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Induction of Postpartum Oestrus in Lakhimi Cows through Hormonal and Nutritional Interventions

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ABSTRACT

Background: Postpartum anoestrus is the most devastating cause of the dairy farming which causes more economic losses to the farmers. The present study was conducted to record the incidence of postpartum anoestrus and to evolve a suitable therapeutic regime for addressing postpartum anoestrus in Lakhimi cows.

Methods: A total of 1000 Lakhimi cows maintained in different villages of Assam were selected and the incidence rate was recorded on the basis of breeding records provided by the farmers, per-rectal examination and ultrasonography screening of genital organ. To study the effect of hormonal and nutritional interventions in post partum anoestrus cows, the cows were divided randomly into seven groups comprising twelve animals in each group. The different treatment regimes were Hydroxyprogesterone caproate + eCG, Clomiphene citrate alone, Clomiphene citrate + Mineral mixture, GnRH analogue alone, GnRH analogue + Mineral mixture, Mineral mixture alone and control. The response to different treatment regimes was based on oestrus response, mean interval from the end of treatment to onset of oestrus and conception rate.

Result: The incidence of postpartum anoestrus was found to be 20.60%. Clomiphene citrate + Mineral mixture administration regime brought about the highest oestrus induction response (100.00%) and conception rate (66.67%).

Key words: Conception rate, Lakhimi cows, Oestrus induction, Postpartum anoestrus.

INTRODUCTION

Lakhimi cattle, the recently registered breed of cattle are scattered in all parts of Assam. Total population of Lakhimi cattle is about 79 lakhs (National Bureau of Animal Genetic Resources, ICAR-Annual Report 2017-18). The Lakhimi cattle are well known for its body characters, heat tolerance, resistance to different diseases and capabilities to survive under scarce feed and fodder. Lakhimi cattle are the pride of Assam which is mostly reared by rural people for milk and draught purposes, facing most commonly postpartum anoestrus among the different gynaecological problems and causes more economic losses to the farmers. Major economic losses occurs through postpartum anoestrus are prolonged inter-calving period, delayed conception and reduction in milk yield. To maintain the recommended calving intervals, the cows need to conceive as soon as possible (85-90 days for cows). The factors mostly responsible for causing anoestrus in the cows are nutritional deficiency, parasitic overload, hormonal imbalance and poor managemental condition (Noakes *et al.,* 2019). During last few years, several attempts have been made to treat the postpartum anoestrus in cows by using various hormones like hydroxyprogesterone, eCG, GnRH and non-hormonal compounds like clomiphene citrate along with mineral mixture supplementation (Bawaskar *et al.,* 2017, Mangrole *et al.,* 2018). Availability of a cost effective therapeutic managemental protocol for overcoming the postpartum anoestrus in Lakhimi cattle is scanty. However, very limited studies had been reported on various reproductive disorders in indigenous and crossbred cattle of Assam (Chhetri, 2018). Therefore the objective of the present study was to record the incidence of postpartum anoestrus and to evolve a suitable therapeutic regime for addressing postpartum anoestrus in Lakhimi cows.

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MATERIALS AND METHODS

Ethical approval

The procedure performed in the present study was ethically approved by Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, Khanpara, Guwahati, India.

Selection of animals

The present study was conducted in a total of 1000 Lakhimi cows maintained in different villages of Assam. The study was conducted from January, 2020 to March, 2021. All the cows in the present study were in 2nd to 4th lactation, body weight ranging from 130-180 kg, body condition score varied

from 2.5 to 3.5, yielding 300 to 350 kg of milk per lactation and maintained in semi-intensive system of rearing (Fig 1). All the animals were fed mostly on straw, green fodder and small amount of concentrate mixture comprising of wheat bran and rice polish.

Incidence of postpartum anoestrus in Lakhimi cows

On the basis of breeding record maintained by the farmers, per rectal palpation and ultrasonographic screening of genital organ the incidence of postpartum anoestrus in Lakhimi cows was recorded and expressed in percentage. A total of 1000 crossbred cows were surveyed and data were recorded on pre prepared questionnaires. A cow was considered to postpartum anoestrus when she failed to show the sign of oestrus even after 90 days of parturition and failing to reveal presence of corpus luteum in the ovaries as per rectal examination and ultrasonographic screening. Ultrasonographic screening was done by using ultrasonographic machine of model M-SONOSITE, FUJIFILM inc Bothwell, WA 98021-3904 USA. On ultasonographic scanning the average follicular diameter measured less than 1.0 cm was selected as anoestrus cow in the present study (Fig 2).

Therapeutic management of postpartum anoestrus

This experiment was conducted to study the efficacy of different treatment protocols for addressing postpartum anoestrus in Lakhimi cows. For this experiment a total of 84 post partum anoestrus Lakhimi cows were selected. All the selected cows were divided randomly in 7 groups and each comprising 12 animals. The treatment protocols were:

Group A: Cows were administered three injections of Hydroxyprogesterone caproate *i.e.* Progesyn injection (Intas Pharmaceutical Ltd., Ahmedabad, India) @ 750 mg intramuscularly at 72 hours interval followed by one injection of Equine chorionic gonadotropin (eCG) *i.e.* Folligon injection (Intervet SPAH) @ 750 I.U. intramuscularly after 72 hours of the last injection of Hydroxyprogesterone caproate.

Group B: The cows were administered Clomiphene citrate bolus *i.e.* Ferty-G (Geevet Remedies, Gujarat, India) @ 300 mg orally once daily for 5 days.

Group C: The cows were administered Clomiphene citrate bolus @ 300 mg orally once daily for 5 days along with Mineral mixture powder *i.e.* Minfa Gold (Intas Pharmaceutical Ltd., Ahmedabad, India) @ 30 g orally once daily for 20 days.

Group D: The cows were injected one GnRH analogue (Buserelin acetate) *i.e.* Gynarich injection (Intas Pharmaceutical Ltd., Ahmedabad, India) @ 20 µg (5 ml) intramuscularly.

Group E: The cows were supplemented mineral mixture powder @ 30 g orally once daily for 20 days and administered one injection of GnRH analogue @ 20 µg (5 ml) intramuscularly on 7th day of Mineral mixture supplementation.



Fig 1: Semi intensive system of rearing of Lakhimi cows in Villages of Assam.

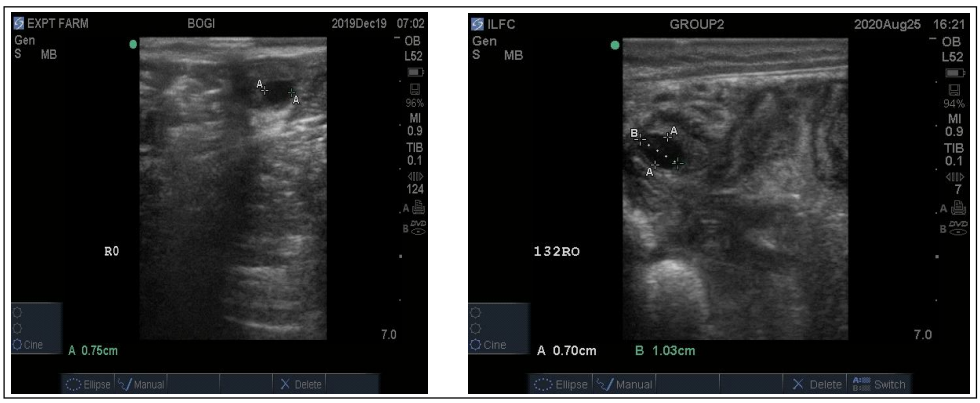

Fig 2: Ultrasonographic screening of ovaries showing follicular diameter in post partum Lakhimi cows.

Group F: The cows were supplemented with Mineral mixture powder @ 30 g orally once daily for 20 days.

Group G: The cows in this group were kept as control *i.e.* without any treatment.

Response to nutritional and hormonal interventions

Following hormonal and nutritional interventions, the responses to various treatment regimes were expressed in terms of onset of oestrus (%), interval from end of treatment to onset of oestrus and conception rate. The cows that came into oestrus were allowed for natural service by locally available breeding bull or artificial insemination was done in absence of breeding bull. Pregnancy diagnosis was done by per rectal palpation of genital organ after 60 days of breeding.

Statistical analysis

Incidence of post partum anoestrus and oestrus responses following different hormonal and nutritional regimens in post partum lakhimi cows were expressed in percentage. Intervals from end of treatment to onset of oestrus were expressed as mean±SE (Standard error). Means were analyzed using one-way analysis of variance followed by post hoc test to determine significance difference between intervals from end of treatment to onset of oestrus, using the SPSS (version 20.0, SPSS, Chicago, IL, USA). Difference with values of $p \leq 0.05$ was considered to be statistically significant. Conception rate was expressed in percentage.

RESULTS AND DISCUSSION

Incidence of postpartum anoestrus

The incidence of postpartum anoestrus in 1000 Lakhimi cows was found to be 20.60%. Almost similar findings (20.30%) in crossbred cows were reported by Dutta *et al*. (2019b). The incidence rate of postpartum anoestrus in indigenous cows of Assam was 18.93% as reported by Chhetri, (2018). However lower incidence rates (16.42%) in crossbred cows were reported by Acharya, (2016). The higher post partum anoestrus (28.00%) in dairy cows was recorded by Selvaraju *et al.* (2009). The variation in the incidences of postpartum anoestrus might be due to feeding practices, genetic makeup of the animals, nutritional status, age, area of study and housing patterns of the animals (Dutta *et al*., 2019b).

Response to various hormonal and nutritional interventions

Oestrus induction response

The oestrus induction responses in various treatment regimes in Lakhimi cows were illustrated in Table 1. It was observed that oestrus induction response was found to be higher in the group treated with Clomiphene citrate + Mineral mixture and the lowest in control group. The oestrus induction response in postpartum anoestrous Lakhimi cows treated with hydroxyprogesterone caproate + eCG estimated in the present study was similar (83.33%) to Chhetri (2018) in anoestrous indigenous cows treated with progesterone + eCG. On the other hand, higher oestrus induction response (90.00%) was reported by Honparkhe *et al.* (2008) in true anoetrous cows treated with hydroxyprogesterone caproate +eCG. The variation in the percentage of induced oestrus in different studies might be due to doses of hormones and resumption of follicular development and its maturation after treatment.

The oestrus induction response for Clomiphene citrate treated group was found to be 33.33% in the present study. Clomiphene citrate bolus orally daily for five days showed oestrus induction response 50.00% (Mangrole *et al*., 2018), which was higher than the present investigation. The percentage of oestrus induction response recorded pertaining to Clomiphene citrate + Mineral mixture treated cows in present findings (100.00%) was higher than the findings of Mangrole *et al*. (2018) in postpartum anoestrous crossbred cows (66.66%) treated with Clomiphene citrate and mineral mixture. In the present study lower oestrus response in the cows treated with Clomiphene citrate alone might be attributed to the non-supplementation of mineral mixture.

The oestrus induction response rate following GnRH treatment cows in the present study was similar (50.00%) with the findings that recorded by Dutta *et al.* (2019a) in anoestrous cows. The rates of oestrus response following GnRH analogue injection were reported as 66.67%

Table 1: Oestrus response following different hormonal and nutritional regimes inpostpartum anoestrous Lakhimi cow.

Treatment regimes	Number of cows treated	Oestrus response (%)
Hydroxyprogesterone caproate + eCG	12	83.33(10)
Clomiphene citrate	12	33.33(4)
Clomiphene citrate + Mineral mixture	12	100.00(12)
GnRH analogue	12	50.00(6)
GnRH analogue + Mineral mixture	12	66.67(8)
Mineral mixture	12	66.67(8)
Control	12	16.67(2)

Table 2: Interval from the end of treatment to onset of oestrus following different hormonal and nutritional regimes in postpartum anoestrous cow.

Treatment regimes	Interval from the end of treatment to onset of oestrus (Mean±SE, Days)
Hydroxyprogesterone caproate + eCG	1.7^{a}±0.09
Clomiphene citrate	7.5^{a}±1.50
Clomiphene citrate + Mineral mixture	5.33^{a}±0.56
GnRH analogue	75.33^{b}±2.60
GnRH analogue + Mineral mixture	30.75^{c}±7.69
Mineral mixture	37.75^{c}±7.69
Control	63.00^{d}±3.00

Means bearing different superscripts in column differ significantly ($P<0.05$).

(Chhetri, 2018) in post partum cows. However, lower oestrus response (33.33%) was recorded by Gupta *et al.* (2010) in cows treated with GnRH. The variation in the oestrus response in different studies might be due to different body condition of cow, feeding management, climatic condition and potency of GnRH hormone used in the study. The oestrus induction response recorded pertaining to GnRH analogue + Mineral mixture treated cows in the present study was similar (66.67%) to the finding of Chhetri, (2018) in postpartum anoestrous indigenous cows treated with GnRH + bypass fat + minerals + vitamins. On the other hand, lower oestrus induction response 50.00% was recorded by Mangrole *et al.* (2018) in crossbred cows treated with GnRH analogue + Mineral mixture. However, higher oestrus response (83.33%) was recorded by Dutta *et al.* (2019a) in true anoestrus crossbred cows treated with GnRH analogue + Mineral mixture. These variations might be due to differences of breed, general health of animal and climatic variations.

Cows treated with Mineral mixture alone in the present study showed 66.67% oestrus induction response which was nearer (70.00%) to the findings of Jana *et al.* (2015). The higher oestrus induction response was recorded by Noonari *et al.* (2016) and reported 100.00% oestrus induction response in crossbred cows treated with mineral mixture. On the other hand, lower oestrus induction response (50.00%) was recorded by Chaudhiry *et al.* (2019) after treated with mineral mixture. Mineral supplementation might show beneficial effect on postpartum ovarian activity by increasing number of ovarian follicles along with follicular growth. But the differences in oestrus response might be due to body condition of animal, parity of animal and season of study.

Interval from end of treatment to onset of oestrus

The mean interval from end of treatment to onset of oestrus in postpartum anoestrous cows treated with different treatment regimes are presented in Table 2. Statistical analysis indicated that the mean interval from end of treatment to onset of oestrus differed significantly between different treatment regimes. In Hydroxyprogestero ne caproate + eCG treatment protocol of present study, the mean interval for onset of oestrus from the end of treatment was in accordance with Chhetri, (2018). The mean interval from the end of treatment to onset of oestrus as 50.00±0.52 hours in the cows treated with progesterone + eCG as reported by Chhetri, (2018). However, Kasthuri (2006) reported higher mean interval from end of treatment to onset of oestrus (3.65±0.23 days) in crossbred cows treated with Folligon injection (PMSG) as compared to the present study. The variation in the values in different findings might be due to doses of eCG (PMSG) and incorporation of different drugs before PMSG therapy.

The mean interval from the end of treatment to onset of oestrus in Clomiphene citrate alone and Clomiphene citrate + Mineral mixture protocol was comparable with findings that observed by Bawaskar *et al.* (2017). The mean interval from the end of treatment to onset of oestrus was recorded to be 4.33±0.91 days (Bawaskar *et al.,* 2017) in the animals treated with Clomiphene citrate bolus @ 300 mg orally daily for 5 days.

The mean interval from the end of treatment to onset of oestrus in GnRH analogue treatment protocol in the present study was found to be higher than that of Dutta *et al.* (2019a). The mean interval from the end of treatment to onset of oestrus was recorded to be 12.67±7.31 days (Dutta *et al.,* 2019a) in cows treated with single injection of GnRH analogue. However, mean interval from the end of treatment to onset of oestrus in GnRH analogue + mineral mixture protocol of present study was found to be higher than the findings recorded by Dutta *et al.* (2019a) in true anoestrus cross bred cows (5.00±1.05 days). GnRH hormone efficacy depends upon availability of recruited ovarian follicles.

The mean interval from the end of treatment to onset of oestrus in mineral mixture alone treatment was nearer with findings that observed by Selvaraju *et al.* (2009).The induction of oestrus in dairy cattle within 2 months while supplemented with area specific mineral mixture as reported by Selvaraju *et al.* (2009). These differences might be due to stage of reproduction of experimental animals, geographical variation, general health of the animal and variation of doses of therapy used.

Conception rate

Conception rates based on number of cows induced to oestrus and cows treated with different treatment regimes are presented in Table 3. The conception rate recorded pertaining to Hydroxyprogesterone caproate + eCG treated cows in the present study was zero which was lower than Chhetri, (2018). The conception rate was 40.00% in cows treated with progesterone + eCG (Chhetri, 2018).

Table 3: Conception rate following different hormonal and nutritional regimes in postpartum anoestrous Lakhimi cow.

Treatment regime	No. of animal induced to oestrus	No. of cows conceived	Conception rate (%)	
			Based on no. of cows induced to oestrus	Based on no. of cows treated
Hydroxyprogesterone caproate + eCG	10	0	0.00	0.00
Clomiphene citrate	4	4	100.00	33.33
Clomiphene citrate + mineral mixture	12	8	66.67	66.67
GnRH analogue	6	4	66.67	33.33
GnRH analogue + mineral mixture	8	6	75.00	50.00
Mineral mixture	8	4	50.00	33.33
Control	2	2	100.00	16.67

The conception rate for Clomiphene citrate alone in postpartum anoestrous cows was found to be 33.33% which was lower than the earlier records (50.00%) in cows treated with clomiphene citrate alone orally daily for 5 days as reported by Mangrole *et al*. (2018). The conception rate based on the number of treated animals following GnRH treatment protocol in the present was similar to that reported by Gupta *et al.* (2010). However higher conception rates 67.70% (Islam *et al*., 2013) and 50.00% (Dutta *et al.,* 2019a) was reported in cows with GnRH treatment. The conception rate based on the number of treated animals following GnRH analogue + Mineral mixture protocol was similar (50.00%) to that reported by Dutta *et al.* (2019). However, there was lower conception rate (33.33%) as compared to the present study with GnRH analogue + Mineral mixture was recorded by Chhetri (2018) in postpartum anoestrous cows.

The cows in the present study treated with mineral mixture protocol showed 33.33% conception rate in postpartum anoestrous cows which was comparable (35.00%) to that reported by Das *et al.* (2016). Sahoo *et al.* (2016) reported 55.00% conception rate in crossbred cows treated with bypass fat @ 100 g/day/animal along with mineral mixture @ 50 g/day/animal.

The lower conception rate in Hydroxyprogesterone caproate + eCG, Clomiphene citrate and GnRH analogue in the present study might be due to non-supplementation of mineral mixture, genetic makeup of animal, environmental and managemental factors and season of study.

CONCLUSION

In conclusion, the incidence of postpartum anoestrus in Lakhimi cows of Assam was 20.60%. Based on oestrus induction response and conception rate, Clomiphene citrate + Mineral mixture protocol was found to be the best therapeutic regime for addressing postpartum anoestrus in Lakhimi cows.

Conflict of interest

All authors declare that they have no conflict of interest.

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