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Effects of intrauterine infusion of *Trueperella pyogenes* on endometrial mRNA expression of proinflammatory cytokines and luteolytic cascade genes and their association with luteal life span in dairy cows

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ABSTRACT

Objectives were to determine the effects of intrauterine infusion (IUI) of *Trueperella pyogenes* on endometrial expression of proinflammatory cytokines and luteal life span. Holstein cows (n = 32) were allocated randomly, in two replicates (15 then 17 cows), to receive one of three treatments on Day 5 of the estrous cycle: TP (n = 13), IUI containing 10^9 colony-forming units/mL of *T pyogenes*; tumor necrosis factor (TNF; n = 9), IUI containing 1 μg of TNFα; and control (n = 10), IUI of saline solution. Five cows per treatment had uterine biopsies collected at 6, 12, and 24 hours after treatment to evaluate the endometrial messenger RNA expression of TNFα (*TNF*), interleukin-1β (*IL1B*), *IL6*, *IL8*, prostaglandin E synthase (*PGES*), prostaglandin F synthase (*PGFS*), and oxytocin receptor (*OXR*), and histologic evidence of inflammation. Messenger RNA expression was measured using quantitative reverse transcription polymerase chain reaction. The remaining cows had ovaries scanned and blood collected for progesterone evaluation; however, only seven, four, and three cows in the TP, TNF, and control groups were used for comparison in replicate 2. The GLIMMIX procedure of SAS was used for statistical analysis. All TP and TNF cows had moderate to severe endometrial inflammation, whereas only one control had mild inflammation. Premature luteolysis occurred in three, one, and zero cows in the TP, TNF and control groups, respectively. Delayed luteolysis occurred in one TP and one TNF cow. *Interleukin-1β* expression was greater in the TP cows than in the TNF cows at 24 hours after IUI. Moreover, *IL6* expression tended to be greater for the TP cows than for the control cows at 12 hours after IUI. *Interleukin 8* expression was greater in the TP cows than in the control and TNF cows at 24 hours after IUI. *Oxytocin receptor* expression tended to be greater for the TP cows and was greater for the TNF cows than for the control cows at 12 hours. The messenger RNA expressions of *TNF*, *PGES*, and *PGFS* were not affected by treatment, time, or their interaction. In conclusion, IUI of *T pyogenes* or TNFα led to histologic evidence of inflammation and early luteolysis in some cows, which may have been caused by increased endometrial expression of proinflammatory cytokines (i.e., *IL1B*, *IL6*), chemokines (i.e., *IL8*), and luteolytic cascade factors (i.e., *OXR*).

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1. Introduction

Endometritis is defined as inflammation of the endometrium diagnosed after 21 days postpartum [1]. It has been reported that endometritis leads to reduced fertilization rates [2], reduced pregnancy per artificial insemination [3], increased pregnancy loss [3], and a longer interval from calving to pregnancy [4]. Endometritis is the leading cause of infertility in high-producing dairy cows, the main reason for disposal of dairy cows [5,6]. Indeed, cows diagnosed with endometritis have a 20% lower pregnancy rate per artificial insemination [3], 30-day longer median time to pregnancy, and 70% greater hazard to be culled than healthy counterparts [7,8]. Considering that the costs of rearing a heifer are recouped only after two lactations [9] and that each day, a cow remains open costs from 1 to 6 dollars, endometritis is a major burden on profitability and sustainability of dairy operations worldwide. Therefore, understanding the underpinning mechanisms of endometritis and the potential role by which microbes subvert host innate immunity disrupting ovarian and uterine function is fundamental to develop surrogate measures to alleviate the negative impacts of endometritis.

Trueperella pyogenes is the major pathogen associated with endometritis [10]. Cows diagnosed with *T pyogenes* in the uterine lumen at Day 35 postpartum have a remarkable 19.8 greater odds of having endometritis with 63.3% of cows diagnosed with endometritis being culture positive for *T pyogenes* [10]. The importance of this bacterium to endometritis is also due to high prevalence in the environment, persistence in the uterus, severity of endometrial lesions, resistance to treatment, and synergistic action with gram-negative anaerobes [11–15]. However, the mechanism by which *T pyogenes* affects the endometrium and reproductive events in dairy cows such as the length of the estrous cycle and concentration of ovarian steroids remains elusive [16–18].

Several studies in the recent years reported that intrauterine infusion (IUI) of live *T pyogenes* disrupts luteal and ovarian function leading to a peak of $PGF_{2\alpha}$ metabolite (PGFM) 3 days later followed by early demise of the CL and ovulation of dominant follicle of first follicular wave [17–19]. However, the mechanism by which *T pyogenes* elicits short cycles disrupting normal luteal function remains unclear.

Recent *in vitro* studies revealed that endometrial cells exposed to bacteria-free filtrate of *T pyogenes* could release $PGF_{2\alpha}$ [20], and live *T pyogenes* can stimulate release of proinflammatory cytokines [21]. This bacterium possesses a number of virulence factors such as pyolysin (PLO), a cholesterol-dependent cytolysin, and peptidoglycan [22]. Peptidoglycan can induce the release of proinflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and IL-6 [23–25], whereas recombinant PLO or *plo*-inactivated *T pyogenes* mutant was unable to elicit release of proinflammatory cytokines [21]. Endometrial synthesis of $PGF_{2\alpha}$ can be stimulated by some proinflammatory cytokines such as IL-1β, IL-6, and TNFα [26–28]. However, the effect of *T pyogenes* on the induction of proinflammatory cytokines and luteolytic factors has never been investigated *in vivo*.

Therefore, the present study was designed to investigate the potential molecular mechanisms by which IUI with live *T pyogenes* leads to shortening of the luteal phase in dairy cows. We hypothesized that IUI of live *T pyogenes* would increase endometrial expression of proinflammatory cytokines and luteolytic cascade factors leading to an acute endometrial production of $PGF_{2\alpha}$ and early demise of the newly formed CL. The objectives of this study were to determine the effects of IUI of *T pyogenes* in dairy cows with newly formed CL on endometrial messenger RNA (mRNA) expression of proinflammatory cytokines, luteolytic factors, plasmatic concentration of progesterone, and CL life span.

2. Materials and methods

2.1. Animals, housing, and diets

The University of Florida Institutional Animal Care and Use Committee approved the use of all animal procedures conducted in this study (IACUC #201105782).

The study was conducted between November of 2012 and March of 2013 in the University of Florida Dairy Unit (Hague, FL, USA). Thirty-two lactating Holstein cows were enrolled in the study in two separate replicates of 15 (replicate 1) and 17 (replicate 2) cows each. The cows were housed in free-stall barns with sand-bedded stalls equipped with sprinklers and fans for forced evaporative cooling and ventilation. The cows were fed twice daily, immediately after the morning milking at 8:30 AM and again at 12:30 PM. Diets were mixed twice daily as base mixture containing corn silage, alfalfa hay and a base-concentrate mix, and the additional grain supplement. This base mixture contained 54% forage and was designed to meet the nutrient needs of a 650-kg cow consuming 23 kg of diet dry matter and producing 40.0 kg of milk with 3.5% fat and 3.0% true protein (CPM-Dairy ver. 3.0.10 software; www.cpmdairy.net).

2.2. Study design and treatments

At 21 ± 3 days postpartum, Holstein cows free of calving-related disorders (dystocia, stillbirth, twins, retained placenta, or metritis) had their estrous cycle synchronized with 100 μg of intramuscular GnRH (2-mL Cystorelin; Merial Ltd., Duluth, GA, USA) followed 7 days later by one injection of $PGF_{2\alpha}$ (25 mg of dinoprost tromethamine, Lutalyse; Zoetis Animal Health, Madison, NJ, USA) at 28 ± 3 days postpartum. Two days later at Day 30 ± 3 postpartum, a second dose of GnRH was given to induce ovulation, as depicted in Figure 1. The day of the last GnRH injection of the Ovsynch was considered estrous cycle Day 0. Ovarian structures were scanned at Days 21 ± 3, 28 ± 3, 30 ± 3, and 32 ± 3 to determine follicle turnover, luteolysis, and ovulation.

Additionally, at Day 30 ± 3 postpartum, all cows had two uterine cytology samples collected for evaluation of subclinical endometritis and bacterial culture using the cytobrush technique as previously described with minor