A comparative therapeutic management of anoestrus in buffaloes using insulin and GnRH

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Abstract

Aim: Anoestrus is one of the most common functional disorders of the reproductive cycle in buffaloes. In spite of technical advancement, there is no single cure for the management of anoestrus. Therefore, the aim of this study was to find out the efficacy of gonadotropic releasing hormone (GnRH) and metabolic hormone for the management of true anoestrus in buffaloes.

Materials and Methods: The experimental animals were selected on the basis of history, gyneco-clinical examinations and progesterone estimation. Deworming was done with Fenbendazole and thereafter mineral mixture was given @ 50 g per animal per day for 10 days in all the selected buffaloes before the start of treatment. The selected buffaloes were randomly divided into four groups (n=25). In Group I, buffaloes were administered 20 μg of buserelin intramuscularly. Buffaloes of Group II were administered long-acting insulin @ 0.25 IU/Kg body weight subcutaneously for 5 consecutive days. In Group III, buffaloes were treated with a combination of insulin and buserelin in the above-mentioned doses whereas buffaloes of Group IV were kept as untreated control.

Results: The higher oestrus induction (64% vs. 28%) was found in Group III and differed significantly (p<0.05) as compared to control group. The conception rate (69.23% vs. 66.66%) was also found higher in Group III but did not differ significantly among the treated groups. The mean time taken for the onset of oestrus was recorded significantly shorter compared to control group. The conception rate (69.23% vs. 66.66%) was also found higher in Group III but did not differ significantly among the treated groups.

Conclusion: The results of this study indicated better fertility response using Insulin plus Buserelin in true anoestrus buffaloes under field conditions.

Keywords: anoestrus, buffalo, buserelin, gonadotropic releasing hormone, insulin.

Introduction

Buffalo is considered as black diamond due to its eminent position among the milk producing animals, but their poor reproductive efficiency, mainly because of anoestrus/sub-anoestrus, affect economy of the farmers in terms of milk yield, net calf crop and additional cost of rearing. In India, the incidence of anoestrus in buffaloes has been reported from 9% to 85.5% [1-4] and economical losses approximately Rs. 372.90 per day per animal [5].

In contrast to earlier literatures, recent reports indicated that normal follicular recruitment and emergence of follicular waves occurs in true anoestrus buffaloes, but dominant follicle fails to ovulate and ultimately regress probably due to derangement during the critical phase of selection, deviation and dominance [6-8]. The growth and maturation of follicle are under the control of hormones (gonadotropic releasing hormone [GnRH], follicle stimulating hormone [FSH] and luteinizing hormone [LH]) as well as intra-ovarian growth factors.

GnRH causes synthesis and release of FSH and LH which in turn control the gametogenesis and steroidogenesis. The effect of GnRH administration depends on the size and functional status of the follicles [9]. It induces ovulation of large dominant follicle and luteinization of small follicles [10]. Metabolic hormone such as insulin promotes folliculogenesis as well as steroidogenesis by increasing the concentration of insulin-like growth factor-1 (IGF-1). Administration of insulin enhances the follicular growth which is a prerequisite to GnRH treatment in anoestrus buffaloes [9]. Insulin has also been used to restore the cyclicity in farm animals with different success rate [11,12].

Hence, the present study was undertaken to access the efficacy of GnRH and Insulin alone and in combination in dairy buffaloes under field conditions.

Materials and Methods

Ethical approval

This study was carried out after approval by the research committee and Institutional Animal Ethics Committee.
Selection of experimental animals

A total of hundred true anoestrus non-suckled Murrah and upgraded Murrah buffaloes between first to fifth parity, aged 5 to 10 years and not exhibited any signs of oestrus up to 75 days post partum were selected for the study. The true anoestrus was confirmed by smooth ovaries on gyneco-clinical examinations at 10 days interval and serum progesterone concentration less than 1 ng/ml. All the animals were maintained at standard management practices during the entire period of study (1-month). Moreover, only those buffaloes were selected who calved normally in previous calving and had no any gynecological affection such as retained fetal membrane, uterine prolapse, metritis, etc. This study was undertaken in the summer season. The average milk production was 8-14 L/animal per day.

Treatment groups and schedule

Following deworming with fenbendazole (dose @ 7.5 mg/kg body weight, orally), mineral mixture was given @ 50 g/animal per day for 10 days in all the selected buffaloes before the start of treatment. All the selected buffaloes were divided into four groups, each comprising 25 (n=25). In Group I, buffaloes were treated with a single intramuscular injection of Buserelin, a GnRH analogue @ 20 μg (day 0). Buffaloes of Group II were treated with subcutaneous injection of long-acting biphasic insulin @ 0.25 IU/Kg body weight for 5 consecutive days (day 0-4). Buffaloes of Group III were received subcutaneous injection of biphasic insulin (day 0-4), followed by an intramuscular injection of GnRHa on day 5 in the above mentioned doses; whereas buffaloes of Group IV were kept as control.

Blood sampling and serum progesterone estimation

About 5 ml blood samples were collected aseptically from a jugular vein from all the selected buffaloes before the start of treatment. The serum was separated and kept at −20°C until progesterone estimation. The concentration of progesterone was estimated using 96 wells ELISA kits (DSI S.r.l, Italy).

Monitoring of animals for fertility response

Following treatment, all the animals were observed twice i.e., early morning and late evening for expression of oestrus signs daily for one month. The time taken for onset of oestrus following withdrawal of treatment was also recorded and analyzed. Animals were bred by natural service at oestrus using fertile buffalo bull. Few buffaloes did not breed because of sub-oestrus, which was confirmed by the presence of corpus luteum on either of the ovaries through rectal palpation. Pregnancy was diagnosed per rectally around 60 days post service. Fertility was recorded and analyzed in terms of oestrus induction rate, time taking for induction of oestrus and conception at induced oestrus.

Statistical analysis

The data obtained for oestrus induction and conception rate among all the treatment groups were analyzed by Chi-square test, whereas, mean time taken (in days) were analyzed by one-way analysis of variance test, using software package SPSS version 16.0, 2010. [13]

Results and Discussion

Oestrus induction

The results indicated higher oestrus induction in insulin plus GnRH (64%) followed by both GnRH and insulin (60% each) treated groups. The oestrus induction in treatment groups found to be significantly higher (p<0.05) than control (Table-1). The oestrus induction was 32% more in insulin-treated than the control group (28 vs. 60%) indicating a beneficial effect of insulin. Similar results have been reported by Kumar et al. [12] and Gupta et al. [14] in buffalo and by Shukla et al. [11] in cattle. Insulin is a metabolic hormone that has a significant role in animal reproduction. It stimulates recruitment of follicles [15] as well granulosa cell proliferation and steroidogenesis by increasing serum IGF-I concentration [16].

Similarly, a good response was observed following GnRH treatment as compared to control group (60% vs. 28%). GnRH is secreted from the hypothalamus in discrete pulsatile bursts, one pulse per 2-6 h [17], which act on anterior pituitary and stimulate synthesis and release of gonadotropin hormone viz. LH and FSH. That is why GnRH has been used to treat the true anoestrus cattle and buffaloes however, response to treatment has been variable ranging from 22 to 87% with mean time interval of 4-29 days [14,18-21]. The variable response may be due to the differential action of GnRH on different stages of follicular development.

Synergistic response has been observed in combined treatment of insulin and GnRH (64%) than the other groups. Others have also reported similar findings using combined therapy of insulin and GnRH [14,22]. Insulin increases the follicular diameter, which is a prerequisite for GnRH treatment to induce oestrus and ovulation in anoestrus buffaloes [9].

Time taken for onset of oestrus

The mean time taken for the onset of oestrus after the end of treatment was found to be minimum (07.66±0.88 days) in group I, which is significantly lower (p<0.05) than the group III and control group (Table-1). The mean time interval for oestrus induction has also been reported 9.5±3.18 days and 10.5±0.63 days following treatment of insulin plus GnRH [14] and GnRH alone [19], respectively. This may be due to the differential action of GnRH on different stages of follicular development.

Conception rate

The result indicated higher conception rate in combined treatment of insulin and GnRH as compared to alone insulin and GnRH (69.23% vs. 60.00% and 66.66%) treated group, respectively (Table-1).

Conception rate
Table-1: Fertility response to treatments in true anoestrus buffaloes.

| Groups                | Animals treated | Induction of oestrus | Time taken for onset of oestrus (days) | Animals bred | Animals conceived |
|-----------------------|-----------------|----------------------|---------------------------------------|--------------|-------------------|
|                       | N   | N  | %       | Mean   | Range       | N   | N  | %       |
| Group I (GnRH)        | 25  | 15 | 60.00   | 7.60±0.92  | 4-16        | 12  | 8   | 66.66   |
| Group II (Insulin)    | 25  | 15 | 60.00   | 8.80±0.69  | 3-15        | 15  | 9   | 60.00   |
| Group III (Insulin+GnRH) | 25  | 16 | 64.00   | 14.43±0.83  | 8-21        | 13  | 9   | 69.23   |
| Group IV (Control)    | 25  | 7  | 28.00   | 20.57±1.69  | 12-22       | 6   | 4   | 66.66   |

Values with different superscripts in column differ significantly (p<0.05).

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