



Hormonal stimulation and some risk factors studied to improve bovine pregnancy rate at the Coastal areas of Barisal district of Bangladesh

Ashit Kumar Paul* and S M Toufiqur Rahman

Department of Medicine, Surgery and Obstetrics, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Barisal-8210, Bangladesh.

Abstract

Hormonal treatment of cows at the coastal region of Barisal district of Bangladesh was performed to assess the improvement of pregnancy rate. A total of 100 cows and heifers with irregular history of cyclicity were selected randomly and divided into five treatment groups. The groups were A (treated with anthelmintic), B (treated with anthelmintic, vitamin ADE and multivitamin powder), C (treated with PGF₂α), D (treated with GnRH) and E (treated with GnRH and PGF₂α). Each group comprised of 20 animals. The age, breed and parity of experimental cows were considered during treatment. In the study, the cows treated with both GnRH and PGF₂α (group E) showed significantly ($p < 0.05$) higher estrus (80%) and pregnancy rate (60%) than that of group A, B, C and D. The overall estrus rates of local and crossbred cows were 64% and 70%, respectively and the pregnancy rates were 40 and 52%, respectively. The crossbred cows responded significantly ($p < 0.05$) to hormonal treatment than that of local cows. Parity-2 cows showed higher estrus sign than that of other parities. However, the pregnancy rates were higher significantly ($p < 0.05$) in parity-2 and parity ≥ 4 cows than that of parity-0, parity-1 and parity-3 cows. The pregnancy rate was also found higher in case of 4 to < 5 years old cows than that of 2 to < 3 , 3 to < 4 , 5 to < 6 , and ≥ 6 years old. It may conclude that the hormonal regimen increases the pregnancy rate as well as decreases the undesired waiting of estrus and conception. Further study with more sample size will reveal the more effective treatment for cows at the coastal areas of Bangladesh.

Keywords: Bovine, Coastal area, hormonal, treatment.

INTRODUCTION

The Livestock sector is an integral part of the agricultural economy of Bangladesh. In Bangladesh, around 8% of total protein for human consumption comes from livestock. Bangladesh possesses a large cattle population, 25.7 million heads cattle (Banglapedia, 2015) out of which 11.49 million are dairy cattle comprised of indigenous, exotic and their crosses with indigenous ones. Huge numbers of people in Bangladesh live near the sea, called 'Coastal area'. Most of them are poor. Major problem of the heifers and cows (both zebu and crossbred) at coastal area is low pregnancy rate. There are many reasons behind this problem such as feed crisis, natural calamities, environmental conditions, management system 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 most of the time it is very difficult to overcome many of these causes in certain situation. To enhance the genetic potential of our na-

tive breeds, several indiscriminate attempts were undertaken in past few decades but no remarkable responses were observed. Most of the cattle in Bangladesh are non-descriptive types, which do not belong to any specific breed. They are smaller in size and their milk production capacity is much lower than that of exotic breeds.

Traditional farming systems in Bangladesh were maintained mainly keeping local cows (Kabir und Islam, 2009). The conception rate of cows is not satisfactory in Bangladesh (Kabir und Islam, 2009). Conception rate is directly associated with the production attribute and responsible for monitoring lifetime productivity of an individual animal. Conception is the first pre-requisite of an animal for entering into the productive life. The reproductive performance of cows with high genetic merit declines in many dairy industries. One of the major constrains of profitable dairy farming is low pregnancy rate in cows (Shamsuddin *et al.*, 2001). The productivity of cattle could be low because of poor nutrition (Alam *et al.*, 2006), and incorrect detection of estrus (Roelofs *et al.*, 2010; Macmillan, 2010; Paul *et al.*, 2011). It is indicated that

*Corresponding author's E-mail address: ashitpaul@pstu.ac.bd

still estrus detection errors cannot be overcome and about 40% cow remained undetected in Bangladesh (Shamsuddin *et al.*, 2001). In coastal area, poor fertility in cows is a major issue in cattle breeding. Perfect detection of estrus is crucial for good husbandry practice of cow. Hormonal interventions have been used to increase the probability of estrous detection and insemination, and to increase pregnancy rates of dairy cattle under a variety of management systems. The hormones used to control the estrous cycle mimic the reproductive hormones found within the normal cow.

Estrus synchronization is the alternative strategy to bypass the critical problem of estrus detection (Paul *et al.*, 2015). Most estrous synchronization systems employ a method for controlling follicular wave development, promoting ovulation in anestrus cows, regressing the corpus luteum in cyclic cows, and synchronizing estrus and (or) ovulation at the end of treatment. To overcome this problem, feasible synchronization/hormonal treatment must be adapted for the specific region of a country for the improvement of pregnancy rate. However, to our knowledge, there is no precise report on the use of estrus synchronization program in zebu and crossbred cows and heifers at coastal areas of Bangladesh. Therefore, the aim of the study was to find out the effective hormonal treatment and pregnancy rate for cows at the coastal region of Bangladesh.

MATERIALS AND METHODS

Animal selection and data collection

A total of 100 experimental animals including cows or heifers were selected randomly at the Barisal district of Bangladesh. During selection of animal the age, parity, breed and body condition as well as utero-ovarian features per rectal examination were considered. The demography of animals was presented in Table 1.

Age

The age of cows was determined by observation of teeth eruption (Banerjee, 1991). According to the age, animals were grouped 2 to <3, 3 to <4, 4 to <5, 5 to <6 and ≥ 6 years old.

Breed

According to the breed, animals were divided into two groups. These were non descriptive local (Desi cow) and crossbred.

Parity

It was determined by the recorded data of calving from farmers. The cows were divided into the following five groups. These were: parity 0 (not calved yet -heifer), parity 1 (one calved), parity 2 (twice calved), parity 3 (thrice calved) and parity ≥ 4 (four or more times calved).

Table 1: The demography of experimental animals

Categorical head	Variables	Number of animals
Age (year)	2 to <3	20
	3 to <4	20
	4 to <5	20
	5 to <6	20
	≥ 6	20
Breed	Local	50
	Crossbred	50
Parity	0	20
	1	20
	2	20
	3	20
	≥ 4	20
Treatment	A	20
	B	20
	C	20
	D	20
	E	20

Animal Management

All animals were dewormed orally using bolus containing triclabendazole 900mg and levamisole 600mg per 75-100

kg body weight (Renadex[®], The Reneta Animal Health Ltd., Dhaka, Bangladesh). The farmers were de-wormed at three months interval. Animals were grazing from early morning up to noon (mid day) and fed 4-5 kg green grasses mixed

with 2-3 kg straw daily as evening meal.

Experimental design

The experimental animals were treated with Ovurelin[®], an analogue of Gonadotropin Releasing Hormone (GnRH), (Ovurelin[®] 20ml Inj. Renata Limited, Dhaka, Bangladesh) and an analogue of PGF2 α (Ovuprost[®] 20ml Inj. Renata Limited, Dhaka, Bangladesh). Experimental animals were randomly divided into five different treatment protocols (Treatment A, B, C, D and E).

Treatment - A (Control group)

The animals were only treated with anthelmintic (one bolus per 75kg of Renadex[®], The Reneta Animal Health Ltd., Dhaka, Bangladesh). Cows were inseminated after observing natural estrus signs.

Treatment - B

Animals were treated orally with anthelmintic (one bolus per 75kg of Renadex[®], The Reneta Animal Health Ltd., Dhaka, Bangladesh), injected 10ml IM vitamin ADE (Renasole[®] AD3E, Renata Limited, Dhaka, Bangladesh) and administered orally 5 tea-spoonful multivitamin powder (Renavit[®] DB, Renata Limited, Dhaka, Bangladesh).

Treatment - C (PGF2 α)

2 ml of Ovuprost (Renata Limited, Dhaka, Bangladesh) was injected intramuscular (IM). The animals were inseminated after observing estrus signs.

Treatment - D (GnRH)

2.5 ml Ovurelin (Renata Limited, Dhaka, Bangladesh) was injected IM. The animals were inseminated after observing estrus signs.

Treatment - E (GnRH +PGF2 α)

At first 2.5 ml Ovurelin (Renata Limited, Dhaka,

Bangladesh) was injected IM followed by injection of 2 ml Ovuprost (Renata Limited, Dhaka, Bangladesh) after 7 days.

Estrous detection

Estrus detection of experimental animals was carried out twice daily by farmer 10-20 minutes in each time. The final confirmation of estrus was detected by research assistant through rectal palpation before insemination.

Pregnancy Diagnosis

The pregnancy was diagnosed by rectal palpation between 60-90 days of post insemination.

Statistical Analysis

The collected data from both control and treatment groups in respect to the return to estrus or pregnancy was organized in Microsoft Excel sheet. The collected data were coded, compiled, tabulated and analyzed in accordance with the objectives of the study by Statistical Programme for Social Science (SPSS[®]) software.

RESULTS

The objectives of present study were to observe the effects of hormonal stimulation on improvement of bovine pregnancy rate in selected coastal areas of Barisal. The fertility problem of heifers and cows in that area is remarkable.

Effect of treatment on estrous and pregnancy rate of cows

The estrous rate of cows in group A, B, C, D and E were 70, 65, 65, 60 and 80% respectively, whereas the pregnancy rate in group A, B, C, D and E were 35, 40, 50, 45 and 60% respectively (Figure 1).

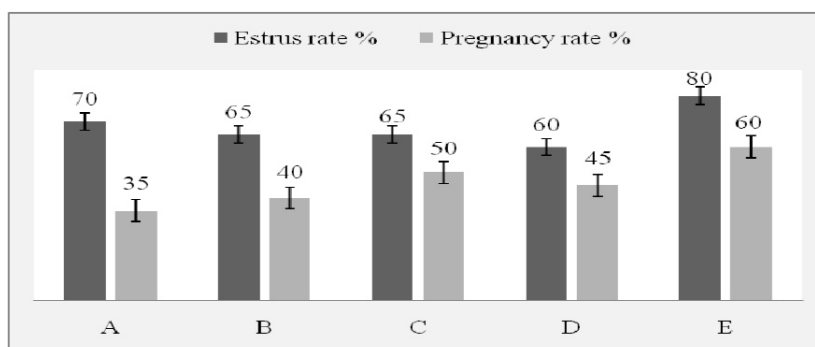


Figure 1: Estrous and pregnancy rates of cows in different groups

Table 2: Estrus and pregnancy rates of cattle of different treatment groups in relation to breed.

Breed	Parameters	A	B	C	D	E	Overall
Local	Estrus rate (%)	70 ^a	70 ^a	60 ^a	50 ^b	70 ^a	64 ^a
	Pregnancy rate (%)	40 ^a	40 ^a	40 ^a	40 ^a	40 ^a	40 ^a
Crossbred	Estrus rate (%)	70 ^a	60 ^a	70 ^a	70 ^a	90 ^b	70 ^a
	Pregnancy rate (%)	30 ^b	40 ^b	60 ^a	50 ^a	80 ^b	52 ^a

^{a-c} Means within a row with different superscripts differ ($P < 0.05$)

Table 3: Estrus and pregnancy rates of cattle of different treatment groups in relation to parity.

Parity	Parameters	A	B	C	D	E	Overall
Parity-0	Estrus rate (%)	75 ^a	75 ^a	75 ^a	75 ^a	75 ^a	75 ^a
	Pregnancy rate (%)	50 ^a	50 ^a	25 ^b	75 ^c	50 ^a	50 ^a
Parity-1	Estrus rate (%)	100 ^a	50 ^b	25 ^c	75 ^b	100 ^a	70 ^b
	Pregnancy rate (%)	25	25	25	50	75	40
Parity-2	Estrus rate (%)	100 ^a	75 ^a	100 ^a	50 ^b	100 ^a	85 ^a
	Pregnancy rate (%)	75 ^a	25 ^b	75 ^a	50 ^a	75 ^a	60 ^a
Parity-3	Estrus rate (%)	75 ^a	50 ^a	75 ^a	75 ^a	50 ^a	65 ^a
	Pregnancy rate (%)	50 ^a	25 ^b	75 ^a	25 ^b	50 ^a	45 ^a
Parity\geq4	Estrus rate (%)	50 ^a	75 ^a	100 ^a	25 ^b	75 ^a	65 ^a
	Pregnancy rate (%)	50 ^a	75 ^a	100 ^a	25 ^b	50 ^a	60 ^a

^{a-b} Means within a row with different superscripts differ ($P < 0.05$)

Effects of breed on estrus and pregnancy rate of experimental animals treated with different treatment protocol

The overall estrus rates of local and crossbred cows were 64 and 70% respectively, whereas the pregnancy rates were 40 and 52% respectively (Table 2). According to the study, estrus rate was the lowest (50%) significantly ($P < 0.05$) in local bred animals of group D and cross bred animals of group B. Although there was no significant ($P > 0.05$) difference in term of pregnancy rate among local bred animals of all groups, significant ($P < 0.05$) variation existed in pregnancy rate of crossbred animals of different groups. The highest pregnancy rate was observed in crossbred animals of group E.

Effects of parity on estrus and pregnancy rate of experimental animals treated with different treatment protocol

Table 3 presents the estrus and pregnancy rates of animals with parity-0, parity-1, parity- 2, parity 3 and parity \geq 4.

Overall estrus rate was the highest (80%) in cattle with parity-0, whereas the pregnancy rate (65%) was observed in cattle with parity-2 and parity- \geq 4. It was observed that the estrus rate was 75% in all heifers (parity-0) of group A, B, C, D and E, whereas estrus rate varied in cows of different treatment groups in relation to parity number (Table-3).

Effects of age on estrus and pregnancy rates of experimental animals treated with different treatment protocol

The overall estrus rates were 70, 55, 90, 65 and 45% and the pregnancy rates were 40, 40, 65, 45 and 45% in cows of 2 to <3, 3 to <4, 4 to <5, 5 to <6, and \geq 6 years old respectively. Results are shown in Table 4.

In case of cows of 2 to <3 years age, the lowest estrus rate (50%) was observed in treatment B and the lowest pregnancy rate (25%) was observed in cows of treatment groups A, B and C. The highest pregnancy rate (75%) was found in group D. In case of cows of 3 to <4 years age, the both estrus rate (100%) and pregnancy rates (75%) were the highest in treatment group E. Similar estrus rate (100%) and pregnancy

rate (75%) were observed in cows 4 to <5 years age in treatment groups A, B, C and E. The estrus rates of cows with 5 to <6 years of age were same (75%) in treatment A, C and D and E, whereas the highest pregnancy rate (75%) in treat-

ment group C. In case of cows of ≥ 6 years age, the estrus rate on treatment A, B, C, D and E were 50, 75, 50, 25 and 75% respectively, whereas the pregnancy rate (50%) was almost similar in groups (Table 4).

Table 4: Estrus and pregnancy rates of cattle of different treatment groups in relation to age.

Age	Parameters	A	B	C	D	E	Overall
2 to < 3	Estrus rate (%)	75 ^a	50 ^b	75 ^a	75 ^a	75 ^a	70 ^a
	Pregnancy rate (%)	25 ^b	25 ^b	25 ^b	75 ^c	50 ^a	40 ^a
3 to < 4	Estrus rate (%)	25 ^b	50 ^a	25 ^b	75 ^c	100 ^c	55 ^a
	Pregnancy rate (%)	25	25	25	50	75	40
4 to < 5	Estrus rate (%)	100 ^a	100 ^a	100 ^a	50 ^b	100 ^a	90 ^a
	Pregnancy rate (%)	75 ^a	50 ^b	75 ^a	50 ^b	75 ^a	65 ^a
5 to < 6	Estrus rate (%)	75 ^a	50 ^b	75 ^a	75 ^a	50 ^b	65 ^a
	Pregnancy rate (%)	50	25	75	25	50	45
≥ 6	Estrus rate (%)	50 ^a	75 ^b	50 ^a	25 ^c	75 ^b	45 ^a
	Pregnancy rate (%)	50 ^a	50 ^a	50 ^a	25 ^b	50 ^a	45 ^a

^{a-c}Means within a row with different superscripts differ ($P < 0.05$)

DISCUSSION

The hormonal treatment program with AI on standing heat for successful pregnancy was implemented to improve the pregnancy rate in zebu and crossbred heifers and cows in this study. The animals of this study were selected after physical observation and rectal palpation. The reproductive features of animal were in different stages. Therefore, the animals were grouped and treated according to their age, breed and parity.

Effect of treatment on estrous and pregnancy rate of cows

In the study, the experimental animals treated with both GnRH and PGF2 α (group E) showed higher estrus and pregnancy rate than that of other groups. Ghosh *et al.* (2012) found that 70% animals had showed estrus with a pregnancy rate of 28.3%. Thatcher *et al.* (1993) stated that the injection of GnRH closer to the onset of estrus may be beneficial in increasing conception rate in both zebu and crossbred heifers.

Effects of breed on estrus and pregnancy rate of experimental animals treated with different treatment protocol

The higher estrus and pregnancy rate was found in treatment group E in crossbred cows. The crossbred cows were more sensitive to hormonal treatment than that of local cows.

Our results conforms with the study of Bhuyian (1990), who reported 57% conception rate in local zebu crosses which were treated with two regimes of cloprostenol at an interval of 10 days. Ghosh *et al.* (2012) also reported that with respect to breed comparison, of the total of local and cross breed heifers, 83.3% and 75% heifers respectively showed estrus. However, these findings were dissimilar with Sarder (2001) who found better estrus and pregnancy rate in cross breed L \times F cows than local cows at Rajshahi district. Moreover, Khatun (2012) obtained higher pregnancy rate in local cows than that of Friesian (F) and Sahiwal (SL) cross cows in Kurigram district. The findings of this study revealed that use of GnRH and PGF2 α analogue improved the conception rate in crossbred cows.

Effects of parity on estrus and pregnancy rate of experimental animals treated with different treatment protocol

In the study, parity-2 showed higher estrus sign than that of other parities. However, the pregnancy rates were higher in parity-2 and parity ≥ 4 cows than that of parity-0, parity-1 and parity-3 cows. The findings of the study are partially agreed with Pryce *et al.* (2004).

Effects of age on estrus and pregnancy rate of experimental animals treated with different treatment protocol

The estrus rate was higher in case of 4 to <5 years old cows than that of other age groups in the study. The pregnancy rate was also found higher in case of 4 to <5 years old cows than that of 2 to <3, 3 to <4, 5 to <6, and ≥6 years old cows. The age related findings of this study did not agree with the report of Spalding *et al.* (1975). They reported a slight increase in the fertility of cow up to 3 to 4 years of age and a decline after 4 years of age, whereas in case of estrus and pregnancy rates in Protocol B have similarity with Spalding *et al.* (1975). No significant difference was obtained in pregnancy rate with respect to age groups of cows in Sirajganj district (Paul *et al.*, 2011). Contrasting to the present finding, variation in pregnancy rate was reported with respect to age of cows in Gaibandha district (Mollah, 2011). Analyzing data from Botswana, one researcher found that fertility rate increased from 69% in 2.5 years old cows to a maximum of 82% in 6 to 7 years old cows and then declined. They also found a marked decline in fertility in cows over 7 years of age and these findings somehow similar with protocol A and protocol C. According to De Kruif (1975), the age of the cow has a marked effect on the pregnancy rate after the first insemination.

It is concluded by nearly all investigators that the pregnancy rate is reduced in those animals which have calved for the first time. They also observed a difference of 5% in pregnancy rate between primiparous and secundiparous cows. The variation in pregnancy rates among studies might be due to variations in management and nutritional factors of cows, skill of AI technicians and agroecological conditions of study areas. In addition to those factors, lactational stress in young growing animals and the ability of older cows to gain body weight and body condition quickly after calving could be another reason. However, GnRH-PGF2 α protocol is more effective than that of other groups for improvement of bovine pregnancy rate in the study. The limitation of this study was the small number of animals per group in different category. For further study large sample size with standard management condition should be considered in designing the experiment.

ACKNOWLEDGEMENT

The authors express their deepest gratitude to University Grant Commission (UGC), Bangladesh for financial support (UGC grants 2016).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

References

- Alam MGS, Azam MS, Khan MJ, 2006. Supplementation with urea and molasses on body weight, milk yield and onset of ovarian cyclicity in cows. *Journal of Reproduction and Development* 52: 529–535.
- Banerjee GC, 1991. A textbook of animal husbandry. 7. Auflage. New Delhi : Oxford & IBH Publishing.
- Banglapedia, 2015. en.banglapedia.org>title=livestock.
- Bhuyian MR, 1990. Synchronization of oestrus in Zebu cattle with Estrumate[®]. MS Thesis, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, Bangladesh.
- De Kruif A, 1975. An investigation of the parameters which determine the fertility of a cattle population and of some factors which influence these parameters. *Tijdschr Diergeneesk* 100: 1089–1098.
- Ghosh T, Paul AK, Talukder AK, Alam MGS, Bari FY, 2012. Use of Two Estrus Synchronization Protocols and Their Success on Pregnancy in Zebu and Crossbred Heifers at Char Areas of Bangladesh. *Journal of Embryo Transfer* 27: 223–228.
- Kabir F, Islam MR, 2009. Comparative study on productive and reproductive performance of local and different cross bred dairy cows at Daulatpur, Khulna, Bangladesh. *Bangladesh Research and Publication Journal* 3: 909–914.
- Khatun MA, 2012. Post Artificial Insemination conception rate in cattle at Rajarhat, Kurigram. MS Thesis, Department of Surgery and Obstetrics. Bangladesh Agricultural University, Mymensingh, Bangladesh.
- Macmillan KL, 2010. Recent advances in the synchronization of estrus and ovulation in dairy cows. *Journal of Reproduction and Development* 56: 42–47.
- Mollah FK, 2011. Post AI conception rate in zebu cows at ShaghataUpazilla of Ghaibandha. MS Thesis, Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.
- Paul AK, Alam MGS, Shamsuddin M, 2011. Factors that limit first service pregnancy rate in cows at char management of Bangladesh. *Livestock Research for Rural Development* 23: 57.
- Paul AK, Ton Y, Bunaparte N, 2015. Hormonal treatment and estrus synchronization in cows: A mini-review. *Journal of Advance Veterinary and Animal Research* 2: 10–17.
- Pryce JE, Royal MD, Garnsworthy PC, Mao IL, 2004. Fertility in high-producing dairy cow. *Livestock Production Science* 86: 125–135.

- Roelofs J, Lopez-Gatius F, Hunter RHF, Van Eerdenburg FJCM, Hanzen CH, 2010. When is cow in estrus, Clinical and practical aspect? *Theriogenology* 74: 327–344.
- Sarder MJU, 2001. Reproductive and productive performance of indigenous cows. *Bangladesh Veterinarian* 18: 123–129.
- Shamsuddin M, Bhuiyan MMU, Sikder TK, Sugulle AH, Chanda, Alam MGS, Galloway D, 2001. Constraints limiting the efficiency of artificial insemination of cattle in Bangladesh. in radioimmunoassay and related techniques to improve artificial insemination programmes for cattle reared under tropical and subtropical conditions. Proceedings of a final research co-ordination meeting organized by the joint FAO/IAEA, Division of nuclear techniques in Food and Agriculture and held in Uppsala, Sweden.
- Spalding RW, Everett RW, Foote RH, 1975. Fertility in New York artificially inseminated Holstein herds in dairy herd improvement. *Journal of Dairy Science* 58: 718–723.
- Thatcher WW, Drost M, Savio JD, Macmillan KL, Entwistle KW, Schmitt EJ, Sota RL, De La, Morris GR, 1993. New clinical uses of GnRH and its analogues in cattle. *Theriogenology* 31: 149–164.