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Physiological, Heamatological, Biochemical and Endocrinological Profiles Changes in Crossbred Cows Affected With Metritis under Tropical Island Ecosystem

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ABSTRACT

Present study was carried out to assess the haematological, physiological, antioxidant, oxidative stress and endocrinological profiles changes in crossbred cows affected with metritis under the humid tropical island ecosystem of Andaman and Nicobar Islands. The cows affected with clinical metritis as group 2 (n=6) and without metritis as group 1 (n=6) were selected for the present study. These cows were selected in the same locality and similar type of management. Physiological profiles, heamatological profiles, antioxidant and oxidative stress profiles and hormone profiles were estimated. The result revealed that the crossbred cows with metritis were suffered severe anaemia. The metritis affected animals had significantly ($p<0.05$) low levels of antioxidant profiles and higher malondialdehyde (MDA) levels than the unaffected animal groups. Similarly, endocrinological profiles revealed that the metritis affected animals had significantly ($p<0.05$) higher levels of cortisol and lower level of estradiol 17β (E2), progesterone (P4), follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroxine (T4) than the unaffected crossbred cows. These heamatological parameters have shown significant ($p<0.05$) positive correlation with follicle stimulating hormone, luteinizing hormone, estradiol 17β, progesterone, thyroxine and antioxidant profiles whereas significant ($p<0.05$) negative correlation was observed with malondialdehyde, cortisol, TWBC and total differential count. It was concluded that the metritis 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. A balanced ration with good quality and quantity of antioxidants, sufficient daily exercise and sufficiently clean drinking water can minimize the metritis percentage in the crossbred cows.

KEYWORDS: Antioxidants, cows, hormones, island ecosystem, metritis, physiological profiles

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1. INTRODUCTION

Metritis is inflammation in all layers of the uterus, from the endometrium through the submucosa, the muscularis and the serosa (Bondurant, 1999). It is characterized by an abnormally enlarged uterus and fetid brown uterine discharge (Sheldon et al., 2006). It is considered as a major etiological factor for poor fertility and delayed conception rate in cattle (Ramoun et al., 2012). Affected cows often have a systemic illness, with fever, loss of appetite, weakness, decreased milk yield, elevated heart rate and depression (Bondurant, 1999; Sheldon et al., 2006). Various reproductive disorders were described in bovine and bubaline species (Perumal et al., 2017). Cattle and buffalo of Andaman and Nicobar Islands are suffered various reproductive disorders such as endometritis (Perumal et al., 2020), retention of placenta (Perumal et al., 2020), post partum anoestrus in cattle (Perumal et al., 2020) and post partum anoestrus in buffaloes (Perumal et al., 2021). Metritis affects 5–20% of cows (Sheldon and Dobson, 2004) and occurs within 21 days and is most common within 10 days of parturition. During peripartum period, sudden nutritional and endocrine changes lead to compromised immune function. Metritis is also associated with severe anaemia with a significant increment of TWBC with neutrophilia (Azawi, 2008; Perumal et al., 2013; Perumal et al., 2019). High milk production, hot season, stress and negative energy balance are the contributing factor for induction of metritis in animals. Metritis/endometritis is correlated with increased oxidative reactions and reduced antioxidant defense capabilities (Hady et al., 2018). Uterine bacterial infections disrupt the endocrine signaling in hypothalamic-pituitary-gonadal axis and the secretion of gonadotrophins (Karsch et al., 2002) and also disrupt the growth of the smaller and less steroidogenic ovarian follicles (Sheldon et al., 2002). Various parameters of blood profiles are varied in association with metritis. Blood is one of the important constituents in the body, which maintains the physiological equilibrium. Thus any disturbance in this equilibrium maintenance will lead to disease or pathological condition, which inturn can be known from the changed haematological, endocrinological and biochemical parameters. TRBC, Hb and PCV are significantly reduced in metritis affected animals. In animals, lack of sufficient quantities of Hb in blood is responsible for reduced oxygen transport to the vital tissues that causes reduced oxidation of nutrients, which in turn affects the whole cellular metabolism in gonadal cells which is metabolically more active and in turn affects the cyclicity leads to metritis (Ramakrishna, 1997). At the same time, the TWBC count is increased in metritis affected animals. It was also reported that deficiency of antioxidants and excess production free radicals leads to infertility disorders in animals (Ahmed et al., 2006) and these authors also observed an association between the oxidative stress and inactivity of the ovaries and reproductive tract. These reports suggested that the metritis in cows is due to stress caused by nutrition deficiency, high temperature humidity index, high level of diseases, etc. Oxidative stress is as a major initiator of tissue damage and could affect enzymatic activity, signal transcription and gene expression (Sen and Packer, 1996). Oxidative stress influences ovarian function by inhibiting the growth of the Graffian follicles, oocyte maturation, ovulation, and fertilization (Megahed et al., 2002) and also it has anti-gonadotrophic and steroidogenic action in the luteal cells (Shimamura et al., 1995). Therefore treatment with antioxidant such as melatonin is advisable to minimize the ROS production (Perumal et al., 2021). Besides, study on the hematological, biochemical and antioxidant profiles and endocrinological profiles are also important in diagnosis and prognosis of metritis status of the crossbred cows. However, no study has been conducted on cross bred cows under the humid tropical island ecosystem. Therefore, the study was designed to assess the changes in physiological, heamatological, biochemical and endocrinological profiles in crossbred cows affected with metritis under tropical island ecosystem in Andaman and Nicobar Islands.

2. MATERIALS AND METHODS

Present study was conducted at South Andaman District, Andaman and Nicobar Islands with average maximum and minimum temperatures were 30.1 and 23°C, respectively. Relative humidity was in the range of 82–94% and annual rainfall is >3100 mm spread over >8 months. Twelve adult crossbred cows of 4–6 years of age were selected from the cattle herd in villages of South Andaman district. The experiment was conducted during the year of 2020–2021. These animals were maintained in the semi-intensive system where they were allowed for grazing from 0700 to 1200 h.

Each six number of cows with clinical metritis (group 2) and without metritis (group 1) was selected. Cows displayed an enlarged uterus and additional aqueous, reddish-brown, malodorous uterine discharge was considered as affected with metritis (Sheldon et al., 2002). In addition, the affected cows often have a systemic illness, with fever, loss of appetite, weakness, decreased milk yield, elevated heart rate and depression. Physiological parameters such as rectal temperature (RT), respiratory rate (RR), pulse rate (PR) and skin temperature (ST) were estimated as per the standard procedure. 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^{-1} of blood) as an anticoagulant. Hematology was carried out in the research laboratory using an automatic

veterinary scan hematological analyzer 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 a refrigerated centrifuge at 3500×g at 4°C for 10 min and 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 Total antioxidant capacity (709001), Superoxide dismutase (706002), Glutathione (703002) and Catalase (707002) in the blood plasma were estimated by using Cayman's Catalase assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) as per the manufacturer's guidelines in 96-well clear polypropylene microplate using Alere Microplate Reader (Alere Medical Pvt Ltd, India, AM 2100). Endocrinological profiles such as FSH (analytical sensitivity: 0.1 mIU ml^{-1}; intra- and inter-assay coefficients of variation: 6.36 and 9.75%, respectively), LH (analytical sensitivity: 13 nmol l^{-1}; intra- and inter-assay coefficients of variation: 5.26 and 8.42%, respectively), E2 (analytical sensitivity: 15 pg ml^{-1}; intra- and inter-assay coefficients of variation: 5.67 and 8.85%, respectively), P4 (analytical sensitivity: 10 pg ml^{-1}; intra- and inter-assay coefficients of variation: 6.12 and 8.43%, respectively), cortisol (analytical sensitivity: 5 nM; intra-and inter-assay coefficients of variation: 5.63 and 9.54%, respectively) and T4 (analytical sensitivity: 10.62 nmol l^{-1}; intra- and inter-assay coefficients of variation: 5.28 and 9.67%, respectively)were measured using commercially available ELISA diagnostic kits (Cayman, USA) as per the manufacturers protocol instructions in 96-well clear polypropylene microplate using Alere Microplate Reader (Alere Medical Pvt Ltd, India, AM 2100).

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

3. RESULTS AND DISCUSSION

Metritis affected animals had lower TRBC, Hb, ESR and PCV significantly ($p<0.05$) at the rate of 11.63, 10.62, 9.98 and16.44%, respectively than the unaffected normal cows (Figure 1). Similarly, the TWBC, neutrophil, lymphocyte, monocyte, eosinophil and platelet were significantly higher in metritis affected cows than in unaffected cows at the rate of 21.37, 22.91, 15.04, 21.38, 27.45 and 10.30%, respectively (Figure 2). Physiological profiles such as RT, PR, RR and ST were lower significantly ($p<0.05$) at the rate of 10.48, 29.12, 20.61 and 6.78%,

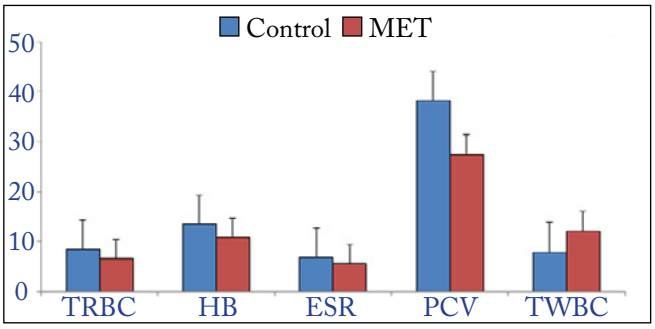

Figure 1: Heamatological profiles in metritis (MET) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant ($p<0.05$) difference between the control and MET affected cows. TRBC: Total Red Blood Cell ($x10^6/mm^3$), HB: Haemoglobin (g dl^{-1}), ESR: Erythrocyte Sedimentation Rate (mm hr^{-1}), PCV: Packed Cell Volume (%) and TWBC: total white blood cell ($x10^3/mm^3$). n=6 cows for control and metritis affected cows

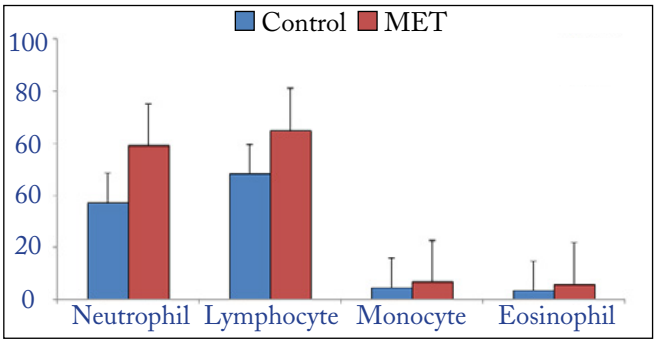

Figure 2: Differential leukocyte count (%) in metritis (MET) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant ($p<0.05$) difference between the control and MET affected cows. n=6 cows for control and MET affected cows

respectively than in unaffected normal cows (Figure 3). Endocrinological profiles revealed that FSH, LH, E2, P4 and T4 was lower and cortisol were higher significantly ($p<0.05$) in metritis affected animals than the normal unaffected cows at the rate of 25.53, 10.96, 9.75, 32.64, 9.21 and 31.54%, respectively (Figure 4). Similarly, antioxidant profiles such as TAC, CAT, GSH and SOD were lower and MDA concentration was significantly ($p<0.05$) higher in metritis affected cows than the unaffected cows at the rate of 30.90, 10.89, 27.71, 34.57 and 15.42%, respectively in the present study (Figure 5). These heamatological parameters have shown significant ($p<0.05$) positive correlation with FSH, LH, E2, P4, T4 and antioxidant profiles whereas significant ($p<0.05$) negative correlation was 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

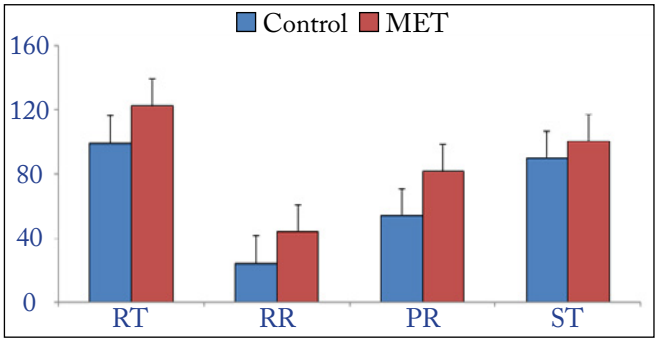

Figure 3: Physiological profiles in metritis (MET) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (p<0.05) difference between the control and MET affected cows. RT: Rectal Temperature (°F), RR: Respiratory rate (beats per min), PR: Pulse rate (beats per min and ST: Skin Temperature (°F). n=6 cows for control and MET affected cows

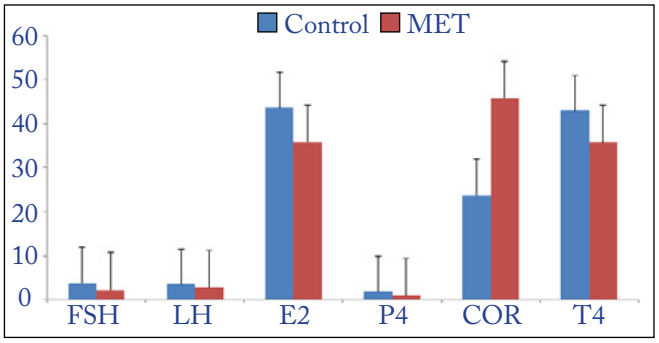

Figure 4: Endocrinological profiles in metritis (MET) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (p<0.05) difference between the control and MET affected cows. FSH: Follicle Stimulating Hormone (mIU/ml), LH: Luteinizing Hormone (mIU ml^{-1}), E2: 17β-Estradiol (pg ml^{-1}), P4: Progesterone (ng ml^{-1}), COR: Cortisol (nmol l^{-1}) and T4: Thyroxin (nmol l^{-1}). n=6 cows for control and MET affected cows

in metritis affected than in unaffected 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 a useful tool in the systemic diagnosis of metritis in early postpartum cows (Chenault et al., 2004).

Metritis affected cows suffered anaemia as indicated by a 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

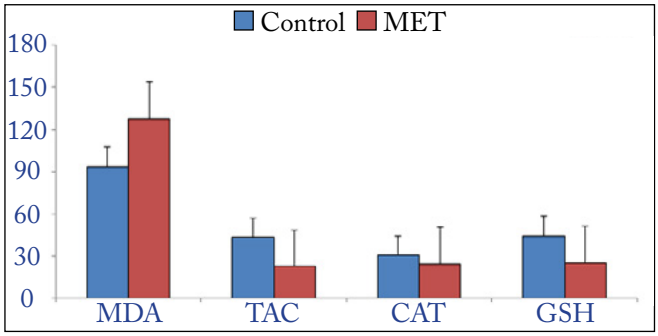

Figure 5: Biochemical profiles in metritis (MET) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (p<0.05) difference between the control and MET affected cows. MDA: Malondialdehyde (nmol l^{-1}), TAC: Total antioxidant capacity (nmol µl^{-1}), CAT: Catalase (nmol min^{-1} l^{-1}), GSH: Glutathione (nmol min^{-1} l^{-1}). n=6 cows for control and MET affected cows

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/metritis (Islam, 2012; Cui et al., 2019). Bacterial toxins circulating in blood result in deformities in the shape of red cells so that it is entrapped in the spleen network and the animals undergo regenerative anaemia. The higher total leukocyte count recorded in the metritis affected cows may be attributed to the increased circulating neutrophils in this study (Kim et al., 2005; Cui et al., 2019). Such changes in cows indicated 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 postpartum endometritis/metritis affected cows than in normal cows has been reported (Islam, 2012). Corticosteroids induce neutrophilia by an increased output of neutrophils from the bone marrow due to neutrophils demargination from the blood vessel wall or by a combination of the two (Lee and Kehrli, 1998).

Cows having metritis 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 Hady et al. (2018). Lipid peroxidation is known to have a role in aging, cancer and many infectious diseases. Malondialdehyde (MDA), by-product of lipid peroxidation has been 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 metritis as compared to healthy animals are apparently due to a marked increase in ROS production during the development of the inflammatory process (Ahmed et al., 2006). A significantly higher MDA level was detected in cows suffering from postpartum endometritis/metritis (Islam, 2012) as similar to the present study. Moreover, greater MDA levels in cyclic cows with subclinical endometritis as compared to non-endometritic cows have also been reported (Binsila, 2011).

Normal hypothalamic and pituitary functioning is critical for postpartum 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 the hypothalamus and pituitary gland respectively and the sensitivity of the pituitary to gonadotrophin releasing hormone, thereby reducing the ability of the dominant follicle to ovulate (Karsch et al., 2002) in the endometriotic or metritis 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 the uterus, bacterial metabolic products and the associated inflammation of uterine layers suppress pituitary LH secretion that leads to disturbance postpartum 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 postpartum dominant follicle was smaller and secreted fewer estradiol as compared with healthy cows (Williams et al., 2007). These cows also had smaller CLs and lower plasma P4 concentrations than healthy cows (Williams et al., 2007). A similar finding has been reported from dairy cattle by Peter et al. (1989). Further due to the shift from PGF2α (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 metritis with inactive ovaries.

Also, cortisol may impair follicular development and ovulation in ruminants (Macfarlane et al., 2000), leading to reproductive insufficiency of such animals. Cortisol is primarily immunosuppressive (Hazeldine et al., 2010). Greater cortisol concentrations could contribute to the overall higher metritis incidence in cows. The plasma estradiol peaks right after calving and decreases after calving (Noakes et al., 2001), but a significant difference was observed between the healthy and metritis affected cows (Gautam et al., 2009). A similar observation was reported by Gundlach et al. (2017). Ultimately the peripheral plasma concentrations of FSH, LH, E2, P4 and metabolites of prostaglandins were affected in the endometritic affected cows.

4. CONCLUSION

The metritis 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. Therefore a balanced ration with good quality and quantity of antioxidants, sufficient daily exercise and sufficiently clean drinking water can minimize the metritis percentage in the crossbred cows.

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