



Peripheral concentrations of metabolic and inflammatory indicators during transition period and their relationship with postpartum clinical endometritis in dairy cattle

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ABSTRACT

The aim of this study was to investigate the peripheral concentration of metabolic and inflammatory indicators during transition period and their relationship in cows that developed clinical endometritis (CE) and remained non-endometritis during later postpartum period. A total of 70 cows were selected and blood was collected from 21 days before calving to 21 days after calving. After adjusting the sampling date based on the actual calving date (-21 ± 2 , -7 ± 1 , 0, 7 ± 1 , 14 ± 1 and 21 ± 2 days) and health status, a minimum of 8 samples each from the CE and non-endometritis cows were used at each time point of transition period for the estimation of acute phase proteins (APPs) (haptoglobin, Hp; serum amyloid A, SAA; and alpha-1 acid glycoprotein, AGP), pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6 and IL-8) and energy indicators (NEFA, BHBA, Leptin and IGF-I) using bovine specific ELISA kits. APPs (Hp, SAA and AGP), cytokines (IL-1 β , TNF- α , IL-6) and energy indicators (NEFA, BHBA, Leptin) levels significantly increased, while IL-8 and IGF-I levels significantly decreased in transition cows that eventually developed to CE compared to the cows which remained non-endometritis during 22–47 days postpartum. It is concluded that cows that diagnosed as CE during postpartum period were experienced negative energy balance and compromised their immune status during transition period.

Key words: Acute phase proteins, Clinical endometritis, Endometritis, Energy indicators, Pro-inflammatory cytokines, Transition period

Majority of the health disorders are occurring around transition period due to nutritional, hormonal and metabolic changes in dairy cows (Drackley 1999, Overton and Waldron 2004). Although more than 95% of the animals get exposed to bacterial contaminations during calving (Sheldon and Dobson 2004), only a few of them develop uterine disease and it could be the associated outcome of pathogens, animal, and environment. However, in a particular environment with equal chances of exposure to pathogens, the greater susceptibility of certain animals in the herd suggested the bigger role of animal factor than others. Postpartum uterine

diseases occur due to greater bacterial contamination with reduced immune function. However, the exact mechanisms by which uterine inflammation are initiated and sustained are not clear at present. An understanding of the uterine defense system during transition period is an important tool for postpartum health management (Dubuc 2011).

Endocrine and metabolic changes around parturition are believed to depress the immune defense mechanism, which consequently favours the development of uterine disease in dairy cattle (Mateus *et al.* 2002, Kim *et al.* 2005). The first response of the innate immune system against uterine infection is the invasion of neutrophils into the uterus, which is determined by pro-inflammatory cytokines and energy balance. For instances, Galvao *et al.* (2011) reported that lower expression of pro-inflammatory cytokines in the endometrium immediately after calving impaired the chemotaxis and neutrophils activity and Negative Energy Balance (NEB) is believed to be an important determinant of neutrophils functionality in uterine infected cows (Hammon *et al.* 2006).

Prevention and early treatment of postpartum uterine infections are more economical than treatment at later stage when diseases get established. Thus, molecules for early

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diagnosis or predictions of uterine infections would be very useful for effective postpartum management. Huzzey *et al.* (2009) reported that haptoglobin (Hp) would be a useful molecule in early detection of metritis and other health disorders (Huzzey *et al.* 2011). Dubuc *et al.* (2010) reported that Hp concentrations >0.8 g/l during first week of postpartum is a risk factor for endometritis. To the best of our knowledge, none of the studies have evaluated the dynamics of major APPs, its regulatory cytokines and energy indicators during transition period in relation to clinical endometritis (CE) conditions in moderate yielding Indian dairy cows. We hypothesized that the indicators of energy and immune status are altered in transition period before the development of CE conditions. The aim of this study was to investigate the relationship between metabolic and inflammatory status during transition period in cows that developed CE and remained non-endometritis during later postpartum period.

MATERIALS AND METHODS

Study area and experiment animals: The study was conducted on Karan Fries crossbred (Holstein Friesian $\times$ Tharparkar) cows maintained at Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal, situated in the Trans-Gangetic plain region of India. The climate of the farm is subtropical in nature. In total, 70 pregnant cows were initially recruited for the study 21 days before calving and all the experimental cows were from multiparous group. Pregnant cows were housed separately (60 days before calving) in paddocks with a brick on edge flooring system. Animals were in good health conditions while inclusions and observed daily during transition period with special attention during calving. The average milk yield of study animals was 13.5 kg/day. The nutrient requirements of the experimental animals were met with *ad lib.* grown green fodder, dry fodder, silage and a measured amount of concentrate. The concentrate mixture was fed at 1.5–2.0 kg/animal for body maintenance and extra concentrate of 1.5 kg/cow was fed 2–3 weeks before the expected date of calving. The concentrate mixture contained maize (33%), groundnut cake (21%), mustard cake (12%), wheat bran (20%), deoiled rice bran (11%), mineral mixture (2%) and common salt (1%) as per National Research Council (2001) standards. Milking cows yielding >5.0 kg milk yield were given additional concentrate @ 1.0 kg for every 2.5 kg milk production. The concentrate mixture was fed in divided allowances during milking. Cows were milked thrice a day using a milking machine.

Blood collection and assays: Blood collection was done through jugular venepuncture using 9 mL blood collection tubes with heparin as an anticoagulant (Vacuette®, Greiner Bio-one GmbH, Austria). Immediately after collection, blood samples were centrifuged at 4°C (1,500 g for 20 min), plasma was separated, and stored in cryovials, at -20°C till assay. The concentration of APPs (Haptoglobin, Hp; serum amyloid A, SAA; and α_1 acid glycoprotein, AGP), pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6, and IL-8) and

energy indicators (NEFA, BHBA, leptin and IGF-I) were estimated using commercially available bovine specific ELISA kits. ELISA kits were purchased from different sources (NEFA from M/s MyBioSource, Inc., San Diego, California, USA; BHBA from M/s EIAab Science Co. Ltd., China; Haptoglobin from M/s Life Diagnostics, Inc., West Chester, Pennsylvania, USA; IGF-I, Leptin, SAA, AGP, IL-1 β , TNF- α , IL-6, and IL-8 were from M/s Usn life science Inc., Wuhan, Hubei, China) and assay was performed as per manufactures recommendations.

Diagnosis of clinical endometritis: Cows with abnormal vaginal discharges between 7 to 47 days postpartum period were examined per rectum as well as per vaginum and the diagnosis was made according to Sheldon *et al.* (2006). Briefly, cows with purulent or mucopurulent uterine discharge after 22 or 26 days to 47 days postpartum, respectively was considered as clinical endometritis. During study period, all the experimental cows were observed daily for any abnormalities. After adjusting the date of sampling with actual calving date (-21 ± 2 , -7 ± 1 , 0, 7 ± 1 , 14 ± 1 , and 21 ± 2 days), cows with inappropriate or a smaller number of sampling and cows suffering from any postpartum complications, uterine infections other than clinical endometritis (metritis, subclinical endometritis) were excluded from study. Thus, 8 cows, each from clinical endometritis and non-endometritis group and had appropriate samples during transition period were used for analysis.

Statistical analysis: The data were analyzed by using mixed-model repeated measure analysis with statistical software package SPSS version 16 (SPSS for windows, V16.0; M/s SPPS Inc., Chicago, IL, USA). Further, the interaction between 2 group's, i.e. endometritis and non-endometritis cows at each time point of intervals (i.e. from -21 to $+21$ days) and interaction within a group was analyzed by using the univariate method. The data were expressed as means $\pm$ SE and the differences between groups were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Hp and SAA are the major APPs, while alpha-1 acid glycoprotein (AGP) is believed to be a moderate APP in bovines (Eckersall and Bell 2010). Cairoli *et al.* (2006) reported that monitoring of APP during pre-partum period could be useful to detect animals prone to develop endometritis during postpartum period. In the present study, plasma concentration of APPs such as Hp (Fig. 1a), SAA (Fig. 1b) and AGP (Fig. 1c) were significantly ($P < 0.05$) increased in transition cows, which eventually progressed to CE condition compared to the cows which remained non-endometritis during postpartum period. In several studies, uterine infection was associated with higher concentrations of APPs during postpartum period (Williams *et al.* 2007, Huzzey *et al.* 2009, Chan *et al.* 2010). Nightingale *et al.* (2015) found that postpartum reproductive performance was impaired in cows with a greater acute phase response (APR). The APR is induced by pro-inflammatory cytokines

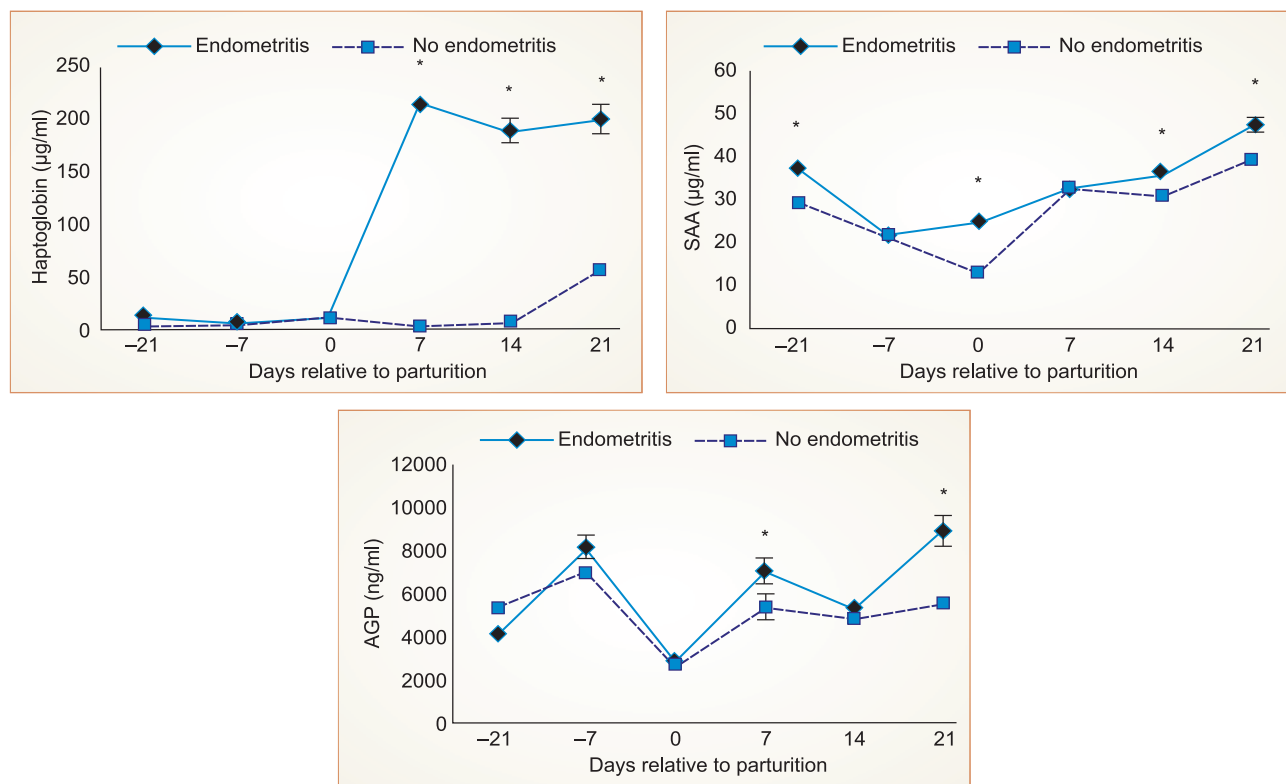


Fig. 1. Changes in plasma concentrations of acute phase proteins [Haptoglobin–Hp (1a); Serum amyloid A–SAA (1b) and Alpha-1 acid glycoproteins–AGP (1c)] in transition cows which eventually developed clinical endometritis (N=8) and remained no-endometritis (N=8). Lines (mean $\pm$ SE) bearing asterisk differ significantly ($P < 0.05$) between the group at each sampling day intervals.

produced by inflammatory cells at the site of infection or inflammation, which subsequently induces hepatocytes to synthesize APPs (Petersen *et al.* 2004). Higher peripheral concentration of pro-inflammatory cytokines in these cows indicated their regulatory role in APP synthesis. In our study, the magnitude of elevation in Hp concentration was higher (3.5 to 65-fold) than SAA (1.2–2 fold) or AGP (1.3–1.6 fold) during early postpartum period and indicated that Hp could be a better indicator of endometritis and it could be due to its bigger and more prolonged response under disease conditions (Horadagoda *et al.* 1994, Carter *et al.* 2002).

We found significantly ($P < 0.05$) higher plasma concentrations of IL-1 β (Fig. 2a), IL-6 (Fig. 2b) and TNF- α (Fig. 2c) in transition cows that developed into CE than remained non-endometritis during postpartum period. It indicated that damage to endometrium and activation of inflammation due to persisted infection in these cows. The observed lower concentration ($P < 0.05$) of IL-8 (Fig. 2d) in transition cows also suggested the impaired uterine immunity as the IL-8 is an important chemoattractant and defense molecule for prevention of uterine infection during early postpartum. Higher concentrations of IL-1 β , TNF- α and IL-6 were reported in CE cows (Chapwanya *et al.* 2009, Galvao *et al.* 2011, Kasimanickam *et al.* 2013).

An excessive pro-inflammatory state during early postpartum period appears to be a key feature for development of endometritis, and postpartum NEB often aggravates the uterine inflammation (LeBlanc 2012). In

our study, NEFA level (Fig. 3a) during pre-partum period and BHBA level (Fig. 3b) during postpartum period were significantly ($P < 0.05$) increased in transition cows that developed CE during later postpartum period as also reported by other researchers (Hammon *et al.* 2006, Giuliodori *et al.* 2013). NEB-mediated induction of uterine pro-inflammatory cytokines and impairment of chemoattraction (Wathes *et al.* 2009, Galvao *et al.* 2010, Hammon *et al.* 2006) might have favoured the development of clinical endometritis.

Leptin plays an important role in regulation of feed intake, metabolic, endocrine and immune functions (Schoenberg *et al.* 2011). We found that leptin concentration was higher during transition period in the cows which developed CE later (Fig 3c). Kasimanickam *et al.* (2013) also reported higher leptin levels in CE cows. In contrast, we found its level below detection limit during early postpartum period in cows which developed CE. Although it is difficult to explain, the compromised energy status could be attributed to persisted higher pre-partum leptin concentration in these cows. Therefore, in quest of increasing their feed intake after parturition, the animals might have undergone adaptive changes to reduce the level of leptin to the non-detectable level in the early postpartum (Schoenberg *et al.* 2011). The nutritional regulation of reproductive function during the postpartum period is mediated to a great extent by circulating IGF-I level (Zulu *et al.* 2002). We observed the significantly ($P < 0.05$) lower

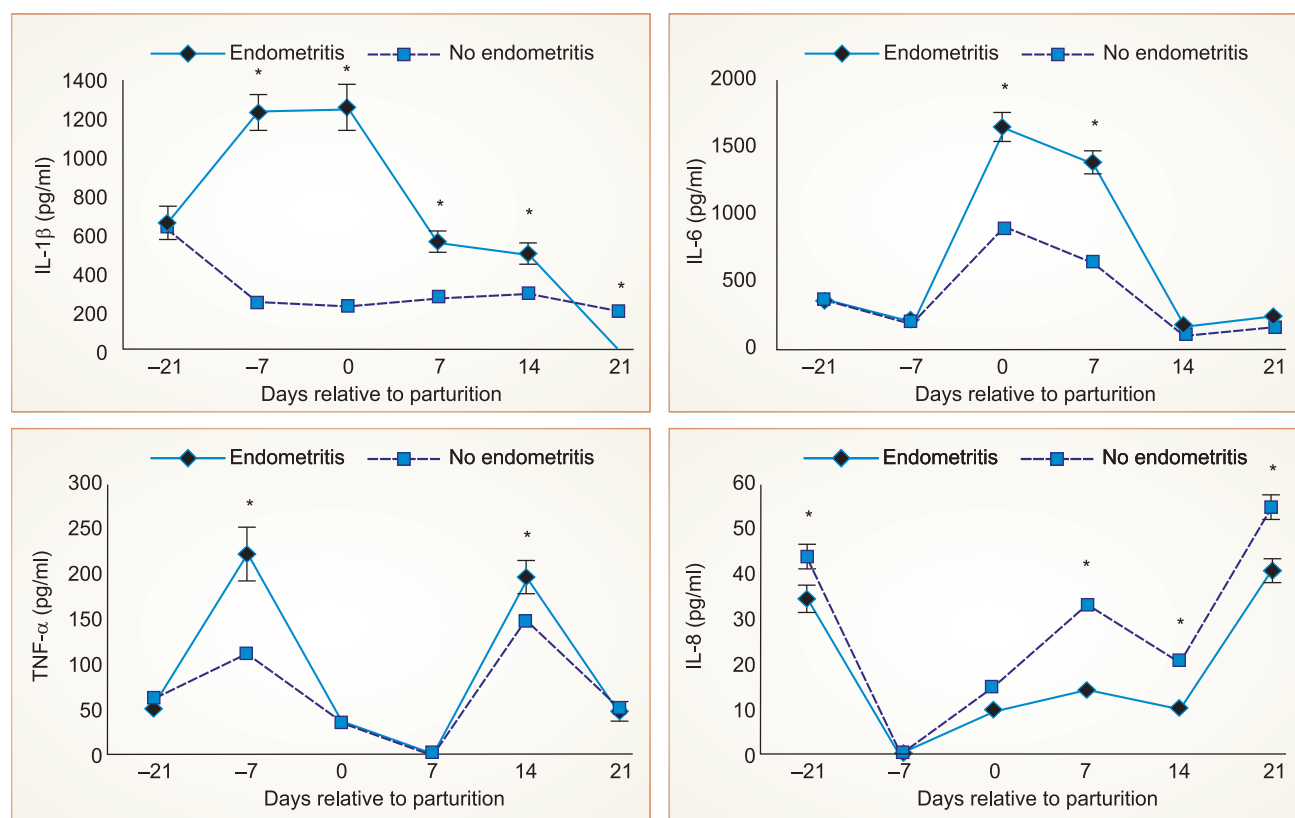


Fig. 2. Changes of plasma concentration of pro-inflammatory cytokines [IL-1 β (2a), IL-6 (2b), TNF- α (2c) and IL-8 (2d)] during transition period in cows which eventually developed clinical endometritis (N=8) and remained no-endometritis (N=8). Lines (mean $\pm$ SE) bearing asterisk differ significantly (P < 0.05) between the group at each sampling day intervals.

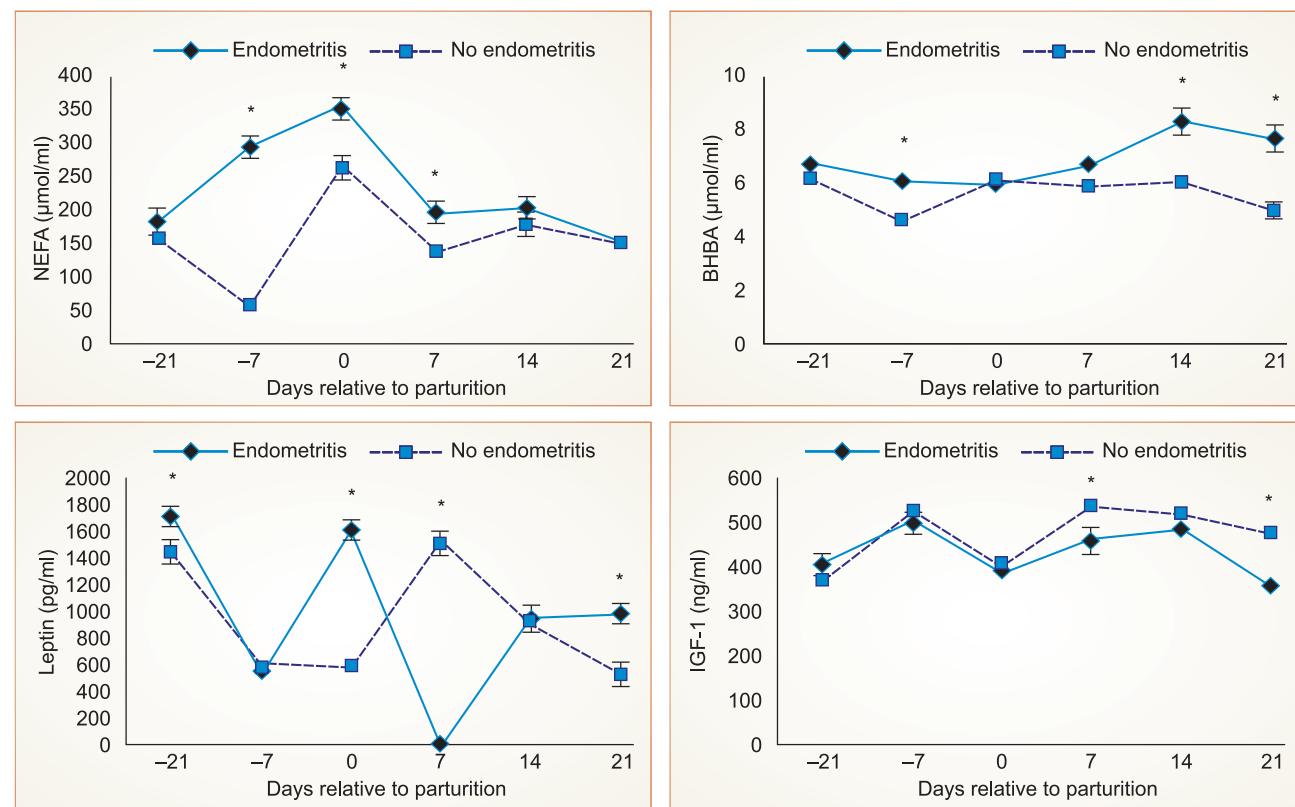


Fig. 3. Changes in concentration of energy indicators [leptin (3a), IGF-1 (3b), NEFA (3c) and BHBA (3d)] during transition period in cows which eventually developed clinical endometritis (N=8) and remained no-endometritis (N=8). Lines (mean $\pm$ SE) bearing asterisk differ significantly (P < 0.05) between the group at each sampling day intervals.

concentration of IGF-I in cows which developed CE later (Fig. 3d). Kasimanickam *et al.* (2013) also reported lower IGF-I levels in CE cows and suggested persistent uterine inflammation in these cows. As observed in the current study, an antagonistic relationship between the pro-inflammatory cytokines and IGF-1 levels generally occurs during disease conditions (Jacobi *et al.* 2006). Therefore, the poor energy status indicated by higher energy metabolites level and lower leptin and IGF-I levels might have reduced the ability of the animal to raise an effective immune response against bacterial infection after calving, making them more susceptible to uterine infections during later postpartum period (Kasimanickam *et al.* 2013).

Since it will be difficult to relate the metabolic and inflammatory variables during transition period with endometritis outcome with small number of cows, further studies are required with optimum sample size. An establishment of threshold value or likelihood ratio of these variables with CE using logistic regression modelling is also required for more understanding. Other biochemical assays are required to confirm the present findings as the absolute values of the ELISA are varied with other studies. Taken together, it is concluded that cows that were diagnosed as clinical endometritis during postpartum period experienced negative energy balance and compromised their immune status during transition period than cows that were not diagnosed with clinical endometritis.

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