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Peripheral blood leukocytes of cows with subclinical endometritis show an altered cellular composition and gene expression

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ABSTRACT

Subclinical endometritis (SCE) is an important postpartum disease in dairy cows, but conventional cytobrush diagnosis often gives imprecise results. The aim of this study was to analyze disease-associated changes in peripheral blood as potential diagnostic parameters. Cellular subpopulations of blood leukocytes from cows with or without SCE (45–55 days postpartum) were flow-cytometrically quantified. Gene expression of whole blood leukocytes was assessed by PAXgene analysis. Subclinical endometritis cows showed significantly higher number of blood mononuclear cells and neutrophils. Among mononuclear cells, numbers of B-cells, NK-cells, and CD172a-positive monocytes were significantly elevated. Compared with non-SCE cows, blood leukocytes of SCE cows significantly expressed higher copy numbers of *CXCL8*, *TNF*, and *IL12*. To test whether circulating plasma factors are responsible for these changes, leukocytes, polymorphonuclear cells, and monocyte subpopulations (classical, intermediate, nonclassical) of healthy cows were stimulated with plasma of SCE and non-SCE cows. Although gene expression of whole leukocytes and polymorphonuclear cells remained unaltered, plasma from SCE animals significantly elevated expressed messenger RNA copy numbers of *CXCL8, CXCL1*, and *IL1B* in intermediate monocytes. In conclusion, elevated number of selected mononuclear subpopulations in peripheral blood and enhanced expression of distinct genes encoding for inflammatory mediators in blood leukocytes reflect the subclinical uterine inflammatory process in cows. Whether the observed changes in the periphery of SCE cows are the consequence of the uterine inflammatory process, or whether they affect the pathogenesis of the disease is currently unknown.

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1. Introduction

Subclinical endometritis (SCE) is defined as endometrial inflammation of the uterus in the absence of purulent material in the vagina [1]. Because of the lack of clinical signs, the most convenient and powerful diagnostic method is still a matter of debate. Although endometrial biopsy may constitute the ideal method of diagnosis of endometritis, the procedure is invasive, expensive, and time-consuming [2–6]. Furthermore, it has been shown that an endometrial biopsy has a potential impairment on future fertility [7]. A standard technique to diagnose SCE is the quantification of polymorphonuclear cell (PMN) among uterine cells by the cytobrush technique [8]. Between 34 to 47 days postpartum (PP), the presence of more than 10% PMN defines the diagnosis SCE [8]. An altered cellular composition in cytobrush samples of cows with SCE is also reflected by a higher messenger RNA (mRNA) expression of lipocalin-type prostaglandin D synthase, *IL1A*, *IL1RN*, *IL6*, *TNF*, and *CXCL8* in uterine cells obtained by cytobrush technique compared with healthy cows [9].

Less invasive methods for the detection and diagnosis of SCE have involved the use of analysis of metabolic blood parameters, serum inflammatory mediators, antibodies, and the cellular composition of the blood: In cows with SCE, Heidarpour, et al. [10] found higher serum concentrations of β-hydroxybutyrate (BHB), haptoglobin, and total sialic acid than in healthy cows. Serum concentrations of nonesterified fatty acids (NEFAs), BHB, bilirubin, and urea at week (wk) −1, at wk +1, and at wk +5 relative to calving, were unsatisfactory for disease prediction [11]. Concentrations of nitric oxide, an inflammatory mediator, in both blood and uterine secretions were higher in animals with subclinical and clinical endometritis when compared with control cows [12].

Neutrophils of SCE cows had reduced glycogen content than healthy cows at Days 7, 28, and 42 in milk, suggesting a diminished phagocytosis activity of PMN in diseased animals [13]. Polymorphonuclear cell of cows with SCE also showed significantly lower PMN myeloperoxidase activity between Day 1 prepartum and Day 8 PP than uterine healthy cows [14].

Data on cellular composition and gene expression profiles of peripheral blood leukocytes of cows with and without SCE are limited. Galvao, et al. [15] analyzed blood monocyte gene expression and secretion of selected cytokines from calving to 42 days after calving in Holstein cows with or without uterine disease. They showed that *Escherichia coli*–stimulated monocytes from cows with metritis expressed fewer key proinflammatory cytokine genes (*TNF*, *IL1B*, and *IL6*) than healthy cows from calving to 14 days after calving.

Cellular subset analyses of peripheral blood leukocytes from cows with SCE are not yet available. Disease-associated changes in peripheral blood have been observed in human endometriosis, a pelvic inflammatory process associated with infertility. Changes involved the increase of selected monocyte subpopulations in blood of patients compared with healthy controls [16], and enhanced the serum concentrations of inflammatory mediators.

To avoid surgical interventions for the diagnosis of endometritis, identification of biomarkers in the periphery for diagnosis and treatment monitoring of bovine SCE would be of significant benefit. The objective of this study was, therefore, to analyze differences in peripheral blood cell composition and the expression of genes associated with inflammation by blood leukocytes between cows with histologically proven SCE and uterine healthy cows.

2. Materials and methods

2.1. Study design, groups of animals, and source of bovine endometrial tissue

The study involved 22 pluriparous Holstein-Friesian cows, housed at the Farm for Education and Research of the University of Veterinary Medicine, Hannover, Germany. Animals between 4 and 10 years were enrolled for this study. All cows were followed from calving (Day 0) to 55 days PP. For both blood sampling and collection of endometrial tissue, a complete general examination was carried out at Days 45 to 55 PP. The gynecologic examination was performed at the same time of sampling.

Only cows considered clinically healthy were included in this study. Cows with postparturient metritis, retained fetal membranes, or mastitis were excluded from the study. A general examination was performed followed by a gynecologic examination. Body condition was scored using a five-point (one = thin to five = fat) system (Table 1). Cows were first inspected for the presence of fresh discharge on the vulva, perineum, or tail. The vulva was wiped clean with damp paper towels. A transrectal palpation of the reproductive tract was performed, and findings were classified as follows: size and contractility of the uterus, symmetry of the uterine horns, size of the ovaries, and palpable ovarian structure (CL, follicle, cyst [>2.5 cm in diameter]). Afterward, all cows were examined by ultrasound to confirm the diagnostic findings followed by a vaginal examination. Collection of uterine specimen by biopsy was performed at 45 to 55 days PP.

Diagnosis and quantification of leukocytes in endometrial tissue was performed as a diagnostic service in the Institute of Pathology, Faculty of Veterinary Medicine, Leipzig, Germany. Based on the histologic diagnosis of the presence or absence of nonpurulent endometritis 45 to 55 days PP in addition to the absence of clinical signs of endometritis (Table 4), cows were divided retrospectively into subclinical (n = 8) and non-subclinical cows (n = 14). All procedures were carried out in accordance with German legislation on animal welfare (AZ 33.14-42502-04-12/0794).

2.2. Collection of cytobrush smears and bacteriologic examination

Cytobrush samples were collected using the modified technique described by Kasimanickam, et al. [8]. In brief, the cytobrush was cut to approximately 5 cm in length threaded onto a solid stainless steel rod, and placed in a stainless steel tube, for passage through the cervix. Inside the uterus, the catheter was retracted, and the brush was rolled along the