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Retention of placenta on physiological, hematological, biochemical and endocrinological profiles in crossbred cows under tropical island ecosystem

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ABSTRACT

A study was carried out to assess the effect of retention of placenta (ROP) on hamatological, physiological, antioxidant and endocrinological profiles in crossbred cows under tropical island ecosystem of Andaman and Nicobar Islands. Each six number of cows was selected which were affected with ROP as group 2 and without ROP as group 1. These cows were in same parity in same locality with similar type management. Both the group of cows delivered healthy live calves normally and the cases were diagnosed as ROP when it failed to expel after 24 h of parturition. The ROP was removed manually as per standard procedure with aseptic measures. These affected cows were treated successfully and allowed to nurse the calves. Physiological profiles such as rectal temperature (RT), pulse rate (PR), respiratory rate (RR) and skin temperature (ST) were measured. Blood samples were collected, analysed for hematological profiles and serum separated, analysed the antioxidant profiles 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 estradiol 17β (E2), progesterone (P4) and cortisol (CORT) were estimated. The result revealed that the crossbred cows of ANI with ROP suffered severe anaemia. The ROP affected animals were shown significantly low level of antioxidant profiles and higher MDA level than the unaffected animal groups. Similarly endocrinological profiles revealed the affected animals were having significantly higher level of CORT, P4 and lower level of E2 than unaffected crossbred cows. It was concluded that the ROP in the present study was due to anaemia, lack of antioxidants, over production of free radicals and disturbances of endocrinological profiles.

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

Retention of placenta is a common complication after the parturition in cattle and buffaloes. In general, the placenta is expelled within 12 hours post-calving in cattle (Mohamed and Amer 2009). If any part of placenta is held for longer period after birth, it is considered to be as pathological or abnormal. The frequency of ROP averages from 5 to 10% under normal ideal conditions in the dairy herd (Stephen 2008). However, abnormal parturition (i.e. twins, caesarean section, fetotomy, dystocia, abortions, prolapse or premature calvings) increases the prevalence of ROP. Cattle herds with infectious diseases increases the prevalence of ROP may go upto 50% (Mohamed and Amer 2009). ROP is one of the most economically important disturbances especially during the post-partum period in dairy herd of cattle and buffaloes as because the ROP affects the herd health, milk production as well as the reproductive efficiency of the herd (Peters and Laven 1996). Although ROP happens due to the imbalance in release of the fetal cotyledon from the maternal caruncle unit and several local factors also play important roles in development of ROP during endocrine cascade events which was initiated by increased secretion of fetal adrenal cortisol associated with fetal hypothalamus-pituitary-adrenal axis maturation (Lye 1996). ROP causes delayed uterine involution, chronic endometritis, followed by pyometra or perimetritis which ends into infertility or sub-fertility or sterility (Beagley *et al.* 2010). Increasing fetal cortisol concentration followed by placental 17β-hydroxylase activity, which causes the placenta converts progesterone into estrogen which causes increase in maternal estrogen: progesterone ratio (Lye 1996). Increase of the E: P4 ratio not only increase the myometrial activity during parturition, but also increases the synthesis and release of stimulatory uterotonins like prostaglandins and oxytocin (Challis and Lye 1986, Lye 1996). These uterotonins show the action through the receptor-mediated pathways to enhance the intracellular calcium and followed by activation of the contractile elements in the uterus (Challis and Lye 1986). However, it was reported that ROP is not the only result of a lack of or poor uterine contractility especially

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during the early stage of the post-partum period (Eiler *et al.* 1984). Grunert *et al.* (1989) expressed that the placentomes maturation processes are very essential for normal placental separation and removal and that placental maturation process is probably controlled by endocrinological factors. Moreover, study on hematological and biochemical profiles will help to prepare the suitable precautionary measurement on feeding and breeding when the animal becomes pregnant to overcome the reproductive problem such as ROP and dystocia in cattle (Perumal *et al.* 2013). However studies on reproductive disorders in crossbred cows under tropical island ecosystem are scanty because of limited geographical distribution. Hence forth, the objective of the present study was to assess the effect of ROP on physiological, hematological, biochemical and endocrinological profiles in crossbred cows under tropical island ecosystem in Andaman and Nicobar Islands.

MATERIALS AND METHODS

Six crossbred cows with ROP and six cows without ROP were selected from Guptapara, Indira Nagar and Chouldhuri of South Andaman district for the present study. These crossbred cows were maintained under similar management. These cows delivered the healthy live calves without any assistance. However, in ROP case, apparently normal placenta was retained partially (most portion was hanging outside the vulva) for 24 h after parturition. The behaviour and physiological parameters (RT, PR, RR and ST) of the animal were measured with standard procedure. Per vaginally exploration with aseptic measures revealed partial placental retention at ovarian end of the gravid right horn. The blood sample was collected at the time of retention of placenta and analysed the hematological profiles such as total red blood cells (TRBC), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), packed cell volume (PCV) and total white blood cell (TWBC) by automatic blood analyser. Antioxidant profiles such as TAC, GSH, SOD and CAT were estimated with commercial available kit and oxidant profile such as MDA were estimated as per method described by Shah *et al.* (1989). Endocrinological profiles such as estradiol, progesterone and cortisol were measured by commercial available diagnostic kits. Statistical analysis of the data was done as per standard procedures. Student "t" test was conducted to assess the significant difference between the with and without ROP affected cows (Statistical Analysis System for Windows, 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.

The affected cows were restrained following low epidural anaesthesia (6 ml, 2% Lignocaine hydrochloride). The hanging placenta was held in right hand and twisted like a rope in order to manage easily during its manual removal. A lubricated left hand was advanced into site of placental retention. Individual cotyledons and caruncles were grasped between thumb and fingers and structures were separated by rolling, pushing and squeezing motion. Simultaneous traction on placenta with right hand helped in easy separation. Gross examination revealed an apparently normal placenta without any inflammatory changes. The cows were treated with four Furea bolus intra uterinely, 2.5 g Streptopenicillin (2.5 g) intramuscularly for five days, 200 mg meloxicam intramuscularly for three days and Replanta powder 50 g BID orally for 3 days. The animal recovered uneventfully.

RESULTS AND DISCUSSION

Physiological parameters such as RT, RR and PR were significantly ($P<0.05$) higher in ROP affected at the rate of 2.80, 30.84 and 20.40%, respectively than in unaffected cows whereas the ST did not differ significantly between them (Fig. 1). ROP affected animals had lower TRBC, Hb, ESR and PCV and higher TWBC significantly ($P<0.05$) at the rate of 20.86, 20.29, 22.40, 13.91 and 16.61%, respectively than in ROP unaffected cows (Fig.2). Endocrinological profiles revealed that E2 was lower and P4 and CORT were higher significantly ($P<0.05$) in ROP affected than in unaffected cows at the rate of 37.53, 28.20 and 25.27%, respectively (Fig. 3). Similarly antioxidant profiles such as TAC, CAT, GSH and SOD were lower and MDA concentration was higher significantly ($P<0.05$) in ROP affected than in unaffected cows at the rate of 26.11, 10.37, 24.63, 35.84 and 20.74%, respectively in the present study (Fig.4). These physiological parameters have shown significant ($P<0.05$) positive correlation with progesterone, cortisol, MDA concentration and TWBC whereas significant ($P<0.05$) negative correlation observed with TRBC, Hb, PCV, ESR, E2 and TAC, CAT, SOD, GSH.

Reproductive disorders such as ROP, endometritis, metritis, toxic puerperal metritis and pyometra are of major importance in dairy cattle and buffaloes (Azawi and Taha 2002). ROP is significant risk factor for induction of toxic puerperal metritis in bovine and bubaline species. Based on available literature, similar results were obtained as reported in buffaloes (Ahmed *et al.* 2009) and cattle (Akar and Yildiz 2005) at the time ROP. In our study, affected cows with ROP suffered from anaemia as indicated by significant decrease in the RBCs, Hb, PCV and other heamatological profiles and also there is a significant leukocytosis associated with monocytosis and lymphopenia. ROP condition may be favoured to inflammation as well as increase of monocytes to scavenge the cells debris. ROP condition in cows induced a significant reduction of Hb content as well as PCV (Sivaraman *et al.* 2003). On the other side, Farzaneh *et al.* (2006) reported significant variation in PCV, total and differential leukocytes counts between cows with and without ROP.

Separation of placenta occurs when foetal cortisol stimulates the production of the 17α-hydroxylase and aromatase enzymes in the placenta which inturn induce oestrogen synthesis at the expense of synthesis of progesterone. Oestradiol-17β level increases suddenly in

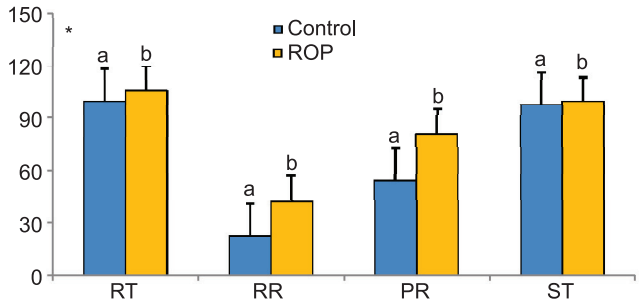

Fig. 1. Physiological profiles in retention of placenta (ROP) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and ROP affected cows. RT, Rectal Temperature (°F), RR, Respiration rate (bpm); PR, Pulse rate (bpm); ST, Skin Temperature (°F). *indicates P<0.05). n= 6 cows for control and ROP affected cows.

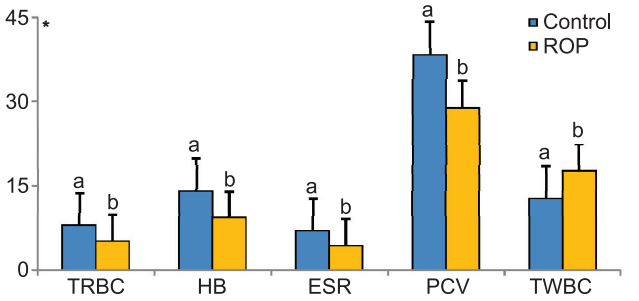

Fig. 2. Heamatological profiles in retention of placenta (ROP) affected animals(mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and ROP affected cows. TRBC, Total Red Blood Cell ($\times 10^6$/mm^3); HB, Haemoglobin (g/dl); ESR, Erythrocyte Sedimentation Rate (mm/hr); PCV, Packed Cell Volume (%); MCV, Mean Corpuscular Volume (µm^3); MCH, mean corpuscular haemoglobin (pg); MCHC, mean corpuscular haemoglobin concentration (g/dl) and TWBC, total white blood cell ($\times 10^3$/mm^3). n= 6 cows for control and ROP affected cows.

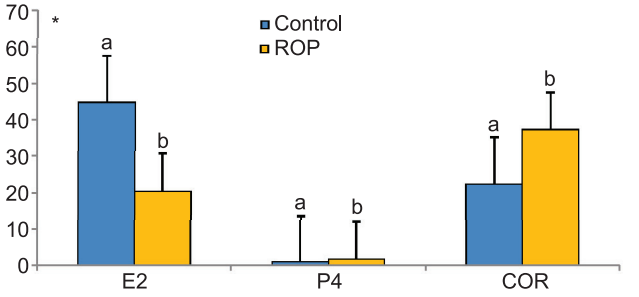

Fig. 3. Endocrinological profiles in retention of placenta (ROP) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and ROP affected cows. E2,17 β-Estradiol (pg/ml); P4, Progesterone (ng/ml);COR, Cortisol (nmol/L).n= 6 cows for control and ROP affected cows.

the maternal plasma, whereas plasma progesterone decreases sharply immediately just prior to calving. This happens during the last week of gestation or a week before

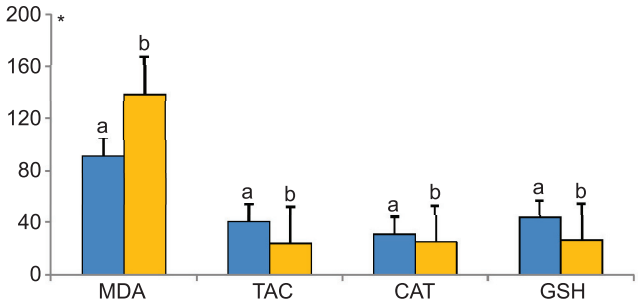

Fig. 4. Biochemical profiles in retention of placenta (ROP) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and ROP affected cows. MDA, Malondialdehyde (nmol/L); TAC, Total antioxidant capacity (nmol/µL); CAT, Catalase (nmol/min/L); GSH, Glutathione (nmol/min/L). n= 6 cows for control and ROP affected cows.

parturition, at that time the estradiol level reaches its maximum to help the uterus to contract and get rid of any remnant of placental membranes. Thus, a decreased level of estrogen is critical factor for enhancing the incidence of ROP in cattle (El-Nemer *et al.* 2000) as reported in the present study. Autocrine and paracrine secretion and release of PGF2α causes spontaneous myometrial contraction which stimulate the parturition process. Disturbance in the endocrine function especially higher progesterone and cortisol concentration and lower oestradiol concentration in blood causes ROP in dairy herd (Michal *et al.* 2006) as observed in our study. Increased progesterone concentration in ROP cases, which may be due to failure to produce the sufficient specific steroidal enzymes by the placenta in progesterone aromatization and its conversion to oestrogen (Ball and Peters 2004).

The delayed luteolysis and severely higher combustion of spare fat enforces to increase concentration of progesterone after parturition (Matton *et al.* 1987). In present study also, ROP cows had a significantly higher concentration of progesterone in compared to control animal group. Increased concentration of progesterone in ROP affected cows may be due to complete failure of the placenta to secrete specific steroidal enzymes which is responsible in progesterone metabolism and also its conversion into estrogen (Matton *et al.* 1987), which in turn may stimulate the accumulation of immunosuppressive proteins in the uterine lumen which inturn make the uterus more susceptible to diseases or infection and also persistence of bacteria inside the uterus (Königsson *et al.* 2002). Cows with ROP had significantly higher cortisol concentration than in healthy ones. Increased cortisol may be associated with the stress in ROP affected cows (Dobson and Smith 2000). Similar results were reported by Kornmatitsuk *et al.* (2000) in ROP cows in induced calving. Similar reports were also reported by other authors also (Sabry *et al.* 1997, Kornmatitsuk *et al.* 2000). In the present study also, the serum estradiol 17β concentration was significantly reduced in ROP cows as compared to the unaffected control group. Similarly Zraly *et al.* (1989) reported that in ROP cows,

the concentration of progesterone was increased between the day 3 and 7 post partum and concentration of cortisol was increased between the day 5 and 8 of post partum in comparison to cows without any postpartum disturbances. El-Nemer *et al.* (2000) reported that the concentration of estradiol reaches its peak level during the week before parturition and this helps the uterus to send out any fetal membranes remnant and also prevent the occurrence of endometriosis. In our study, results were also very similar. Therefore, a reduced concentration of estrogen may be suggested as an important factor for enhancing ROP (Farzaneh *et al.* 2006). Similar to previous observation reported on ROP in bovine and high concentration of progesterone and cortisol (Sabry *et al.* 1997, Michal *et al.* 2006) and low concentration of estrogens (Kankofer *et al.* 1998) levels in the blood in the present study clearly indicated a homogeneous increase of the progesterone and cortisol level and a decrease of estradiol 17β concentrations.

Enzymatic antioxidants such as GSH-Px, SOD and CAT and non-enzymatic antioxidants such as Vit A, E, and ß-carotene and GSH protect the living organisms against free radicals (Miller *et al.* 1993). In the current study, the cow with ROP showed decreased CAT, SOD, GSH and TAC values and increased MDA values, proving that these ROP affected animals were under severe oxidative stress. The present study results were similar with reports submitted by Kankofer (2001) as he reported that the cows affected with ROP had significantly lower GSH-Px level in the maternal as well as in the placental tissues than cows without affected ROP. The antioxidative defense system is the complex which is used to scavenge the free radicals. It was reported that some sort of oxidative stress was observed in ROP case as compared to the animals that properly shed placenta (Kankofera *et al.* 2005) and also observed that ROP in cattle is associated with imbalance between production and scavenging or neutralization of ROS. It was also reported that the GSH-Px activity was significantly lower in the dystocia affected animals than the animals showing the normal labour whereas MDA level was significantly higher in the dystocia affected group than in the normal labour animals (Yildiz *et al.* 2011). The present observations were similar to the earlier reports of Gupta *et al.* (2005) and Ahmed *et al.* (2009). MDA is measured to assess the lipid peroxidation level and is one of the products of the final decomposition of process of the lipid peroxidation. MDA is formed as a final product of the cyclooxygenase reaction in the prostaglandin metabolism process. High level of MDA was observed in the ROP affected animals in the present study. Higher levels of MDA in ROP affected cows may be due to higher levels of eicosanoids, glucocorticoids and adrenaline-stimulated mechanism of aerobic production of energy which was associated with calving, which causes more production of metabolites of reactive oxygen species and lipid peroxidation. In ROP affected cows, there is a decreased level of antioxidants activity and enhanced MDA concentrations which clearly indicated that the free radical formation and the beginning of an oxidative stress process in the ROP affected animals. Moreover, it was also suggested that changes in antioxidative capacity are associated to alterations in endocrines levels which occurs close to calving, parturition itself creates changes in oxygen consumption and pressure together may stimulate the increased production of ROS. The important clinical symptoms are observed in the imbalance of steroid hormones as well as the prostaglandin F2α concentration (Heuwieser and Grunert 1987).

It was reported that the reduction of the antioxidant enzyme activity in placenta during the stage of pregnancy may also significantly contribute to the causative of ROP in bovine and bubaline species (Wischral *et al.* 2000, Gupta *et al*. 2005). Lower levels of pre-partum placental antioxidant such as SOD as well as the hormone such as plasma estrogen were induced ROP in the cows (Wischral *et al.* 2001). Researchers proposed a mechanism for placental retention starting with disturbances in the antioxidant capacity at the level of placenta, followed by reduction in the estrogen production which resulted in reduced PGF2α production and accumulation of arachodonic as well as linoleic acids within placental tissue (Wischral *et al.* 2001). A meta-analysis study comprises of 44 research investigations on incidence of ROP in cows supplemented with Vit E as antioxidant and control untreated cows indicated that Vit E supplementation has reduced the ROP incidence in cows (Bourne *et al.* 2007), however, the benefits of Vit E treatment could depend on either cattle having adequate or marginal blood Vit E before treatment (LeBlanc *et al.* 2002). However, such type of parallel studies on cause and incidence of retained placenta in crossbred cows in Andaman and Nicobar Islands is scanty thus, alarming the research scope on cows.

It could be concluded that ROP is the most important factor leading to uterine infection and toxic puerperal metritis occurring during the early post-partum period. In the present study, the ROP was due to anaemia, lack of antioxidants, overproduction of free radicals, and imbalance of endocrinological profiles. The optimum is to maintain a healthy, contented and active cow prior to, during and after parturition. A balanced, limited ration with good source of antioxidants during the 6–8 week dry period; sufficient daily exercise; sufficiently large, clean and comfortable calving areas; and proper sanitary procedures during the calving period minimize the chances of retention and infections of the reproductive tract.

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