estrus protocol in terms of time, logistics and convenience to both farmers and technicians may not justify its use under the smallholder farmer level. Therefore, ovulation synchronization for fixed-time artificial insemination without estrus detection could be a feasible option for smallholder farmers.

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