Theriogenology 82 (2014) 427–432

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Inflammatory cytokine concentrations in uterine flush and serum samples from dairy cows with clinical or subclinical endometritis

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ARTICLE INFO

Article history:
Received 16 January 2014
Received in revised form 28 April 2014
Accepted 28 April 2014

Keywords:
Endometritis
Cytokines
Uterine flush
Serum
Dairy cows

ABSTRACT

The objective of this study was to compare the concentrations of inflammatory cytokines in uterine flush and serum from healthy postpartum dairy cows and cows with clinical or subclinical endometritis. Clinical endometritis was diagnosed by observation of vaginal discharges (>50% pus) and subclinical endometritis was diagnosed by evaluation of uterine cytology (neutrophils >18%) at 4 weeks postpartum. Uterine flush was obtained from 48 cows at 4, 6, and 8 weeks postpartum for evaluation of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-8, and IL-10 concentrations. Serum samples were obtained from 34 cows just after calving and at 1, 2, 4, 6, and 8 weeks postpartum for evaluation of TNF-α, IL-1β, and IL-6 concentrations. Concentrations of TNF-α, IL-6, and IL-10 were greater ($P < 0.05$) in cows with clinical endometritis than in cows with subclinical endometritis and healthy controls, whereas concentrations of IL-8 in both cows with clinical and subclinical endometritis were greater ($P < 0.005$) than in controls. Overall, IL-6 and IL-10 concentrations decreased during the postpartum period. IL-1β concentrations in cows with clinical endometritis decreased ($P < 0.0005$) during the postpartum, whereas concentrations in cows with subclinical endometritis and controls did not change significantly with time; at 4 weeks postpartum, concentrations were greater ($P < 0.0001$) in cows with clinical endometritis. There were no significant effects of group, sampling time, or interaction on serum cytokine concentrations. In conclusion, cows with endometritis have greater inflammatory cytokine concentrations in uterine flush than healthy cows, but no differences were observed in serum.

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1. Introduction

Most cows are exposed to bacterial infection after calving [1,2]. More than 70% of cows clear the uterine bacteria *via* innate immune responses; however, 17% to 37% of cows develop clinical endometritis, whereas 14% to 53% develop subclinical endometritis, which results in reduced fertility [3–9]. Proper regulation of immune responses during the weeks after calving is important for subsequent uterine health [10–13]. The development of bovine endometritis is associated with very complex signaling processes involving the detection of bacterial components by innate immune cells *via* Toll-like receptors, the production of tumor necrosis factor-α (TNF-α) and other proinflammatory cytokines (e.g., interleukins (ILs)), and the mobilization of neutrophils followed by phagocytosis of invading pathogens within the uterine lumen [14–21]. Proinflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6) and

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http://dx.doi.org/10.1016/j.theriogenology.2014.04.022

chemokines (e.g., IL-8) stimulate neutrophil and monocyte diapedesis and chemoattraction and promote increased phagocytosis [22].

Previous studies have used uterine biopsy [23] and the cytobrush technique [24] to examine the expression of inflammatory cytokine mRNA in uterine tissue collected from cows with endometritis. Others used endometrial tissue scraping from buffalos with endometritis [25]. The results revealed that the expression of TNF-α, IL-1β, and IL-6 (all proinflammatory cytokines), as well as IL-8 (the primary chemokine that regulates neutrophil activity) and IL-10 (an anti-inflammatory cytokine), was related to the development of bovine clinical or subclinical endometritis [23,24,26]. Moreover, a recent study suggests that the cytokine profile in uterine tissue is associated with the severity and persistency of uterine inflammation [25]. In addition, other studies found that serum obtained from cows with endometritis and from healthy cows during the peripartum period contained different levels of inflammatory cytokines [26,27]. Likewise, higher expression of inflammatory cytokine genes in uterine tissue and/or an increase in the levels of those cytokines in the serum are thought to predict the development of bovine endometritis [23,24,27]. However, no study has measured the levels of inflammatory cytokines in uterine flush from cows with endometritis. Measuring inflammatory cytokine levels in uterine flush and serum from dairy cows with clinical or subclinical endometritis during the voluntary waiting period may provide valuable information that can serve as a diagnostic tool for the uterine inflammation.

The objective of the present study was to compare uterine flush and serum concentrations of inflammatory cytokines in healthy postpartum dairy cows with cows that develop clinical or subclinical endometritis.

2. Materials and methods

2.1. Animals

All experiments were performed with the approval of the Institutional Animal Care and Use Committee of Chungbuk National University, Korea. This experiment was conducted on two dairy farms located in Chungcheong Province, Korea, during the period from March 2012 to September 2013. Forty-eight and 34 Holstein cows, with 2.3 ± 1.4 lactations (mean $\pm$ standard deviation; range: 1–7 lactations), were used to obtain uterine flush and serum samples, respectively. The cows were maintained in a loose-housing system, fed a total mixed ration, and milked twice daily.

2.2. Diagnosis of clinical and subclinical endometritis

All of the cows were evaluated for clinical endometritis at week 4 postpartum by examining vaginal discharges that are removed using the Metricheck tool [28]. Briefly, after cleaning the vulva with the disinfectant (chlorhexidine gluconate), the Metricheck device was inserted until it reached the vaginal fornix and then retracted for evaluation of the vaginal mucus contained in the cup. Cows with a mucopurulent uterine discharge (>50% pus) were diagnosed with clinical endometritis [1,3]. At the same time, cows were evaluated for subclinical endometritis by uterine cytology [29]. Briefly, after cleaning the vulva, a cytobrush and stainless steel rod (which was guarded by a stainless steel sheath and covered with a protective plastic sheath) were introduced into the vagina. At the external end of the cervix, the plastic sheath was pulled back, and the stainless steel sheath and stainless steel rod and cytobrush were passed into the body of the uterus. The stainless steel sheath was then retracted to expose the cytobrush. The cytobrush was rotated clockwise to obtain cellular material from the endometrium. After removal from the vagina, the brush was rolled onto a glass slide that was allowed to air-dry. All slides were stained using Diff-Quick stain (Sysmex Inc., Kobe, Japan) according to the manufacturer's guidelines. Each slide was examined under a microscope (×200 magnification) by the same examiner. The numbers of epithelial endometrial cells and neutrophils were counted (up to 200 cells per slide), and the percentage of neutrophils was calculated. Subclinical endometritis was defined as a neutrophil proportion more than 18% in the absence of clinical endometritis [1,30]. Cows not diagnosed with clinical or subclinical endometritis were classified as healthy controls.

2.3. Sampling of uterine flush and blood

Uterine flush samples were collected at weeks 4, 6, and 8 postpartum. Briefly, after thoroughly cleaning the vulva with disinfectant, a two-way Foley catheter (20 French, 30 mL) was placed into the previously pregnant uterine horn (determined as the horn with the greater diameter and length) and inserted approximately 5 cm past the point of bifurcation of the uterus. The cuff of the catheter was inflated with 8 to 10 mL of air (depending on the diameter of the uterine horn), and 20 mL of isotonic saline solution was infused into the uterine horn and recovered using a 60-mL sterile syringe. Aspirated fluid was transferred to a sterile 50 mL sterile disposable centrifuge tube and immediately placed in an ice bath. The samples were then centrifuged at 2000× *g* for 10 minutes at 4 °C, and the supernatant was transferred to 2 mL microcentrifuge tubes and stored frozen at −80 °C until analysis.

Blood samples were collected from the tail vein just after calving (1.0 ± 0.1 hours; range: 30 minutes to 3 hours) and then again at weeks 1, 2, 4, 6, and 8 postpartum. Ten milliliters of blood were placed into a plastic centrifuge tube without additives and immediately placed in an ice bath. The samples were then centrifuged at 2000× *g* for 10 minutes at 4 °C, and the serum was harvested and frozen at −80 °C until required.

2.4. Measurement of cytokine concentrations in uterine flush and serum samples

The concentrations of TNF-α, IL-1β, IL-6, IL-8, and IL-10 in uterine flush and TNF-α, IL-1β, and IL-6 in serum samples were determined using commercially available kits (a bovine TNF-α ELISA kit (R&D Systems, Minneapolis, MN, USA), bovine IL-1β and IL-6 ELISA kits and a human IL-10

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