

## Effect of Non-hormonal Treatments on Postpartum True Anoestrus Crossbred Dairy Cows

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### Abstract

The present study was conducted to study the efficacy of different medicaments on postpartum anoestrus crossbred cows. Total of 20 post partum anoestrus crossbred cows were selected based on the case history and rectal palpation from the private dairy farm in and around the Khanapara, Guwahati, Assam. The animals were split into two groups (n=10) and treated with Vitamin A+Tonophosphan (Group 1), Vitamin A+Tonophosphan+Genital Massage (Group 2). Result of experiment revealed that number of animal responded and conceived were higher and duration between the initiation of treatment and expression of heat was shorter in group 2 treatment and expression of various heat signs were more prominent in this group 2 treatment. Similarly the biochemical profiles were higher in responded animal than non- responded and in group 2 treatment. Moreover, the biochemical profiles were significantly higher in day of induced oestrus, day 20 than day 10 or 0 of treatment in responded animal and higher in group 2 treatment. It was concluded that group 2 (Vitamin A+Tonophosphan+Genital Massage) treatment was given better response to the postpartum anoestrus in crossbred cows.

### 1. Introduction

Cattle productivity depends largely on reproductive efficiency, and is often measured by number of off-spring per breeding animal per unit of time. Crossbred cattle suffer to a large extent from the reproductive disorder of post partum anoestricity (about 75%) which causes a great inflict on the economy to the dairy farmers (Kutty and Ramachandran, 2003) and is often attributable to deficiency of nutrients in the ration or stress (Short et al., 1990). A period of anoestrus following parturition is a normal physiological event but it becomes abnormal when its duration extends more than accepted average (Hopkins, 1986). Lack of minerals especially calcium and phosphorus and vitamin A (Sane, 1994) upsets the proper functioning of reproductive organs, poor follicular development and post partum anoestrus in cows. Estimation of blood cholesterol and total serum protein were useful parameters in diagnosis and treatment of infertility problem (Patil, 1976). During the last few years, several studies have been attempted to treat the prolonged postpartum anestrus in cows by using hormonal treatments such as gonadotropin releasing hormone (GnRH), estrogen, prostaglandin (PGF<sub>2</sub>α) and progesterone (Edwell et al., 2004) and non hormonal substances such as lugol's

iodine, ovarian and uterine massage (Edwell et al., 2004). Since treatment with hormone is costly and not free from detrimental effects on reproductive health, investigations for alternative therapy are justifiable. Therefore the present study was proposed to observe the effect of vitamin A, phosphorus, and genital massage in different combinations on different reproductive and serum biochemical parameters in anoestrus crossbred cows.

### 2. Materials and Methods

#### 2.1. Animals, ration and experimental design

This study was conducted at private dairy cows farm in and around Khanapara area of Guwahati, India. Forty commercial Jersey crossbred cows aged between 3 to 6 years old weighing 350-400 kg average body weight were used in the present study. All the animals were fed green fodder, hay, compounded concentrate and mineral mixture as per the standard feeding schedule on the farm. These dairy cows had BCS  $\geq 3$  [37]. All animals were in healthy condition and kept under strict control measures for internal and external parasitism, as they undergo a periodical deworming and prophylactic vaccination against the endemic diseases. A full case history of each animal was recorded. The animals were apparently health good. Those



animals did not show any visible signs of oestrus after day 90 parturition were selected for the study. All cows in the present study were subjected to rectal examination of genitalia twice weekly for three successive weeks to follow up the reproductive status of each animal. According to the ovarian findings, the cows without any structure on their ovaries (true anoestrus) were selected.

Treatment cows without any structure on their ovaries (true anoestrus). Cows (n=20) had smooth structureless ovaries without CL. They were diagnosed as true- anoestrus. The forty animals were split equally into four groups.

Group 1 (n=10): Cows were treated with a combination of 6 lakh IU Veta A (TTK Healthcare Limited, Vijayawada) and 5 ml Tonophosphan (Intervet India Pvt. Ltd, Pune) injection given intramuscularly at alternate days for five occasions, group II (n=10): cows were received intramuscular injections of 6 lakh IU Veta A (vitamin A) and 5 ml Tonophosphan at alternate days for five occasions. In addition genital organs were massaged on the days of treatment.

#### 2.2. *Estrus evaluation and fertility traits*

Response of different animals groups to various treatments was evaluated. Cows were observed twice daily at a 12 hr interval by experienced herdsman for at least one hour for estrous signs, especially the acceptance of cows to the bull, mounting, bellowing, restless and sniffing of external genitalia. The day at which the female stand to be mounted was considered the day of estrus. The females were checked for pregnancy 90 days after artificial insemination by palpation per rectum. Fertility measures including the number of animal responded the mean interval between initiation of treatment and onset of oestrus and conception rates were recorded.

#### 2.3. *Biochemical analysis*

Blood samples for biochemical analysis of serum calcium, inorganic phosphate, total protein, and total cholesterol were simultaneously collected from jugular vein into test tubes on day 0, 10, 20, 30 of experiment. The collected blood was immediately centrifuged and serum samples transferred into sterilized test-tubes and stored at -20°C until assayed. Serum calcium, serum inorganic phosphorous, total protein and total cholesterol were estimated with the diagnostic kit (Crest Biosystems, Goa).

#### 2.4. *Statistical analysis*

Data were expressed as mean±SEM. The data were analyzed statistically by ANOVA method and student's 'test' was used to detect differences among means using SPSS 15 (SPSS, Chicago, IL, USA). Reproductive performance percentages were analyzed by the Chi-square test.

### 3. Results and Discussion

The problem of postpartum infertility is one of the well known drawbacks in cattle production, resulting in substantial financial losses due to prolongation of the service period and culling (Bailey et al., 1999). Group 2 had significantly ( $p<0.05$ ) shorter onset of estrus and increased the incidence of estrus and conception rate in comparison to the other treatment group. This result is in agreement with some earlier studies (Mathur et al., 2005) in cow and buffaloes (Zaabel et al., 2009). This indicates that group 2 treatment of smooth inactive ovary in cows could be profitable.

In the present study intramuscular injections of vitamin A and Tonophosphan might have corrected true anoestrus condition in the experimental cows of group I and II by stimulating gonadal and genital activities and thus promoting the secretion of gonadotropic hormones through hypothalamo-pituitary-gonadal-axis. The present findings gained support from the observation of Palmer et al. (1941) who stated that ovarian functions are interfered with phosphorus deficiency through a decrease in secretion of FSH. Moreover phosphorus and Vitamin A deficiency is known to be frequently associated with poor follicular development, atresia, postpartum anoestrus condition and impaired fertility in cows (Sane, 1994).

Higher response was obtained in anoestrous crossbred cows of group II in respect of induction of oestrus and conception as compared to that in group I, which might be due to additional genital massage resorted to in the animals of treatment group II. Positive response with genital massage in treating anoestrus condition along with other drug combination was reported by Zaabel et al. (2009). Massage of the genital organs might have caused stimulation of the anterior pituitary that resulted in release of gonadotropic hormones which could help in folliculogenesis through the utero-pituitary-ovarian-axis.

#### 3.1. *Interval between initiation of treatment and onset of oestrus*

Almost similar interval for induction of oestrus in postpartum anoestrous cows with the treatment combination of vitamin A and Tonophosphan was reported by Kumar (1986) in buffalo cows (22.30 days) and Mathur et al. (2005) in Sahiwal cows (21.00±6.43 days). Although the mean interval between treatment and induced oestrus was found to vary non-significantly between the groups (Table 1), the interval was shorter in group II which indicated that the efficacy of genital massage rendered in that group. Critical difference test revealed that there was earlier occurrence of post treatment oestrus in group I than group II. Earlier induction of oestrus in group I could be attributed to influence of genital massage used on gonadotropic hormone activity.



### 3.2. Signs of oestrus

In the present study, the various signs of oestrus recorded in the crossbred cattle at induced oestrus were mounting, bellowing, restlessness, sniffing of external genitalia, reduced milk yield and loss of appetite (Table 2). Bellowing and restlessness were two most prominent signs of oestrus in the groups of cows that responded treatment. The frequency of occurrence of bellowing and restlessness was found highest in the cows of group II (85.71% and 100% respectively) followed by group I cows (83.33% and 83.33% respectively). Other signs of oestrus viz., mounting, sniffing of external genitalia, reduced milk yield and loss of appetite were observed comparatively in lesser percentages in both the groups in comparison with the two premier signs of oestrus viz., bellowing and restlessness. However, reduced milk yield was exhibited by moderately higher percentages of cows in II (71.43) than in group I (66.67) cows.

Higher frequencies of occurrence of the signs of oestrus were observed in the cows of group II treated with the combination of vitamin A, Tonophosphan and genital massage followed by the cows of group I treated with vitamin A and Tonophosphan which could be due to the incorporation of additional genital massage which had stimulated the hypothalamo pituitary ovarian axis which could cause difference in endocrine status. The signs of oestrus observed in the present study in the two treatment groups appeared to be physiological and comparable with the classical signs of oestrus as stated by McDonald (1977).

### 3.3. Biochemical analysis

#### 3.3.1. Serum calcium

The mean serum calcium level was found to be the lowest on day 0 of treatment among all the days of observation in anoestrous crossbred cows of the two groups that responded to treatment (Table 3). Similar concentration of serum calcium was reported by Yadav et al. (2004). Low serum calcium level in the postpartum anoestrous crossbred cows could be consequential to drainage of calcium during lactation (Pareek and Deen, 1985), since calcium played a vital role in GnRH stimulation for the release of LH from pituitary cells, the concentration of which is required to rise for approaching oestrus (Wagner, 1980). Release of LH was known to be prevented in absence of calcium or in the presence of calcium blocking agents (Hurley

and Doane, 1989). Low level of calcium was found to be associated with reduced steroidogenesis in the ovaries leading to the condition of anoestrus in cows (Shemesh et al., 1984).

Analysis of variance showed that there was significant difference ( $p < 0.01$ ) in the serum calcium level between the days of observation in the two groups (Table 3). Critical difference test revealed no significant difference in mean serum calcium level between day 0 and day 10 of treatment in both the experimental groups. Serum calcium level increased significantly ( $p < 0.01$ ) on day 20 of treatment as compared to day 10 in the two groups. The highest level of serum calcium was obtained on the day of induced oestrus in both the treated groups, which was significantly higher ( $p < 0.01$ ) than that on day 20. Increasing trend of serum calcium concentration after treatment with significant difference between the condition of anoestrus and oestrus was also reported by Hazarika (2001) in cows. However, non-significant increase in the serum calcium level of post partum cattle following treatment was reported by Shah et al. (2003). The highest level of serum calcium in the cows of different groups at induced oestrus in the present study might be due to increased level of estrogen which mobilized calcium from different body depots to blood that is necessary for genital tract contraction (Hafez, 1987). The serum calcium level obtained in the cows of different groups at induced oestrus was almost similar to that of Yadav et al. (2004).

Although there was no significant difference in mean serum calcium level on different days of observation between the treatment groups, it was slightly higher in group II on day 20 of treatment and the day of induced oestrus. This might explain better response in inducing oestrus and earlier induction of oestrus in group II cows treated with vitamin A, Tonophosphan along with genital massage. Sarkar et al. (1990) reported that serum calcium concentration was increased following Tonophosphan therapy due to mobilization of calcium from bone by the stimulation of the parathyroid gland.

In the animals that did not respond to treatment for induction of oestrus the mean serum calcium level showed non-significant difference between the days of observation in both the groups (Table 3). This might imply individual differences to treatments applied in both the groups that led to non-significant gradual rise in serum calcium level during the period of observation. In the non-responded cows, the calcium level in the circulation

Table 1: Effect of different treatment protocols on the estrus evaluation and fertility traits (mean±S.E.) in crossbred cows

Group	Treatment applied	No. of cows treated	No. of cows responded	No. of cows Conceived	Interval (days) between initiation of treatment and onset of oestrus
I	Vitamin A+Tonophosphan	10	6 (60.00%)	3 (50.00%)	21.17±1.08 (18-25)
II	Vitamin A+Tonophosphan +Genital massage	10	7 (70.00%)	4 (57.14%)	19.86±1.10 (15-23)

might not have reached optimal level essential for showing oestrus symptoms, as calcium is necessary to sensitize the tubular genitalia for the action of hormones (Moddie, 1965).

3.3.2. Serum inorganic phosphorus

The concentration of the mean serum inorganic phosphorus was found to be the lowest on day 0 of treatment among all the days of observation in anoestrous crossbred cows of all the groups that responded to treatment (Table 4). Similar figures for serum concentration of phosphorus were recorded by Ramakrishna (1997). The low level of serum inorganic phosphorus in the anoestrous crossbred cows obtained in the present study might be due to secretion of large quantity of phosphorus into milk in lactating cows and the resulting exhaustion of phosphorus reserve which might cause anoestrus. Association of low blood phosphorus level and anestrus condition has been documented (Dindorkar and Kohli, 1979). Lower level of phosphorus might be a contributing factor in inhibiting the anterior pituitary resulting in suppression of ovarian activity (Behera et al., 1993). Marginal deficiency of phosphorus in serum might cause disturbance in pituitary-ovarian axis without manifestation of

deficiency symptoms (Das et al., 2002).

Analysis of variance showed that the level of inorganic phosphorus varied significantly between the days of observation in the two groups (Table 4). Critical difference test revealed that the value was significantly higher ( $p < 0.01$ ) on day 20 of treatment as compared to day 0 in both the groups. The serum phosphorus concentration was significantly higher ( $p < 0.01$ ) on the day of induced oestrus as compared to that on day 20 of treatment in both the groups. The significantly higher inorganic phosphorus level on day 20 of treatment in comparison with that on day 0 in both the groups might be due to use of therapy. Similar increasing pattern in phosphorus level after treatment was also reported by Shah et al. (2003). Rise in estrogenic activity as a result of stimulation due to massage of genitalia might explain significantly higher level of serum inorganic phosphorus in group II on day 20 of treatment than that on anoestrus state on day 0. Significantly elevated concentration of serum inorganic phosphorus obtained on the day of induced oestrus in both the groups might reflect a concomitant increase of the mineral level with increase in estrogen concentration during oestrus which was responsible for increased phosphorus absorption and retention during reproductive phase (Mc Dowell, 1985). The level of serum inorganic phosphorus at induced oestrus in cows recorded in the present investigation was comparable with that reported by Das et al. (2002).

In the non-responded anoestrous cows the mean serum inorganic phosphorus level increased gradually but non-significantly (Table 4) from day 0 of treatment during all the days of observation in both the treatment groups, which might be due to difference in individual physiological status of animals. Similar non-significant increase in phosphorus level was also reported in the non-responded anoestrous heifers after treatment by Behera et al. (1993).

Table 2: Frequency of occurrence of different signs of oestrus in different groups of anoestrous crossbred cows responded to treatment

Signs of oestrus	Frequency of occurrence (%)	
	Group I (n=6)	Group II (n=7)
Mounting	50.00 (3)	57.14 (4)
Bellowing	83.33 (5)	85.71 (6)
Restlessness	83.33 (5)	100.00 (7)
Sniffing of external genitalia	33.33 (2)	57.14 (4)
Reduced milk yield	66.67 (4)	71.43 (5)
Loss of appetite	50.00 (3)	57.14 (4)

Table 3: Level of serum calcium (mean±S.E.) at different days of observation in different groups of anoestrous crossbred cows

Group	Respond				Non-respond			
	Day 0	10	20	Day of induced oestrus	Day 0	10	20	Day of induced oestrus
I	10.32 <sup>a</sup> ±0.06	10.47 <sup>a</sup> ±0.06	10.72 <sup>b</sup> ±0.11	11.10 <sup>c</sup> ±0.09	10.15±0.09	10.19±0.10	10.23±0.10	10.26±0.10
II	10.37 <sup>a</sup> ±0.10	10.52 <sup>a</sup> ±0.10	10.90 <sup>b</sup> ±0.09	11.15 <sup>c</sup> ±0.09	10.20±0.07	10.25±0.08	10.28±0.08	10.32±0.07

Figures with same superscript (a, b, c) do not differ significantly ( $p < 0.01$ ) in rows within the responded group

Table 4: Level of serum inorganic phosphorus (mean±S.E.) at different days of observation in different groups of anoestrous crossbred cows

Group	Respond				Non- Respond			
	Day 0	10	20	Day of induced oestrus	Day 0	10	20	Day of induced oestrus
I	4.53 <sup>a</sup> ±0.18	4.74 <sup>ab</sup> ±0.19	4.96 <sup>b</sup> ±0.21	5.52 <sup>c</sup> ±0.23	4.28±0.08	4.34±0.07	4.37±0.07	4.40±0.08
II	4.56 <sup>a</sup> ±0.16	4.76 <sup>ab</sup> ±0.15	4.99 <sup>b</sup> ±0.25	5.56 <sup>c</sup> ±0.15	4.31±0.10	4.38±0.11	4.40±0.12	4.44±0.12

Figures with same superscript (a, b, c) do not differ significantly ( $p < 0.01$ ) in rows within the responded group

### 3.3.3. Serum total protein

The mean serum total protein level of the responded anoestrous crossbred cows on day 0 of treatment was found to be lower than that of the other days of observation in both the groups (Table 5). Present findings in the anoestrous cows are in agreement with the level of serum total protein reported by Vhora et al. (1995) in anoestrous crossbred cows. Lower blood protein concentration was reported to be associated with anoestrus condition in cows (Sane et al., 1994) which could be due to resultant lesser functioning of pituitary and reproductive organs (Maynard, 1979). The lower level of serum protein might cause deficiency of certain amino acids required for synthesis of gonadotropin, thereby causing reproductive disturbance of failure of oestrous cycle (Vhora et al., 1995). Production of higher quantum of milk in the experimental cows that required higher level of protein might affect reproduction during post partum period.

After treatment the mean level of serum total protein on day 20 was found to be significantly higher ( $p < 0.01$ ) than that of anoestrous cows on day 0 of treatment in both the groups (Table 5). This could be due to positive effect of treatment since administration of vitamin A and phosphorus might improve serum protein level as depletion of vitamin A, phosphorus and protein in blood was interrelated (Roberts, 1971). It was observed that the level of serum total protein increased gradually and reached the peak level on the day of induced oestrus in all the responded cows of both groups. Similar increasing trend was also reported by Shah et al. (2003). Serum total protein level on the day of induced oestrus was found to be significantly higher ( $p < 0.01$ ) than that of day 20 of treatment in both the groups. Significantly higher level of serum total protein during the oestrous period of the experiment might be one of the contributory factors causing rising of estrogen level resulting in induction of oestrus. Synthesis of steroid was

found to be related to energy status of animals (Hafez, 1987). A certain amount of optimal protein level was necessary for expression of oestrus in cows (Zaabel et al., 2009).

Although mean level of the non-responded anoestrous cows did not vary significantly between the days and between the treatments groups it was observed that there was a gradual increase of serum total protein level during the whole period of investigation in both the groups (Table 5). However, the marginal rise in serum total protein level was not enough to bring about an increase in the synthesis of gonadotropin and thus not associated with exhibition of oestrus. The non-significant rise in serum total protein level following treatment on different days of observation in both the groups with higher level in group II might be attributed to genital massage owing to inherent difference in endocrine status.

### 3.3.4. Serum cholesterol

In the present study, the mean serum cholesterol concentration in both the groups of responded anoestrous crossbred cows (Table 6) on day 0 of treatment was found to be least in comparison with the values obtained on the other days of observation. Low cholesterol level in the anoestrous cows on day 0 in all the groups might be correlated with energy status of the animals since subnormal energy status could induce hypocholesterolemia (Pareek and Deen, 1985). Velhankar (1973) also reported a positive correlation between blood cholesterol concentration and energy status of the animals. Low serum cholesterol concentration in all the groups of cow on day 0 of treatment also could be due to higher progesterone level during anoestrus state because of more conversion of cholesterol to progesterone.

Analysis of variance (Table 6) showed significant difference ( $p < 0.01$ ) in serum cholesterol level between different days of observation in both the groups as the level registered an

Table 5: Level of serum total protein (mean±S.E.) at different days of observation in different groups of anoestrous crossbred cows

Group	Respond				Non-respond			
	Day 0	10	20	Day of induced oestrus	Day 0	10	20	Day of induced oestrus
I	7.08 <sup>a</sup> ±0.15	7.32 <sup>ab</sup> ±0.15	7.62 <sup>b</sup> ±0.15	8.16 <sup>c</sup> ±0.18	6.81±0.16	6.83±0.16	6.86±0.16	6.89±0.16
II	7.16 <sup>a</sup> ±0.12	7.38 <sup>ab</sup> ±0.13	7.71 <sup>b</sup> ±0.09	8.21 <sup>c</sup> ±0.11	6.87±0.18	6.92±0.19	6.95±0.19	6.99±0.19

Figures with same superscript (a, b, c) do not differ significantly ( $p < 0.01$ ) in rows within the responded group

Table 6: Level of serum cholesterol (mean±S.E.) at different days of observation in different groups of anoestrous crossbred cows

Group	Respond				Non-respond			
	Day 0	10	20	Day of induced oestrus	Day 0	10	20	Day of induced oestrus
I	185.40±3.43	199.87±4.85	213.10±1.88	243.16±3.29	170.98±4.09	171.95±3.95	173.31±3.89	175.86±3.99
II	187.68±2.48	202.36±3.01	218.58±3.98	244.58±3.52	172.82±1.70	174.85±2.23	177.05±1.96	178.80±1.84

Figures with same superscript (a, b, c, d) do not differ significantly ( $p < 0.01$ ) in rows within the responded group

increasing trend with increase in the period after treatment. Increase in the level of serum cholesterol after treatment was also observed in anoestrous cows that responded to treatment by Prasad et al. (1984). The serum cholesterol level was observed to be highest on the day of induced oestrus in both the groups which might be due to low progesterone concentration at oestrus because of minimal conversion of serum cholesterol to progesterone. Ahmed (1998) also reported significant higher level of serum cholesterol at induced oestrus than anoestrus in crossbred cows. Tandle et al. (1997) observed higher serum cholesterol level in cows during estrogen dominance at oestrus.

In the anoestrous crossbred cows that did not respond to treatment gradually increased mean serum cholesterol level was obtained on day 10, 20 and 30 of treatment although the increase was non-significant (Table 6). Similar non-significant post treatment increase in serum cholesterol level of anoestrous crossbred heifers was reported by Behera et al. (1993). The mean serum cholesterol level obtained in the non-responded cows of both the groups on different days of observation was lower than that found in anoestrous cows that responded to treatment. This might indicate difference in physiological status between two categories of animals, which could suggest that non-responded cows of the groups would require a different dosage of treatment regime. Present findings were also suggestive of lower energy status in anoestrous cows that did not respond to treatment.

#### 4. Conclusion

Based on percentage and interval of induction of oestrus, conception rate, signs of oestrus and concentration of serum calcium, inorganic phosphorous, total protein and cholesterol at induced oestrus, treatment of anoestrous crossbred cows with vitamin A and Tonophosphan was found to be effective. The efficacy of the treatment was higher when it was coupled with genital massage.

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