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Effect of clinical endometritis on physiological, hematological, biochemical and endocrinological profiles in crossbred cows under tropical island ecosystem

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ABSTRACT

The present study was carried out to assess the effect of endometritis on hematological, physiological, antioxidant, oxidative and endocrinological profiles in crossbred cows under tropical island ecosystem of Andaman and Nicobar Islands. Each 12 number of cows affected with clinical endometritis was selected as group 2 and without endometritis as group 1. These cows were in same parity in same locality with similar type management. Physiological profiles, hematological profiles, antioxidant profiles and hormone profiles were estimated. The result revealed that the crossbred cows with endometritis were suffering severe anaemia. The endometritis affected animals were shown significantly low level of antioxidant profiles and higher MDA level than the unaffected animal groups. Similarly endocrinological profiles revealed that the endometritis affected animals have significantly higher level of CORT and lower level of E2, P4, FSH, LH and T4 than the unaffected crossbred cows. It was concluded that the endometritis was due to anaemia, lack of antioxidants, over production of free radicals and disturbances of endocrinological profiles in crossbred cows of Andaman and Nicobar Islands.

Keywords: Andaman and Nicobar islands, Antioxidants, Blood, Crossbred cows, Endometritis, Hormone, Island ecosystem, Physiological profiles

Endometritis is defined as a local inflammatory condition of the endometrium and it is considered as a major etiological factor for poor fertility and delayed conception rate (Sharma *et al.* 2017). It affects 10–20% of dairy cows between 21 to 40 days postpartum. During peri-partum period, sudden nutritional and endocrine changes lead to compromised immune function. Endometritis is also associated with severe anaemia with significant increment of TWBC with neutrophilia (Azawi 2008). Endometritis is correlated with increased oxidative reactions and reduced antioxidant defense capabilities (Behiman *et al.* 2001). Uterine bacterial infections disrupt the endocrine signalling in hypothalamic-pituitary-gonadal axis and the secretion of gonadotrophins (Karsch *et al.* 2002) and also disrupt growth of the ovarian follicle and its function with smaller and less steroidogenic ovarian follicles (Sheldon *et al.* 2002). However, no study was in crossbred cows under tropical island ecosystem. Therefore, the study was to assess effect of endometritis on physiological, hematological, biochemical and endocrinological profiles in crossbred cows under tropical island ecosystem in Andaman and Nicobar Islands.

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MATERIALS AND METHODS

Present study was conducted at South Andaman district, Andaman and Nicobar Islands with average maximum and minimum temperature were 30.1°C and 23°C, respectively. Relative humidity was in range of 82–94% and annual rainfall is >3,100 mm spread over >8 months. Twenty four healthy adult crossbred cows of 4–6 year of age were selected from the cattle herd in these villages.

Each 12 number of cows was selected which were affected with clinical endometritis as group 2 and without endometritis as group 1. Clinical endometritic was diagnosed as expulsed purulent (>50% pus) uterine discharge detectable in the vagina more than 21 days after calving or muco-purulent (50% pus – 50% mucus) uterine discharge detectable in the vagina after 26 days after calving. Approximately 10 mL of jugular blood sample was collected from each experimental animal in 15 mL sterile polypropylene centrifuge tube containing heparin (20 IU/mL of blood) as anticoagulant. Hematology was carried out using automatic veterinary scan hematological analyzer directly after the samples were received by the research laboratory and within 30 min after samples were collected. Hematological profiles 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 were estimated.

Plasma was separated in refrigerated centrifuge at 3,500×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 profiles such as TAC, GSH, SOD and CAT and MDA were estimated with commercial available kit (Cayman, USA). Endocrinological profiles such as FSH, LH, E2, P4, cortisol and T4 were measured by commercial available ELISA diagnostic kits (Cayman, USA). Protocol for assay was as per manufacturer's instructions.

Statistical analysis of the data was done as per standard procedures (Statistical Analysis System for Windows, SPSS (Version 10) Inc., Chicago, Illinois, USA). Differences with values of P<0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Endometritis affected animals had lower TRBC, Hb, ESR and PCV significantly (P<0.05) at the rate of 18.12, 21.15, 20.51 and 19.90%, respectively than in unaffected normal cows (Fig. 1). Similarly the TWBC, neutrophil, lymphocyte, monocyte, eosinophil and platelet were significantly higher in endometritis affected cows than in unaffected cows at the rate of 21.50, 32.69, 13.33, 29.39, 30.97 and 12.14%, respectively (Fig. 2). Physiological profiles such as RT, PR, RR and ST were lower significantly (P<0.05) at the rate of 10.11, 22.04, 21.60 and 4.65%, respectively than in unaffected normal cows (Fig. 3). Endocrinological profiles revealed that FSH, LH, E2, P4 and T4 was lower and cortisol were higher significantly (P<0.05) in endometritic animals than in normal unaffected cows at the rate of 34.39, 30.55, 27.60, 31.85, 17.47 and 26.44%, respectively (Fig. 4). Similarly antioxidant profiles such as TAC, CAT, GSH and SOD were lower and MDA concentration was higher significantly (P<0.05) in endometritic cows than in unaffected cows at the rate of 31.08, 13.50, 30.51, 30.97 and 27.47%, respectively in the present study (Fig. 5). These hematological parameters showed 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, cortisol, TWBC and total differential count.

Physiological profiles such as rectal temperature, pulse rate, respiratory rate and heart rate were significantly increased in endometritic affected cows than in unaffected

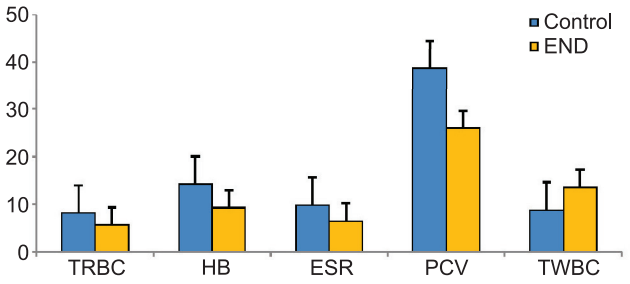

Fig. 1. Heamatological profiles in endometritis (END) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and endometritis affected cows. TRBC,Total Red Blood Cell (×10^6/mm^3); HB, Haemoglobin (g/dl); ESR, Erythrocyte Sedimentation Rate (mm/h); PCV, Packed Cell Volume (%) and TWBC, total white blood cell (×10^3/mm^3). n= 12 cows for control and endometritis affected cows.

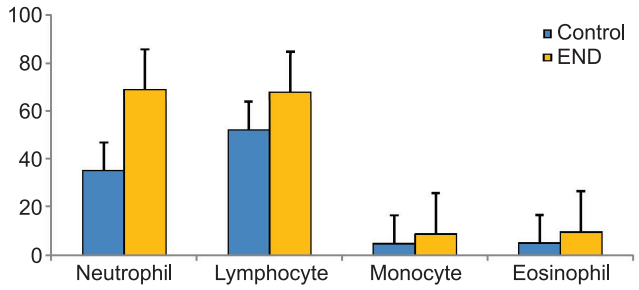

Fig.2. Differential leukocyte count in endometritis (END) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and END affected cows.

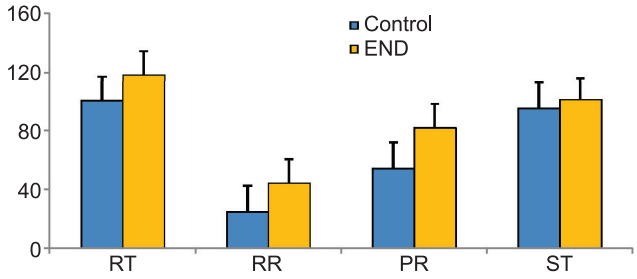

Fig. 3. Physiological profiles in endometritis (END) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and END affected cows. RT, Rectal Temperature (°F); RR, Respiratory rate (beats per min); PR, Pulse rate (beats per min and ST, Skin Temperature (°F). n= 12 cows for control and END affected cows.

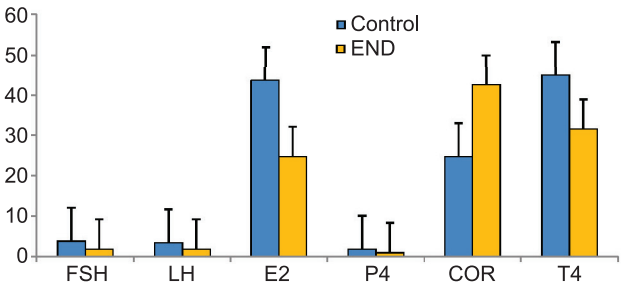

Fig. 4. Endocrinological profiles in endometritis (END) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and END affected cows. FSH, Follicle Stimulating Hormone (mIU/ml); LH, Luteinizing Hormone (mIU/ml); E2, 17β-Estradiol (pg/ml); P4, Progesterone (ng/ml); COR, Cortisol (nmol/L) and T4, Thyroxin (nmol/L). n= 12 cows for control and END affected cows.

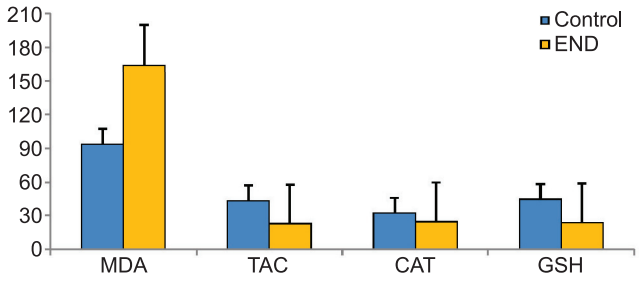

Fig. 5. Biochemical profiles in endometritis (END) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and END affected cows. MDA, Malondialdehyde (nmol/L); TAC, Total antioxidant capacity (nmol/µL); CAT, Catalase (nmol/min/L); GSH, Glutathione (nmol/min/L). n= 12 cows for control and END affected cows.

cows. This may occur from the interaction between the host immune system and bacterial endotoxins which trigger the cascade of events that lead to elevated temperature. Measurement of physiological profiles has been considered as the useful tools in systemic diagnosis of endometritis in early post-partum cows (Chenault *et al.* 2004).

Endometritis affected cows suffered anaemia as indicated by significant decrease in TRBCs, Hb, PCV and ESR as normocytic hypochromic anaemia, neutrophilia and monocytosis. During peri-partum period, cows experience sudden nutritional and endocrine changes, leading to compromised immune function (Sordillo 2016). This lowered immune response predisposes the cows to uterine infection (Senosy *et al.* 2011). The increases of TWBC and granulocytes in cows were probably caused by the stress response at parturition (Azawi 2008). The TWBC, granulocytes and monocytes continued to increase in cows with endometritis (Islam *et al.* 2014). Bacterial toxins circulating in blood results deformities in the shape of red cells so that it is entrapped in the spleen network and the animals go in regenerative anaemia. The higher total leukocyte count recorded in the endometritis affected cows may be attributed to the increased circulating neutrophils in this study (Kim *et al.* 2005). Such changes in cows indicate a potential persistent peripheral inflammatory response in the cows with uterine infection (Islam 2012).

On the other hand, higher neutrophil percentage and TLC might also be due to the increased cortisol level in the cows as the MDA level in these cows was higher than in the normal unaffected cows. The simultaneous increase of MDA and cortisol in the post-partum endometritis affected cows than in normal cows has been reported (Islam 2012). Corticosteroids induce neutrophilia by an increased output of neutrophils from the bone marrow, by neutrophils demargination from the blood vessel wall or by a combination of the two (Lee and Kehrli 1998).

Cows having endometritis revealed disturbed oxidative status with increased MDA and decreased CAT, SOD, GSH and TAC values as compared to healthy animals in the present study. It is well known that inflammatory diseases are associated with enhanced oxidative reactions and reduced antioxidant defense capabilities. Similar reports were also reported by Behiman *et al.* (2001). Lipid peroxidation is known to have a role in aging, cancer and many infectious diseases. Malondialdehyde (MDA) is a by-product of lipid peroxidation and is used as an index of the rate of tissue reaction chain. MDA is also used as an indicator of oxidative stress in cells and tissues (Madebo *et al.* 2003). The higher blood MDA concentrations in cows affected with endometritis as compared to healthy animals is apparently due to a marked increase in ROS production during development of the inflammatory process (Ahmed *et al.* 2010). Significantly higher MDA level was detected in cows suffering from postpartum endometritis (Islam 2012) similar observation was reported in the present study. Moreover, greater MDA level in cyclic cows with subclinical endometritis as compared to non-endometritis cows has also been reported (Binsila 2011).

Normal hypothalamic and pituitary functioning is critical for post-partum resumption of ovarian cycles. In animals with uterine infection, ovarian follicular waves arise during first few weeks of post-parturition as follicle stimulating hormone (FSH) concentrations remain unaffected in such animals (Sheldon *et al.* 2002). However, it has been reported that *E. coli* derived LPS (endotoxin) suppresses GnRH and LH release from hypothalamus and pituitary gland respectively and the sensitivity of the pituitary to gonadotrophin releasing hormone, thereby reducing the ability of dominant follicle to ovulate (Karsch *et al.* 2002) in the endometritic affected cows.

Furthermore, endotoxin inhibits the responsiveness of the pituitary to GnRH (Williams *et al.* 2001), which in turn could affect ovulation and luteal development. It has been mentioned that bacterial load in uterus, bacterial metabolic products and the associated inflammation of uterine layers causes to suppress pituitary LH secretion that leads to disturbance in post-partum ovarian follicular growth and function (Herath *et al.* 2009). In dairy cattle, metabolic stress, most often negative energy balance is the main cause of reduced LH pulse frequency (Cheong *et al.* 2016). In addition to smaller CLs in the first postpartum estrous cycle, bacterial contamination of the postpartum uterus has also been shown to be associated with lower plasma P4 concentrations (Williams *et al.* 2007). Uterine infections negatively affect ovarian activity. In cows with severe bacterial uterine contamination, the first post-partum dominant follicle was smaller and secreted less estradiol compared with healthy cows (Williams *et al.* 2007). These cows also had smaller CLs and lower plasma P4 concentrations than in healthy cows (Williams *et al.* 2007). A similar finding has been reported from dairy cattle by Peter *et al.* (1989). Further due to shift from PGF2á (luteolytic) to PGE2 (anti-inflammatory), luteolysis is disrupted, since the endometrial epithelial cells of animals affected with uterine disease secrete PGE2 instead of PGF2α, this leads to extended luteal phases and hence infertility. Progesterone level was undetectable

in animals having endometritis with inactive ovaries.

Also, cortisol may impair follicular development and ovulation in ruminant (Macfarlane *et al.* 2000), which might enhance reproductive insufficiency of such animals. Cortisol is primarily immunosuppressive (Hazeldine *et al.* 2010). Greater cortisol concentrations could contribute to the overall higher endometritis incidence as observed in cows. The plasma estradiol peaks right after calving and decreases quickly after calving (Noakes *et al.* 2001), but significant difference was observed between the healthy and meteoritic affected cows (Gautam *et al.* 2009). Ultimately the peripheral plasma concentrations of FSH, LH, E2, P4 and metabolites of prostaglandins were affected in the endometritic affected cows.

It was concluded that the endometritis in the present study was due to anaemia, lack of antioxidants, over production of free radicals and disturbances of endocrinological profiles in crossbred cows of Andaman and Nicobar Islands.

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