

## **Ultrasound guided diagnosis of anoestrus and its treatment in postpartum crossbred Holstein-Friesian cows**

**MU Ahmed, MM Rahman, J Bhattacharjee and MMU Bhuiyan\***

Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

### **Abstract**

This study was undertaken to determine the feasibility of using ultrasonography for diagnosis of postpartum anoestrus followed by its effective management and treatment in crossbred (Holstein-Friesian X zebu) cows. A total of 57 crossbred lactating cows with unobserved oestrus for  $\geq 60$  days postpartum was examined by ultrasonography at Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka. Cows were divided into silent oestrus and true anoestrus groups by ultrasonography. The pregnancy was diagnosed transrectally using ultrasonography 28 - 35 days after AI. Cows with silent oestrus were treated with Cloprostenol (500  $\mu\text{g}$ ) followed by AI or with 2 doses of Cloprostenol 10 days apart followed by AI at observed oestrus. The true anoestrus cows were supplied with anthelmintic and vitamin ADE injection with balanced nutrition, treated with Gonadorelin (500  $\mu\text{g}$ ) followed by AI or with Gonadorelin followed by injecting Cloprostenol at 7 days interval and AI at observed oestrus. All cows with silent oestrus and true anoestrus were divided into different body condition score (BCS), milk yield, age and parity groups to determine their influence on outcome of treatment. Out of 57 anoestrus cows examined, 56% were confirmed as silent oestrus and 44% were confirmed as true anoestrus. In silent oestrus, 71% cows showed cyclicity and 43% became pregnant after treatment with Cloprostenol and 78% cows showed cyclicity and 50% became pregnant after treatment with 2 doses of Cloprostenol 10 days interval ( $P > 0.05$ ). In true anoestrus group, 60% cows showed cyclicity and 40% became pregnant when balanced diet was supplemented. Around 78% cows showed cyclicity and 44% became pregnant after treatment with Gonadorelin and 91% cows showed cyclicity and 55% became pregnant when Gonadorelin was followed by Cloprostenol at 7 days interval ( $P > 0.05$ ). Significant ( $P < 0.05$ ) influence of parity was observed in post-treatment cyclicity in true anoestrus cows. (*Bangl. vet.* 2019. Vol. 36, No. 1 - 2, 33 - 41)

### **Introduction**

Postpartum anoestrus is a major cause of economic loss in dairy and beef industries resulting in increased inter-calving interval (Dziuk and Bellows, 1983). Lengthening the postpartum interval reduces the financial returns by reducing the number of pregnancies, and the cost of treatment. Due to poor nutrition and oestrus detection, prolonged postpartum anoestrus is common and pregnancy rate is low. Around 40%

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\*Corresponding author:- E-mail: mmubhuiyan@gmail.com; mmubhuiyan@bau.edu.bd

of postpartum cows were not detected in oestrus when they completed one or more ovarian cycles (Ghosh *et al.*, 1993; Shamsuddin *et al.*, 2001). Poor oestrus detection and prolonged calving interval are major problems for the dairy industry (Shamsuddin, 1995), and missing one oestrus extends calving interval by 21 days causing economic losses of \$11 (Shamsuddin *et al.*, 2006). In order to achieve an ideal calving interval of one year, a voluntary waiting period of 60 days is required followed by conception within 85 to 90 days after calving (Opsomer *et al.*, 1998).

The calving interval in cows is influenced by the resumption of postpartum ovarian activity (Shrestha *et al.*, 2004). Inactive ovaries are the most important cause of delayed resumption of ovarian cyclicity (Opsomer *et al.*, 1998; de Vries and Veerkamp, 2000). Ovarian cyclicity along with pregnancy and condition of uterus can easily be detected by ultrasonography. Application of ultrasound for management of reproduction in cows is limited in Bangladesh (Rahman, 2010; Islam *et al.*, 2013; Kamal *et al.*, 2014), but it can improve reproductive management (Ginther, 2014). The present study was undertaken to determine the feasibility of using ultrasonography for diagnosis of anoestrus and to determine the effective treatments in crossbred Holstein-Friesian cows.

## Materials and Methods

The investigation was conducted at the Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka from January to May 2018.

### *Selection and management of cows*

A total of 57 crossbred (Holstein-Friesian X zebu) lactating cows with unobserved oestrus for  $\geq 60$  days postpartum were selected. Information on farm management, general and reproductive health were collected from register. Age of animals was 4 to 14 years with one to nine parities. Routine deworming against roundworms and liver fluke was done by oral administration of anthelmintics 6 monthly at the rate of 1 bolus per 75 Kg body weight (Levamisole 600 mg + Triclabendazole 900 mg; Bolus Renadex®, Renata Ltd., Dhaka, Bangladesh). The cows were vaccinated routinely against foot and mouth disease {Trivalent FMD Vaccine, 6 ml, s/c, 6 monthly, Livestock Research Institute (LRI), Mohakhali, Dhaka Bangladesh}, anthrax (Anthrax Vaccine, 1 ml, s/c, yearly, LRI, Mohakhali, Dhaka) and haemorrhagic septicaemia (HS vaccine, 2 ml, s/c, 6 monthly, LRI, Mohakhali, Dhaka). Cows were housed 24 h in sheds with natural ventilation. Animals were fed concentrate, green grass and straw twice a day. Cows were milked by hand or milking machine twice daily at an interval of 8-10 h.

### *Collection of data*

Daily milk yield per cow was taken from the register. Body condition score (BCS) of cows using 1 - 5 scales (0.5 fraction between 2 scores) was measured as described by Nicholson and Butterworth (1986). The cows with  $<2.5$  BCS were not selected.

### ***Examination of reproductive organs by ultrasonography***

The ovaries were examined transrectally using transducer (Easi-Scan Linear, 4.5 to 8.5 MHz frequency probe, BCF Technology, UK) placed alongside the operator's arm after evacuating faeces. The transducer was lubricated with a coupling medium (Ultrasonic Gel® for Medical use) and covered by a lubricated plastic sleeve. Cows were divided into two groups (silent oestrus and true anoestrus). Cows with corpus luteum (CL) with normal uterus were regarded as silent oestrus. Cows without CL and follicles, smooth relatively small ovaries and normal uterus when examined 10 days apart were considered as having true anoestrus.

### ***Treatment protocol and artificial insemination (AI)***

AI was done at observed oestrus by trained technician using frozen semen. Pregnancy was confirmed by ultrasonography (Easi-Scan Linear) 28 to 35 days after AI. Fourteen cows with silent oestrus were treated with PGF<sub>2</sub>α analogue (Cloprostenol sodium 500µg; Ovuprost®, Bayer New Zealand Ltd., Auckland, New Zealand) injection (i/m) followed by AI at observed oestrus. Other 18 cows were treated with two injections of PGF<sub>2</sub>α analogue at an interval of 10 days followed by AI at observed oestrus. Of the 25 true anoestrous cows, five were treated with anthelmintic (Bolus Renadex®, Renata Ltd., Dhaka, Bangladesh) and vitamin ADE injection (10 ml i/m, weekly for 4 weeks, Injection Renasol® AD<sub>3</sub>E, Vitamin A 500000 IU, D<sub>3</sub> 75000 IU and E 50 mg per ml, Renata Ltd., Dhaka, Bangladesh) and fed concentrate supplement [Maize (50%), Wheat bran (30%), Oil cake (10%), Rice polish (8%) and Vitamin-Mineral premixes (2%)]; nine cows were treated with GnRH analogue i/m (Gonadorelin 500 µg, Ovurelin®, Bayer New Zealand Ltd.) followed by AI at observed oestrus; and 11 cows were treated with GnRH analogue followed by PGF<sub>2</sub>α analogue i/m at 7 days interval and AI was done at observed oestrus.

Moreover, all cows with silent oestrus and true anoestrus were grouped into 2.5, 3.0 and 3.5 BCS, 6-8, 9-11 and 12-19 litres milk yield daily, 4-6, 7-9, 10-14 years of age, and 1-3, 4-6 and 7-9 parities for determining their influence on treatment response.

### ***Statistical analysis***

The data were entered in Microsoft Excel 2007 and descriptive statistics were performed. The data were analysed by paired *t test* using MINITAB version 13 statistical programme. The difference between values was considered significant when the P value was less than 0.05.

## **Results and Discussion**

Out of 57 anoestrus cows examined by ultrasonography at 60 or more days postpartum, 32 (56%) were confirmed as silent or unobserved oestrus and 25 (44%) as true anoestrus. More than half of the anoestrus cows missed one or more cycles. Pregnancy was confirmed using ultrasonography at 28 to 35 days, earlier than rectal examination at 60 to 90 days. Earlier identification of non-pregnancy allows earlier

re-insemination. This indicates the feasibility of using ultrasonography in cows under field conditions in Bangladesh. Application of ultrasound as a tool to improve reproductive management of cattle is documented (Ginther, 2014). Application of ultrasound for management of reproductive problems and pregnancy diagnosis in cows has been reported in Bangladesh (Rahman, 2010; Islam *et al.*, 2013; Kamal *et al.*, 2014).

Treatment regime of anoestrus cows is presented in Table 1. Cows with silent oestrus showed cyclicity (71%) and became pregnant (43%) when treated with PGF<sub>2</sub>α followed by AI at observed oestrus. Cows showed cyclicity (78%) and became pregnant (50%) when treated with two injections of PGF<sub>2</sub>α at an interval of 10 days followed by AI. True anoestrus cows showed cyclicity (60%) and became pregnant (40%) when supplemented with concentrates. When GnRH was administered, 78% cows showed cyclicity and 44% became pregnant after AI at observed oestrus. Around 91% cows showed cyclicity and 55% became pregnant after GnRH administration followed by PGF<sub>2</sub>α at 7 days interval and AI at observed oestrus. Cows not detected in oestrus, but with CL were treated with PGF<sub>2</sub>α. Smith *et al.* (1998) reported that treatment of anoestrus cows with detectable CL with PGF<sub>2</sub>α resulted in oestrus in 55% of animals within six days of treatment. When the CL was detected following rectal palpation, oestrous response rate within six days of treatment was 52% (Whittier *et al.*, 1989). Further, treatment of true anoestrus can be done by Ovsynch protocol, GnRH followed by PGF<sub>2</sub>α at 7 days interval and AI at observed oestrus with another injection of GnRH (Stevenson *et al.*, 1996; Peters *et al.*, 1999). Similar cyclicity was reported by Kamal *et al.* (2012) in true anoestrus cows when Ovsynch protocol were used, but lower pregnancy rate was reported.

Table 1: Effects of treatment of anoestrus crossbred cows on induction of cyclicity and pregnancy

| Type of anoestrus | Treatments   | Number of cows treated | Response to treatment            |                                 |
|-------------------|--|------------------------|----------------------------------|---------------------------------|
|                   |  |                        | No. (%) of cows showed cyclicity | No. (%) of cows became pregnant |
| Silent oestrus    | PGF <sub>2</sub> α + AI at observed oestrus                                    | 14                     | 10 (71)                          | 6 (43)                          |
|                   | PGF <sub>2</sub> α + after 10 days PGF <sub>2</sub> α + AI at observed oestrus | 18                     | 14 (78)                          | 9 (50)                          |
|                   | Total  | 32                     | 24 (75)                          | 15 (47)                         |
| True anoestrus    | Deworming +Nutrition+ADE   | 5                      | 3 (60)                           | 2 (40)                          |
|                   | GnRH + AI at observed oestrus  | 9                      | 7 (78)                           | 4 (44)                          |
|                   | GnRH + after 7 days PGF <sub>2</sub> α + AI at observed oestrus                | 11                     | 10 (91)                          | 6 (55)                          |
|                   | Total  | 25                     | 20 (80)                          | 12 (48)                         |

Percentage values within same column did not vary significantly between each other (P>0.05).

Influence of BCS of treated anoestrus crossbred cows on induction of cyclicity and pregnancy is presented in Table 2: 50 to 100% cows showed cyclicity and 25 to 67% became pregnant ( $P>0.05$ ). The nutrition has tremendous impact on reproduction (Bindari *et al.*, 2013). Malnutrition results in the loss of body weight and body condition, interfering with ovarian cyclicity by decreasing gonadotrophin secretion followed by prolonged post-partum anoestrus (Boland *et al.*, 2001). Chronic nutritional deficiencies may lead to subfertility by interrupting hypothalamo-pituitary-ovarian axis function in dairy cows (Dobson and Alam, 1987). Affected cows not only delay ovarian cyclicity, but have poor oestrus signs resulting in error in oestrus detection and breeding time, and reduced pregnancy rate (Gumen *et al.*, 2003). As cows with less than 2.5 BCS were excluded, all treated cows had good cyclicity and pregnancy rates.

Table 2: Influence of BCS of treated anoestrus crossbred cows on induction of cyclicity and pregnancy

| Type of anoestrus | BCS | Number of cows treated | Response to treatment            |                                 |
|-------------------|-----|------------------------|----------------------------------|---------------------------------|
|                   |     |                        | No. (%) of cows showed cyclicity | No. (%) of cows became pregnant |
| Silent oestrus    | 2.5 | 9                      | 7 (78)                           | 6 (67)                          |
|                   | 3.0 | 20                     | 14 (70)                          | 7 (35)                          |
|                   | 3.5 | 3                      | 3 (100)                          | 2 (67)                          |
| True anoestrus    | 2.5 | 8                      | 4 (50)                           | 2 (25)                          |
|                   | 3.0 | 13                     | 11 (85)                          | 8 (62)                          |
|                   | 3.5 | 4                      | 4 (100)                          | 2 (50)                          |

Percentage values within same column did not vary significantly between each other ( $P>0.05$ ).

Influence of milk yield of treated anoestrus crossbred cows on induction of cyclicity and pregnancy is presented in Table 3: 56 to 100% cows showed cyclicity and 29 to 71% became pregnant ( $P>0.05$ ). Higher milk yield may delay onset of oestrous cycles if associated with exacerbated negative energy balance. However, milk yield is closely associated with dry matter intake (Liefers *et al.*, 2003), and energy intakes accounted for most of the variation in energy balance in postpartum cows (Villa-Godoy *et al.*, 1988). Milk yield had no influence on the treatment response in crossbred anoestrus cows. This may be because cows selected were not suffering from negative energy balance during lactation (2.5 to 3.5 BCS).

Influence of age of treated anoestrus crossbred cows on induction of cyclicity and pregnancy is presented in Table 4: 55 to 100% cows showed cyclicity and 36 to 67% became pregnant ( $P>0.05$ ). Influence of age on onset of postpartum cyclicity in bovine has been reported by Williams and Griffith (1995). It is likely that age of animals has little influence on reproductive performance if the animal does not suffer from

malnutrition or negative energy balance, or stress due to suckling and environment. Nutrition supplementation and restricted suckling were followed.

Table 3: Influence of milk yield of treated anoestrus crossbred cows on induction of cyclicity and pregnancy

| Type of anoestrus | Milk yield (L) | Number of cows treated | Response to treatment            |                                 |
|-------------------|----------------|------------------------|----------------------------------|---------------------------------|
|                   |                |                        | No. (%) of cows showed cyclicity | No. (%) of cows became pregnant |
| Silent oestrus    | 6 - 8          | 8                      | 6 (75)                           | 5 (63)                          |
|                   | 9 - 11         | 17                     | 12 (71)                          | 5 (29)                          |
|                   | 12 - 14        | 7                      | 6 (86)                           | 5 (71)                          |
| True anoestrus    | 6 - 8          | 9                      | 5 (56)                           | 4 (44)                          |
|                   | 9 - 11         | 13                     | 11 (85)                          | 7 (54)                          |
|                   | 12 - 14        | 3                      | 3 (100)                          | 1 (33)                          |

Percentage values within same column did not vary significantly between each other ( $P>0.05$ ).

Table 4: Influence of age of treated anoestrus crossbred cows on induction of cyclicity and pregnancy

| Type of anoestrus | Age (Years) | Number of cows treated | Response to treatment            |                                 |
|-------------------|-------------|------------------------|----------------------------------|---------------------------------|
|                   |             |                        | No. (%) of cows showed cyclicity | No. (%) of cows became pregnant |
| Silent oestrus    | 4 - 6       | 8                      | 6 (75)                           | 4 (50)                          |
|                   | 7 - 9       | 16                     | 12 (75)                          | 7 (44)                          |
|                   | 10 - 14     | 8                      | 6 (75)                           | 4 (50)                          |
| True anoestrus    | 4 - 6       | 3                      | 3 (100)                          | 2 (67)                          |
|                   | 7 - 9       | 11                     | 10 (91)                          | 6 (55)                          |
|                   | 10 - 14     | 11                     | 6 (55)                           | 4 (36)                          |

Percentage values within same column did not vary significantly between each other ( $P>0.05$ ).

Influence of parity of treated anoestrus crossbred cows on induction of cyclicity and pregnancy is presented in Table 5: 60 to 100% cows showed cyclicity and 20 to 50% became pregnant. The proportion of true anoestrus cows cycling varied significantly ( $P<0.05$ ) between 1 - 3 and 4 - 9 parity groups. The result in parity 1 - 3 is not consistent with earlier reports, because primiparous cows had greater concentrations of blood non-esterified fatty acids (NEFA) than multiparous cows one week before parturition (Meikle *et al.*, 2004) and early postpartum cows (Meikle *et al.*, 2004; Wathes *et al.*, 2007). Elevated NEFA and ketone bodies before parturition are related to reduced periparturient immunological function and uterine disease (Hammon *et al.*, 2006), which are known to influence postpartum ovulation (Sheldon *et al.* 2002).

Primiparous cows typically have more early postpartum uterine problems (Goshen and Shpigel, 2006), which can delay resumption of ovulation. Cows diagnosed with uterine infection have dominant ovarian follicles that grew at a slower rate than those without uterine problems (Sheldon *et al.*, 2002). It is possible that primiparous cows might be more sensitive to metabolic and endocrine signals during the periparturient period such as those influenced by the nutrient balance and by alterations in uterine health, thereby delaying the resumption of postpartum ovulation. It is noteworthy to mention that most of the cows were with parity 2 - 3 and only two true anoestrus cows with 1<sup>st</sup> parity were included in 1 - 3 parity group which might influence the present results.

Table 5: Influence of parity of treated anoestrus crossbred cows on induction of cyclicity and pregnancy

| Type of anoestrus | Parity | Number of cows treated | Response to treatment            |                                 |
|-------------------|--------|------------------------|----------------------------------|---------------------------------|
|                   |        |                        | No. (%) of cows showed cyclicity | No. (%) of cows became pregnant |
| Silent oestrus    | 1 - 3  | 18                     | 14 (78)                          | 8 (44)                          |
|                   | 4 - 6  | 12                     | 8 (67)                           | 6 (50)                          |
|                   | 7 - 9  | 2                      | 2 (100)                          | 1 (50)                          |
| True anoestrus    | 1 - 3  | 10                     | 10 (100) <sup>a</sup>            | 6 (50)                          |
|                   | 4 - 6  | 10                     | 6 (60) <sup>b</sup>              | 5 (50)                          |
|                   | 7 - 9  | 5                      | 3 (60) <sup>b</sup>              | 1 (20)                          |

<sup>a,b</sup> Percentage values with superscripts within same column varied significantly between each other (P<0.05).

## Conclusions

Use of ultrasonography is feasible to diagnose anoestrus and pregnancy in cows. The treatment protocol initiated cyclicity in >60% cows with >40% pregnancy in anoestrus crossbred Holstein-Friesian cows. Significant influence of parity was observed in post-treatment cyclicity in true anoestrus cows. This study will help the practising veterinarians to deal with the anoestrous cows efficiently. Further investigation with a greater number of animals is needed to draw more definite conclusions.

## Acknowledgements

The authors acknowledge Bangladesh Agricultural University Research System (BAURES) for funding through the research project # 2017/240/BAU. Department of Livestock Services (DLS), People's Republic of Bangladesh is also acknowledged for allowing us to conduct the study at the Central Cattle Breeding and Dairy Farm, Savar, Dhaka.

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