J. Emb. Trans. (2012) Vol. 27, No. 4, pp. 223～228
http://dx.doi.org/10.12750/JET.2012.27.4.223

Use of Two Estrus Synchronization Protocols and Their Success on Pregnancy in Zebu and Crossbred Heifers at *Char* Areas of Bangladesh

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ABSTRACT

This study was designed to adopt two estrus synchronization protocols in zebu and crossbred heifers and their effects on pregnancy rate after timed artificial insemination (TAI). A number of 120 cyclic heifers were allotted for two different treatment groups and one control group. Heifers under protocol A were injected with GnRH at first day followed by a single dose of $PGF_{2\alpha}$ at Day 11 and injection of GnRH at the day of AI; and heifers belonged to protocol B were treated with GnRH, two $PGF_{2\alpha}$ injections at 11 days apart and injection of GnRH at AI. AI was done at fixed time (within 72～96 hours after $PGF_{2\alpha}$ injection) in both protocols and pregnancy was confirmed by rectal palpation on 80～120 days of post AI. In control group; local heifers were conceived higher (30%) proportion than that of crossbred heifers (25%; $p<0.05$). In protocol A, the local breed were conceived higher (38.9%) proportion compared with crossbred (25%; $p<0.05$). In protocol B, local breed heifers were conceived higher (38.9%) proportion compared with crossbred heifers (33.3%; $p<0.05$). The overall pregnancy rate in protocol A and protocol B was 33.3% and 36.6%, respectively. The proportion of pregnancy rate of local heifers (38.9%; Protocol A) was significant ($p<0.05$) in comparison with local heifers (30%) in control group ($p<0.05$). The overall pregnancy rate between pooled control group (28.3%) and treatment group (35%) was significantly ($p<0.05$) differ from each other's. Results of present study concluded that estrus synchronization followed by fixed time AI could be applied for higher pregnancy rate in zebu and crossbred heifers.

(Key words : estrus synchronization, pregnancy rate, zebu and crossbred heifer)

INTRODUCTION

Millions of people in Bangladesh live in the island of river, called '*char*' (Low lying flood and erosion-prone areas in or adjacent to major rivers). Most of them are ultra-poor. The donor organization come forward to stand beside the *char* dwellers and donated cattle as a living asset. Paul *et al*. (2011) reported that the major problem of the heifer (both zebu and crossbred) at *char* area is low pregnancy rate. There are many reasons behind this problem such as feed crisis, natural calamities, and limited access to veterinary service, negative energy balance, genetic variation and poor body condition leading to non-functional ovaries, which is considered important causes of low pregnancy rate. But it is most of the time very difficult to overcome many of these causes in certain situation. For example, still estrus detection errors cannot be overcome and 40% cow remained undetected in Bangladesh (Shamsuddin *et al*., 2001). As a result, researchers have thought about the alternative reproductive managements for successful achievement of the reproductive goals. Stevenson *et al*. (1996) and Peters *et al*. (1999) stated that hormonal treatment with timed artificial insemination (TAI) is one of the encouraging assistance technologies to develop elsewhere.

TAI with estrus synchronization is an effective tool for improving reproductive management in dairy cows, since it avoids the need for estrus detection. Donaldson *et al*. (1980) reported

† Financial assistance from the Department of International Development (DFID) and Government of Bangladesh (GoB) under the Char livelihoods Program (CLP) is gratefully acknowledged.
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that the $PGF_{2\alpha}$-based TAI has the advantages of facilitating AI programme. Moreover, it is not always possible to determine the functional status of the corpus luteum by rectal palpation (Chowdury *et al*., 1998). To avoid this problem, Alam et a1. (1993) and Butler *et al*. (2011) suggested that the double injection of $PGF'_{2\alpha}$ at 10 days apart may be used for estrus synchronization in cows. According to opinion of Thatcher and Chenault (1976), Alam and Dobson (1987) and Voss *et al*. (1989), administration of GnRH in the case of $PGF'_{2\alpha}$ induced TAI increases pituitary responses for pregnancy. GnRH treatment could be adapted to the timed insemination protocol as it increases pregnancy rate (Martinez *et al*., 1997). Several researchers investigated the effect of GnRH on fertility using different approaches in *Bos taurus* cows (Alam *et al*., 1993). However; at *char* in Bangladesh, there is no precise report on the use of estrus synchronization program in zebu and crossbred heifers followed by TAI. Therefore; the present study was designed to introduce and adopt two different estrus synchronization protocols and their effects on pregnancy rate in local and crossbred heifers.

MATERIALS AND METHODS

1. Animal Managements

A total number of 120 heifers were selected for the study whose body condition score (BCS) were $\geq$2.5 and age ranged from 2 to 3.5 years old. The animals were divided into two groups; control and treatment group. In control group, a total number of 60 heifers (n=40 local and n= 20 crossbred) were used and another 60 heifers were used for experimental group. Within experimental group heifers were again subdivided in equal number for treatment protocol A (n=30; n=18 local and n=12 crossbred) and B (n=30; n=18 local and n=12 crossbred). These heifers were de-wormed by broad spectrum anthelmintics with a combination of Trichlobendazole 900 mg and Levamizole 600 mg (L-T vet®, Techno drugs Ltd. Dhaka, Bangladesh) and vaccinated against Foot and mouth disease virus (FMD), Black quarter (BQ), Haemorrhagic septicemia (HS) and Anthrax (Livestock Research Institute, Dhaka, Bangladesh). The heifer was maintained under semi stall feeding system. All these heifers were housed under straw made shed as a common animal housing in Bangladesh. All the heifers used in this study had well developed reproductive organ which we were confirmed by manual palpation transreactally, and palpably free from any diseases or abnormality i.e. cervicitis, pyometra, tumor. The cyclicity of heifers were confirmed through observing clear vaginal discharged and by rectal palpation and only the cyclic heifers were selected for this study.

2. Semen Used for AI

In this study, frozen semen were used for both control and treatment groups. The semen was collected from Department of Livestock services (DLS, Ministry of Livestock and Fisheries, Dhaka, Bangladesh) and preserved in Liquid Nitrogen tank. All the semen used in this study was from Local × Shahiwal because of its high pregnancy rate in aspect of *char* area of Bangladesh (Paul *et al*., 2011).

3. Insemination of Heifers

The heifers under control group were observed closely by the trained owners and inseminated within 10～12 h after observing estrus by skilled technician. Heifers belonged to treatment group received AI service at fixed time.

4. Experimental Design

The heifers were synchronized for estrus with Gonadorelin, an analogue of gonadotrophin releasing hormone (GnRH), (Fertagyl® Inj., Intervet Ltd., Netherland) and an analogue of $PGF_{2\alpha}$ (Dinoprost® Inj., Techno Drugs Ltd., Dhaka, Bangladesh). Two different treatment protocols (Protocol A and B) were used for the study.

5. Treatment Protocol A (GnRH-$PGF_{2\alpha}$-GnRH)

First 2.5 ml Fertagyl were injected IM followed by injection of 5 ml Dinoprost after 11 days. The heifers were inseminated within fixed time; first AI at 72 hours and second AI at 96 hours of Dinoprost injection. Further 2.5 ml Fertagyl was injected within one hour of AI.

6. Treatment Protocol B (GnRH-$PGF_{2\alpha}$-$PGF_{2\alpha}$-GnRH)

2.5 ml of Fertagyl were injected IM followed by injection of 5ml Dinoprost at Day 11 of Fertagyl injection. A repeated injection of 5 ml Dinoprost was given at Day 11 day of first Dinoprost injection. The heifers were inseminated at fixed time; first at 72 hours and second at 96 hours of second Dinoprost injection. A further of 2.5ml GnRH was injected within one hour of AI.

7. Pregnancy Diagnosis

The pregnancy was diagnosed by rectal palpation between

80～120 days post of AI.

8. Statistical Analysis

The data collected from both control and treatment groups in respect to the return to estrus or pregnancy were organized in Microsoft Excel 2003. The collected data were coded, compiled, tabulated and analyzed in accordance with the objectives of the study by SPSS® software (Version 12). The chi-square test was done to test the hypothesis. The difference between values was considered significant when the *P* value was less than 0.05 ($p<0.05$).

RESULTS

1. Effects of Treatment Protocol A for Induction of Estrus and Pregnancy in Heifers

In the control group; the number of heifers showed estrus and the pregnancy rate (both local and crossbred) are shown in Table 1. Among a total number of 60 heifers in control group, 70% heifers were showed estrus with a pregnancy rate of 28.3%. The 70% heifer in both local and crossbred showed estrus.

The treatment effect of Protocol A for induction of estrus and pregnancy is presented in Table 2. Out of 30 heifers, the number of heifers showed estrus was 24 of which 33.3% were pregnant. With respect to breed comparison, among the total of local and cross breed heifers, the 83.3 and 75%, heifers respectively showed estrus.

Table 1. Pregnancy rate in heifers following insemination at natural estrus

Group	No. of heifers showing estrus	No. of heifers pregnant	Pregnancy rate (%)
Zebu (n=40)	28	12	30[a]
Crossbred (n=20)	14	5	25[b]
Total (n=60)	42	17	28.3

[a,b] The values within the same column differed significantly from each other ($p<0.05$).

Table 2. Effects of treatment protocol A on heifers for induction of estrus and pregnancy

Breed	No. of heifers	No. of heifers in estrus	No. of heifers pregnant (%)
Zebu	18	15	7 (38.9)[a]
Crossbred	12	9	3 (25)[b]
Total	30	24	10 (33.3)

[a,b] The values within the same column differed significantly from each other ($p<0.05$).

The comparison between control group and treatment group (Protocol A) on estrus and pregnancy rate is given in Table 3. In treated group, zebu heifers showed highest (38.9%) proportion pregnancy than that of control group (30%).

2. Effects of Treatment Protocol B for Induction of Estrus and Pregnancy in Heifers

The effect of treatment protocol B on induction of estrus and pregnancy is presented in Table 4. Twenty six heifers out of 30 showed estrus in respond to treatment. The pregnancy

Table 4. Effects of treatment protocol B on heifers for induction estrus and pregnancy

Breed	No. of heifers	No. of heifers in estrus	No. of heifers pregnant (%)
Zebu	18	16	7 (38.9)[a]
Crossbred	12	10	4 (33.3)[b]
Total	30	26	11 (36.6)

[a,b] The values within the same column differed significantly from each other ($p<0.05$).

Table 3. Comparisons of pregnancy rate between control and treatment protocol A group

Group	Breed	No. of heifers	No. of heifers in estrus	No. of heifers pregnant (%)
Control	Zebu	40	28[a]	12(30%)[a]
	Crossbred	20	14[c]	5(25%)
Treatment protocol A	Zebu	18	15[b]	7(38.9)[b]
	Crossbred	12	9[d]	3(25%)

[a～d] The values within the same column differed significantly from each other ($p<0.05$).

rate in treated heifers was 36.7%. When compared with local and crossbred heifers, 16 local and 10 crossbred heifers showed estrus out of 18 and 12, respectively. The pregnancy rate in local and cross breed heifers was 38.9 and 33.3%, respectively.

The comparison of pregnancy rate between control and treatment group (pooled, both A and B protocol) is presented in Table 5. The overall pregnancy rate for heifers in control and treatment group was 28.3 (17/60) and 35% (21/60), respectively.

The comparison of pregnancy rate between two treatment groups (protocol A and protocol B) is shown in Table 7. The pregnancy rates in heifers belonged to protocol A and B were 33.3 (10/30) and 36.7% (11/30), respectively.

Table 6. Comparisons of pregnancy rate between pooled control and treatment group

Group	No. of heifers	No. of heifers pregnant (%)
Control group	60	17 (28.3)[a]
Treatment group	60	21 (35)[b]

[a,b] The values within the same column differed significantly from each other ($p<0.05$).

Table 7. Comparisons of pregnancy rate between treatment protocol A and B

Treatment protocol	No. of heifers	Pregnancy rate (%)	*P*-value
Protocol A	30	10 (33.3)	$p>0.05$
Protocol B	30	11 (36.7)	

DISCUSSION

This study was done in the island area of Jamuna River, called *char* areas, at the northern part of Bangladesh. In this study, the estrus synchronization program with fixed time AI for successful pregnancy was introduced and implemented to improve the pregnancy rate in zebu heifers and crossbred heifers.

The fertility problem of heifers and cows in that area is remarkable. Earlier, Paul *et al*. (2011) indicated that the lack of balanced feed and poor knowledge of farmer regarding cattle rearing were the core setback of low pregnancy rate in this area. The first service pregnancy rate of cows in that area was promoted to 42.7% after improving the management system (Paul *et al*., 2011). Success of induction of estrus and pregnancy rate of local and crossbred heifers were found significantly ($p<0.05$) differ from each other which is partially agreed with the Alam and Dobson (1987).

Higher proportion of zebu heifers showed coming into estrus than that of crossbred heifers. Similarly, the pregnancy rate was significantly higher ($p<0.05$) in local breed heifers compared with crossbred heifers. Chenault *et al*. (1990) and Stevenson *et al*. (1993) stated that the administration of an exogenous GnRH influenced directly the anterior pituitary to initiate ovulation and subsequently conception. The difference for pregnancy between control and treatment group was significantly ($p<0.05$) differ from each other whereas in cross breed heifers between control and treated group did not show any differences 56 *vs* 86% (Twangiramungu *et al*., 1992).

The pregnancy rate between zebu and crossbred heifers under protocol-B was significantly ($p>0.05$) differ each other. The pregnancy rate was higher in local breed heifers than that of crossbred. The number of heifer in control group has showed significantly ($p<0.05$) higher estrus signs, compared than that of treatment group. But the pregnancy rate was significantly ($p<0.05$) higher under treatment group in both local and crossbred heifers than that of control group. The difference in pregnancy rate between treatment protocol-A and B was not significantly

Table 5. Comparison of pregnancy rate between control and treatment protocol B group

Group	Breed	No. of heifers	No. of heifers in estrus	No. of heifers pregnant (%)
Control group	Zebu	40	28[a]	12(30)[a]
	Crossbred	20	14[c]	5(25)[c]
Treatment group (Protocol B)	Local	18	16[b]	7(38.9)[b]
	Crossbred	12	10[d]	4(33.3)[d]

[a vs b; c vs d] The values within the same column differed significantly from each other, respectively ($p<0.05$).

(p>0.05) differ each other which is agreed with Hardin *et al*. (1980) who reported following an induced estrus by $PGF_{2\alpha}$ injection in zebu cattle and their crosses, only 30% cows were conceived. But, Bhuyian (1990) reported that 57% conception rate in zebu and zebu crosses which were treated with two regimens of closprostenol at an interval of 10 days. In protocol-B, the double injection of GnRH and $PGF_{2\alpha}$ had significantly increased the tendency of zebu heifers to conceive compared with crossbred heifers and the overall pregnancy rate was 36.6%. This pregnancy rate is significant in context with local zebu heifers and their crosses in char areas. This study supports the fact that, local zebu cows and their crosses may be placed under the synchronization programme using only $PGF_{2\alpha}$, as these animals have been in suffering from persistent luteal tissue in the ovary (Alam and Ghosh, 1993). The heifers showed more tendencies to pregnant in double GnRH and double $PGF_{2\alpha}$ treatment but were not significantly higher than double GnRH and single $PGF_{2\alpha}$ treatment. From the result of the present study, after synchronization of estrus with $PGF_{2\alpha}$ and GnRH, total 21 heifers were conceived out of 60 heifers and in control group 17 heifers were pregnant out of 60 heifers which is statistically significant (p<0.05). Pregnancy rate following this GnRH-$PGF_{2\alpha}$-GnRH treatment and fixed time AI was assessed in two other studies. Pregnancy rates in lactating cows are similar to those of controls (Pursley *et al*., 1997), whereas Schmitt *et al*. (1996) reported a reduction in pregnancy rate in heifers. $PGF_{2\alpha}$ treatment would reduce the incidence of sub clinical uterine infection and hasten the uterus in return to a suitably environment for fertilization (Ott and Gustafsson, 1981). It is not always possible to maintain the proper hygienic management in char areas of Bangladesh which may result the sub clinical uterine infection with persistent corpus luteum.

It is necessary to develop a programme using exogenous hormones for fixed time AI to eliminate the need for estrus detection within estrus synchronization programme especially in zebu heifers and their crosses. Before the selection of a hormonal approach one should realise the economic status of the farmers. Hormonal drugs specially the analogues of $PGF_{2\alpha}$ and GnRH are expensive. GnRH-$PGF_{2\alpha}$-GnRH treatment at day 0, 7 and 8/9 respectively regardless of the stage of estrus and then fixed timed AI is an economically advantageous method for controlling reproduction (Esslemont and Mawhinney, 1996). In this study, AI on fixed timed estrus resulted increased number of conception rate than that at AI on natural estrus. But some researchers observed higher conception rate after AI on natural estrus than fixed time (Seguin *et al*., 1983; Zeroual, 1994). In this study single dose of 500 μg of GnRH was used intramuscularly immediately after AI with a view to ensure synchrony between estrus and insemination. It has been reported that conception rate improves when GnRH is administered at insemination with a body condition score <3.0 (Burke *et al*., 1996) at insemination regardless of parity. Conception rate may be influenced positively by body condition of the treated animals with $PGF_{2\alpha}$ and GnRH (Burke *et al*., 1996). All heifers used in this study had ≥2.5 body condition score. In this study, GnRH is given within 72 to 96 hours after last $PGF_{2\alpha}$ injection which was partially agreed with Thatcher *et al*. (1993) who was stated that the injection of GnRH closer to the onset of estrus may be beneficial in increasing conception rate in in both zebu and crossbred heifers.

It may be concluded that the zebu and crossbreed heifers could be treated with estrus synchronization protocols for higher pregnancy rate in Bangladesh. Protocol A (GnRH-$PGF_{2\alpha}$-GnRH) might be good as it would be economic than using protocol B (GnRH-$PGF_{2\alpha}$-$PGF_{2\alpha}$-GnRH) However, to get a hold consistent and accuracy of result, protocol A may be tested with a large population of heifers with hormonal assay and ultrasonography for field application.

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(received: 2012. 10. 13 / revised: 2012. 10. 15 / accepted: 2012. 12. 15)