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Endometrial expression of selected transcripts in postpartum of primiparous Holstein cows with clinical and subclinical endometritis

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ABSTRACT

Postpartum endometritis compromises milk production and fertility in high-producing dairy cows. Infection of the endometrium induces an inflammatory response with secretion of cytokines that lead to polymorphonuclear cells (PMN) influx and bacterial clearance. Considering that only a portion of cows with endometritis is eligible for clinical diagnosis, there is an increasing effort for developing reliable tools and protocols for diagnosis of subclinical endometritis. Recent reports have indicated that primiparous cows are at greater risk of uterine infection and primiparous cows with subclinical endometritis produce less milk compared to healthy cows. In the present study, gene expression profiles were compared for selected cytokine and hormone endometrial transcripts in the postpartum of primiparous Holstein cows with clinical and subclinical endometritis. Cows were classified as healthy (no signs of clinical endometritis), cows with subclinical endometritis (PMN < 5% in the cytological sample) and cows with clinical endometritis (PMN > 5%). Although, cows with clinical endometritis had greater ($P < 0.05$) relative amounts of mRNA for the *IL1A*, *IL6*, *IL17A*, *TNFα*, *PGES* and *PGHS2* genes compared to healthy cows; no significant differences were detected between clinical and subclinical endometritis groups. Spearman correlation coefficients were positive between relative amounts of gene expression as indicated by amount of these transcripts and PMN percentages and ranged from 0.74 to 0.93 ($P < 0.05$). Relative amounts of cytokine mRNA suggest similar inflammatory response in the endometrium of cows with subclinical and clinical endometritis. Moreover, differential relative amounts of hormone transcripts suggest dysregulation of the luteolytic mechanism and PG synthases but not ERα in cows with endometritis.

1. Introduction

Postpartum endometritis caused by persistent bacterial infection is prevalent in high-producing dairy cows and leads to reduced milk yield and fertility (LeBlanc

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et al., 2002). Presence of pathogenic bacteria in the uterus causes inflammation, histological lesions of the endometrium, and perturbs uterine involution, ovulation and embryo survival (Sheldon et al., 2006). Whereas, clinical endometritis is associated with the presence of sufficient polymorphonuclear cells (PMN) influx to result in purulent or mucopurulent uterine exudates through the cervix; subclinical endometritis is characterized by abnormally larger numbers of PMN in the uterine lumen without clinical signs, including purulent material in the vagina (Gilbert et al., 2005). Bacterial infection of the endometrium induces an inflammatory response with secretion of chemokines and cytokines including tumor necrosis factor α (TNFα), interleukin 1A and 6 (IL1A and IL6) (Roach et al., 2002; Chapwanya et al., 2012). TNFα and IL1A stimulate gene expression for potent chemotactic factors (IL8, monocyte chemotactic protein-1, (MCP-1)), and adhesion molecules on vascular endothelial cells, leading to PMN recruitment to the site of inflammation (Sica et al., 1990; Roach et al., 2002). Similarly, greater expression of the IL6 gene has been detected in cows with endometritis and elevated amounts of IL6 in serum before parturition has been detected in cows susceptible for developing endometritis postpartum (Ishikawa et al., 2004; Galvao et al., 2011). Furthermore, interleukin17A (IL17A) is produced by the lymphocyte T helper 17 (Th17) cells and has been involved in host defence in epithelial and mucosal barriers against several pathogens (Jin and Dong, 2013). Its role in endometritis in cattle has not been characterized; however, IL17A has been described in various immune responses and inflammation conditions (Kolls and Linden, 2004).

The inflammatory response in the endometrium against bacterial infection is also mediated by pro-inflammatory molecules such as prostaglandins (PG), which have roles as multifunctional factors that regulate production of cytokines and mediate the luteolytic mechanism during the estrous cycle (Arosh et al., 2002). Prostaglandin G/H synthases (PGHS2) is an enzyme involved in the conversion of arachidonic acid into PGH2, a common precursor for various forms of PG including PGE2 and PGF2α. The downstream enzyme PGE synthase (PGES) catalyzes the conversion of PGH2 to PGE2, which regulates production of various cytokines including TNFα and IL6 and is also involved in maternal recognition of pregnancy (Bos et al., 2004; Arosh et al., 2002). PGF2α secretion from the endometrium is an important regulator of the estrous cycle as it initiates the regression of the corpus luteum (McCracken et al., 1999). Luteolysis in cows is preceded by activation of an estrogen receptor (ERα) which influences the timing of upregulation of endometrial oxytocin receptor and subsequently the release of PG (Robinson et al., 1999).

Elucidating the molecular mechanism controlling the local immune response is important for diagnosing and controlling postpartum uterine infection and potentially for identifying prognostic indicators for cows undergoing clinical and subclinical endometritis. The objective of the present study was to compare selected cytokine and hormone endometrial gene expression profiles in postpartum of primiparous Holstein cows with clinical and subclinical endometritis.

2. Materials and methods

2.1. Experimental animals

All procedures were approved by the Bioethical Committee of the Faculty of Veterinary Sciences at University of Chile (Certificate No. N21-2014). Cows on a large scale (~1000 cows) commercial dairy farm in the central zone of Chile were examined at days 29–36 postpartum (pp) by palpation of the uterus per rectum, manual examination of the vagina and vaginoscopy. Approximately, 38% of lactating cows were primiparous and from this group, 25% were diagnosed with endometritis by clinical examination. Three groups ($n = 6$ cows per each group) were defined. Group 1, included primiparous healthy cows with no signs of clinical endometritis (i.e. presence of purulent or mucopurulent vaginal discharge) and no signs of subclinical endometritis (PMN < 5% in the cytological sample; Gilbert et al., 2005). Group 2, consisted of primiparous cows with subclinical endometritis. These cows had no clinical signs for endometritis but the percentage of PMN in the cytological sample was >5. Group 3 consisted of primiparous cows with signs for clinical endometritis (i.e. purulent or mucopurulent vaginal discharge).

2.2. Cytological tests

For each animal, an endometrial epithelium sample was collected from the uterine horn using a trans-cervical guarded swab (Noakes et al., 1989). The swab comprised a long plastic rod bearing a cotton wool tip sheathed in a plastic guard tube. The guard tube was covered by sterile plastic sheath to prevent contamination of the swab during the cervix insertion. After restraining the animal and securing its tail, the perineal region was washed and cleaned. The cervix was grasped per-rectum and the sterilized catheter was passed through the cervix into the right uterine horn. The inner rod of the catheter was pushed forward to expose the swab to the endometrium and was rotated against the uterine wall and withdrawn within the catheter. Smears were prepared for cytological examination by rolling swabs on glass slides. The smears were then allowed to dry at room temperature for 30–35 min. Slides were transported to the laboratory and a differential cell count of each smear was done on Giemsa-stained slides. Cells ($n = 200$) were counted in each of 20 microscopic fields (900×).

2.3. Endometrial biopsy

Endometrial epithelium samples were collected from the uterine horn *ex vivo* for quantitative-PCR (Q-PCR) analyses using a Hauptner biopsy instrument (Kevorkian's uterine biopsy forceps) (Galvao et al., 2011). Briefly, after cytology sample collection, the biopsy instrument covered with a protective sheath was introduced into the vagina and guided into the cervix by manipulation *per rectum*. The instrument alone was introduced into the uterus after rupturing the sheath at the external cervical orifice and guided into the right horn past the uterine bifurcation. After collection, samples were immediately fixed in 500 μL of RNAlater (Qiagen Incorporated, Valencia, CA, USA) and