
Outcome of Gonadotropin Releasing Hormone Treatment 12 Days Post AI and Double Insemination on Conception Rate of Repeat Breeding Cows in Mekelle, Tigray, Ethiopia

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Abstract: The aim of the present study was to determine outcome of gonadotropin releasing hormone analogue given 12 days post AI and double inseminations during estrous period of dairy cattle and improvement of conception rate. Field trial was carried out on repeat breeding cross breed dairy cows which are lactating, apparently healthy and exhibits estrus regularly. A total of Sixty three repeat-breeding cows from twenty eight herds were selected and assigned randomly in to three equal groups G (1, 2 and 3); two treated and one control group. Group1 (n=21) cattle were treated intramuscularly with 10 µg busserelin acetate 12 days post AI. Group2 (n=21) cattle were inseminated twice during estrus exhibition with 6-8 hours interval. Group3 (n=21) cattle were receive no treatment, inseminated once as heat was detected and considered as a control group. Dairy cows were examined for pregnancy after three months through rectal palpation. Pregnancy rates recorded were 52%, 38% and 28% in G1, G2 and G3 respectively. Conception rates of treated groups (G1 and G2) exceed control group by 24% and 10% respectively and showed a significant statistical variation ($P<0.05$) as compared to control groups. Comparison between treated groups also indicated that G1 has statistical significant difference from G2. From this experiment, it is recommended that use of GnRH 12 days can improve pregnancy rate as compared to double insemination and single service.

Keywords: Crossbred Cattle, Estrus, Double Insemination, GnRH Analogue, Pregnancy Rates

1. Introduction

Fertility problems in dairy cattle are caused by several factors. Reproductive disorder is most costly problems facing dairy industry and occurs frequently in lactating dairy cows. Repeat breeding is among the most frustrating problems which are defined as cows' failure to conceive from three or more services (Royal *et al.*, 2000; Lucy, 2001 and McDougall, 2006). These are cows that cycle normally and have no clinical abnormalities after a minimum of 3 inseminations (Levine, 1999). The economic losses associated with this problem are highly considerable resulted from increased veterinary expenses, insemination costs, reduced productivity, and losses due to involuntary culling. Reproductive problems have been the primary cause of culling in animal husbandry for many years (Coleman, 1985

and Opsomer *et al.*, 2000).

Several investigations were carried out to find out possible solutions to reduce the number of repeat breeder dairy cows. Some of the possible suggestions that were tried to reduce the incidence of repeat breeding were; strengthening estrus detection, embryo transfer, administration of GnRH at insemination, continued artificial insemination, resynchronization of non-pregnant cows. Double insemination and gonadotropin-releasing hormone treatment twelve days post AI of repeat-breeding dairy cattle were also among the most suggested solutions for this problem by Stevenson *et al.*, 2000. Double insemination increases chances of pregnancy rate due to hidden or error of estrus detection of the dairy men.

Physiologically it is known that gonadotropin releasing hormone (GnRH) is produced by hypothalamus which controls synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). These two hormones have combined effects on follicular development, ovulation and corpus luteum functions (Douglas, 1998). The efficacy of synthetic gonadotropin releasing hormone GnRH analogue mimics the function of this natural hormone in modifying reproductive efficiency of normal as well as repeat breeder dairy cows.

This hormone has improved half life in blood circulation, greater stability to enzymatic degradation, increased receptor affinity, prolonged biological potency and less antigenic than other molecules for repeated use (Cline, 2002). The exogenous administration of these hormones at different period of estrus to the cow may lead to treat the disease condition or cysts in the ovary or may rectify hormonal problem or insufficiency to these cows (Morgan, *et al.*, 1993 and Bearden and Fuquay, 1997).

GnRH has generally been used as a therapeutic agent in reproductive management. The GnRH had been used as treatment on rates of fertilization, embryonic mortality and as a therapeutic treatment for the cystic ovaries (Gustafsson *et al.*, 1986). It had been used as treatment along with or 12 days post AI to increase rates of fertilization, reduce embryonic mortality and as a therapeutic treatment for the cystic ovaries, ovarian follicular cysts and for triggering the LH surge to stimulate ovulation (Gustafsson *et al.*, 1986; Thatcher, 1991).

Double insemination with some hours interval reduces incidence of repeat breeding by increasing probability of site and time of semen deposition and estrus detection.

Limited knowledge was available about use of synthetic hormones and double insemination for management of repeat breeding dairy cows in Ethiopia and in Tigray region in particular. Nothing was done to investigate problems of repeat breeding using GnRH analogue on day 12 post AI and double insemination for enhancement of conception rate of cross breed repeat breeding dairy cattle. Therefore, the present study was conducted with the aim of enhancing the fertility rates in cross breed cattle with the specific objectives of: evaluating effects of GnRH analogue (buserelin) 12 days post AI and double insemination with eight hours interval on enhancement of conception rate as compared to control groups.

2. Materials and Methods

2.1. Description of the Study Areas

This controlled trial was conducted in the capital of Tigray regional state, Mekelle. It is one of the seven Administrative Zones and is located at 783 km north of Addis. It is geographically located 39° 29'E and 13° 3'N longitude. Mekelle is surrounded by south east zone in the south and east, Eastern zone from north, central zone from west. It has an average temperature of 20°C and experiences an average

annual rain fall of 600 mm. Livelihoods of the people of the city depends on trade, civil servants and agricultural activities. Dairy farming is one of the major farming systems practiced and small holder farmers in vicinity of the city supply moderate amount of milk to the urban dwellers. Some of the farmers in the nearby area practice a mixed crop livestock production system. The area is moderately covered with savanna, bushy and low weed vegetations. Both small holder farmers and commercial dairy farms owners were included the study.

2.2. Study Population and Management Practices

The study was conducted in Mekelle and experiment was conducted in cross breed dairy herds owned by small holder farmers and private commercial dairy farms in the city. The experimental cows were kept in stall facility and feed grass hay, concentrate composed of wheat by products, locally available crop residues, forages and other locally prepared fermented alcohol by products. The cows have good body condition and apparently healthy. They are with history of more than two services and milked twice per day. They were vaccinated regularly against common infectious diseases. Generally, dairy cows were managed in similar agro ecological and climatic conditions. AI was exercised based on visual observation of standing heat and mucosal discharges from vulva by herd keepers.

2.3. Experimental Design and Selection Process

Randomized controlled trial was used to study effects of GnRH analogue given 12 days post AI and double inseminations with 6-8 hours interval on enhancement of pregnancy rates as compared to dairy herds which receive single service. The study period was from December 2009 to February 2010 in and around Mekelle. A total of 63 dairy cows were selected from twenty eight farms. Cows with normal cycling, no clinical abnormalities, failed to conceive after two successive inseminations, not previously assigned for any study and aged from three to ten years were included in the study. Cows that were treated at the first AI and returned to estrus couldn't be reassigned for the study. Sixty three cross breed dairy cows were selected based on this and equally divided in to three groups each consisting of twenty one dairy cows. These repeat breeders were randomly assigned into three protocols of treatment groups.

2.4. Experimental Protocol

This protocol trial consists of three main phases: In the first phase, training was provided for twenty eight herd owners to create awareness about symptom of estrus cycle, behavioral changes observed during estrus exhibition. Some of the signs to be observed by herd owners were mucosal discharges from their vulva, restlessness, frequent urination; allow to be mounted by other animals, raised tails and others. Herd owners were informed that as soon as these symptoms were observed, they would phone to inseminator immediately and these cows were included in the study. The second phase

was random assignment of these dairy herds in to three groups; control and treatment groups. The first group G₁ (n=21) was inseminated and 10 µg (2.5ml) buserelin acetate was given intramuscularly 12 days post AI. The second group G₂ (n=21) was inseminated twice with 6-8 hours interval during estrus exhibitions. The last group G₃ (n=21) was given only single service and considered as control group. The last phase of the study was pregnancy diagnosis and was done after 60 days post AI using a rectal palpation in these herds which failed to return to estrus. The cows were considered as pregnant if we were able to palpate or sense fetal membrane, amniotic vesicles, cotyledons and fetus otherwise the cows were classified as negative.

2.5. Data Collections and Statistical Analysis

In addition to recording of data concerning inseminations and owners name, dairy herd owners were asked about breed type, insemination and service history, parity, management condition and milk yield, time and day of estrus exhibition and previous condition of the dairy herds. During study period, pregnancy diagnosis was made through rectal palpation and positive and negative results were recorded. Secondary data was collected from farm records about animals' previous milk yield, clinical cases, origin of the animal and service type. This field trial was of a randomized type in which data was collected from treated and controlled study animals. Collected data was fed to Microsoft Excel spread sheet and process of data cleaning, handling, validating and coding was done on this spread sheet. Difference in pregnancy proportion, age and parity among different treated groups against control one was compared using descriptive statistics and Pearson chi-square tests (SPSS16.0; 2005). The significance level of difference was observed at P value of less than 5%.

3. Results and Discussion

From 63 dairy cows included in the study, 25 cows were found to be pregnant. A total of 11 cows from 21 of G₁ were pregnant with conception rate of 52%. In the G₂, 8 cows out of 21 (38%) were conceived. The results were indicated in Table 1. G₁ treated with buserelin acetate 12 days post AI exceeds in conception rate to G₂ by 14% that was given double insemination. In general treated groups recorded higher pregnancy rate as compared to the G₃.

Conception rate of G₁ (treated) exceeds conception rate of G₃ (control) by 24% with statistical significant variation ($\chi^2=7.636, P=0.006$). The pregnancy rates of G₂ were greater than group G₃ by 10 % with statistical significant variation

($\chi^2=13.650, P=0.000$). Comparison of conception rate between two treated groups: G₁ scores more conception rate with statistical significant difference as compared to G₂ ($\chi^2=11.748, P=0.001$). Comparison among the three groups, conception rate in both G₁ and G₂ were significantly higher than G₃. The overall comparison of treated and controlled group was shown in table 2. Management condition, season and parity variation doesn't show any significant variation in the treated groups as compared to controlled group.

Repeat-breeding dairy cows are sources of frustration and economic loss to dairy producers and the present study was carried out to see effects of double insemination and treatment of GnRH analogue 12 days post AI. G₁ and G₂ scores more 52% and 38% respectively than G₃ that scored 28%. These treated groups vary significantly ($p<0.05$) than control groups. This indicates that incidence of repeat breeding can be reduced through double insemination and GnRH treatment. There is a significance difference between GnRH treatment and double insemination ($P<0.05$) but numerical difference between double inseminated and controlled group. This may be due to insufficient or defective corpus luteum development (Pursley *et al.*, 1997) and treatment might have produced a healthy CL. This could maintain high progesterone concentration in the serum which reduces embryonic mortality in the early stages.

This result was in agreement with the study by Blowey (1992) that administration of GnRH analogue 12 posts AI improves fertility of cows by 9 % to 12 % and by Drew and Peter (1994) which was already reported. Other study by Beckelr *et al.*, (2006) observed that there was no significant difference between treated and controlled groups which were given 12 days post AI on reproductive performance of dairy cows which is opposite to the present study. Double insemination showed statistical significance variation as compared to control. Time interval at which two inseminations given was six to eight hours. So the observed results may be due to either insemination time is not followed by the owner or by the inseminator, otherwise it may be due to delayed ovulation.

Reduction in incidence of repeat breeding following these treatments results in financial return to farmers as compared to animals with single service. Intramuscular injection of GnRH analogue 12 days post AI exceeds pregnancy rate by 24% from controlled group. Repeat breeding dairy cows with double AI exceed the controlled group by 10%. Still GnRH treated groups overwhelm double inseminated groups by 14% with statistical significance variation. So, all these variations indicated that hormonal treatment will result in a great financial benefit to the farmers.

Table 1. Results of various protocols with their respective percentages of conception.

Group	No. animals in each protocol	Type of Protocol followed	Pregnancy rate
1	21	10µg(5ml) buserelin acetate 12 days post AI	11/21(52%)
2	21	Double insemination with 6-8 hours interval	8/21(38%)
3	21	Controlled group(receive no treatment, single service)	6/21(28%)

Table 2. Cross tabulation of the treated groups with controlled animals.

Cross tabulated groups			Group 3			Significance Level
			Negative	Positive	Total	
G1*G3	Group 1	Negative	10	0	10	$(\chi^2=7.636,P=0.006)$
		Positive	5	6	11	
		Total	15	7	21	
			Group 3			
G2*G3	Group 2	Negative	13	0	13	$(\chi^2=13.650,P=0.000)$
		Positive	2	6	8	
		Total	15	6	21	
			Group 2			
G1*G2	Group 1	Negative	10	0	10	$(\chi^2=11.748,P=0.001)$
		Positive	3	8	11	
		Total	13	8	21	

4. Conclusion and Recommendations

The finding our study indicated that double insemination and intramuscular injection can be used as treatment options for repeat breeding dairy cows. Both treated groups; GnRH analogue treated 12 days post AI and double inseminations scores 52% and 38% respectively. There is statistical significance variation between treated and control groups. This experiment provides evidence for continued recommendation of the GnRH treatment to improve conception rates of repeat breeders and farmers could be benefitted financial from these alternative treatment options. Based on these results, the following points are recommended.

- The GnRH analogue administration 12 days post AI could improve the reproductive performance of repeat breeding dairy cows.
- Double insemination can achieve more conception rate for those cows with silent estrus or for the missed detection of heat.
- Farmers should be aware of treatment options to be benefitted from their dairy cows
- Given the cost of GnRH analogue, we continue to recommend the use of this hormone to rectify the problem of repeat breeding crossbred dairy cows to prevent financial losses

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