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## Therapeutic management of true anoestrous crossbred cows through nutritional and hormonal intervention

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### Abstract

A study was conducted to compare the efficacy of GnRH and GnRH fortified with bypass fat, minerals and vitamins for treatment of true anoestrus in crossbred cows based on oestrus induction response, post treatment conception rate and blood biochemical constituents. Per cent response to different treatment regimen in terms of manifestation of external signs of oestrus was recorded as 83.33, 50.00 and 83.33 for supportive, GnRH and GnRH + supportive treatment groups respectively. The corresponding post treatment oestrus intervals for the cows under the three treatment groups were  $10.60 \pm 2.11$ ,  $12.67 \pm 7.31$  and  $5.00 \pm 1.05$  days and the conception rates were 50.00, 50.00 and 50.00 per cent. As regards to blood biochemical constituents studied in true anoestrus crossbred cows it could be mentioned that serum levels of most of the minerals (phosphorus, iron and zinc); reproductive hormone (Oestrogen) and metabolic hormones (leptin, ghrelin and IGF-1) were not found to vary significantly following treatment with different regimens. It can be concluded that Supportive treatment was found to be effective as GnRH in addressing true anoestrus in crossbred cows.

**Keywords:** GnRH, true anoestrus, crossbred cows, bypass fat, conception rate

### 1. Introduction

The optimum production from dairy animals is dependent upon efficient reproduction and is often measured by number of offspring per breeding animal per unit of time <sup>[1]</sup>. The major reproductive disorders of economic importance in cattle are repeat breeding and anoestrus including silent oestrus which cause increase in the span of days open in cows. Increase in open span by one cycle even accounts for loss of production in addition to cost of treatment and labour involved in the vacant days. Anoestrus is a multicausative factors associated problem but its occurrence signals the inadequate nutrition, environmental stress, uterine pathology and improper managerial practices <sup>[2]</sup>. Anoestrus condition is generally observed after parturition when the animal suffers from negative energy balance. Underfeeding for prolonged period causes failure of follicular development. Inactive ovary, also called as true anoestrus is a condition in which the ovaries are quiescent without signs of cyclicity or cycle related ovarian structures <sup>[3]</sup>. Till today, extensive efforts had been put forward to eliminate this infertility problem from the herd. But in spite of all these efforts, anoestrus is a significant problem amongst dairy herds. In this study, attempts have been made to treat true anoestrus crossbred cows by using some hormonal preparations and nutritional supplement. As because there appears to have certain gaps in our knowledge in understanding the interactions among different factors leading to infertility in animals, such knowledge is important for proper application of therapeutic techniques in the animals for elimination of the infertility problems completely. It is felt necessary to conduct studies for enhancing the efficacy of common therapeutic techniques through Physio-nutritional intervention in cattle under varied field conditions. It is also known that energetic status of the animal modulates secretion of hormones that play key role in growth of ovarian follicle, ovulation, corpus luteum formation and fertilization <sup>[4]</sup>. Addition of fat, minerals and vitamins in the diet improves body condition of the animals. Increased level of fat in the diet also increases secretion of reproductive hormones. Discovery of the metabolic hormones leptin, ghrelin and IGF-1 opened a new dimension in the study of energy balance in animal. These hormones are considered as the indicators of energy level in animals. An increase in the level of leptin and IGF-1 and decrease in the level of ghrelin indicate positive energy balance <sup>[5-7]</sup>.

Very little information is available on characteristic genital changes in relation to different types of treatment of true anoestrus in cattle as well as variations in different blood biochemical constituents. Therefore, the aim of the current investigation was to compare the efficacy of GnRH and GnRH fortified with bypass fat, minerals and vitamins for treatment of true anoestrus in crossbred cows based on oestrus induction response, post treatment conception rate and blood biochemical constituents.

**2. Materials and Methods**

**2.1 Ethical approval**

The procedure performed in this study was ethically approved by Institutional Animal Ethics Committee (IAEC) and approval number 770/ac/CPCSEA/FVSc/AAU/IAEC/15-16/291 dated 10/04/2015.

The present study was conducted in crossbred cows maintained in 30 private farms of Assam and out of which a total of 18 number of true anoestrous animals were selected on the basis of cows not showing signs of oestrus even after four months of calving and failing to reveal distinctly palpable corpus luteum in the ovaries with apparently normal uterus as observed in rectal examination. The true anoestrous animals were divided into 3 groups and treated as per the following plan-

**Group A:** Supportive treatment comprising of bypass fat, minerals and vitamins as described below.

1. Bypass Fat (Fatomax, Intas Pharmaceuticals limited, Ahmedabad, India) @ 150 gm/day/animal for 20 days.
2. Mineral mixture (Minfa Gold, Intas Pharmaceuticals limited, Ahmedabad, India) @ 30 gm/day/animal for 30 days.
3. Injectable Phosphorus (Tonophosphan Vet, containing sodium salt of 4-dimethyl amino-2-methyl phenyl-phosphinic acid, MSD-Animal Health, Pune, India) @ 2 gm intramuscularly per day for three occasions at alternate days
4. Vitamin A (Intavita H, Intas Pharmaceuticals limited, Ahmedabad, India) @ 5ml intramuscularly for three occasions at alternate days.

**Group B:** GnRH Buserelin acetate equivalent to 20 µg of Buserelin intramuscularly on the day of treatment

**Group C:** GnRH with Supportive treatment:

Buserelin acetate equivalent to 20 µg of Buserelin intramuscularly on the day of treatment and Supportive treatment as mentioned under Group A.

Cows under true anoestrus group were examined on day 0, day 14 and day of subsequent oestrus to record presence of vaginal discharge, uterine tone and ovarian structures. Signs of oestrus in the cows responding to treatment were also recorded. Cows showing signs of oestrus following treatment were inseminated and pregnancy was confirmed on per rectum examination within 3 months and conception rates in different treatment groups were worked out. Efficacy of different treatment regimen was found out on the basis of per cent animal showing signs of oestrus and subsequent conception rate. Blood samples were collected on day 0 and day 14 and analyzed for blood biochemical parameters like calcium (Ca), phosphorus (P), iron (Fe) and zinc (Zn) by colorimetric method as per manufacturers' instructions using specific kit. Reproductive hormones like Oestrogen and Progesterone and metabolic hormones like leptin, ghrelin and insulin like growth factor-I (IGF-I) were estimated by using a solid phase enzyme linked immunosorbant assay kit following manufacturers' instructions.

**2.2 Statistical analysis**

The statistical analysis of the data was done using software IBM-SPSS-20 (<http://www.spss.co.in>) and Micro Soft Excel-2010 (<http://office.microsoft.com>).

**3. Results and Discussion**

**3.1 Therapeutic techniques for true anoestrus**

Treatment regimen used in this study was supportive treatment alone, GnRH and GnRH with supportive therapy. Frequency of occurrence of characteristic genital changes in true anoestrous crossbred cows at day 0 and day 14 of treatment with different treatment regimen has been shown in Table 1. Per cent response to treatment, post-treatment oestrus interval and subsequent conception rates have been represented Table 2.

**Table 1:** Characteristics of vaginal discharge, uterine tone and presence of ovarian structures in True anoestrous crossbred cows at different days of treatment with different treatment regimen

| Characteristic genital changes      | Frequency of occurrence (%) |          |                        |            |           |                        |                         |           |                        |
|-------------------------------------|-----------------------------|----------|------------------------|------------|-----------|------------------------|-------------------------|-----------|------------------------|
|                                     | Supportive treatment (n=6)  |          |                        | GnRH (n=6) |           |                        | GnRH + Supportive (n=6) |           |                        |
|                                     | Day 0                       | Day 14   | Post treatment oestrus | Day 0      | Day 14    | Post treatment oestrus | Day 0                   | Day 14    | Post treatment oestrus |
| Vaginal discharge Present           | 0.00(0)                     | 16.66(1) | 83.33(5)               | 0.00(0)    | 0.00(0)   | 50.00                  | 0.00(0)                 | 0.00(0)   | 83.33(5)               |
| Vaginal discharge Absent            | 100.00(6)                   | 83.33(5) | 16.66(1)               | 100.00(6)  | 100.00(6) | (3)50.00(3)            | 100.00(6)               | 100.00(6) | 16.66(1)               |
| Uterine tone Good                   | 0.00(0)                     | 0.00(0)  | 33.33(2)               | 0.00(0)    | 0.00(0)   | 50.00(3)               | 0.00(0)                 | 0.00(0)   | 50.00(3)               |
| Uterine tone Moderate               | 16.66(1)                    | 16.66(1) | 50.00(3)               | 0.00(0)    | 0.00(0)   | 0.00(0)                | 16.66(1)                | 0.00(0)   | 33.33(2)               |
| Uterine tone Absent                 | 83.33(5)                    | 83.33(5) | 16.66(1)               | 100.00(6)  | 100.00(6) | 50.00(3)               | 83.33(5)                | 100.00(6) | 16.66(1)               |
| Ovarian structure Palpable follicle | 83.33(5)                    | 50.00(3) | 83.33(5)               | 66.66(4)   | 100.00(6) | 50.00(3)               | 66.66(4)                | 66.66(4)  |                        |
| Ovarian structure Palpable CL       | 0.00 (0)                    | 50.00(3) | 0.00(0)                | 0.00(0)    | 50.00(3)  | 0.00(0)                | 0.00(0)                 | 83.33(5)  |                        |

Figures in the parentheses indicate number of observation

**Table 2:** Level of response, post treatment oestrus interval and conception rate in true anoestrous crossbred cows treated with different treatment regimen

| Treatment Regimen    | No. of cow treated | Level of response             |       | Post treatment oestrus interval (Mean ±S.E.) in days | Conception rate      |       |
|----------------------|--------------------|-------------------------------|-------|--|----------------------|-------|
|                      |                    | No. showing vaginal discharge | %     |  | No. of cows pregnant | %     |
| Supportive treatment | 6                  | 5                             | 83.33 | 10.60 ± 2.11   | 3                    | 50.00 |
| GnRH                 | 6                  | 3                             | 50.00 | 12.67 ± 7.31   | 3                    | 50.00 |
| GnRH+ Supportive     | 6                  | 5                             | 83.33 | 5.00 ± 1.05  | 3                    | 50.00 |

Figures in the parentheses indicate observed value of individual cows.

NS, Not significant, \*, P<0.05, \*\*, P<0.01

Results obtained in the present study on treatment of true anoestrus with supportive, GnRH and GnRH with supportive treatment showed that oestrus response in true anoestrus cows treated with GnRH + Supportive treatment regimen was higher (83.33 per cent) than that treated with GnRH alone (50.00 per cent). Supportive treatment alone was equally effective for induction of oestrus in true anoestrus animals (83.33 per cent). Subsequent conception rate obtained with the three treatment regimens was however similar (50.00 per cent each). Post treatment oestrus interval was shortest 5.00±1.01 days in cows treated with GnRH + Supportive treatment against 12.67±7.31 days in GnRH and 10.60±2.11 days in supportive treatment regimen group. These findings led to a conclusion that fortification of GnRH was advantageous over GnRH alone for the treatment of true anoestrus in crossbred cows and supportive treatment was equally effective. Perusal of available literature revealed some information on improvement of the efficacy of GnRH for treatment of true anoestrus in cows using different treatment protocols. In a study conducted by Kamal *et al.* [8] obtained 60.00 per cent conception rate in anoestrus crossbred dairy cows using timed A.I. with GnRH administration protocol following prostaglandin treatment. Islam *et al.* [9] using GnRH and PGF<sub>2</sub>α for the treatment of postpartum anoestrus in cows obtained 75.00 per cent oestrus response rate and 57.50 per cent conception rate. Karmakar *et al.* [10] who have reported 85.00 per cent oestrus response rate by using Vitamin A injection along with GnRH for treatment of true anoestrus in crossbred heifers. Inclusion of mineral mixture in ovsynch protocol resulted in 100.00 per cent ovulation response and 87.50 per cent conception rate in postpartum cows [11]. Kumar *et al.* [12] using single dose of GnRH for treatment of anoestrus

in cows obtained 84.62 per cent oestrus induction response rate, which was similar to that obtained in the present study. Use of Ovsynch protocol resulted in 100.00 per cent oestrus induction response in anoestrus Gir cows but the subsequent conception rate was only 50.00 per cent (Ramakrishnan *et al.*) [13] Which was similar to that obtained using GnRH alone or GnRH with supportive treatment in the present study. Results obtained in earlier studies as reviewed above supported the view generated in the present study that GnRH with supportive treatment was the chosen protocol for treatment of true anoestrus in crossbred cows.

Genital changes recorded in the true anoestrus cows before (0 day) and after treatment with the three treatment regimens also supported superiority of GnRH + Supportive and only supportive treatment protocol over GnRH alone for treatment of true anoestrus in crossbred cows. Presence of vaginal discharge, moderate to good uterine tone and presence of palpable follicle in the ovary were considered as the genital changes indicative of oestrus in the animals under the present study. Available literature revealed very little information on characteristic genital changes in relation to different types of treatment of true anoestrus in cattle for effective comparison with the findings of the present study.

### 3.2 Blood biochemical constituents

The blood levels of minerals, ovarian hormones and metabolic hormones in true anoestrus crossbred cows at day 0 and day 14 of treatment with different treatment regimens. Mean serum levels of calcium, phosphorus, iron and zinc have been presented in Table 3. The values for oestrogen, progesterone, leptin, ghrelin and IGF-1 are shown in Table 4.

**Table 3:** Level of calcium, phosphorus, iron and zinc in the serum of true anoestrus cross bred cows at different days of treatment with different treatment regimen.

| Treatment Regimen     | Calcium (mg/dl)          |                          | Phosphorus(mg/dl) |            | Iron (µg/dl)  |               | Zinc(µg/dl)   |               |
|-----------------------|--------------------------|--------------------------|-------------------|------------|---------------|---------------|---------------|---------------|
|                       | Day 0                    | Day 14                   | Day 0             | Day 14     | Day 0         | Day 14        | Day 0         | Day 14        |
|                       | Mean± S.E.               | Mean ± S.E.              | Mean± S.E.        | Mean± S.E. | Mean± S.E.    | Mean ± S.E.   | Mean± S.E.    | Mean ±S.E     |
| Supportive (3)        | 9.90 <sup>a</sup> ±1.19  | 12.80 <sup>a</sup> ±0.71 | 3.24±0.89         | 4.44±0.16  | 308.90 ±43.30 | 234.80 ±32.30 | 107.90 ±11.70 | 114.70 ±16.70 |
| GnRH (3)              | 12.60 <sup>a</sup> ±2.40 | 14.10 <sup>a</sup> ±1.51 | 2.60±0.95         | 2.40±0.34  | 261.70 ±3.10  | 325.30 ±14.30 | 82.88 ±14.50  | 92.83 ±33.10  |
| GnRH + Supportive (3) | 11.80 <sup>a</sup> ±1.02 | 15.60 <sup>b</sup> ±0.67 | 1.98±0.29         | 2.72±0.59  | 277.10 ±9.74  | 287.60 ±29.30 | 320.90 ±17.20 | 90.07 ±9.77   |

Figures in the parentheses indicate number of animals studied.

Means bearing similar superscript of small letter in a column and similar subscript of capital Letter in a row within a specific blood biochemical constituent do not differ significantly.

**Table 4:** Level of oestrogen, progesterone, leptin, ghrelin and IGF-1 in the serum of true anoestrus cross bred cows at different days of treatment with different treatment regimen

| Treatment Regimen     | Oestrogen (pg/ml)       |                          | Progesterone (ng/ml)    |                          | Leptin(ng/ml) |             | Ghrelin(pg/ml) |               | IGF-1(ng/ml) |              |
|-----------------------|-------------------------|--------------------------|-------------------------|--------------------------|---------------|-------------|----------------|---------------|--------------|--------------|
|                       | Day 0                   | Day 14                   | Day 0                   | Day 14                   | Day 0         | Day 14      | Day 0          | Day 14        | Day 0        | Day 14       |
|                       | Mean ± S.E.             | Mean ± S.E.              | Mean ± S.E.             | Mean ± S.E.              | Mean ± S.E.   | Mean ± S.E. | Mean ± S.E.    | Mean ± S.E.   | Mean ± S.E.  | Mean ± S.E.  |
| Supportive (3)        | 8.38 <sup>a</sup> ±1.33 | 11.90 <sup>a</sup> ±1.14 | 0.82 <sub>A</sub> ±0.34 | 1.05 <sub>A</sub> ± 0.29 | 2.85 ±0.35    | 3.77 ±0.44  | 232.30 ±54.70  | 578.90 ±13.20 | 54.43 ±5.12  | 58.28 ±6.620 |
| GnRH (3)              | 5.42 <sup>a</sup> ±3.81 | 3.65 <sup>b</sup> ±2.24  | 1.40 <sub>A</sub> ±0.38 | 5.93 <sub>A</sub> ±0.11  | 2.21 ±0.44    | 2.09 ±0.56  | 152.40 ±25.80  | 137.90 ±89.40 | 46.92 ±5.77  | 59.01 ±3.60  |
| GnRH + Supportive (3) | 9.38 <sup>a</sup> ±2.39 | 9.64 <sup>a</sup> ±1.79  | 0.48 <sub>A</sub> ±0.35 | 14.10 <sub>B</sub> ±5.91 | 1.94 ±0.56    | 2.56 ±0.23  | 933.10 ±82.20  | 799.90 ±93.40 | 47.40 ±11.60 | 56.93 ±6.370 |

Figures in the parentheses indicate number of animals studied.

Means bearing similar superscript of small letter in a column and similar subscript of capital letter in a row within a specific blood biochemical constituent do not differ significantly.

As regards to blood biochemical constituents studied in true anoestrus crossbred cows it could be mentioned that serum levels of most of the minerals (phosphorus, iron and zinc), reproductive hormones (Oestrogen) and metabolic hormones (leptin, ghrelin and IGF-1) were not found to vary significantly following treatment with different regimens. Level of serum calcium also did not vary significantly following treatment with supportive and GnRH treatment regimen but in case of anoestrus cows treated with GnRH +

Supportive treatment regimen it increased to a significantly higher level at day 14 of treatment. Level of serum progesterone was also found to be significantly higher in true anoestrus cows treated with GnRH + Supportive treatment only. These findings indicated that blood biochemical constituents included in the present study were not the indicators for comparing efficacy of different treatment regimens used for addressing true anoestrus. Hadiya *et al.* [14] reported that level of calcium and phosphorus in crossbred

cattle varied significantly due to supplementation with minerals and enzymes and also due to period of supplementation. According to Shakkarpude *et al.* [15] pattern of reproduction in crossbred cows was influenced by certain blood biochemical parameters of physiological significance. Calcium: phosphorus ratio alteration was also found to affect ovarian function leading to reproductive disorders [16].

#### 4. Conclusion

From the present study, it can be concluded that Supportive treatment was as effective as GnRH in addressing true anoestrus in crossbred cows. In regards to blood biochemical constituents included in the present study it can be commented that, as we found some inconsistent values in some parameters, therefore those could not only be considered as the exact or true indicators for comparing efficacy of different treatment regimens used for addressing true anoestrus under the environmental conditions of Assam.

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