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Transcriptional abundance of antioxidant enzymes in endometrium and their circulating levels in Zebu cows with and without uterine infection

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ABSTRACT

Oxidative stress during peripartum period may compromise the uterine immunity. In the present study, we assessed the oxidative stress and antioxidant status during peripartum period and studied their relationship with postpartum uterine infection in dairy cows. Peripheral blood concentrations of total antioxidant capacity (TAC), malondialdehyde (MDA) and nitric oxide (NO) were determined (day −21, −7, on the day of calving and day +7, +21, +35) in normal (n = 11), puerperal metritic (n = 7) and clinical endometritic (n = 6) cows. Endometrial biopsy was performed on the day of calving and expression of CAT, GPx4 and SOD2 genes was studied using qRT-PCR. Puerperal metritic cows had significantly ($P < 0.05$) lower TAC (on day −7, day 0, day +7, +21 & +35), higher MDA (on day −21, −7 & on the day of calving) and NO (on day 0, +7 & day +35) concentrations compared to normal cows. Similarly, clinical endometritic cows had significantly ($P < 0.05$) lower TAC (on day −7, 0, +7 & +21), higher MDA (on day −21, −7, +7 and +35) and NO (on day +7, +21 & +35) concentrations compared to normal cows. The expression of CAT and GPx4 genes was lower ($P < 0.05$) and SOD2 gene was higher ($P < 0.05$) in endometrial tissue of cows that developed uterine infection compared to normal cows. The relationship of peripheral levels of MDA and NO with antioxidant enzymes expression in endometrial tissue was found significant. Receiver operator characteristic analysis revealed that the concentrations of TAC on day −7 to day +35, MDA on day −21 to day +7 and NO on the day of calving to day +35 were highly correlated to the development of postpartum uterine infection in cows. It may be inferred that the low serum TAC level and high level of lipid peroxidation and NO during peripartum period influenced the endometrial expression of anitioxidative genes that compromised the uterine health during postpartum period.

1. Introduction

In dairy cattle, postpartum uterine infection is one of the most important causes of sub-fertility and infertility leading to increased involuntary culling and economic

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losses (Bartlett et al., 1986; Sheldon, 2004). The incidence of uterine infection is quite high ranging from 12.7 to 47.9% in cattle and buffaloes including metritis 20% (8–>40%); clinical endometritis 20% (5–>30%) and subclinical endometritis 30% (11–>70%) (Le Blanc et al., 2002; Hammon et al., 2006; Goshen and Shpigel, 2006; McDougall et al., 2007).

During peripartum period, dairy animals undergo numerous physiological stresses leading to generation of pro-oxidative and oxidative molecules. The production of low or moderate concentrations of reactive oxygen or nitrogen species (ROS and RNS) is especially essential for a number of normal physiological processes related to innate and acquired immune response. For instance, ROS is necessary for the oxygen-dependent destruction of invading pathogens (Valko et al., 2007) and can control the magnitude and duration of the inflammatory response since it is involved in signal transduction pathways leading to the expression of cytokines, eicosanoids and other immunoregulatory factors (Finkel, 2011). However, very high production of oxidative molecules surpassing the endogenous level of antioxidative molecules in the host results oxidative stress situation where uncontrolled release of oxidants modify and denature functional and structural molecules of cells bringing cell injury and dysfunctions (Vaziri, 2008). Several endogenous antioxidant defense mechanisms tightly regulate ROS accumulation within tissues. Regulation of redox system is mainly provided by antioxidative enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Young and Woodside, 2001; Willcox et al., 2004).

Several studies suggested that uncontrolled oxidative stress leads to host immune dysfunction and aggravate inflammatory responses that can increase the incidence and severity of infectious diseases (Miller et al., 1993; Sordillo and Atiken, 2009). Recent reports on redox changes during peripartum period have drawn attention to correlate these changes with uterine health. Kizil et al. (2010) reported that acute uterine infection in cows was associated with increased level of lipid peroxidation and altered levels of antioxidative molecules. However, the influence of these changes on uterine endometrial redox environment especially in relation to postpartum uterine health is poorly understood.

We hypothesized that alterations in oxidative and antioxidative molecules in peripheral circulation may influence the antioxidative defense mechanism in uterine endometrium and contribute to development of uterine infection during postpartum period in cows. Thus the aim of the present study was (i) to estimate peripheral concentrations of total antioxidative capacity (TAC), malondialdehyde (MDA) and nitric oxide (NO) during peripartum period and (ii) to study the expression of catalase (CAT), glutathione peroxidase (GPx4) and superoxide dismutase (SOD2) genes in uterine endometrium in Zebu cows that did and did not develop postpartum uterine infection.

2. Materials and methods

The study was conducted on Zebu (Sahiwal) cows maintained at Livestock Research Centre of National Dairy Research Institute, Karnal, India. Sahiwal is one of the most important descript dairy breed known for its high milk yield (average 2270 kg of milk during a lactation), heat tolerance and disease resistance.

2.1. Experimental animals

A total number of 40 cows were selected during prepartum period and out of these, 24 cows calved exactly one day before or after the expected date of calving, were considered for the experiment. All the cows used in the study were of 2–4 parity, apparently healthy, undergone normal calving without the occurrence of dystocia, retention of fetal membrane and any other parturient problems and were maintained under iso-managerial system under loose housing system. The nutrient requirements of the animals were mostly met with ad lib green fodder and measured amount of concentrate as per National Research Council (NRC, 2001) requirement. Animals for present experiment were duly approved by Institute Animal Ethics Committee (1705/Go/ac/13/CPCSEA).

2.2. Blood sampling protocol

Blood samples were collected from all the 24 experimental cows on day −21, −7 (before calving), on the day of calving (day 0) and day +7, +21 and

+35 (post calving) through jugular venipuncture using 9 mL blood serum collection tubes (Vacuette®, Greiner Bio-one Gmbh, Austria). Blood samples were kept at room temperature for 1 h and centrifuged at 3000*g* for 15 min, serum was separated and stored in cryovials at −20 °C till assay.

2.3. Endometrial biopsy sampling

Endometrial biopsy was performed using uterine biopsy forceps (54 cm; M/S Hauptner, Solingen, Germany) on the day of calving immediately after expulsion of fetal membranes. All biopsies were performed by the same veterinarian during the entire period of the study. Briefly, animal was restrained and epidural anesthesia was administered. Biopsy instrument protected by sanitary sheath was introduced into the vagina through the vulval opening. The forcep was passed through the cervix, then to the previously gravid horn (site of biopsy i.e. at the level of bifurcation of uterine horns) by *trans*-rectal palpation. Endometrial tissue was clipped off by closing jaws and the instrument was withdrawn. The tissue was washed with nuclease free water to remove any contamination of blood and placed in RNA later (Qiagen, Austin, US) at room temperature for 2 h and then samples were stored in −80 °C till further processing. Uterine fluid samples were obtained by using universal gun with blue sheath.

2.4. Grouping of animals

All cows were evaluated for postpartum uterine infection on day 4, 10, 14, 17, 21, 24, 28, 35 and 42 postpartum based on *trans*-rectal ultrasonography of uterus, uterine fluid scoring and other clinical signs including rectal