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Efficacy of GnRH-PGf2$_\alpha$ - GnRH in Synchronizing Estrus in Cross Bred Dairy Cows

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Abstract
An investigation on the efficacy of Ov-synch and modified Ov-synch with single dose of PGF2$_\alpha$ in combination with GnRH in Synchronization of estrus in HF x local cross bred cows was conducted in and around Mekelle, From November 2013 to May 2014. A total of 20 animals, non-cyclic (presence of well-developed CL in either ovary) were inducted in to the experiment. Based on clinical examination animals were divided in to two groups. Group I animals: in clued both types of cycling with a CL and non-cycling without CL and received an IM dose of 75µg GnRH on day 0, 25mg PGF$_{2\alpha}$ on day 7 followed by a second dose of 75µg GnRH on day 9, all the animals were given a single insemination 24hrs after the second GnRH administration. Group II animals: were all cycling animals with a well-developed CL in either ovary, received a simultaneous administration of 25mg PGF$_{2\alpha}$ IM and 100µg GnRH IM and give two fixed time insemination at 48 and 72 hrs after the treatment Five animals were excluded from the study due to various reasons. There for, a total 15 animals (8 cows and 7 heifers) were retained in the study and inseminated, Out of which 5 (33.3%) [3 cow (37.5%) and2 heifer (28.6%)] were diagnosed pregnant. The differences in conception rate of 28.6% and 37.5% in Group I and Group II respectively. The conception rates in the two treated groups were similar. The overall conception rates of 28.6 and 37.5% in the two groups were poor. For further confirmation of the results obtained in these protocols, another study; under controlled conditions of management and feeding is necessary before it can be recommended for wide spread application.
Keywords: Cattle, CL, GnRH, PGF$_{2\alpha}$, Synchronization, AI, Pregnancy rate,

1. INTRODUCTION

Livestock production has been considered as major economic sector and still to be continued in the future in most parts of the world. These animals and their products play a great role for the livelihood of the society. They serve as source of milk, meat, hide and the products or processed forms of these raw materials. Animals also serve as social and cultural functions in some segments of the society of some parts of the world (Banerjee, 1998). These production sectors can significantly reduce the poverty, particularly in recent time; they may play their own role to reduce the economic crisis of the world. Livestock production systems have played a role in both the rural and urban areas by generating regular income to the smallholder farmers. Cattle production is one of live stock production system and the major one to serve people as source of milk, meat and their products unlike other domesticated animals, which give service other than food. Ethiopia has the potential of cattle production, which is the main component for the agricultural development (Tegegn *et al*., 1999).

Ethiopia holds large potential for dairy development. The country currently manages the largest livestock population in Africa, with estimated cattle population at about 29 million (Ahmed *et al*., 2004). Azage *et al.* (2000) has shown that the estimated number of milking cows of the country is about 9 million and is in the hands of small holder farmers and pastoralists under traditional management system.

However, production efficiency of cattle is low in Ethiopia despite their large population. Although Ethiopia is sufficient enough in meat production, still imports many dairy products. Milk produced from the animals provides an important dietary source for the majority of rural and periurban population. However, the country's per capita milk consumption is estimated to be about 19.2kg per year, which is far below the average per capita consumption of Africa 37.2kg per year (FAO, 2000).

The major factor limiting reproductive performance in many dairy herds is failure to detect estrus in a timely and accurate manner. Efficient and accurate estrus detection is essential to optimize the economic management of individual cows to yield a profitable dairy operation (Nebel and Jobst, 1998). As dairy herds expand having inadequate labor to pay attention to the important details of heat detection, AI becomes a larger problem. The time available to observe the cows in estrus often becomes inadequate to detect cows in estrus accurately. Furthermore, it is often more difficult to detect cows in estrus when they are constantly on slippery concrete surfaces, often found in large herds (Miller, 1999).

Estrus synchronization can minimize the amount of time and labor required to accurately detect estrus. Many reproductive hormones are used in estrous synchronization. Developing a basic understanding of several

reproductive hormones is necessary when trying to determine which protocol will work best for your herd. Progesterone (P_4), prostaglandin ($PGF_{2\alpha}$), gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) are a few of the hormones involved in the estrous cycle. Depending on the estrous synchronization protocol, these hormones can be used independently or in combination with one another (Timothy, 2003).

Protocols based on the administration of a combination of hormones like GnRH and $PGF_{2\alpha}$ have been used over the last two decades. Mainly three protocols namely Ov-synch, co – synch and select – synch are being used either alone or in combination with progesterone supplementation. Observed a higher pregnancy rate when progesterone was supplemented with Ov-synch (Melendez *et al.,* 2006).

Ov-synch protocol uses sequential injections of GnRH, $PGF_{2\alpha}$, and GnRH prior to timed AI. GnRH causes the pituitary to release LH that acts to transform a large follicle containing an unfertilized egg in to a CL. Recently GnRH was incorporated into synchronization programs. GnRH was used 6-7 days prior to $PGF_{2\alpha}$ because it increased the percentage of cows that were synchronized in response to $PGF_{2\alpha}$. Usually the GnRH analogues Cystorelin, Factrel, or Fertagyl are used. The most frequently used combination of GnRH and $PGF_{2\alpha}$ is referred to as the Ov-synch procedure. It is called Ov-synch because it controls ovulation and so timed AI without estrus detection, can be performed successfully. The first injection of GnRH is given to all cows without any knowledge of the stage of the estrous cycle. Depending on the stage, GnRH will either luteinize or ovulate the largest follicle in about 85% of all cows, forming a CL. After a 7 day waiting period, $PGF_{2\alpha}$ is given. Administration of $PGF_{2\alpha}$ regresses the CL induced by GnRH. A new dominant follicle grows and is available for ovulation by the second GnRH injection. This injection is given 36-48 hours after the $PGF_{2\alpha}$ injection. The cows are time bred 16-20hrs after this GnRH injection, without watching for estrus (Miller, 1999; Geary and Whittier, 1999).

Recently a modified GnRH – $PGF_{2\alpha}$ protocol (Gbrehiwot *et al.*, 2009) was successfully used in cross bred dairy cows and heifers in Mekelle.Since no other studies are available in the country on these aspects the present investigations was carried out with the following objective.

- To evaluate the efficacy of Ov – synch (GnRH _$PGf_{2\alpha}$ _ GnRH) in synchronizing estrus in cross bred dairy cow in and around Mekelle.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted from November 2013 to May 2014 in Mekelle and it's vicinity. The town is the capital city of Tigray region which is located at the northern part of Ethiopia, 783 Km from Addis Ababa. In general the region is bordered by Eritrea in the north, Sudan in western part of region, Afar in eastern and Amhara region in southern part of Tigray. The Tigray region is located at 12^0 13' – 14^0 54'N and 30^0 27' to 44^0 100' E latitude and Mekelle is located at 39^0 29' E and 13^0 3'N of longitudes, having an average temperature 20^0C. The mean annual rain full also ranges from 990mm in the south western part of region to less than 200mm in the Dankell depression. Rainy season occur mainly between June to September, although a short rainy season occur on March and April along the eastern and southern high lands (MCPPP, 2007).

2.2 Study Animals

A total of 20 cross bred cattle were selected from different organized farms, small scale unorganized farms of the town and around the town. The animals were reported to be non cyclic by owner. However, on trans rectal palpation a majority of the females were diagnosed to be cycling (presence of well developed CL in either ovary). The selected animals were apparently clinically healthy, aged 2 to 9 year with parity of 0 to 3 with the body condition score varying between 1.5-5 and feeding of straw, hay, concentrates, alfalfa were included in the feeding system.

2.3 Study design

Animals were divided in to two groups (I and II). Group I animals (n=10) includes both types of females (cycling with a CL and non cycling with out a CL) and received an intramuscular dose of 75µg GnRH (cystorelin) on day 0, 25mg $PGF_{2\alpha}$ (lutalyse) on day 7 followed a second dose of 75µg GnRH on day 9. All the animals were given a single insemination 24hrs after the second GnRH with frozen thawed semen. Group II animals (n= 10) were all cycling animals with a well developed CL in either ovary. These animals received a simultaneous administration of 25mg $PGF_{2\alpha}$ IM and 100µg GnRH IM. The animals were given two fixed time inseminations at 48 and 72 hrs after the treatment, with frozen thawed semen.

Pregnancy status was confirmed between 60-90 days after insemination by trans rectal palpation.

Figure 1.GnRH – $PGF_{2\alpha}$ combination synchronization protocols, Ov – synch.

Day 0	7	9	10
GnRH	$PGF_{2\alpha}$	GnRH	Time breed

Figure 2. $PGF_{2\alpha}$ - GnRH combination estrus synchronization protocol modified. Single dose of $PGF_{2\alpha}$ - GnRH combination IM, on day 0 (group II animals).

Day 0	48	72
$PGF_{2\alpha}$ + GnRH	Time breed	Time breed

2.4 Statistical Analysis

The data was analyzed using JMP-S Statistical Soft Ware(SAS Institude,2002) .

3. RESULITS

The present study was conducted on a total of 20 HF x Local Crossbred cattle, in different small and medium size cattle farms in and around Mekelle. Group I animals (n=10) were treated with Ov-Synch (GnRH 75 µg on day 0, $PGF_{2\alpha}$ 25 mg on day 7 and GnRH 75 µg on day 9). A single insemination was performed 24 hrs after the second GnRH administration. Group II animals on rectal palpation had revealed the presence of a well developed corpus luteum on either of the ovaries, were treated with "Modified Ov-Synch". All the animals received a simultaneous administration of 25 mg $PGF_{2\alpha}$ and 100 µg GnRH, IM. Group I animals were given a single insemination on day 10, whereas Group II animals received two fixed time inseminations at 48 and 72 hrs post treatment. All animals were subjected to evaluation of pregnancy status between 60 and 90 days post insemination by trans-rectal palpation. The results of the study are presented in Table I.

In the Ov-Synch group, only 7 of the 10 treated animals could be successfully inseminated at the appropriate time. In one animal the cervical canal was still closed at the time of AI, whereas in the other two animals the inseminator was not available for inseminating the animals, although the animals were reported to be showing estrus symptoms. Therefore, only seven animals were inseminated, out of which only 2 (28.6%) were ultimately confirmed pregnant.

In the modified Ov-Synch group estrus was induced in 8 out of 10 animals treated, giving an estrus synchronization response of 80%. Three animals (37.5%) were confirmed pregnant. The conception rates in the two treatment groups were similar.

An analysis of the overall response of the animals in the two treatment groups shows the estrus synchronization rate was 75%, whereas the total conception rate in the two groups was 33.3 percent.

Analysis of the data on the basis of parity, irrespective of the treatment given, revealed that out of eight heifers treated, seven (87.5 percent) were inseminated and two (28.6%) became pregnant. From the 12 adult, parous cows treated, 8 (75%) were inseminated and 3 of them conceived (37.5%).

Table 1. Fertility Response of Holstein Friesian X Local Crossbred Cattle to OV-Synch and Modified OV-Synch.

Groups	Treatment	No.Treated	No. Inseminated	No. Pregnant
I	Ov- Synch	10	7(70)	2(28.6)
II	Modified Ov-synch	10	8(80)	3(37.5)
	Total	20	15(75)	5(33.3)

Table 2. Fertility Response of HF X Local Crossbred Cattle on Parity Basis.

S.No	Parity	No. Treated	No. Inseminated	No.Pregnant
1	Heifers	8	7(87.5)	2(28.6)
2	Adult cow	12	8(75)	3(37.5)
	Total	20	15(75)	5(33.3)

4. DISCUSSION

Ovarian cyclicity failure, whether due to smooth, non-functional ovaries;or because of inaccurate heat detection , which may in turn be caused by weak or total absence of external heat symptoms or even due to human errors of observation, is a major cause of reproductive failure in cattle breeding programs. In Ethiopia, (Shiferaw *et al*., 2003) reported an incidence of 38% infertility in cross bred cows and heifers, a substantial proportion of these animals being reported as non-cyclic. Anoestrus in pubertal heifers translates into late joining of such animals in

the breeding program and in post partum females; it is responsible for increasing the number of days open, with a consequent increase in the calving interval. All these undesirable events are directly and indirectly responsible for economic losses, which keep on accumulating as the time lag between various reproductive events remains uncontrolled. The major economic losses are due to loss of milk, loss of calf crop and reduced life time production. It is, therefore, important that all pubertal heifers and post partum cows, after the voluntary waiting period, are clinically examined to ascertain their ovarian status; and appropriate corrective measures put in place, to ensure early resumption of reproductive function. During the previous years our experience has shown that a large number of cross bred cows and heifers, in and around Mekelle town, in most of the cattle farms ; and reported to be non-cycling were in fact already going through the various stages of estrus cycle as evidenced by the presence of functional structures on the ovaries. Apparently, estrus detection failure was the dominant factor contributing to the reproductive failure in these animals.

The present investigation was designed to evaluate the efficacy of Prostaglandins in combination with GnRH in enhancing the reproductive efficiency of Cross bred heifers and cows by using the Ov-Synch protocol. Additionally another protocol, developed in the department (Modified Ov-Synch) in the previous years, and found to be quite successful with an acceptable conception rate (Gebrehiwot *et al.,* unpublished data) was also used. This modified protocol is based on the clinical findings that either of the ovaries of the female contains an active, functional CL, which eliminates the administration of the first dose of GnRH. Both treatment groups had 10 animals each.

Over the past several decades strong efforts have been made, across the world, to eliminate the need of heat detection in cattle (Geary and Whittier, 1999; Timothy, 2003; Wichtel, 2004) by using different hormonal combinations and to breed the females either according to estrus or by fixed time AI. Prostaglandins alone either as a single or double dose schedule has been used for a long time for estrus synchronization in cattle (MacMillan, 1978; Seguin *et al.,* 1978, Plunkett *et al*,1984, Rosenberg *et a*l.,1990, Kristula *et al*., 1992) with varying results of estrus and conception. Several reports (Thatcher *et al.,* 1989,Stevenson *et al.,* 1999) have reported a higher rate of estrus synchronization and (80%) when PGF2α is combined with GnRH than when Prostaglandins are used alone (50%).The currently used protocols of GnRH and Prostaglandins (OV-Synch , Co-Synch)include two administrations of GnRH and one of prostaglandins. The second administration of GnRH is supposed to synchronize ovulation and a single insemination 16 to 20 hours after second administration of GnRH is recommended.

In the Ov-Synch treated animals (6 adult cows and 4 heifers) two animals (one cow and heifer) did not have any functional structure on the ovaries; whereas the remaining eight animals did have an apparently functional CL.Both of these animals were not synchronized 70% after the treatment and could not be inseminated at the fixed time. Another cow also did not allow insemination at the appointed hour, as the cervix was observed to be still closed. Therefore, only seven of the 10 treated animals (70%) were inseminated, giving an estrus response. Only two of the inseminated animals were later on diagnosed pregnant, with a conception rate of 28.6 %.

In the modified Ov-Synch group, all the ten animals (6 adult cows and 4 heifers), 8 animals (4 cows and 4 heifers) were synchronized to estrus within the next three days after the end of the treatment, giving an estrus response of 80%. The two non responding cows were excessively fat animals, which were also totally unproductive for the last two years. In spite of the fact that these animals were excessively fat, some other explanations need to be given for the failure of the animals to respond to the action of Prostaglandins. Prostaglandins are effective only when they are administered between day 6 to day15 of the estrus cycle, the diestrus stage of the estrus cycle (Kristula *et a*l., 1992). It is quite possible that in these animals the CL may have been at an earlier developmental stage, when prostaglandins are not effective. Additionally the simultaneously administered dose of GnRH given to these animals would have triggered the release of additional LH from the anterior pituitary to provide further luteal protection. The remaining eight animals were successfully twice at 48 and 72 hrs after the treatment, and 3 of the inseminated animals were diagnosed pregnant (37.5 %).

In both treatment groups the estrus response of 70 and 80 % are more or less acceptable, although (Gordon, 1996) maintains that for a treatment to be considered successful 90% of the treated females should show estrus within a few days after the end of the treatment. However, the conception rates in both treatment groups are very low in comparison to the earlier reports. (Geary and Whittier, 1999) reported conception rates of 52% with Ov-Synch and 55%with Co-Synch protocols. (Gebrehiwot *et al*., unpublished data) reported an estrus response varying between 63 to 93% and conception rates of 69% in cows and 72% in heifers treated with modified OV-Synch protocol. However, (Murugavel *et al*., 2003) reported that Ov-Synch program is not effective in heifers because of their inconsistent pattern of follicular wave development. In the present study four out of 10 animals treated with Ov-synch were heifers, out of which three failed to respond to the treatment. It is quite possible that after the prostaglandin treatment in these heifers, an already emerging new follicular pool was not available and, therefore, the second dose of GnRH was not in a position to synchronize estrus and ovulation. However, from the other six older cows also, only one animal was diagnosed pregnant. Another possible

explanation for the low conception rates might be the reduced dose of GnRH used in the present study. Although (Fricke *et al*., 1998; Yamada *et al*., 2002 and Hall *et a*l., 2002) have reported that the reproductive performance of dairy cows is not affected when the dose of GnRH is reduced to half (50 µg instead of 100 µg), in the Ov-Synch protocol.

The low conception rates observed with the modified Ov-Synch protocol are a cause for concern. This protocol was included in the present study only to confirm the results of the previous study conducted in the department. In the original study conception rates of 68% in cows and 72% in heifers were recorded. The present conception rate of 37.5% is considerably lower. (Stevens *et al*., 1993) reported that simultaneous administration of prostaglandins and GnRH during the diestrus stage of dairy cows delays the return to estrus. When cows were treated with a simultaneous administration on day 8 of the cycle, only one of the eight treated animals was synchronized. However, when the treatment was given on day 10, 3 of the 8 animals were synchronized. These observations would suggest that simultaneous administration of the two hormones in the early diestrus phase does not produce the desired result. However, when the hormone combination is used in the later half of the diestrus phase, the response improves. The differences in the conception rates of the present and the previous study conducted in the department, may lie with the stage of the cycle at which the animals were treated in the two experiments. Possibly the animals in the present study may have been in the early diestrus phase. Another possible explanation for low conception rates in this treatment group could be that 5 of the ten treated animals belonged to one particular farm. One month after our treatment in the farm, one of the treated animals died of an infectious disease. Post mortem of the dead animal was conducted and the animal was found not pregnant. Later on when the final pregnancy check was conducted other animals were also observed showing symptoms of the same infectious condition and only one of the four remaining animals was diagnosed pregnant. It is interesting to speculate whether the possible presence of the infection in the herd could have affected the fertility results obtained with the modified Ov-Synch protocol.

In conclusion it can be said that although acceptable estrus response rates were obtained in both Ov-Synch and modified Ov-Synch protocols, the conception rates in both treatment groups were poor. Whereas, with the quality of veterinary services presently available in the country as well as absence of proper "Record Keeping" in the cattle farms of the region, Ov-Synch protocol can not be recommended for use under field conditions. For further confirmation of the results obtained in the modified Ov-Synch protocol, another study, under controlled conditions of management and feeding is necessary, before it can be recommended for wide spread application.

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