



Original Research

Comparative Studies on Haemato-Biochemical Parameters of Anoestrus Crossbred Cows Treated with Different Therapeutic Regimen

Vinita Mangrole*, Anavil Bhardwaz, Ranjit Aich, Madhu Shivhare, Sudarshan Kumar and Ritika Mandhwani

Department of Veterinary Gynaecology & Obstetrics and Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, Mhow, Indore, Madhya Pradesh, INDIA

*Corresponding author: vinitamangrolevet@gmail.com

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Abstract

The present study was conducted on 24 crossbred cows with history of postpartum anoestrus randomly divided into four treatment groups: Clomiphene citrate (Gp I), GnRH analogue (Gp II), GnRH analogue + mineral mixture (Gp III) and Clomiphene citrate + mineral mixture (Gp IV). The mean Hb value (g/dl) was found significantly ($p < 0.05$) higher during post-treatment (induced oestrus) period in Group II, III and IV. The mean value of neutrophil was significantly ($p < 0.05$) higher during pre-treatment period (anoestrus). The mean value of lymphocyte (%), PCV (%) and serum calcium (mg/dl) was significantly higher ($p < 0.05$) during post-treatment period in all treatment groups as compared to pre-treatment values. The mean value of total protein (g/dl) was found significantly ($p < 0.05$) higher during post-treatment period in Group I and III.

Key words: Clomiphene Citrate, GnRH Analogue, Mineral Mixture, Postpartum Anoestrus

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Introduction

Livestock rearing is one of the most important economic activities in the rural areas as it provides supplementary income for most of the families dependent on agriculture. Anoestrus is the most common single cause of infertility in cattle. Postpartum anoestrus is the period after parturition during which cows do not show behavioral signs of oestrus. Post-partum anoestrus in cows occurs mainly due to nutritional deficiency and poor management of the cows but hormonal imbalance may also aggravate this condition (Hussain *et al.*, 2009). Productive and reproductive performance is one of the important factors for





determining the profitability of the dairy farmers. Subfertility in cows is an important hindrance to dairy farmers (Jainuddin and Hafez, 1993). The knowledge of haematological and biochemical values is useful in diagnosing various pathological and metabolic disorders, which can adversely affect the productive and reproductive performance of cows, resulting in great economic losses to dairy farmers (Dutta *et al.*, 1988). Normal levels of biochemical constituents are of utmost importance for maintaining the functional integrity of the reproductive system (Niazi *et al.*, 2003). The minerals also play an intermediate role in the action of hormones and enzymes at the cellular level in an integrated fashion. Calcium works as a cofactor or activator of various enzyme systems. Disturbance in calcium and phosphorus ratio has been associated with subnormal fertility and anoestrus conditions (Moddie, 1965).

Materials and Methods

The study was carried out on clinical cases brought to the Teaching Veterinary Clinical Complex of College of Veterinary Science and A.H., Mhow and in villages adapted for college ambulatory clinics.

Experimental Design

The postpartum anoestrus dairy cows (n=24) were equally divided into four groups on the basis of history and gynaeco-clinical examination. The cows were apparently healthy with history of postpartum anoestrus for more than 60 days. These cows were subjected to gynaeco-clinical examination twice with an interval of 10 days.

Group I

In this group (n=6) cows were administered Clomiphene citrate (Ovulanta-Kit) orally @ 300 mg daily for five days. Treatment was discontinued if cow exhibited oestrus earlier than 5 days.

Group II

The cows in this group (n=6) were treated with GnRH analogue (Buserelin acetate) @ 0.0042 mg (5ml) intramuscularly once only as single dose.

Group III

The cows in this group (n=6) were supplemented with mineral mixture powder (Agrimine-Fort) @ 30 gm orally daily for 20 days and treated with GnRH analogue (Receptal VET) @ 0.0042 mg (5ml) intramuscular on 7th day of mineral mixture supplementation.

Group IV

The cows in this group (n=6) were supplemented with mineral mixture powder @ 30 gm orally daily for 20 days and Clomiphene citrate orally @ 300 mg daily for first five days. Oestrus was detected by visual observation twice a day in morning and evening and confirmed by heat detector [Vaginal Electrical Impedance (VEI)].



Collection of Blood Sample

7 ml of blood from each cow was collected aseptically from jugular vein in sterile vials on the day of treatment and on subsequent oestrus. The sample was divided into two parts, 1st part for haematological examination containing anticoagulant (EDTA @ 2 mg/ml) and 2nd part was allowed to clot in test tube for serum separation and stored in a sterilized vial at -20°C until biochemical analysis.

Haematological Parameters

The following haematological parameters were estimated-

1. Haemoglobin (g/dl) by Sahli's acid haematin method
2. Differential leukocyte count (percentage) by Wright stain
3. Packed cell volume (percentage) by microhaematocrit method

Biochemical Parameters

1. Total protein (gm/dl) estimated by Biuret method (Burtis *et al.*, 2012)
2. Inorganic phosphorus (mg/dl) analyzed by Modified Metol method (Gomori, 1942)
3. Serum calcium (mg/dl) analyzed by OCPC method (Moorhead and Briggs, 1974)

These biochemical parameters were analyzed with semiautomatic analyzer.

Statistical Analysis

Analysis was done by the standard statistical method Completely Randomized Designs (CRD) described by Snedecor and Cochran (1994).

Result and Discussion

Haematological Parameters

Haemoglobin (g/dl)

Hb levels during anoestrus (pre-treatment) was recorded to be 9.03 ± 0.23 , 9.76 ± 0.43 , 9.4 ± 0.17 and 9.7 ± 0.29 g/dl in Group I, II, III and IV, respectively. The respective values during oestrus (post-treatment) were 9.73 ± 0.37 , 10.65 ± 0.19 , 10.5 ± 0.20 and 10.53 ± 0.09 g/dl (Table 1). The level of Hb was significantly higher during oestrus in Group II, III and IV however, non-significantly differ in anoestrus and on the day of oestrus in Group I at ($p < 0.05$). The overall mean values in pre-treatment and post-treatment period were 9.47 ± 0.29 and 10.35 ± 0.21 g/dl, respectively. The present findings are slightly comparable with Ahmad *et al.* (2003) who stated that Hb concentration was slightly lower (9.26 ± 0.34) at anoestrus as compared to cyclic crossbred cows (12.4 ± 0.44). The Hb values in Clomiphene citrate group are in agreement with Bawaskar (2004) who stated that Hb concentration non-significantly differ in anoestrus cows before and after treatment with Fervivet.

The difference in Hb value between pretreated and post treated cows can be concluded that lower level of haemoglobin might be representing some systemic disarrangement due to deficiencies of certain trace minerals which in turn has depressed the physiological reproduction. Though the important of the level of haemoglobin has not been directly implicated in reproductive disorders, yet the decrease in its value is indicative of certain systemic disorder which can indirectly affect the functional activity of the reproductive organ.

Table 1: Haemoglobin and PCV values in anoestrus and induced oestrus crossbred cows (Mean $\pm$ S.E.)

Group No.	Haemoglobin (gm %)		Packed Cell Volume (%)	
	Anoestrus	Induced oestrus	Anoestrus	Induced oestrus
I	9.03 $\pm$ 0.28 ^a	9.73 $\pm$ 0.37 ^a	27.1 $\pm$ 0.84 ^b	30.2 $\pm$ 1.40 ^a
II	9.76 $\pm$ 0.43 ^b	10.65 $\pm$ 0.19 ^a	30.13 $\pm$ 0.78 ^b	33.46 $\pm$ 1.17 ^a
III	9.4 $\pm$ 0.17 ^b	10.5 $\pm$ 0.20 ^a	28.83 $\pm$ 0.26 ^b	31.5 $\pm$ 0.6 ^a
IV	9.7 $\pm$ 0.29 ^b	10.53 $\pm$ 0.09 ^a	29.56 $\pm$ 0.62 ^b	32.8 $\pm$ 0.68 ^a
Overall Mean	9.47 $\pm$ 0.29	10.35 $\pm$ 0.21	28.90 $\pm$ 0.62	31.99 $\pm$ 0.96

Mean with similar superscript do not differ significantly from each other at ($p < 0.05$)

Packed Cell Volume (%)

In the present study, the PCV level was 27.1 $\pm$ 0.84, 30.13 $\pm$ 0.78, 28.83 $\pm$ 0.26 and 29.56 $\pm$ 0.62 percent during anoestrus and 30.2 $\pm$ 1.40, 33.46 $\pm$ 1.17, 31.5 $\pm$ 0.61 and 32.8 $\pm$ 0.68 percent on the day of oestrus in Group I, II, III and IV, respectively (Table 1). The overall mean of PCV was 28.90 $\pm$ 0.62 and 31.99 $\pm$ 0.96 in anoestrus and induced oestrus, respectively. Significantly ($p < 0.05$) higher value of PCV was observed in oestrus cows as compared to anoestrus cows in all treatment groups. The present findings are in agreement with Ahmad *et al.* (2003) and Majumder (2014) who reported 27.72 $\pm$ 0.84 and 29.7 $\pm$ 0.59 percent PCV during anoestrus. Ahmad *et al.* (2003) observed PCV level 28.76 $\pm$ 0.66 percent during oestrus.

Differential Leukocyte Count (%)

Neutrophil

The neutrophil count during anoestrus (pre-treatment) state was 41.16 $\pm$ 0.94, 39.83 $\pm$ 0.74, 39.33 $\pm$ 0.49 and 40.16 $\pm$ 1.01 percent in Group I, II, III and IV, respectively. The respective values during oestrus (post-treatment) were 35.5 $\pm$ 1.25, 35.66 $\pm$ 0.66, 34.33 $\pm$ 1.30 and 34.33 $\pm$ 0.95 percent (Table 2). The overall mean value of neutrophil during anoestrus and induced oestrus was 40.12 $\pm$ 0.79 and 34.95 $\pm$ 1.04 percent, respectively. Significantly higher ($p < 0.05$) values of neutrophil was observed during anoestrus as compared to oestrus in all treatment groups.

Table 2: DLC (%) in pre-treatment and post-treatment period (Mean $\pm$ S.E.)

Groups	Differential Leukocyte Count (%)							
	Neutrophil		Lymphocyte		Monocyte		Eosinophil	
	Anoestrus	Induced oestrus	Anoestrus	Induced oestrus	Anoestrus	Induced oestrus	Anoestrus	Induced oestrus
I	41.16 $\pm$ 0.94 ^a	35.5 $\pm$ 1.25 ^b	48.66 $\pm$ 0.55 ^b	53.16 $\pm$ 1.04 ^a	4.16 $\pm$ 0.60 ^a	3.83 $\pm$ 0.70 ^a	6.33 $\pm$ 0.66 ^a	7.50 $\pm$ 0.34 ^a
II	39.83 $\pm$ 0.74 ^a	35.66 $\pm$ 0.66 ^b	50.16 $\pm$ 0.40 ^b	54.16 $\pm$ 0.94 ^a	3.66 $\pm$ 0.49 ^a	4.50 $\pm$ 0.22 ^a	6.34 $\pm$ 0.66 ^a	5.66 $\pm$ 0.33 ^a
III	39.33 $\pm$ 0.49 ^a	34.33 $\pm$ 1.30 ^b	50.83 $\pm$ 0.30 ^b	53.5 $\pm$ 1.25 ^a	3.66 $\pm$ 0.42 ^a	5.0 $\pm$ 0.51 ^a	6.16 $\pm$ 0.40 ^a	5.50 $\pm$ 0.50 ^a
IV	40.16 $\pm$ 1.01 ^a	34.33 $\pm$ 0.95 ^b	51.0 $\pm$ 0.36 ^b	55.66 $\pm$ 0.66 ^a	3.33 $\pm$ 0.42 ^a	4.50 $\pm$ 0.88 ^a	7.16 $\pm$ 0.79 ^a	5.60 $\pm$ 0.61 ^a
Overall mean	40.12 $\pm$ 0.79	34.95 $\pm$ 1.04	50.16 $\pm$ 0.40	54.12 $\pm$ 0.97	3.70 $\pm$ 0.48	4.45 $\pm$ 0.57	6.08 $\pm$ 0.55	6.48 $\pm$ 0.51

Mean with similar superscript do not differ significantly from each other at ($p < 0.05$)

Lymphocyte

Lymphocyte count during anoestrus (pre-treatment) was recorded to be 48.66 ± 0.55 , 50.16 ± 0.40 , 50.83 ± 0.30 and 51.0 ± 0.36 percent in Group I, II, III and IV, respectively. The respective values during oestrus (post-treatment) were 53.16 ± 1.04 , 54.16 ± 0.94 , 53.5 ± 1.25 and 55.66 ± 0.66 percent (Table 2). The overall mean value of lymphocyte during anoestrus and induced oestrus was 50.16 ± 0.40 and 54.12 ± 0.97 percent, respectively. Significantly higher ($p < 0.05$) lymphocyte count was observed during oestrus as compared to anoestrus cows in all treatment groups.

Monocyte

Monocyte count during anoestrus (pre-treatment) was recorded to be 4.16 ± 0.60 , 3.66 ± 0.49 , 3.66 ± 0.42 and 3.33 ± 0.42 percent in Group I, II, III and IV, respectively. The respective values during oestrus (post-treatment) were 3.83 ± 0.70 , 4.50 ± 0.22 , 5.0 ± 0.51 and 4.50 ± 0.88 percent (Table 2). Overall mean values of monocyte count was recorded to be 3.70 ± 0.48 and 4.45 ± 0.57 percent during anoestrus and induced oestrus, respectively. No significant difference was recorded between anoestrus and induced oestrus monocyte percentage.

Eosinophil

Eosinophil count during anoestrus (pre-treatment) was recorded to be 6.33 ± 0.66 , 6.34 ± 0.66 , 6.16 ± 0.40 and 5.50 ± 0.50 percent in Group I, II, III and IV, respectively. The respective values during oestrus (post-treatment) were 7.50 ± 0.34 , 5.66 ± 0.33 , 7.16 ± 0.79 and 5.60 ± 0.61 percent (Table 2). Overall mean values of eosinophil count was recorded to be 6.08 ± 0.55 and 6.48 ± 0.51 percent during anoestrus and induced oestrus, respectively. Statistically no significant difference was recorded between anoestrus and induced oestrus values.

In the present study, the mean value of neutrophil are in agreement with Singh and Singh (2006) who recorded 41.67 ± 2.45 and 32.33 ± 1.88 percent neutrophil in anoestrus and oestrus condition, respectively.

Bawasker (2004) and Majumder (2014) recorded 34 ± 1.23 and 33.1 ± 0.8 percent neutrophil, 59.5 ± 1.31 and 57.0 ± 1.22 percent lymphocyte, 4.66 ± 0.55 and 4.25 ± 0.25 percent eosinophil and 2.05 ± 0.4 and 5.0 ± 0.40 percent monocyte in anoestrus group, in which mean value of neutrophil is lower and mean value of lymphocyte is higher than the present study. The significantly higher ($p < 0.05$) percentage of neutrophils were recorded in postpartum anoestrus cows as compared to induced oestrus cows indicated that animals in postpartum anoestrus group were suffering from neutrophilia indicating infection.

Biochemical Parameters

Total Protein (g/dl)

In present study, the total protein values was 6.96 ± 0.24 , 6.43 ± 0.63 , 5.83 ± 0.38 and 6.68 ± 0.38 g/dl during anoestrus in Group I, II, III and IV, respectively. The respective values during induced oestrus were 8.25 ± 0.18 , 7.30 ± 0.61 , 7.52 ± 0.44 and 7.73 ± 0.37 g/dl (Table 3). The overall mean of total protein was 6.47 ± 0.40 and 7.7 ± 0.40 g/dl in anoestrus and induced oestrus, respectively. The serum total protein was significantly lower ($p < 0.05$) in anoestrus cows as compared to oestrus cows in Group I and III however, no significant difference found in Group II and IV. The present findings are in close approximation with Yadav *et al.* (2004) who also observed similar concentration of total protein in anoestrus (6.18 ± 0.18 g/dl) and oestrus cows (7.30 ± 0.24 g/dl). Lower levels of total protein in cows than the present study was reported by Pariza *et al.* (2013) who observed 3.4 ± 0.8 and 5.2 ± 0.8 g/dl total protein in anoestrus and cyclic cows, respectively. The differences in total protein levels in anoestrus and induced oestrus may be due to variation in environment, level of nutrition and health status of animals. Excessive intake of protein in the feed can reduced the fertility and increases the number of services per conception.

Serum Calcium (mg/dl)

In the present study, the serum calcium level was 8.44 ± 0.37 , 9.12 ± 0.23 , 8.66 ± 0.34 and 9.40 ± 0.23 mg/dl during anoestrus and 9.88 ± 0.35 , 10.41 ± 0.33 , 10.19 ± 0.32 and 10.91 ± 0.10 mg/dl on the day of oestrus in Group I, II, III and IV, respectively (Table 3). The overall mean value of serum calcium during anoestrus and induced oestrus was 8.90 ± 0.29 and 10.34 ± 0.27 mg/dl, respectively. On statistical analysis, significantly higher ($p < 0.05$) value of serum calcium was observed in oestrus as compared to anoestrus cows in all treatment groups. The present study in Group I is in agreement with More (2012) and Nagare (2014) they found the serum calcium level before treatment as 8.41 ± 0.27 and 8.51 ± 0.46 mg/dl and after treatment 9.18 ± 0.30 and 8.56 ± 0.29 mg/dl. The present findings in Group II are in agreement with the More (2012) and Nagare (2014) who found the serum calcium level before treatment was 7.37 ± 0.21 and 8.24 ± 0.30 mg/dl and after treatment 10.02 ± 0.57 and 10.04 ± 0.55 mg/dl. The overall mean value of serum Ca in present findings are in agreement with Ray *et al.* (2016) who observed the serum Ca level as 8.31 ± 0.51 mg/dl in anoestrus and 11.17 ± 0.88 mg/dl in cyclic cows, respectively.

In the present study the significant increase in the calcium level in crossbred cows after treatment (induced oestrus) may be due to supplementation of mineral mixture or due to fluctuating levels of estrogen in oestrus and the lower levels of serum calcium in anoestrus cows might be due to failure of endocrine system to mobilized the body calcium which leads to reproductive failure.

Serum Inorganic Phosphorus (mg/dl)

In the present study, the serum inorganic phosphorus level was 4.7 ± 0.12 , 4.35 ± 0.21 , 4.94 ± 0.18 and 5.11 ± 0.14 mg/dl during anoestrus and 5.36 ± 0.21 , 4.96 ± 0.26 , 5.84 ± 0.35 and 6 ± 0.23 mg/dl on the day of oestrus in Group I, II, III and IV, respectively (Table 3). The overall mean values of serum inorganic phosphorus was 4.77 ± 0.16 and 5.54 ± 0.26 mg/dl during anoestrus and induced oestrus, respectively. On statistical analysis there was no significant difference ($p < 0.05$) was noted in the post-treatment (anoestrus) values of serum inorganic phosphorus over their pre-treatment (induced oestrus) values in all treatment groups. The overall mean value of IP in present study are in agreement with Raj *et al.* (2006) and Mahour *et al.* (2011) observed non-significant difference in serum IP level during anoestrus and induced oestrus cows. The result of present study in Group I are in slight agreement with Nagare (2014) who observed the serum IP level as 3.75 ± 0.44 mg/dl before treatment and 5.82 ± 0.43 mg/dl after treatment with Clomiphene citrate. The result of present study in Group II are in slight agreement with Nagare (2014) who observed the serum IP level as 3.47 ± 0.20 mg/dl before treatment and 6.36 ± 0.44 mg/dl after treatment with GnRH analogue. The overall mean value of IP in present study is comparable with Yadav *et al.* (2004), Ray *et al.* (2016) and Singaram and Jacob (2017) recorded as 4.48 ± 0.06 , 4.31 ± 0.35 and 4.81 ± 0.33 mg/dl during anoestrus condition.

Table 3: Biochemical indices in anoestrus and induced oestrus (Mean $\pm$ S.E.)

Groups	Total Protein (g/dl)		Inorganic Phosphorus (mg/dl)		Serum Calcium (mg/dl)	
	Anoestrus	Induced oestrus	Anoestrus	Induced oestrus	Anoestrus	Induced oestrus
I	6.96 ± 0.24^b	8.25 ± 0.18^a	4.7 ± 0.12^a	5.36 ± 0.21^a	8.44 ± 0.37^b	9.88 ± 0.35^a
II	6.43 ± 0.63^a	7.30 ± 0.61^a	4.35 ± 0.21^a	4.96 ± 0.26^a	9.12 ± 0.23^b	10.41 ± 0.33^a
III	5.83 ± 0.38^b	7.52 ± 0.44^a	4.94 ± 0.18^a	5.84 ± 0.35^a	8.66 ± 0.34^b	10.19 ± 0.32^a
IV	6.68 ± 0.38^a	7.73 ± 0.37^a	5.11 ± 0.14^a	6.0 ± 0.23^a	9.40 ± 0.23^b	10.91 ± 0.10^a
Overall Mean	6.47 ± 0.40	7.70 ± 0.40	4.77 ± 0.16	5.54 ± 0.26	8.90 ± 0.29	10.34 ± 0.27

Mean with similar superscript do not differ significantly from each other at ($p < 0.05$).

Conclusion

Low energy, leukocytosis with neutrophilia, hypoproteinemia and hypocalcaemia may be the possible etiological factors for postpartum anoestrus in crossbred cows. Mineral mixture supplementation for management of postpartum anoestrus is best remedial measures.



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