

Impact of hormonal intervention on fertility enhancement in cyclic non-breeder cows

C. Acharya^{1,2*}, B.C. Deka¹, M. Sarma², M. Sarma², N.A. Kader², P.C. Mishra², M. Bhuyan¹

¹College of Veterinary Science Khanapara, Assam Veterinary and Fisheries University, Khanapara, Guwahati, Assam-781022

²Institute of Veterinary Science and Animal Husbandry, Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar, Odisha-751003

*Corresponding author email: chiranacharya2003@gmail.com

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Abstract

The current research work was carried out in the crossbred cattle population of Instructional Livestock Farm (ILF) of College of Veterinary Science (CVSc), Khanapara, Assam Agriculture University (AAU), Guwahati and private dairy farms located in and around Guwahati, Assam to investigate the effect of some hormonal combinations against repeat breeding. For the purpose of investigating the impacts of hormonal therapies on the rate of conception and estimation of mineral and cholesterol profile, repeat breeder cows were segregated into 4 groups. Group I and II were treated with buserelin acetate and Human Chorionic Gonadotropin (hCG) respectively on 0th and 11th day after Artificial Insemination (AI). Animals in Group III were treated with hydroxyl progesterone caproate on 5th and 11th day after AI, whereas Group IV acted as untreated control. Following hormonal treatments, the rates of conception in Group I, II, III and IV was 60, 70, 50 and 20 per cent respectively. Statistical analysis did not exhibit any significant difference between pre and post treatment values with respect to serum cholesterol and magnesium, levels irrespective of treatment adopted including untreated control. However, significant difference existed between mean values of serum magnesium on the day of oestrus, prior to administration of hormones. On 11th day of oestrus cycle, the amount of serum cholesterol as well as magnesium varied significantly following treatment among treatment groups.

Key words: Repeat Breeding, Buserelin Acetate, Human Chorionic Gonadotropin, hCG, Hydroxy progesterone Caproate

Introduction

India is the leading milk producer since 1998 and harbours world's largest bovine population. The total Milk production in the country is estimated as 239.30 million tonnes during 2023-24 registered a growth of 5.62% over the past 10 years which was 146.3 million tonnes in 2014-15. Further, the production has increased by 3.78% during 2023-24 over the estimates of 2022-23 (PIB, 2024). India's global position in dairy sector can be attributed to a plethora of intensive crossbreeding programmes, carried out across the country, over the past few decades. However, massive intensification has negative effects on reproduction. The dairy cattle are expected to calve once a year, which means that every cow is expected to conceive after Artificial Insemination (AI), and deliver at the end of gestation i.e. around 280 days and then wait for 60-90 days for successful insemination. However, this is not achieved always and the animals need to be inseminated again and again in subsequent oestrus cycles without conception. Conception failures may be attributed to various reproductive disorders. It is seen that about 40.3 % of dairy cattle has at least one clinical reproductive disturbance (Dawit et al. 2013). Reproductive disorders as well as disorders of remote body system lead to reproductive and artificial insemination failure (Gilbert, 2019). Repeat breeder refers to female cattle with apparently normal oestrus cycle without detectable abnormalities that has failed in conception following three or more fertile services (natural mating or AI) (Jeong and Kim, 2022). Cattle rearing are less rewarding in Assam, due to multitudes of problems like scarcity of green fodders, increased feed cost, increased parasitic diseases, bottle necks in transportation facility, change in climatic condition, etc. Owing to low output of milk in Assam, Government have given considerable importance to increase milk production through cross breeding local cattle with high yielding superior germplasm of registered Indian and Exotic breeds. Reproductive failure owing to repeat breeding has emerged one of the expensive obstacles in profit making dairy industry. As it lengthens the calving interval, thus it negatively impacts per year milk yield and overall production (Tóth et al., 2023). Different hormonal protocols like double ovsynch (Macias-Munoz et al., 2026) and FSH (Kumar et al., 2025) have been tried with varying success rate.

Repeat breeding had multifactorial complex aetiology. Many extrinsic and intrinsic factors are involved (Saraswat and Purohit, 2016). Luteal dysfunction resulting in insufficient production of progesterone during post breeding luteal phase might be one of the reasons of embryonic death leading to repeat breeding.

Although a few studies were conducted on certain aspects of repeat breeding, in crossbred cattle of Assam, a systematic study on improving the production of progesterone during critical luteal phase of the oestrous cycle by incorporating certain hormones has not been undertaken. Taking these facts into consideration, the current research work was undertaken to investigate into the effect of hormonal intervention on fertility of cyclic non breeder cows.

Materials and methods

A total of 40 repeat breeding cattle were selected to study the efficacy of administration of hormones on conception rate, serum cholesterol and mineral profiles. Selection of animals was based on history collected from the owner and clinico-gynaecological examination. The animals which failed to conceive after breeding for more than three consecutive times were included in the study. The clinico-gynaecological examination of the animals was performed on the day of oestrus. The animals having regular oestrous cycle and oestrous period, clear vaginal discharge, detectable regressed corpus luteum on the ovary, moderate to good tone in uterus, were only selected for the study. Anovulatory and animals with infected reproductive tract were not included in the study. The selected animals were divided into 4 groups each consisting of 10 cows.

The animals in Group I were administered with buserelin acetate 2.5 ml (0.004mg/ml) i.m. per animal on the day of AI and day 11 post AI. The animals in Group II received 2000 I.U. of hCG i.v. per animal at the time of AI and day 11 post AI and the animals in group III were administered 500 mg of Progesterone (hydroxy progesterone caproate) i.m. per animal on Day 5 and 11 post AI. Group IV animals were kept as untreated control. In all the groups A.I. was done 12-15 hrs after the onset of oestrus.

Study to evaluate the blood biochemical profiles

A total of twenty repeat breeding crossbred cows as well as heifers comprising five animals from each of the four groups of animals used for studying the effect of hormonal treatments were selected at random for studying the blood biochemical profile on 0th, 5th and 11th day of oestrus cycle/AI.

Collection of blood and preservation of serum

Before administration of hormone, blood samples were obtained from the jugular vein by utilising an 18 gauze sterile needle.

Six ml of whole blood were collected into a sterile 8.5 ml vacutainer and was kept in slanted position till the completion of clotting. Following this, separation of serum was done and the serum was collected in a clean dry sterile plastic vial using a sterile pipette and stored at (-) 20° C. The serum so obtained was subjected to centrifugation as and when necessary.

Estimation of serum biochemical constituents

The cholesterol and magnesium levels were estimated using automatic biochemical analyzer (BeneSphera™C61) and compatible kits from AGD Biochemical (P) Ltd. (Griener diagnostic GmbH, Griener diagnostic GmbH).

Pregnancy diagnosis

Pregnancy diagnosis was performed at 60 to 90 days post AI in all the animals per rectum on those who did not return to oestrus and then conception rate was calculated.

Results

The repeat breeders were divided into 4 groups in order to evaluate the effects of some hormones on conception rate and serum mineral and cholesterol profile. Group I and II was administered with buserelin acetate and human chorionic gonadotropin (hCG) respectively on 0th and 11th day following AI. Group III animals received hydroxyl progesterone caproate on 5th and 11th day following AI, whereas Group IV was used as untreated control.

Conception rate

The conception rates achieved after hormonal treatment are shown in Table 1. The conception rate recorded in Gr. I, II, III and IV were 60, 70, 50 and 20 % respectively. The rate of conception was found to be higher in treatment groups in comparison with the untreated control. Out of the treated groups the conception rate in hCG treated group was the highest (70.00 %).

Serum cholesterol

The mean serum cholesterol levels in repeat breeder cattle before and after administration of buserelin acetate (Group I), hCG (Group II), hydroxyl progesterone caproate treatments (Group III) and in control (Group IV) are presented in Table 2. The mean of serum cholesterol levels on 0th day of oestrous cycle before administration of hormone in Gr. I, II and IV were recorded to be 336.60 ± 22.55 , 281.00 ± 23.33 and 313.20 ± 22.26 mg/dl respectively and for Gr. III (hydroxyl progesterone group) on 5th day of oestrous cycle, pre-treatment value was 364.60 ± 30.37 mg/dl. The mean serum cholesterol level post treatment on 11th day of oestrous cycle in Group I, II, III and IV was 334.40 ± 18.43 mg/dl, 512.20 ± 73.33 , 431.80 ± 25.42 and 390.80 ± 20.27 mg/dl respectively (Table 2).

Statistical analysis showed that values of serum cholesterol level before treatment in repeat breeding crossbred cattle did not vary significantly between groups, whereas it differed significantly ($P < 0.05$) between groups post treatment (Table 2, 3 and 4). Duncan's Multiple Range Test revealed that serum cholesterol level on 11th day of oestrous cycle after treatment although significantly higher in hCG treated group than in buserelin acetate group, the values of both the groups did not differ significantly from that of control group. Furthermore, it was observed from the results of the t- test that the values of serum cholesterol before and following treatment did not vary significantly in all the treatment groups (Table 2)

Serum magnesium

The mean serum magnesium level on the 0th day of oestrous cycle in repeat breeding crossbred cattle before administration of busereline acetate, hCG and in control group was 2.92 ± 0.16 , 2.74 ± 0.02 , and 1.19 ± 0.01 mg/dl respectively and for hydroxy progesterone group the serum magnesium level on 5th day of oestrous cycle was 1.15 ± 0.01 mg/dl. Whereas, on 11th day of oestrous cycle *i.e.*, following treatment, the mean of serum magnesium level varied between 1.15 ± 0.01 mg/dl (Group IV) and 2.80 ± 0.02 (Group I & II), while Group III animals recorded an intermediate value of 1.21 ± 0.09 mg/dl (Table 2).

Serum magnesium level in repeat breeding crossbred cattle before and after treatment varied significantly ($P < 0.01$) between treatments (Table 2, 3 and 4). Duncan's Multiple Range Test revealed the serum magnesium levels on day 0 (before treatment) and 11th day of oestrous cycle (following treatment) were significantly higher in buserelin acetate and hCG treated groups than in control group. The results of the t-test revealed that the mean values of serum magnesium before as well as after treatment did not vary significantly in all treatments (Table 3 & 4).

Table 1. Conception rate in cyclic non-breeders treated with different hormonal regime

Groups	No. of animals		Conception Rate (%)
	Inseminated	Conceived	
Group I (Buserelin acetate @2.5ml/animal on day 0 and day 11 post A.I.)	10	6	60.00
Group II (hCG @ 2000 I.U./animal on 0th day and 11 th day post A.I.)	10	7	70.00
Group III (Hydroxy progesterone Caproate @ 2ml/animal on 5 th day and 11 th day post A.I.)	10	5	50.00
Group IV (Control)	10	2	20.00

Table 2. Mean ($\pm$ SE) pre and post treatment serum cholesterol and magnesium levels in repeat breeder cows with different hormonal treatment

Treatment Parameters	Buserelin acetate	hCG	Progesterone	Control
Cholesterol (mg/dl)				
Day 0	336.60 $\pm$ 22.55	281.00 $\pm$ 23.33	364.60 $\pm$ 30.37*	313.20 $\pm$ 22.26
Day 11	334.40 ^b $\pm$ 18.43	512.20 ^a $\pm$ 73.33	431.80 ^{ab} $\pm$ 25.42	390.80 ^{ab} $\pm$ 20.27
t- value	0.158 ^{NS}	2.94 ^{NS}	1.249 ^{NS}	9.67 ^{NS}
Magnesium (mg/dl)				
Day 0	2.92 ^a $\pm$ 0.16	2.74 ^a $\pm$ 0.02	1.15 $\pm$ 0.01*	1.19 ^b $\pm$ 0.01
Day 11	2.80 ^a $\pm$ 0.02	2.80 ^a $\pm$ 0.02	1.21 ^b $\pm$ 0.09	1.15 ^b $\pm$ 0.01
t- value	0.777 ^{NS}	2.08 ^{NS}	1.567 ^{NS}	4.19 ^{NS}

* Values on day 5 of oestrous cycle ^{NS} Non significant Means having different superscripts within rows varies significantly (p<0.05).

Table 3: Analysis of variance of serum cholesterol and magnesium levels on day 0 of oestrous cycle following hormonal treatments in repeat breeding crossbred cattle

Source	d.f.	Cholesterol		Magnesium	
		MS	F value	MS	F-value
Treatment	2	3896.4670	1.51 ^{NS}	4.5232	110.09**
Error	12	2580.333		0.042	

*P<0.05 **P<0.01 NS - non significant

Table 4: Analysis of variance of serum cholesterol and magnesium levels on day 11 of oestrous cycle following hormonal treatments in repeat breeding crossbred cattle

Source	df	Cholesterol		Magnesium	
		MS	F value	MS	F- value
Treatment	3	27984.8660	3.31*	4.3820	1750.92**
Error	16	8466.9750		0.0025	

*P<0.05, **P<0.01, ^{NS} Non significant

Discussion

Days 11 to 16 post-AI are critical for the maternal recognition of pregnancy. If progesterone is insufficient, the uterus releases prostaglandin, triggering luteolysis (the destruction of the CL) and causing early embryonic death, a primary cause of repeat breeding. Day 11 coincides with the mid-luteal phase when the corpus luteum (CL) should be producing high levels of progesterone to maintain pregnancy. Hormonal treatment like Buserelin, hCG) around mid-cycle induces the ovulation or luteinization of a dominant follicle from the second follicular wave. This creates an accessory corpus luteum, which adds functional luteal tissue and significantly increases maternal progesterone levels (Besbaci et al, 2018)

The rate of conception in crossbred repeat breeding cattle treated with buserelin acetate, hCG and hydroxyprogesterone caproate was 60, 70 and 50 per cent respectively as compared to 20% in the control. A higher conception rate in crossbred repeat breeders following hormonal treatment as compared to the untreated control has also been reported by earlier workers (Ergene, 2012; More *et al.*, 2012; Mathew *et al.*, 2013; Parmar *et al.*, 2013; Senthil *et al.*, 2014 and Pandey *et al.*, 2016). In the present study, in buserelin acetate treated group the rate of conception was found to be 60.00 per cent. More *et al.* (2012) has reported a higher CR following administration of buserelin acetate. Further, various researchers have reported lower conception rate following buserelin acetate administration than that was recorded in our study. (Ergene, 2012; Mathew *et al.*, 2013; Parmar *et al.*, 2013; Senthil *et al.*, 2014 and Pandey *et al.*, 2016).

The rate of conception in repeat breeding cattle following hCG treatment in the present study was found to be 70.00 %. Similar rate of conception in repeat breeding cattle treated with hCG was reported by Pandey *et al.* (2016). In contrary, higher rates of conception following hCG administration in repeat breeding cattle have also been reported by Parmar *et al.*, (2013). Senthil *et al.* (2014) and Mathew *et al.* (2013) have reported lower conception rates after hCG treatment. Better conception rate in hCG than buserelin might be due to the fact that, hCG acts directly as a substitute for Luteinizing Hormone (LH) by binding immediately to LH receptors on the ovaries. In contrast, buserelin acetate is a GnRH analogue, meaning it must first signal the cow's anterior pituitary gland to secrete its own endogenous LH. In repeat breeder cows, the pituitary response can sometimes be sub-optimal or sluggish. Since day 11 is the exact threshold for maternal recognition of pregnancy, the stronger progesterone boost from hCG is more effective at preventing early embryonic mortality, leading to a higher overall conception rate (Pandey *et al.*, 2016).

The rate of conception in crossbred repeat breeding cattle after hydroxyprogesterone caproate treatment in the present study was found to be 50.00 per cent. Similar rate of conception in repeat breeding cattle treated with hydroxyprogesterone caproate was reported by More *et al.* (2012) and Parmar *et al.* (2013). However, Senthil

et al. (2014) and Pandey *et al.* (2016) reported lower conception rate after hydroxy progesterone caproate treatment than that obtained in the current study. The rate of pregnancy in untreated control group in the present study was found to be 20.00. Similar rate of conception in untreated repeat breeding cattle was reported by several workers (Ergene, 2012; More *et al.*, 2012; Mathew *et al.*, 2013 and Parmar *et al.* 2013). On the other hand, Senthil *et al.* (2014) and Pandey *et al.* (2016) reported higher conception rates in untreated group than the present study.

The findings of the present study indicate that all the three hormonal treatments effectively improved pregnancy rate in repeat breeding crossbred cattle. Out of the three treatment groups, the conception rate was found to be the highest in hCG treated group (70 %) followed by that in buserelin acetate treated group (60 %) and was the lowest in hydroxyl progesterone caproate treated group (50 %).

The increased conception rate observed in buserelin acetate and hCG treated groups as compared to the progesterone treated group might be owing to the fact that the first injection of hCG and buserelin acetate have improved ovulation rate and timing with respect to AI, apart from stimulating and maintaining corpus luteum, leading to increased progesterone concentration.

Serum cholesterol

The mean serum cholesterol level in repeat breeding crossbred cattle on the day of oestrus before treatment with buserelin acetate, hCG, and in control group was 336.60 ± 22.55 , 281.00 ± 23.33 and 313.20 ± 22.26 mg/dl, respectively. It was 364.60 ± 30.37 mg/dl on day 5 of oestrous cycle before treatment in hydroxyl progesterone caproate treated group. The values did not vary significantly between treatment groups. After treatment on 11th day of oestrous cycle the cholesterol concentrations in group I, II, III and IV were 334.40 ± 18.43 , 512.20 ± 73.33 , 431.80 ± 25.42 and 390.80 ± 20.27 mg/dl, respectively. Although hCG treated group had significantly ($P < 0.05$) higher serum cholesterol concentration on 11th day of oestrous cycle than in buserelin acetate treated group, the serum cholesterol concentration in all the treatment group did not vary significantly from that in control group. Increased concentration of cholesterol in hCG treated group is indicative of increased stimulation of corpus luteum by hCG as compared to buserelin acetate in the given dosage.

Since cholesterol is the precursor of steroid hormone, its increased concentration might have produced more amount of progesterone, the hormone responsible for maintenance of pregnancy (Bishop *et al.*, 2022). The current study reported a higher serum cholesterol level.

The difference in the level of serum cholesterol observed in various studies may be due to a plethora of factors, such as individual variation, age of the animal, feeding and nutritional status of the animals, fertility status of the animal and the techniques used for estimation.

Serum magnesium

The mean of serum magnesium level in crossbred repeat breeding cattle before administration of buserelin acetate or hCG including control on the day of oestrus was 2.92 ± 0.16 , 2.74 ± 0.02 , and 1.19 ± 0.01 mg/dl respectively and it was 1.15 ± 0.01 mg/dl for hydroxyl progesterone caproate group on 5th day of oestrous cycle. On 11th day of oestrous cycle following treatment, the mean of serum magnesium level in group I, II, III and IV was 2.80 ± 0.02 , 2.80 ± 0.02 , 1.21 ± 0.09 and 1.15 ± 0.01 mg/dl, respectively. The level of magnesium in control and progesterone treated group was observed to be lower than buserelin acetate and hCG treated groups. As in these two groups, the magnesium levels were low even prior to treatment; therefore it can be perceived that the difference might not be due to treatment, but individual variations. In all the four groups, there was no difference in magnesium levels before and after the treatment.

Conclusion

Out of the three treatment regimens using buserelin acetate, hCG and hydroxyl progesterone caproate in repeat breeding crossbred cattle, the rate of conception was found to be the highest (70 %) when treated with injection of 2000 I.U of hCG (i.v.) at the time of A.I. and repeated 11 days post AI.

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