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Efficacy of a Modified Gnrh-PGF$_{2\alpha}$ Combination for Estrous Synchronization in Dairy Cattle

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Abstract: A total of 52 HF X Local crossbred cattle (28 Heifers around 2 years of age and 24 adult cows greater than 90 days postpartum) were examined by trans-rectal palpation to confirm their ovarian status. Animals with a mature, fully developed corpus luteum (15 Heifers and 12 Cows- Group I) were given a simultaneous I/M administration of 25 mg PGF2α (Lutalyse) and 20 microgram GnRH (Receptal). Animals with a small, soft developing corpus luteum or where the presence of a luteal structure was doubtful (13 Heifers and 12 Cows-Group II) received a double dose of 25 mg PGF2α, 11 days apart. On the second PGF2α administration, the animals also received a simultaneous administration of 20 microgram GnRH. Animals in both groups were given fixed time AI at 48 and 72 hours post GnRH administration. Pregnancy status was confirmed through trans-rectal palpation 60 to 90 days post AI. In group I 14 heifers (93%) and 8 cows (67%) were inseminated with conception rates of 78.6% for heifers (11/14) and 50% (4/8) for cows, respectively. In group II 11 out of 13 heifers (84.6%) and 11 out of 12 cows (91.7%) were inseminated with conception rates of 63.6% (7/11) and 81.8% (9/11) for heifers and cows, respectively. The overall estrus response of 88 percent and conception rate of 72.7 percent in group II was non-significantly higher in comparison to group I animals. Two cows in group II, diagnosed pregnant were observed to suffer embryonic losses between 60 and 90 days post AI. The overall conception rate for both groups together was 70.45 percent (31/44). Twenty nine animals (18 Heifers and 11 cows) calved with a calving percentage of 65.9 percent. Although the estrus response, conception rates and calving rate is quite high, a larger trial needs to be conducted to confirm the findings.

Key words: Corpus luteum · Estrus · GnRH · PGF2α · Synchronization

INTRODUCTION

Ethiopia holds considerable potential for dairy development with an estimated population of about 9 million milking cows [1]. However, the production potential of indigenous zebu cattle being very low [2]; the onus for meeting the milk requirements of the country lies with exotic and cross bred cattle. Coupled with the twin problems of inadequate supply of quality feed and poor estrus detection efficiency; the majority of breedable female cattle, even among the crossbreds, either have a high age at first calving or long inter calving intervals or both [3-6]. All these factors combine together to culminate in low reproductive efficiency, resulting into huge economic losses.

Failure to detect estrus in dairy herds, in a timely and accurate manner, is a major factor limiting reproductive efficiency [7]. Efficient and accurate estrus detection is essential to optimize the economic management of individual dairy cows to yield a profitable dairy operation [8]. As dairy herds expand, having inadequate labour to pay attention to the important details of heat detection, artificial insemination becomes a larger problem. Furthermore, it is often more difficult to detect cows in estrus when they are constantly on slippery, concrete surfaces; often found in large, intensive farming systems [9].

Prostaglandins in combination with GnRH are being widely used for induction and synchronizing estrus and ovulation in dairy and beef cattle [10]. All of these involve sequential administration of GnRH, PGF2α, GnRH at fixed intervals followed either by timed breeding (OV-Synch and CO-Synch) or breeding according to estrus (Select-Synch). These protocols, therefore, either involve a large number of visits to the cows and extra expenditure on both drugs and visits or the need of estrus

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detection is not eliminated completely. Since the treatments are administered without ascertaining the actual ovarian status of the animals, conception rates varying between 52 to 61 percent have been reported [11- 13].

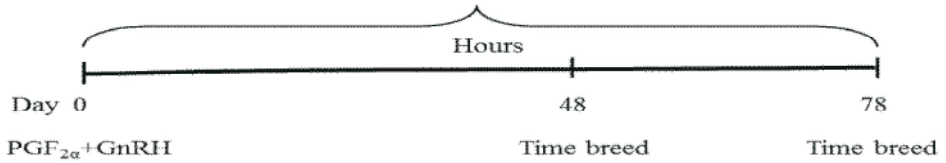
In Ethiopia exotic and crossbred cattle are the main contributors of milk to the growing dairy industry, in the face of low yields from local zebu. Confronted with the problems of poor heat detection and less than adequate AI facilities and services, the optimum exploitation of the genetic potential of the crossbred cattle is a big challenge. The present study was, therefore, aimed at testing the efficacy of a modified GnRH- PGF2α combination in synchronizing estrus in crossbred cows and heifers; as well as to try to reduce the cost of treatment to make it more affordable for the livestock farmers of developing countries, like Ethiopia.

MATERIALS & METHODS

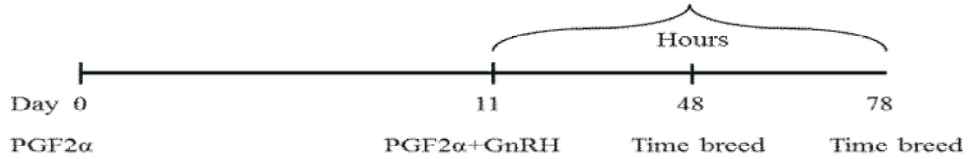
Study Area: The study was conducted in and around Mekelle town, located at 13 28N and 39 119E latitude. The altitude of the area ranges between 2100-2260 m.a.s.l and the agro-ecology of the region is arid with mean annual rainfall (Short rainy season in May and June and long rainy season from July to September) ranging from 11.3 to 39.1 mm with temperatures ranging from 12 C to 27 C. The study was conducted from October 2008 to May 2009, with calvings taking place from august to mid-September 2009.

Study Animals and Study Design: The study was conducted on heifers and cows belonging to organized dairy farms in and around Mekelle town and some unorganized, back yard, farms. The treatment protocol is described in Figure.1.

The study was conducted on HF X Local crossbred heifers (n=28), around two years of age and parous, adult cows (n=24) more than 90 days postpartum. All animals had no previous history of any breeding dates. The animals were initially examined to ascertain the normalcy of the genital tracts and their ovarian functional status. Animals with abnormalities of the genital tracts and smooth, non-functional ovaries were excluded. The selected animals were divided into two groups (I and II) on the basis of ovarian findings. Animals with a well-developed, functional corpus luteum (15 Heifers and 12 cows Group I) were assigned to receive a single, I/M, administration of 25 mg PGF2α (Lutalyse, Upjohn company, USA), followed immediately by I/M administration of 20μg GnRH (Receptal, Intervet International, Boxmeer, The Netherlands). Group II animals (13 Heifers and 12 cows) with a soft, small developing or a doubtful, not easy to palpate, corpus luteum; were assigned to receive a double dose of 25 mg $PGF_2\alpha$, 11 days apart. The second dose was immediately followed by an IM administration of 20ug GnRH. All animals were given fixed time inseminations, twice at 48 and 72 hours after GnRH administration. Since our preliminary examination of the frozen semen had revealed a low post Thaw motility (about 30%), the inseminators were advised to use two straws at each insemination to ensure the availability of sufficient numbers of motile spermatozoa. Pregnancy status of the animals was confirmed by trans-rectal examination, 60 to 90 days post AI. The fertility data of the animals was analyzed with Fisher's Exact Test, using computer software "GraphPad Prism-5".



a). Single administration of PGF2α+GnRH



b). Double administration of PGF2α and GnRH on the last PGF2α

Fig. 1: Experimental protocol used for estrus synchronization in cattle: a) protocol used for heifers and cows with a well-developed, functional corpus luteum; b) protocol used in heifers and cows with a small, soft or doubtful corpus luteum.

Table 1: Effects of doses of $PGF_{2\alpha}$ and GnRH on fertility of cows and heifers

	Single Dose $PGF_{2\alpha}$ + GnRH			Double Dose $PGF_{2\alpha}$ + GnRH			
Observations	Heifers	Cows	Total	Heifers	Cows	Total	Group I + II Combined
No. Treated	15	12	27	13	12	25	52
No. Eliminated	1(6.7)	4(33.3)	5(18.5)	2(15.4)	1(8.3)	3(12)	8(15.4)
No. in Estrus and Inseminated	14(93.3)	8(66.7)	22(81.5)	11(84.6)	11(91.7)	22(88)	44(84.6)
No. Conceived	11(78.6)	4(50)	15(68.2)	7(63.6)	9*(81.8)	16(72.7)	31(70.5)
No. Calved	11/14(78.6)	4/8(50)	15/22(68.2)	7/11(63.6)	7/11(63.6)	14/22(63.6)	29/44(65.9)

Values in parentheses are percentages.

Table 2: Estrus and conception rates of dairy heifers and cows based on farming systems.

Observation	Organized Farms	Backyard Unorganized Farms	Total
No. Treated	34	18	52
No. Eliminated	2(5.9)	6(33.3)	8(15.4)
No. in Estrus and Inseminated	32*(94.1)	12*(66.7)	44(84.6)
No. Pregnant	25*(78.1)	6*(50)	31(70.5)
No. Calved	25/25	4/6	29/31

Values in parentheses are percentages.

RESULTS

The results obtained in the present study are summarized in Tables 1 and 2. A total of 52 animals (28 heifers and 24 cows) were inducted into the experiment. Two cows at the time of clinical examination were diagnosed with luteal cysts and included in the single dose group, as they were either sold or not inseminated, even though observed in estrus. Another six animals (one heifer and two cows from group I and two heifers and one cow from group II) were excluded from the study due to the failure of the inseminator to inseminate at the scheduled time, post treatment. Therefore, a total of 44 animals (25 heifers and 19 cows) were effectively left in the study. The fertility results of cows and heifers treated with single and double dose of PGF2α, in combination with a single administration of GnRH is summarized in Table 1.

Estrus and conception rates of heifers and cows treated with single/double dose of $PGF_{2\alpha}$ in combination with GnRH. From a total of 52 treated animals, 44 (84.6%) were inseminated twice at 48 and 72 hours after treatment. Across groups the estrus response was similar (81.8% v/s 88%). The conception rates in the two groups were also similar (68.2% v/s63.6%). However, a comparison of the estrus response between heifers and cows showed a non-significantly. Higher response in heifers in Group I (93.3%) in comparison to cows (66.7%). In group II the marginally higher response in cows was not significant ($P > 0.05$).In group I although much higher conception rates were obtained from heifers, in comparison to cows, the differences again were not significant ($p > 0.05$).

All the 15 pregnant animals in Group I delivered normal, live calves between 270 and 290 days post AI. In group II two cows suffered embryonic losses, between 60 and 90 days post AI and only 14 of the 16 pregnant females calved. Overall 29 out of 31 pregnant animals in the two groups calved. The fertility data with respect to the farming systems of all animals is presented in Table 2.

From a total of 34 animals treated at organized, well managed farms; 32 were in estrus and inseminated, out of which 25 (78.1%) conceived. From the unorganized, back yard farming system, 18 animals were treated, out of which 6 had to be eliminated due to various reasons and only 12 animals were inseminated. From the inseminated animals only six (50%) conceived and two suffered embryonic losses.

DISCUSSION

Employing strategic breeding programs for controlled reproduction in cattle needs to consider several factors. One of the most important aspects is the probable diversity of the population, with regard to ovarian function at the start of the treatment. Some animals would be expected to be at early or late stages of the cycle; others at mid cycle and still others may be non-cycling. Synchronization of estrus and ovulation in such a diverse population, without actually ascertaining and categorizing the animals is a challenging task. Protocols like Ov-Synch and Co-Synch aim at either inducing luteal development or luteinization of the largest available follicle and/or prolongation of the life of an already available CL for the next seven days [11, 13]. This ensures the availability of

sufficient luteal tissue for the action of prostaglandins on day 7 to precipitate estrus. The administration of second dose of GnRH on day 9 is expected to synchronize ovulation, to pave way for timed insemination either on day 9 or day 10. However, such combinations have been known to produce conception rates of about 50 percent [11] and would also involve multiple visits and a high cost of treatment. The present investigation was, therefore, undertaken to examine the possibility of enhancing the fertility rates, on the one hand and try to reduce the cost of treatment, on the other hand, to make the program affordable to the livestock farming community.

Failure of cyclicity, irrespective of whether it is real or due to failure of heat detection; either due to silent estrus or lack of proper observation, is a major constraint in successful cattle breeding programs. Shiferaw [3] recorded a 38 percent incidence of infertility in Ethiopian crossbred heifers and cows, with anoestrus and long calving intervals being the main problems. This is, therefore, directly responsible for high economic losses resulting from increase in the age at first calving and a long calving interval. The loss of calf crop and reduced life time milk yield are, therefore, two of the most important factors which need to be addressed if dairy farming is to be profitable.

Strong efforts have been made across the world to eliminate the need of heat detection in cows [10, 14, 15], by using different hormonal combinations and to breed the animals either according to estrusor through timed matings/inseminations. During the previous years, our experience has shown that a large number of cows and heifers, in and around Mekelle, both at organized farms as well as individually owned small back yard dairy units, reported to be non-cycling are actually, regularly going through the various stages of estrus cycle, but were probably not detected by the herdsmen or the owners. Therefore, the present study was planned to investigate the efficacy of GnRH-PGF2α combination vis-à-vis timed inseminations, through a modified protocol.

In the present investigation conducting the initial clinical examination not only helped in eliminating animals with genital pathology from the study, but also helped in identifying the animals at different stages of the cycle. Thus the requirement of administering the first dose of GnRH was eliminated. The simultaneous administration of PGF2α and GnRH in Group I and administration of GnRH along with the second PGF2α dose in Group II, helped in reducing the duration of treatment, number of visits to the animals and to a certain extent the total cost of the treatment.

In our modified protocol, we envisaged that by simultaneous PGF2α and GnRH administration, whereas the prostaglandins would bring about regression of the corpora lutea, GnRH would, at the same time help in the endogenous release of pituitary gonadotropins. This gonadotropic surge would be expected to lead to development of ovarian follicles and ovulation in the next three to four days. The estrus response of the heifers and cows, in the present study, varying between 67 to 93 percent compares favorably with the previous observations [16]. The conception rates of 72 percent in heifers and 68 percent in cows are quite promising and somewhat higher than those previously reported for Ov-synch or Co-Synch protocols [11, 12, 13]. Higher conception rates have also been reported for animals bred according to estrus, rather than timed matings [17]. However, Burke *et al.* [18] recommended timed matings as an effective tool for improving reproductive management of dairy cows, as it avoid the need of estrus detection. All the animals in the present study were supposedly non-cycling, for the owners/farm managers; although on clinical examination they were found to be actually cycling normally. This highlights the problems of heat detection both in the organized as well as unorganized, back yard, dairy units of Ethiopia.

Prostaglandins alone, either as single or double dose schedule, have been extensively used for a long time for synchronizing estrus in cattle [19, 20, 21] with varying results of estrus and conception. For PGF2α to be effective, the animals need to be in diestrus stage of the cycle; whereas many animals between day one to five of the cycle are not responsive. Therefore, a double dose schedule was developed to overcome this problem [22, 23, 24]. However, variations in the interval to estrus have also been reported [25]. Young and Henderson [26] reported a conception rate of 47 percent after synchronization with single or double dose of Prostaglandins.

In view of these limitations and to improve the post treatment fertility various hormonal combinations have been tried. Several reports [27, 16] have described a higher rate of estrus synchronization (80 percent) when Prostaglandins are combined with GnRH than when Prostaglandins are used alone (50 percent). The currently used protocols include two administrations of GnRH and one of PGF2α. The effectiveness of these protocols has been well established [28]. The second dose of GnRH synchronizes the ovulation [11]. However, such combinations also increase the cost of treatment, so as to become out of reach of the average farmer in a developing economy.

In conclusion, it can be said that, the modified GnRH-PGF2α protocol used in the present study to synchronize estrus in crossbred dairy heifers and cows was quite successful. The overall conception rates of 72 percent in heifers and 68 percent in cows are higher in comparison to others. However, to further validate the results; a controlled study on larger numbers of animals is advocated.

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