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Secretion of prostaglandins and leukotrienes by endometrial cells in cows with subclinical and clinical endometritis

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ABSTRACT

The aims of this study were (1) to measure the secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), and leukotriene C_4 (LTC_4) by endometrial cells collected by a cytobrush from healthy cows and cows with subclinical and clinical endometritis in the fourth week postpartum, and (2) to evaluate the relationship between the mediators' levels of secretion and the number of polymorphonuclear neutrophils (PMNs) in the uterine smears of cows with subclinical endometritis. The study included cows without any signs of clinical endometritis ($n = 63$) and cows with clinical endometritis as a positive control (ENDOM, $n = 12$). Two different threshold ratios (>5% and >18% of PMNs) were used to categorize the cows without clinical signs as with or without cytologic endometritis (CE). Considering the first or second threshold, the animals with CE were included in group CE POS I or CE POS II, whereas the healthy cows were assigned to group CE NEG I or CE NEG II, respectively.

The prevalence of CE was 68.25% (42/63) and 57.14% (36/63) according to the first and second thresholds, respectively. The highest level of secretion of all of the measured mediators occurred in the ENDOM group and differed significantly ($P < 0.05$) from the CE POS and CE NEG groups, regardless of the threshold. $PGF_{2\alpha}$ secretion in the CE POS II group (1629 pg/mL) was significantly lower ($P < 0.05$) when compared with the CE NEG II group (2797 pg/mL), whereas there was no significant difference between the CE POS I and CE NEG I groups. PGE_2 secretion differed between both groups with CE; higher concentrations were measured in the CE POS II group (6.68 ng/mL) when compared with the CE POS I (2.4 ng/mL) and CE NEG II (2.37 ng/mL) groups ($P < 0.05$). No significant differences were observed in the LTB_4 and LTC_4 secretion between the CE POS and CE NEG groups, considering both thresholds. It seems that CE does not fully mimic the inflammatory cascade associated with clinical signs. The response in the subclinical cases was limited to enhanced production of PGE_2, which was particularly well-pronounced in cows with high numbers of PMNs (>18%) in the endometrial scrapings.

1. Introduction

Endometritis is one of the most important disorders in dairy cows during the postpartum period and detrimentally impacts reproduction [1,2]. Currently, endometritis is classified into clinical and subclinical endometritis. The main criterion for the clinical inflammation of the endometrium is the presence of vaginal (muco) purulent discharge without systemic signs of illness after the 21st day following parturition. The diameters of the horns and that of the cervix are also considered [1,3]. The prevalence of clinical endometritis ranges from 10% to 27% in dairy cows [3–5].

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Subclinical endometritis is the inflammation of the uterus without clinical signs of endometritis that results in a significant reduction of reproductive performance in dairy cows [1]. Subclinical endometritis can be diagnosed only by cytologic examination of uterine smears or flushing and counting the proportion of polymorphonuclear leukocytes (polymorphonuclear neutrophils [PMNs]) to epithelial cells. Subclinical endometritis is also called cytologic endometritis (CE) depending on the diagnostic method used. Previous studies on CE have developed certain diagnostic criteria and investigated the prevalence and impact of CE on fertility and treatment. The studies have also used different thresholds for diagnosing CE. Three diagnostic limits have been suggested at the fourth week postpartum: over 5%, 8%, or 18% of PMNs [2,6–8]. The prevalence of CE ranges from 45% to 70% in the fourth postpartum week [6,9,10].

PMNs mediate the uterine defense mechanisms [11]. An influx of PMNs can be caused by bacteria, especially *Escherichia coli* and other coliforms [12], which initiate a uterine immune system response. The inflammatory response is mediated by proinflammatory factors, including eicosanoids ($PGF_{2\alpha}$, prostaglandin E [PGE], and leukotrienes [LTs]) and cytokines (tumor necrosis factor α, interleukin 1, and interleukin 6; for reviews, see refs. [13,14]).

Several studies have determined the levels of prostaglandins (PGs) in blood plasma or uterine fluid during clinical endometritis. Del Vecchio et al. [15] and Bekana et al. [16] observed higher plasma levels of PGFM in cows with endometritis compared than in uninfected herdmates. The duration of postpartal release of $PGF_{2\alpha}$ was prolonged in cows with uterine infections [17,18]. Mateus et al. [19] reported that the level of PGFM in peripheral blood was significantly higher in plasma but not in the uterine fluid in cows with severe endometritis than in cows with mild endometritis. Manns et al. [20] observed high concentrations of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) in the uterine fluid of cows with pyometra, whereas Mateus et al. [19] measured significantly higher levels of PGE_2 in the uterine secretion of cows with severe clinical endometritis than in cows with mild endometritis.

Limited information concerning LTs in cows with endometritis is available. In previous studies, higher levels of leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) were found in endometrial tissue from cows with clinical endometritis than from healthy cows [21,22]. Zerbe et al. [23] induced a significant influx of neutrophils after infusion of LTB_4 into the uterine lumen and described it as a model for experimental endometritis. LTC_4 modulated the secretion of progesterone and PGs in bovine ovarian cells [24].

Recently, several researchers [25–27] successfully applied the endometrial cytobrush technique to obtain a material with which to perform gene expression analysis of proinflammatory cytokines. However, no previous studies have evaluated the production of PGs and LTs by cells harvested by a cytobrush from the uteri of cows with or without different types of endometritis.

The aims of this study were (1) to measure the secretion of $PGF_{2\alpha}$, and PGE_2, LTB_4, and LTC_4 by endometrial cells collected by a cytobrush from healthy cows and cows with subclinical and clinical endometritis in the fourth week postpartum and (2) to evaluate the relationship between the mediators' levels of secretion and the number of PMNs in the uterine smears of cows with subclinical endometritis.

2. Materials and methods

2.1. Animals and study design

This study was performed in one dairy herd consisting of Polish Holstein-Friesian cows. The cows ($n = 75$) were aged 2 to 7 years with an average milk yield of 9000 L. The cows were maintained in a loose-housing barn and fed grass and maize silage, concentrates, and vitamin and mineral supplements. A partial mixed ration feeding system was used.

Cows were examined between the 21st and 28th day postpartum. During this examination, cows that were suitable for enrollment in the study were identified. The examination procedure included inspection of the vulva, tail, and perineum; vaginoscopy; transrectal palpation; and ultrasonography of the uterus and ovaries. The study included cows without any signs of clinical endometritis ($n = 63$) and cows with clinical endometritis as a positive control (presence of purulent uterine discharge, $n = 12$). Purulent uterine discharge was defined as an exudate containing at least 50% of pus originating from the uterine cervix. This group of animals was described as ENDOM. The cows without clinical endometritis according to the cytologic evaluation were assigned to particular experimental groups (see Section 2.3).

2.2. Samples collection

Two samples from the larger uterine horn were collected by a cytobrush, as described previously [28]. The first sample was used to evaluate the number of PMNs in the smear, and the second sample was used to evaluate the secretion of proinflammatory mediators. To obtain samples from the cows, sterile brushes for cytologic examinations in human gynecology (Cervical Rambrush type I C, Shanghai International Holding Corp. GmbH, Germany) were used. The brush was slid over the mandrel, and both were inserted into a stainless steel catheter. To protect the brush from vaginal contamination, the entire setup was inserted into a sterile glove for transrectal examination (Kruuse, Denmark). The setup was inserted into the cows' genital tracts. The cervix was manipulated by a hand inserted in the rectum to allow the catheter to pass through the cervix. After the catheter passed through the cervix, the glove protecting the catheter was punctured. The catheter was moved into the uterine horn, and a sample was taken by pushing the brush out of the catheter and rotating it three times. The brush was pulled back through the catheter and out of the cow. The material collected by the cytobrush was transferred to a microscope slide by rolling the brush on the slide to determine the ratio of PMNs to epithelial cells. Another brush was slid over the mandrel and inserted into the catheter, and a second sample was collected. After the second sample was collected, the brush was pulled back into the catheter, and the entire setup was removed from