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Haematological, biochemical and endocrinological profiles in crossbred postpartum anestrus cows under tropical island ecosystem

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ABSTRACT

A study was carried out to assess the effect of postpartum anestrus (PPA) on haematological, antioxidant and endocrinological profiles in crossbred cows under tropical island ecosystem of Andaman and Nicobar Islands. Based on the records, the cows that had shown anestrous for more than 120 days were selected in PPA group while cows coming in estrous before 65 days of postpartum for more than three successive lactations involving present lactation were selected in normal cyclic group (control group). Blood samples were collected, analysed for haematological profile, antioxidant profile such as catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), total antioxidant capacity (TAC) and free radical such as malondialdehyde (MDA), and hormone profiles such as follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol 17β (E2), progesterone (P4), cortisol (CORT) and thyroxin (T4). Crossbred cows of ANI with PPA had suffered severe anaemia. PPA affected cows showed significantly low level of antioxidant profiles and higher MDA level than the unaffected animals. Similarly, endocrinological profiles revealed the PPA affected cows had significantly higher level of CORT and lower level of E2, P4, FSH, LH and T4 than the unaffected crossbred cows. PPA was due to anaemia, lack of antioxidants, over production of free radicals and disturbances of endocrinological profiles. A balanced, limited ration with good quality and quantity of antioxidants, sufficient daily exercise and sufficiently large, clean drinking water can minimize the PPA in crossbred cows.

Keywords: Andaman and Nicobar Islands, Antioxidants, Blood, Crossbred cows, Hormone, Island ecosystem, Physiological profiles, Postpartum anestrus

Reproductive performance is one of the important markers to estimate the profitability of the dairy farm (Lucy 2007). Anestrus in dairy cattle is a kind of infertility broadly defined as absence of estrus expression at the expected time frame. It is more appropriately defined as functionless, quiescent ovaries and reproductive tract. Anestrous is associated with several factors like season, breed, suckling, parity, infection, dystocia, mineral and nutritional deficiency, etc. (Khan *et al.* 2014). Incidence of PPA is very high in high-yielding milch cows (Opsomer *et al.* 2000). The incidence of PPA was reported as 49.70 (Rao 1993), 24.73 (Patel *et al.* 2007), 53.15 (Pandit 2004), 2.13–45.97 (Kulkarni *et al.* 2002) in India.

Haematological and biochemical profiles may differ in normal cycling and anestrous acyclic animals (Geneser 1986). TRBCs and TWBCs increase significantly during estrous phase of the cycle in bovine species. Similarly, Hb reduces in anestrous than in normal cycling buffaloes (Dhoble and Gupta 1981) and cycling cows (Nadiu and Rao 1982). Further, TRBC, Hb and PCV reduce whereas MCV and TWBC increase significantly in anestrus acyclic cows (Kumar and Sharma 1991). Besides, study on the haematological and biochemical profiles is also important in diagnosis and prognosis of healthy and diseased status of the animals.

Alteration of seasonality and nutritional deficiency has resulted into derangement of hormone secretory pattern in milking bovine and bubaline species (Qureshi *et al*. 2000, Khodaei-Motlagh *et al*. 2011). Anestrus condition may be due to insufficient secretion of E2 which plays a key function in expression of the characteristic estrus behaviour and signs. P4 is another key important player in the induction of estrus and potentiates the action of estrogen for manifestation of estrus behaviour. Thyroid gland activity or its secretion is having very essential functions on the reproductive performance which is directly or indirectly associated with infertility in animals.

Oxidative stress markers increase in the dairy animals when they affected with disorders like anestrum, repeat breeding syndrome, cystic ovary and metabolic disorders (Atakisi *et al.* 2010). Antioxidant defense system reduces

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the damage caused by ROS through neutralization or minimization of production of ROS or cessation of free radical chain reaction to keep the animals in healthy condition. During the postpartum period of dairy cows, antioxidants play a significant role in changes in reproductive tract and ovary in cyclic animals which is lacking in anestrus animals (Derar *et al.* 2011) and no study was conducted in crossbred cows in Andaman and Nicobar Islands. Therefore, the present study was undertaken to assess the effect of PPA on haematological, biochemical and endocrinological profiles in crossbred cows under tropical island ecosystem in Andaman and Nicobar Islands.

MATERIALS AND METHODS

Location: Present study was conducted at Guptapara, Indira Nagar and Chouldhuri of South Andaman district, Andaman and Nicobar Islands and is placed in between 6º45′ to 13°41′ North Latitude and in between 92°12′ to 93°57′ East Longitude. Average maximum and minimum temperature were 30.1 and 23°C, respectively. Relative humidity was in range of 82–94% and annual rainfall is >3,100 mm spread over 8 months. Crossbred cows of ANI were maintained in the semi-intensive system where they were allowed for grazing from 0700 to 1200 h.

Experimental animals: Apparently healthy adult crossbred cows (40) of 4–6 year of age with good body condition (score 5–6) were selected from the cattle herd in these villages. They were maintained under hygienic managemental conditions. Prophylactic measures like deworming and vaccination were done as per the schedule.

Experimental procedure: Crossbred cows (20) with PPA and cyclic cows (20) were selected on the basis of their reproductive history obtained from their records. Crossbred cows that had shown anestrous for more than 120 days were selected in postpartum group (PPA) and animal coming in estrous before 65 days of postpartum for more than three consecutive lactations including present lactation were selected in normal cyclic group. The animals selected in the current lactation were having average postpartum anestrous period of 212.76±19.56 days and those in normal cyclic had 62.65±8.78 days. Current status of reproductive organs of all animals in the study was also examined and verified by per rectal examination.

Haematological analysis: Jugular blood sample (10 mL) was collected from each experimental animal in 15 mL sterile polypropylene centrifuge tube containing heparin (20 IU/mL of blood) as anticoagulant. Haematology was carried out using automatic veterinary scan haematological analyzer directly after the samples were received by the research laboratory and within 30 min after samples were collected. Haematological profile such as total red blood cells (TRBC), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), packed cell volume (PCV), total white blood cell (TWBC), lymphocytes, monocytes, neutrophils, eosinophils and platelets was determined.

Biochemical analysis: Plasma was separated in refrigerated centrifuge at 3500×g at 4°C for 10 min and stored in aliquots at –20°C until analysis. Separated plasma was aliquoted into small aliquots in micro cryo-tubes and placed frozen at –20°C until further analysis of biochemical and antioxidant profiles with commercial diagnostic kits. Antioxidant profile such as TAC, GSH, SOD and CAT was estimated with commercial available kit (Cayman, USA) and oxidant profile such as MDA was estimated as per method described by Shah *et al*. (1989). Endocrinological profile such as FSH, LH, E2, P4, cortisol and T4 was measured by commercial available ELISA diagnostic kits (Cayman, USA).

Statistical analysis: Statistical analysis of the data was done as per standard procedures. Student "t" test was conducted to assess the significant difference between the with PPA affected and cyclic crossbred cows SPSS (Version 10) Inc., Chicago, Illinois, USA). Tables present the non-transformed data. Pearson's correlation coefficient was estimated between the experimental parameters. Differences with values of $P<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

PPA affected animals had significantly lower TRBC, Hb, ESR, PCV and TWBC ($P<0.05$) at the rate of 19.50, 22.02, 24.71, 14.82 and 14.55%, respectively than in cyclic cows (Fig. 1A). Similarly, neutrophil, lymphocyte, monocyte, eosinophil and platelet were significantly higher in PPA affected than in unaffected cows at the rate of 22.24, 11.30, 25.84, 33.28 and 9.89%, respectively (Fig. 1B). Endocrinological profile revealed that FSH, LH, E2, P4 and T4 was lower and CORT were significantly higher ($P<0.05$) in PPA animals than in cyclic cows at the rate of 50.89, 49.89, 33.19, 34.33, 15.25 and 21.79%, respectively (Fig. 1C). Similarly, antioxidant profiles, such as TAC, CAT, GSH and SOD were lower and MDA concentration was significantly higher ($P<0.05$) in PPA cows than in cyclic cows at the rate of 27.11, 15.54, 26.81, 34.54 and 20.71%, respectively (Fig. 1D). These haematological parameters has shown significant ($P<0.05$) positive correlation with FSH, LH, E2, P4, T4 and antioxidant profiles whereas significant ($P<0.05$) negative correlation observed with MDA and cortisol.

Anestrus is an important reproductive functional disturbance in cattle and buffaloes which is significantly affecting the dairy enterprise and its economy to a large extent (Kumar *et al.* 2014) through increased open days, calving to conception intervals, poor net calf crops, production and reproduction losses, higher treatment expenses and increased cost of replacing mature animal with first calving heifer (Pawshe *et al.* 2011). Anestrus is caused by various factors including lack of sufficient nutrients, hormonal imbalance, lack of antioxidants, anaemia, etc (Barile 2005). Further, percentage of anestrus was higher in adult than in heifers (Markandeya and Bharkad 2003).

In our study, TRBCs, Hb and PCV values were significantly ($P<0.05$) decreased in anestrus group than in cyclic animal group (Kumar *et al.* 2014). Similar

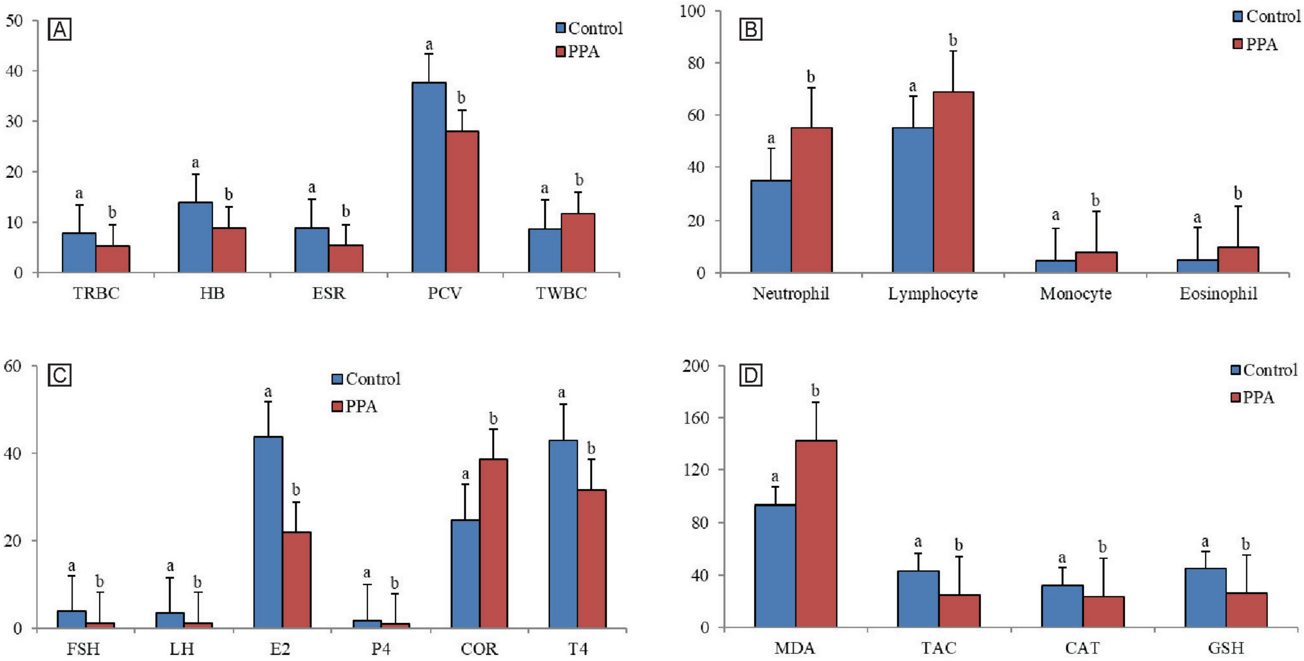

Fig. 1. **A**. Haematological profile in postpartum anestrus (PPA) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and PPA affected cows. TRBC, Total red blood cell ($\times10^6$/mm^3); HB, haemoglobin (g/dl); ESR, erythrocyte sedimentation rate (mm/hr); PCV, packed cell volume (%); TWBC, total white blood cell ($\times10^3$/mm^3). n= 6 cows for control and PPA affected cows. **B**. Differential leukocyte count in postpartum anestrus (PPA) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and PPA affected cows. **C**. Endocrinological profile in postpartum anestrus (PPA) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and PPA affected cows. FSH, Follicle stimulating hormone (mIU/ml); LH, luteinizing hormone (mIU/ml); E2, 17 β-Estradiol (pg/ml); P4, progesterone (ng/ml); COR, cortisol (nmol/L) and T4, thyroxin (nmol/L). n= 6 cows for control and PPA affected cows. **D**. Biochemical profile in postpartum anestrus (PPA) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and PPA affected cows. MDA, Malondialdehyde (nmol/L); TAC, total antioxidant capacity (nmol/µL); CAT, catalase (nmol/min/L); GSH, glutathione (nmol/min/L). n=6 cows for control and PPA affected cows.

observation was reported by Ahmad *et al.* (2004). Increased RBCs in normal cyclic animals might be due to increased metabolic rate which in turn increased the RBC production which causes an increase in the values of other blood profiles (Patil *et al.* 1992). Again (1986) reported that increase of RBC in cyclic oestrus cows is due to higher excitement and hyperactivity under favour of oestrogen. Further, in-sufficient Hb in blood which inturn reduced the oxygen carrying capacity of blood to the vital tissues especially to the gonads and reproductive organs causes reduced oxidation of nutrients, which directly affects the cellular metabolism which inturn affects the cyclicity of the female as these gonads and reproductive organs are metabolically more active during cycle (Ramakrishna 1997).

Total WBC was significantly increased in non-cyclic than in cyclic animals with marked increases of neutrophil count. Leukocytosis occurs as a result of infection and the degree of leukocytosis depends upon nature of the causative agent, severity of infection, resistance of animal and localization of inflammatory response (Benjamin 1978). Moreover, it was also observed that non-cyclic cows had significantly higher total WBC and platelet counts as compared to cyclic ones which might be probably due to bacterial or any other infection in the anestrus cows (Connell *et al.* 2008). Total and DLC in anestrus group had significantly (P<0.05) higher TLC which was clearly indicated by marked neutrophilia than in normal cyclical group. Similarly, eosinophil and lymphocyte were also significantly (P<0.05) increased in anestrus than in control group.

In our study, lipid peroxidation (plasma MDA production) was significantly (P<0.005) higher in PPA cows than in normal cyclic cows. Antioxidant-oxidant activity revealed that significant increases of serum MDA and significant decreases of serum TAC, SOD, CAT and GSH in anestrus than in normal cyclic cows indicated that PPA cows suffered severe oxidative stress (Ahmad *et al.* 2004). Total antioxidant capacity in the PPA group was significantly (P<0.05) lower than in normal cyclic cows (Aydilek *et al.* 2014). Oxidative stress is an important working factor to increase the disease susceptibility in dairy cattle (Sordillo 2005) as there is increased metabolic demands in late pregnancy, energy consuming parturition process and initiation and continuation of lactation would increase the production of free radical which results into oxidative stress. Reactive oxygen metabolites and free radicals are generated in animal body systems. Enzymatic and non-enzymatic antioxidant molecules detoxify the free radicals/ ROS and also minimize damage caused by them to the biomolecules. Further, it was also stated that animal suffered with anestrus due to oxidative stress which inturn decreased the fertility (Kahlon *et al.* 2003). In our study, authors clearly indicated that ROS causes infertility in animal species due to it adversely affecting the gametogenesis, folliculogenesis, steroidogenesis, endocrine disruption, fertilization process and preimplantation of embryo and all these processes are very sensitive to ROS and easily damaged by them. Moreover, inadequate nutrients intakes also impair function of the immune and antioxidant systems (Sharma *et al.* 2011).

Insufficient LH pulse frequency impede the growth of follicles and decreases the ovulation chance and these conditions occurred due to lack of proper nutrition/ malnutrition (Ramoun *et al.* 2012) and negative energy balance (Beever *et al.* 2001). Moreover, high temperature and heat stress also had adverse effect on feed intake and consequently affect endocrine profiles and serum biochemical profiles (Hady *et al.* 2018). Higher temperature-humidity index was also significantly affected TRBCs, Hb, ESR, T4, SOD, glutathione peroxidase (GSHPx), lipid peroxide (MDA) and TAC (Chaudhary *et al.* 2015).

Endocrine profile in our study revealed that FSH, LH, E2 and P4 concentrations were significantly decreased in PPA animals than in normal cyclic animals. Similar observation was also reported that plasma FSH was significantly lower in cows with smooth inactive ovaries (acyclic) than in normal cyclic cows (Saleh *et al.* 2013). Pituitary FSH is important for ovarian follicles development and its maintenance in ovulating species (Taya *et al.* 1991). It was also reported that FSH and P4 were significantly ($P \leq 0.05$) decreased in anestrus group than in control group. GnRH from the hypothalamus induces the release of FSH and LH from the anterior pituitary and stimulates the normal cyclicity. FSH and LH directly act on the gonads, ovaries to initiate and stimulate the follicular growth followed by maturation leads into estrus. It was also stated that reduced energy intake was associated with decreased P4 concentrations in the follicular fluid of small and medium follicles and also decreased the size of the large follicles in cyclic heifers (Spicer *et al.* 1991). In the present study, the non-cycling PPA cows have low P4 and E2 concentrations because these cows lacked a CL and had very small antral follicles. The ovarian inactivity and anoestrus risk are associated with reduced concentration of blood E2 and antioxidant enzymes such as SOD, GSHPx and CAT (El-Bayomi *et al.* 2018). Reduced concentrations of E2 are sufficient to block the pulsatility of GnRH and LH, thus impeding the follicle growth leading to non-cyclicity in PPA cows (Santos 2011). Animals with acyclic smooth inactive ovaries revealed a significant reduction in serum P4 than in cyclic animals (Ahmad *et al.* 2004).

Postpartum anestrus is one of the most prevalent reproductive disorders in Andaman and Nicobar Islands. Biochemical parameters and haematological profiles in PPA cows are characterized by significant decrease in RBCs, Hb, PCV, FSH, LH, progesterone, estradiol, thyroxin and antioxidants whereas there was an increase in TLC, neutrophil, eosinophil, lymphocyte and ROS.

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