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Alteration in peripheral blood concentration of certain pro-inflammatory cytokines in cows developing retention of fetal membranes

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ABSTRACT

Retention of fetal membranes (RFM) adversely affects the production and reproduction potential of the affected cows leading to huge economic loss. Physiological separation of fetal membranes is reported to be an inflammatory process. The present study compared the concentrations of certain pro inflammatory cytokines [Interleukin 1β (IL-1), Interleukin 6 (IL-6), Interleukin 8 (IL-8) and Tumor necrosis factor α (TNF-α) between the cows that developed RFM ($n = 10$) and the cows that expelled fetal membranes normally ($n = 10$) to find out if they could serve as a predictive tool for RFM. Blood samples were collected from the cows from 30 days before expected parturition through day −21, day −14, day −7, day −5, day −3, day −1, on the day of parturition (day 0), day 1 postpartum and the pro-inflammatory cytokines were estimated in blood plasma by ELISA method. The IL-1β concentration was significantly lower ($P < 0.05$) in cows that developed RFM compared to those that expelled fetal membranes normally from 3 days before calving till the day of calving. The plasma concentrations of IL-6 and IL-8 were also lower ($P < 0.05$) in cows that developed RFM than those calved normally. On the day of calving, significantly ($P < 0.05$) lower concentrations of TNF-α was observed in cows that developed RFM compared to those expelled fetal membranes normally. It may be inferred that the concentrations of IL-1, IL-6, IL-8 and TNF-α around parturition were altered in cows developing RFM compared to those expelled fetal membranes normally.

1. Introduction

Fetal membranes are generally expelled within 12 h after delivery of the calf in dairy cattle (Drillich et al., 2006; El-Malky et al., 2010; Hanafi et al., 2011). Failure to expel the fetal membranes within 12 h after parturition lead to retention of fetal membranes (RFM), the incidence of which in dairy cows has been reported to range from 3 to 12% (Sheldon, 2004). However, higher incidences of RFM have been reported in crossbred (26%) and Zebu (16%) cattle (Kumari et al., 2014). High incidence of RFM in a herd incurs huge economic loss as productive and reproductive performance of the dairy animals is seriously compromised during postpartum period. During parturition, the dam

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executes a series of cellular and molecular events resulting in expulsion of fetal membranes (Gunnink, 1984). Parturition is an inflammatory process in the utero-placental tissues and activation of the maternal immune response against the fetal membranes plays an important role in the separation of fetal membranes (Norman et al., 2007; Jabbour et al., 2009). In dairy cattle, it has been shown that RFM is associated with impaired neutrophil function and reduced chemotaxis (Kimura et al., 2002). Thus, the release of pro-inflammatory cytokines from uterine epithelial cells (Tzianabos, 2000; Butterfield et al., 2006) during parturition is important for the migration of inflammatory cells to the site of feto-maternal junction, which is a critical step for fetal membrane separation. Pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) and the chemokine IL-8 stimulate neutrophil and monocyte diapedesis, chemo attraction and promote phagocytosis (Semnani et al., 1993; Kabbur et al., 1995; Kimura et al., 2002; Salanga and Handel, 2011).

Several studies indicated that altered inflammatory process and lower levels of pro-inflammatory mediators are associated with RFM in cows (Walter and Boos, 2001; Boos et al., 2003; Takagi et al., 2007; Beagley et al., 2010; Ghai et al., 2012; Streyl et al., 2012). Further, IL-8, an important chemotactic agent for neutrophils, was also reported to be lower in cows with RFM than cows that expelled fetal membranes normally (Beagley et al., 2010). From the above studies, it is evident that a cytokine mediated immune mechanism operates at feto-maternal interface for normal placental separation while any altered or delayed release of such cytokines may impair the separation process resulting into the development of RFM. We hypothesized that an altered pro-inflammatory cytokine mediated immune response/changes around pre-partum is responsible for the development of RFM in cows. Thus the aim of the present study was to estimate some important pro-inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) in peripheral blood plasma of cows that developed RFM and to compare with cows that expelled fetal membranes normally.

2. Materials and methods

The study was conducted on zebu (Sahiwal) and KF crossbred (Holstein Friesian X Tharparkar) cows maintained at Livestock Research Centre of National Dairy Research Institute, Karnal, situated in the Trans-Gangetic plain region of India. Sahiwal is one of the most important descript Zebu dairy breed known for its high milk yield (average 2270 kg of milk during a lactation) and heat tolerance. Due to its unique characteristics, this breed has been recognized by other countries also and played role in development of several breeds like Australian milking Zebu and Australian Friesian Sahiwal.

2.1. Experimental animals and their management

A total number of 64 pregnant crossbred ($n = 33$) and Sahiwal ($n = 31$) cows (40 days prior to expected date of calving) were initially recruited for the present study. All the cows used in the study were of 3–5 parity, apparently healthy and vaccinated against common diseases (like Brucellosis, Foot and Mouth Diseases, Hemorrhagic Septicemia and Black Quarter) as per the standard management practices of the farm. Cows were maintained in loose housing system under group management practice. Dry and lactating cows were housed separately in paddocks with brick on edge flooring. The space in the paddock, feeding manger and watering trough was as per BIS standard. The cows were fed as per NRC (2001). Milking cows were given additional concentrate at the rate of 1.0 kg for every 2.5 kg milk production, above 5.0 kg milk yield. The concentrate was fed to the cows in divided allowances milking (3 times a day). Pregnant animals and nearing parturition animals were maintained separately. Before one week of the expected date of calving, cows were moved to separate calving pens where the animals were retained up to 5 days post-calving.

2.2. Blood sampling protocol and grouping of animals

Blood samples were collected from all the 64 experimental cows from 30 days before expected parturition through day −21, day −14, day −7, day −5, day −3, day −1, on the day of parturition (day 0), day 1 postpartum till day 2 postpartum through jugular venipuncture using 9 mL blood collection tubes with EDTA as anticoagulant (*Vacuette®*, *Greiner Bio-one Gmbh*, Austria). Immediately after collection, blood samples were centrifuged at 4 °C (1500 × *g* for 20 min), plasma was separated, and stored in cryovials at −20 °C till assay.

All the experimental cows were observed for expulsion of fetal membranes. The time of calving and the time of expulsion of fetal membranes were noted. Cows that expelled the fetal membranes within 12 h after delivery of the calf were grouped under normal parturition (NP) group while the cows that failed to expel the fetal membranes within 12 h after delivery of calf were grouped under retained foetal membrane (RFM) group. Cows with abnormal calving like dystocia were excluded from the study. After eliminating the cows that calved 3 days or earlier than the expected date of calving and cows that had abnormal calvings, we selected 10 RFM cows (5 crossbred and 5 Sahiwal) and 10 normal cows (5 crossbred and 5 Sahiwal) for estimation of pro-inflammatory cytokines.

2.3. Estimation of pro-inflammatory cytokines in blood plasma

Pro-inflammatory cytokines IL-1, IL-6, IL-8 and TNF-α were estimated in blood plasma using ELISA kits (USCN, Life Science Inc.) as per the protocols provided by the manufacturers. The standard curve and the concentration of IL-1β, IL-6, IL-8 and TNF-α were calculated using GraphPad PRISM® 3.0 software package (GraphPad software, USA). The minimal detectable concentration of IL-1β, IL-6, IL-8 and TNF-α using the kit was 0.1 ng/mL.

2.4. Statistical analysis

Descriptive statistics were calculated for concentrations of all the interleukins for breeds, RFM/NP and different days; the results are expressed as mean ± SEM. Differences in blood plasma concentrations of IL-1β, IL-6,