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Changes in endometrial transcription of TLR2, TLR4, and CD14 during the first-week postpartum in dairy cows with retained placenta

Telma M. Martins [a], Clarice S. Muniz [a], Virgílio B. Andrade [a], Tatiane A. Paixão [b], Renato L. Santos [a], Álan M. Borges [a,*]

[a] Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

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ABSTRACT

Changes in the endometrial transcription of pattern recognition receptors may increase the susceptibility to postpartum uterine infections in Holstein cows with retained placenta. To test this hypothesis, nine cows with retained placenta and ten cows without retained placenta were submitted to endometrial biopsies at the first and seventh days postpartum. Cows were monitored weekly with clinical and gynecological examinations until 42 days postpartum. Samples of the uterine contents were collected weekly for aerobic bacteria isolation. All cows had endometrial transcription of Toll-like receptors (*TLRs*) *1/6*, *2*, *4*, *5*, and *9*; nucleotide-binding oligomerization domain (*NOD*)-like receptors *1* and *2*; and the coreceptors cluster of differentiation 14 (*CD14*) and myeloid differentiation protein-2 (*MD-2*), as measured on the first and seventh days postpartum. *Escherichia coli* was the most common bacterium isolated from the uterine contents of cows with or without retained placenta until 21 days postpartum. Transcription levels of *TLR2*, *TLR4*, and *CD14* in Holstein cows with retained placenta significantly decreased ($P < 0.05$) between the first and the seventh day postpartum. Conversely, cows without retained placenta did not have any significant changes in transcription levels between these time points.

1. Introduction

Postpartum uterine infections and inflammation are important causes of reproductive failure in dairy cows. Nearly all cows acquire ascending bacterial contamination of the uterus after parturition because of the opening of anatomic barriers including the vulva, vagina, and cervix [1,2]. Endometrial cells express receptors that recognize microbe-associated molecular patterns (MAMPs), previously known as pathogen-associated molecular patterns [3]. Once activated, these receptors trigger an inflammatory response aimed at eliminating the invading bacteria [4–6]. Innate immune responses, including inflammation, are modulated, along with other mechanisms through the expression and activation of Toll-like receptors (TLRs) or nucleotide-binding oligomerization domain (NOD)-like receptors, which sense MAMPs. These receptors are known as pattern recognition receptors (PRRs). Failure of the innate immune response favors the multiplication of potentially pathogenic bacteria in the uterus, which may lead to persistent infection and impaired fertility [7–9].

Acute and chronic postpartum uterine infections occur frequently in dairy cows, and retained placenta is a major risk factor for these diseases [10–12]. Retained placenta

* Corresponding author. Tel.: +55-31-34092259; fax: +55-31-34092230.
E-mail address: alanmborges@ufmg.br (Á.M. Borges).

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impairs myometrial contractility, which prevents a complete elimination of uterine contents and inhibits uterine defense mechanisms, thus favoring the establishment of infections [13,14]. Endometrial transcription of *TLRs* and *NOD*-like receptors in Holstein cows at variable postpartum time points has been previously described [7,8]. Although immunological factors play a significant role in the etiology of placental retention in cows [15,16], the effect of retained placenta on transcription of the genes associated with uterine innate immunity remains to be investigated [17].

Considering the economic significance of the dairy industry and the negative impact of uterine diseases in dairy cows, a better understanding of the factors affecting uterine innate immunity may lead to novel approaches that prevent uterine infections and infertility [7,18,19]. Therefore, the goal of this study was to assess endometrial transcription of the PRRs responsible for recognizing invading aerobic bacteria in the uterine environment in Holstein cows with or without retained placenta. Endometrial transcription levels of *TLRs 1*, *2*, *4*, *5*, *6*, and *9*; *NODs 1* and *2*; and the coreceptors cluster of differentiation 14 (*CD14*) and myeloid differentiation protein-2 (*MD-2*) were compared between the first and the seventh day postpartum (dpp) and between cows with or without retained placenta.

2. Materials and methods

All experimental procedures were approved by the Ethics Committee on Animal Experimentation at the Universidade Federal de Minas Gerais (CETEA-UFMG, Protocol number 049/2011).

2.1. Cows

Nineteen multiparous Holstein cows, ranging from the second to the fifth parturition, were divided into two groups: with retained placenta (n = 9) and without retained placenta (n = 10). All cows gave birth between February and May 2012. The placenta was considered retained if it was not released until 12 hours after fetal delivery. Body condition scores at parturition were, on average, 3.5 ± 0.5 on a scale of 1 to 5, as described by Edmonson et al. [20]. The cows were kept in free-stall barns and received total mixed ration twice a day. Among the nine cows that had retained placenta, two had twins, one of which required obstetric manipulation during delivery. All cows without placental retention had normal parturition. Gestation length was 286.3 ± 6.3 and 283.1 ± 7.4 days for cows without and with retained placenta, respectively. There was no occurrence of fetal death.

2.2. Uterine biopsies

Endometrial biopsies were performed at 1 dpp (24–30 hours after the parturition) and at 7 dpp. These time points correspond to higher incidences of uterine infections during the first week postpartum [1,2,12]. Endometrial samples were obtained from intercaruncular areas using a biopsy forceps (Hauptner, Solingen, North Rhine-Westphalia, Germany), which was introduced through the vagina and cervix protected by a sterile plastic wrap. Manual removal of fetal membranes was not performed under any circumstance. Two samples, approximately 8 × 4 mm in surface area, were collected from each cow. Samples were place in RNase-free sterile cryovials, snap frozen in liquid nitrogen and stored at −80 °C until further processing.

2.3. Postpartum monitoring

The reproductive tract of experimental cows was monitored weekly from the first to the 42nd dpp by transrectal palpation, ultrasonography, and vaginoscopy. Parameters that were analyzed included uterine infections, uterine involution, and resumption of ovarian activity. Determination of occurrences of uterine infections was adapted from Sheldon et al. [21]. Briefly, cows with hemorrhagic/purulent and fetid vaginal secretion at 7 dpp, associated with signs of systemic illness such as dehydration, apathy, decreased milk yield, and fever (rectal temperature ≥ 39.5 °C), were diagnosed with acute puerperal metritis. Cows without signs of systemic illness but with an abnormally enlarged uterus and purulent vaginal secretion at 14 dpp were diagnosed with clinical metritis. The presence of abnormal uterine contents and purulent secretion at 21 dpp or mucopurulent secretion at 28 dpp were diagnosed with clinical endometritis.

Cows that had uterine infections were treated with antibiotics according to the protocols adopted on the farm. Puerperal metritis was treated with oral hydration and three doses of long-acting oxytetracycline (Terramicina LA, 20 mg/kg, im) with 2-day intervals between the doses. Clinical metritis and endometritis were treated with three doses of ceftiofur hydrochloride (Ceftiomax, 2 mg/kg, im) for three consecutive days. Uterine involution was considered complete when the uterus was entirely located in the pelvis, the uterine horns were symmetrical and there were no uterine contents. Resumption of ovarian activity was characterized by the detection of the first CL.

After gynecological examinations, samples of uterine contents were aseptically collected weekly for aerobic bacteriologic culture. Disposable sterile 20-mL syringes were connected to disposable sterile pipettes protected by a sterile plastic wrap, and then they were introduced into the uterus. Collected samples were placed in a tube containing Stuart medium for bacterial transport and processed for aerobic bacteria isolation as described by Koneman et al. [22].

2.4. RNA extraction and cDNA synthesis

Total RNA was extracted from endometrial samples using Trizol solution (Invitrogen, São Paulo, Brazil) according to the manufacturer's instructions. RNA purity and concentration were assessed by spectrophotometry. RNA integrity was evaluated by electrophoresis in 1% agarose gel.

For cDNA synthesis, the total RNA was diluted in diethylpyrocarbonate-treated water to a concentration of 500 ng/μL. cDNA synthesis was performed using a