

EFFECTS OF GnRH ANALOGUE AND VITAMIN AD₃E ON INDUCTION OF CYCLICITY IN ANOESTRUS HEIFERS

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ABSTRACT

The purposes of this investigation were to determine the effects of different drugs such as GnRH analogue, vitamin AD₃E and combination of vitamins and minerals on induction of cyclicity followed by conception in crossbred anoestrus heifers. The effects of body condition score (BCS) and age at anoestrus heifers during treatment on induction of cyclicity and conception were also determined. Sixty crossbred anoestrus heifers having > 2.5 BCS at >24 months age at Potiya Upazilla of Chittagong district were used. The cyclicity of the cows was confirmed by using real time ultrasonography on the basis of presence or absence of corpus luteum. The anoestrus heifers were allotted for 1 to 4 different treatment groups with GnRH analogue, vitamin AD₃E and vitamin mineral solution including one no-treatment control group. AI was done in observed oestrus heifers and the conception was determined transrectally by using B-mode real time ultrasonography between 28-35 days post-AI. Heifers showed oestrus at higher (46.67%) proportion in treatment groups than that in no-treatment counterpart (6.67%) (P<0.05). The conception rate was 35.56% in treatment received heifers. The GnRH treated heifers showed the highest (60.00%) proportion of cyclicity and conception rate (46.67%) whereas vitamin mineral solution treated heifers showed the lowest cyclicity (33.33%) and conception rate (26.67%) (P<0.05). Heifers with BCS 3.5 had the highest (57.14%) rate of oestrus induction and (57.14%) rate of conception (P<0.05) whereas heifers with BCS 2.5 had the lowest (41.67%) rate of oestrus induction and (20.83%) rate of conception (P>0.05). Heifer showed highest rate (50.00%) of cyclicity and (38.89%) rate of conception when their age ranges from 36 to 42 months where as heifer showed lowest (37.50%) rate cyclicity and (31.58%) rate conception when their age ranges from 24 to 29 months (P>0.05). In conclusion, treatment of crossbred anoestrus heifers with drugs has positive effect on induction of cyclicity followed by conception. GnRH may be the drug of choice among the drugs tested in this study. The BCS of heifers during treatment influences the treatment outcome with respect to induction of cyclicity followed by conception.

Keywords: Heifer, GnRH analogue, vitamin AD₃E, ultrasonography

INTRODUCTION

Reproductive efficiency is a major determinant of profitability in both dairy and beef production and is one of the most costly problems facing the dairy industry today. Anoestrus is one of the major causes of economic losses in both the dairy and beef industries (Dziuk and Bellows, 1983). The goal of one calf annually per cow is not often achieved because too many heifers and cows do not become pregnant in time (Bellows *et al.*, 1979). A major reason for this is inadequate nutrient intake and the resultant anoestrus. Indeed, reduced energy intake increases the interval from calving to conception (Wiltbank *et al.*, 1964; Dunn *et al.*, 1969; Spitzer *et al.*, 1995) and delays puberty (Kinder *et al.*, 1994; Yelich *et al.*, 1995). Anoestrus is a period of sexual quietude in which the animal fails to exhibit normal oestrus cycles and no manifestation of heat (Boyd, 1977). There are four different clinical forms of anoestrus, namely: (1) silent heat; (2) ovarian afuction (hypofunction); (3) cystic ovarian disease; (4) corpus luteum pseudograviditatis (Arthur, 1973; Ax *et al.*, 1984). Extensive efforts, worldwide, have been put into research to limit the occurrence of anoestrus. However, despite all these efforts, infertility is still a significant problem in dairy herds. Nonetheless, few advances have been made in reducing the condition through proper nutrition, hormonal therapy and application of other non hormonal remedies including physical massage of the reproductive system (Jeong *et al.*, 1996). Efficient and profitable reproductive performance of a dairy herd requires routine but conscientious heat detection and proper timing of artificial insemination. Failure to detect estrus (heat) is a major factor contributing to low fertility. The purposes of this investigation were to determine the effects of administration of GnRH analogue, vitamin AD₃E and vitamin

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mineral solution on induction of cyclicity and conception in anoestrus crossbred heifers. This study also determines the effects of body condition score and age of animals during treatment on induction of cyclicity and conception in anoestrus crossbred heifers.

MATERIALS AND METHODS

The investigation was conducted in the selected dairy farms at Potiya Upazilla of Chittagong district. The farms were the registered farms of the Community based Dairy Veterinary Foundation (CDVF) project, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh. The study period was from July to November 2010.

Description of animals and their management

For this study, 60 crossbred (Friesian X non-descript indigenous zebu) anoestrus heifers were selected from 26 dairy farm families. All heifers were above 24 months old. The heifers were vaccinated routinely against foot and mouth disease, anthrax and hemorrhagic septicemia, and routine deworming against round worms and liver flukes was in practice. The heifers were housed almost 24 hours in their rearing sheds having the facilities of natural ventilation and were fed concentrate, green grasses and straw. Total feeds and forages were given in two splits per day.

Determination of body condition score and age of animals

The nutritional status were determined by scoring the body conditions of the heifers using 1-5 scales (0.5 fraction between 2 scores) on the basis of bony prominence and deposition of subcutaneous fat. The heifers with < 2.5 body condition score (BCS) were not selected for this study. The ages of heifers were determined by interviewing the farmers and by dental examination.

Examination of genital organs by ultrasonography

All selected heifers were examined by transrectal ultrasound using B-mode real time ultrasonography for determination of ovarian cyclicity on the basis of scanning views on the monitor. Tringa Linear (Esaote, Piemedical, USA) ultrasound scanner was used for ultrasonography. After adequate restraining, the scanner was placed at a sensible distance from the heifers on the side opposite to the operator's arm engaged in rectal palpation. All fecal materials from the rectum were evacuated prior to introduction of the transducer. The transducer face was lubricated with a suitable coupling medium (Ultrasonic Gel® for Medical use-250 mg) and was usually covered by a lubricated plastic sleeve before insertion. The transducer was then progressed cranially along the rectal floor to overlie the reproductive tract. The ultrasound screen and the human eye were at similar level for accurate interpretation of ultrasound images.

Heifers having corpus luteum (CL) on either of the ovaries and with normal uterus were regarded as heifers with undetected oestrus. Heifers having small follicle (Fig. 1) and no CL on either of the ovaries and with normal uterus were examined transrectally by using B-mode real time ultrasonography twice at 10 days interval. Only heifers having small follicle (Fig. 1) and no CL on ovaries at twice examinations at 10 days interval were regarded as heifers with true anoestrus and were selected for induction of cyclicity.

Treatment protocol

The selected heifers with true anoestrus were divided into 4 groups containing 15 animals in each group. Heifers in group 1 were treated with single intramuscular (IM) injection of 500 µg GnRH analogue (Each ml contains 100µg synthetic gonadorelin, Fertilon, Techno Drugs Bangladesh Ltd., Dhaka). Heifers in group 2 were treated with 10 ml IM injection of vitamin AD₃E (Renasol, each ml contains, vita A: 3lac, vita D: 10000 IU, vita E: 50mg, Reneta Limited, Bangladesh, Dhaka) three times at four days interval. Heifers in group 3 were treated with 15 ml oral administration of vitamin mineral solution (Capsiton, each 10 ml contain vita A: 12 lac IU, vita D₃: 60000 IU, vita E: 10mg, Zinc: 180mg, Cobalt: 1mg and Selenium: 1mg, Navana Pharmaceutical Ltd., Dhaka) daily for 20 days. Heifers in group 4 were used as control which received no treatment interventions. All animals were under close observation until 30 days post treatment for the detection of occurrence of cyclicity.

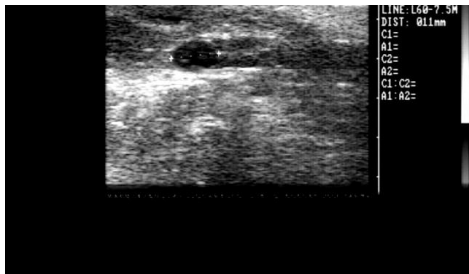


Fig. 1. Ultrasonographic image of an ovarian follicle



Fig. 2. Ultrasonographic image of a 30 days old fetus

Artificial insemination

All heifers were inseminated by skilled AI technicians using frozen semen at observed oestrus. Oestrus was detected by the farmers on the basis of clinical manifestation of oestrus signs.

Pregnancy Diagnosis

The heifers were examined by transrectal ultrasound using B-mode real time ultrasonography for pregnancy diagnosis between 28-35 days after AI and results were recorded (Fig. 2).

Statistical analysis

The data collected on heifers' information and response to treatment interventions with respect to occurrence of cyclicity and conception were entered in Microsoft Excel 2003 and descriptive statistics were performed. The data collected were coded, scored, compiled, tabulated and analyzed in accordance with the objective of the study by LSD using SPSS® software. The difference between values was considered significant when the P value was less than 0.05.

RESULTS AND DISCUSSION

Effects of treatment of anoestrus heifers on occurrence of cyclicity and conception

The effects of treatment of anoestrus heifers on occurrence of cyclicity and conception are presented in Table 1. When treatment was given for induction of cyclicity, higher (46.67%) proportion of heifers showed oestrus within 30 days post treatment than that of no-treatment counterpart (6.67%). The difference in occurrence of cyclicity in heifers between treatment and no-treatment groups was statistically significant ($P < 0.05$). When conception was determined by ultrasonography, 35.56% heifers conceived after receiving treatment compared to 0.0% conception in heifers received no treatment. .

Lack of ovarian activity or true anoestrus is considered as one of the major problems to efficient cattle reproduction. This is still one of the most prevalent reproductive disorders in dairy cows despite significant technological advances made in animal husbandry (McLeod and Williams, 1991). Heifers with true anoestrus may have complete ovarian inactivity with virtually no functional structures on the surface of both ovaries (Kalis and Van De Wiel, 1980). It may occur, among others, due to hormonal imbalance, nutritional deficiency and disease conditions. The nutritional deprivation lowers pulse frequency of GnRH by initiating hypothalamic inactivity rather than by altering pituitary response to hypothalamic secretions (Richards *et al.*, 1989).

Effects of different drugs used for treatment of anoestrus heifers on occurrence of cyclicity and conception

Effects of different drugs used for treatment of anoestrus heifers on occurrence of cyclicity and conception are presented in table 2. When compared, the highest (60.00%) proportion of heifers showed cyclicity after receiving treatment with GnRH and the lowest (33.33%) proportion of heifers showed cyclicity after receiving treatment with vitamin mineral solution. The difference in occurrence of cyclicity between treatment groups was significant ($P < 0.05$). Similarly, the highest (46.67%) proportion of heifers conceived after receiving treatment with GnRH and the lowest (26.67%) proportion of heifers conceived after receiving treatment with vitamin mineral solution. The difference in conception rate between treatment groups was significant ($P < 0.05$).

Table 1. Effects of treatment of anoestrus heifers on occurrence of cyclicity and conception

| Treatment Type | Number of heifers treated | Number of heifers responded | Proportion of heifers responded (%) | Number of heifers conceived | Proportion of heifers conceived (%) |
|-----------------------|---------------------------|-----------------------------|-------------------------------------|-----------------------------|-------------------------------------|
| Received treatment | 45 | 21 | 46.67 ^a | 16 | 35.56 ^a |
| Received no-treatment | 15 | 1 | 6.67 ^b | 0 | 0.0 ^b |

^{a, b} The values with superscript within the same column differed significantly from each other (P<0.05).

Table 2. Effects of different drugs used for treatment of anoestrus heifers on occurrence of cyclicity and conception

| Drugs used for treatment | Number of heifers treated | Number of heifers responded | Proportion of heifers responded (%) | Number of heifers conceived | Proportion of heifers conceived (%) |
|--------------------------|---------------------------|-----------------------------|-------------------------------------|-----------------------------|-------------------------------------|
| GnRH inj | 15 | 9 | 60.00 ^a | 7 | 46.67 ^a |
| AD ₃ E inj | 15 | 7 | 46.67 ^{ab} | 5 | 33.33 ^{ab} |
| Vitamin mineral solution | 15 | 5 | 33.33 ^b | 4 | 26.67 ^b |

^{a, b} The values with superscript within the same column differed significantly from each other (P<0.05)

In the present study, GnRH analogue induced oestrus in higher proportion of heifers than that of vitamin AD₃E and vitamin mineral solution. Heifers with earlier episodic peaks of LH ovulate sooner than other heifers (Stevenson and Britt, 1979). In abnormal heifers, the LH peak could be delayed further. The GnRH evokes LH release which increases response to come into estrus (Kesler *et al.*, 1977; Zaied *et al.*, 1980; Rick, 1982; Azzazi *et al.*, 1983; Richardson *et al.*, 1983). Moreover, treatment with GnRH may assist to first ovulation in heifers (Britt *et al.*, 1974; Boestedt *et al.*, 1980; Zaied *et al.*, 1980; Boestedt and Maurer, 1982; Rick, 1982).

Administration of vitamin AD₃E or vitamin mineral solution also induced cyclicity and conception in higher proportion of anoestrus heifers compared to no treatment group. This means, other than hormone, vitamin AD₃E or vitamin mineral solution can be used to induce cyclicity and conception in some proportion of anoestrus heifers. Administration of AD₃E or vitamin mineral solution to heifers is preferable to GnRH analogue for induction of cyclicity in heifers. Because, administration of vitamin AD₃E or vitamin mineral solution to heifers is more natural approach than administration of GnRH analogue.

Saxena *et al.* (1991) found a positive correlation with minerals concentration in serum and age at puberty. However, the mechanism by which nutrition influences reproduction in bovine has not been fully established. But the amount and quality of digestible nutrient intake is very important in the prepartum and postpartum periods and most crucial in the last trimester of gestation (Rutter and Manes, 1987). The positive effect of vitamin AD₃E and vitamin mineral solution in the present study may be explained by the fact that calcium and phosphorus metabolism are interrelated and complex. Controlling factors for calcium and phosphorus metabolism include vitamin D, parathyroid hormone, thyrocalcitonin, and the dietary levels of calcium and phosphorus. The absorption of calcium is regulated to a large extent by calcium intake. The extent of dietary phosphorus that is absorbed depends not only on the source of phosphorus, but also vitamin D levels, and the level of other minerals such as aluminum, manganese, and potassium in the diet (Challa *et al.*, 1989; Martz *et al.*, 1990). Developing heifers on a high plane of nutrition (both energy and protein) from weaning to breeding results in earlier puberty (Wiltbank *et al.*, 1969), improved udder development (Bond and Wiltbank, 1970) and increased conception percentage compared to a low plane counterpart. This difference in conception is probably at least partially due to differences in pituitary function of heifers fed a low-energy versus a high-energy diet (Day *et al.*, 1986).

Effects of BCS of treatment received anoestrus heifers on occurrence of cyclicity and conception

The effects of BCS of treatment received anoestrus heifers on occurrence of cyclicity and conception are presented in the Table 3. The highest (57.14%) proportion of heifers showed cyclicity after receiving treatment when their BCS was 3.5 and the lowest (41.67%) proportion of heifers showed cyclicity after receiving treatment when their BCS was 2.5. However, the difference in occurrence of cyclicity in heifers between BCS groups was not significant ($P>0.05$). Similarly, the highest (57.14%) proportion of heifers conceived after receiving treatment when their BCS was 3.5 and the lowest (20.83%) proportion of heifers conceived after receiving treatment when their BCS was 2.5. The difference in conception rate in heifers between BCS groups was significant ($P<0.05$).

Table 3. Effects of body condition score (BCS) of treatment received anoestrus heifers on occurrence of cyclicity and conception

| Body condition score | Number of heifers treated | Number of heifers responded | Proportion of heifers responded (%) | Number of heifers conceived | Proportion of heifers conceived (%) |
|----------------------|---------------------------|-----------------------------|-------------------------------------|-----------------------------|-------------------------------------|
| 2.5 | 24 | 10 | 41.67 | 5 | 20.83 ^a |
| 3.0 | 14 | 7 | 50.00 | 7 | 50.00 ^b |
| 3.5 | 7 | 4 | 57.14 | 4 | 57.14 ^b |

^{a, b} The values with superscript within the same column differed significantly from each other ($P<0.05$).

The BCS is widely used for the evaluation of nutritional status of animals which influences the fertility indexes. Accordingly, the present study determined the effects of BCS of animals during treatment on induction of cyclicity and conception in anoestrus crossbred heifers. The present study indicates that the BCS of heifers during treatment influenced the conception rate in crossbred anoestrus heifers. Heifers with less than 2.5 BCS was not included in this study and no heifer was found to have more than 3.5 BCS. Heifers with BCS 3.5 during treatment conceived at a higher rate than that of heifers with BCS 2.5. This may be explained by the fact that the BCS and the onset of cyclicity in heifer are correlated. Meeting but not grossly exceeding the target weight is important for heifer's fertility and production. The puberty in the beef heifer reaches when she is able to express estrous behavior, ovulate a fertile oocyte and obtain normal luteal function (Moran *et al.*, 1989). The BCS in the same group of 1,863 heifers showed the same result with improving first-service conception rates as body condition increased up to a score of 6 (1-9 scales) and then declining in fat heifers (Stewart *et al.*, 1985).

Effects of age of treatment received anoestrus heifers on occurrence of cyclicity and conception

The effects of age of treatment received anoestrus heifers on occurrence of cyclicity and conception are shown in the Table 4. The highest (50.00%) proportion of heifers showed cyclicity after receiving treatment when their age ranged from 36 to 42 months and the lowest (37.50%) proportion of heifers showed cyclicity after receiving treatment when their age ranged from 24 to 29 months.

Table 4. Effects of age of treatment received anoestrus heifers on occurrence of cyclicity and conception

| Age (Month) | Number of heifers treated | Number of heifers responded | Proportion of heifers responded (%) | Number conceived | Proportion of heifers conceived (%) |
|-------------|---------------------------|-----------------------------|-------------------------------------|------------------|-------------------------------------|
| 24 – 29 | 8 | 3 | 37.50 | 3 | 37.50 |
| 30 – 35 | 19 | 9 | 47.37 | 6 | 31.58 |
| 36 – 42 | 18 | 9 | 50.00 | 7 | 38.89 |

However, the difference in occurrence of cyclicity in heifers between age groups was not significant ($P>0.05$). Similarly, the highest (38.89%) proportion of heifers conceived after receiving treatment when their age ranged from 36 to 42 months and the lowest (31.58%) proportion of heifers conceived after receiving treatment when

their age ranged from 30 to 35 months. However, the difference in conception rate in heifers between age groups was significant ($P < 0.05$).

The heifers with less than 24 months old were not included in the present study and no anoestrus heifer was found with more than 42 months old in the study area. The present study indicates that the age of heifers during treatment had no effect on induction of cyclicity and conception in crossbred anoestrus heifers. Contrasting to the present finding, it has been reported that age has positive influence on onset of cyclicity in anoestrus bovine (Williams and Amstalden, 2000). The onset of puberty is primarily influenced by age and weight within breed (Nelsen *et al.*, 1982; Oeydipe *et al.*, 1982; Nelsen *et al.*, 1985). The target-weight concept is based on reports that *Bos taurus* breed heifers such as Angus, Hereford, Charolais or Limousin are expected to reach puberty at about 60 percent of mature weight (Dziuk and Bellows, 1983; Wiltbank *et al.*, 1985). Dual purpose breed heifers such as Braunvieh, Gelbvieh or Red Poll tend to reach puberty at about 55 percent of mature weight and *Bos indicus* heifers, most commonly Brahma or Brahma-cross, are older and heavier at puberty than the other beef breeds; about 65 percent of mature weight (Laster *et al.*, 1972; Stewart *et al.*, 1985). Nevertheless, further studies with more number of animals are needed to confirm the effects of BCS and age of animals during treatment on induction of cyclicity and conception in anoestrus crossbred heifers as number of animal used in this study was very low.

In the present study, real-time ultrasonography was used for determination of cyclicity and pregnancy of heifers. Real-time ultrasonography has gained tremendous popularity in recent years as a diagnostic as well as a research tool in veterinary and animal science. As a diagnostic aid, ultrasonography is well suited for bovine practice, particularly for the examination of reproductive organs (Rajamahendran *et al.*, 1994). The technique is noninvasive, relatively simple and effective, safe to both the subject and the operator, portable and ultra-rapid, and it facilitates immediate interpretation and diagnosis in most circumstances. Accordingly, applications of ultrasound in diagnostic veterinary medicine and research on cattle and other farm animals have been documented elsewhere (Pierson and Ginther, 1988). However, still the application of ultrasonography as diagnostic tool in veterinary practices in Bangladesh is limited (Akter *et al.*, 2010; Hoque *et al.*, 2011; Rahman, 2010). In the present study, when the farmers were interviewed, silent oestrus heifers were regarded as anoestrus since they did not observe any strong clinical manifestation of oestrus signs. Moreover, the veterinarians with average skill may fail to detect silent oestrus by per rectal palpation of genital organs. Under this situation, ultrasonography can be effectively used to detect silent or undetected oestrus accurately (Kahn and Leidl, 1986; Pieterse *et al.*, 1990; Ribadu *et al.*, 1994).

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