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Effects of intrauterine infusion of *Escherichia coli* lipopolysaccharide on uterine health, resolution of purulent vaginal discharge, and reproductive performance of lactating dairy cows

João G. N. Moraes,*†[1] Paula R. B. Silva,*† Luís G. D. Mendonça,* Alexandre A. Scanavez,* Joseane C. C. Silva,* and Ricardo C. Chebel*†‡§[2]
*Department of Veterinary Population Medicine, and
†Department of Animal Science, University of Minnesota, St. Paul 55108
‡Department of Large Animal Clinical Sciences, and
§Department of Animal Sciences, University of Florida, Gainesville 32608

ABSTRACT

The objectives of the current experiment were to evaluate the effects of intrauterine infusion of *Escherichia coli* lipopolysaccharide (LPS) in cows diagnosed with purulent vaginal discharge (PVD) on intrauterine cell population, resolution of PVD, uterine health, and reproductive performance. Jersey cows (n = 3,084) were examined using the Metricheck device to diagnose PVD at 35 ± 6 d postpartum. Purulent vaginal discharge was defined as the presence of purulent (≥50% pus) discharge detectable in the vagina. Of the 310 cows positive for PVD, 267 cows were enrolled in the current experiment. To ensure proper timing of treatment and collection of samples, only 9 PVD-positive cows were treated per day. Selected cows were balanced at 35 ± 6 d postpartum for lactation number, body condition score, and milk yield and were randomly assigned to receive an intrauterine infusion of 20 mL of phosphate-buffered saline (PBS; control, n = 87), 20 mL of PBS with 150 μg LPS (LPS150, n = 91), or 20 mL of PBS with 300 μg of LPS (LPS300, n = 89). Uterine cytology was performed immediately before treatment and 1, 2, and 7 d after treatment to evaluate the effect of LPS treatment on intrauterine cell population. Cows were examined with the Metricheck device at 7 and 28 d after treatment to evaluate the effects of treatment on resolution of PVD. Reproductive status was recorded up to 200 d postpartum. Cows diagnosed with PVD had greater incidence of twinning, dystocia, retained placenta, and metritis after calving than cows without PVD. Count of polymorphonuclear leukocytes (PMNL) in uterine cytology 1, 2, and 7 d after intrauterine infusion was not statistically different among treatments. From d 0 to 1, however, PMNL count in uterine cytology of PBS cows increased by 5%, whereas the PMNL count in uterine cytology of LPS150 and LPS300 cows increased by 54 and 48%, respectively. Treatment did not affect the likelihood of cows being diagnosed with PVD 7 and 28 d after intrauterine infusion. Cows without PVD and LPS150 cows were more likely to be pregnant after the first postpartum AI than PBS cows. After the second postpartum AI, cows without PVD were more likely to be pregnant than PBS and LPS300 cows. Hazard of pregnancy up to 200 d postpartum was decreased for PBS and LPS300 cows compared with cows without PVD, and it tended to be decreased for LPS150 cows compared with cows without PVD. Intrauterine treatment with 150 μg of *E. coli* LPS of cows diagnosed with PVD improved likelihood of pregnancy after the first postpartum AI, but further research is needed to elucidate the mechanism by which LPS treatment improved fertility.

Key words: lactating dairy cow, purulent vaginal discharge, lipopolysaccharide

INTRODUCTION

Purulent vaginal discharge (**PVD**), formerly known as clinical endometritis (Dubuc et al., 2010), has a negative effect on profitability of cattle industries because of infertility, increased culling for reproductive failure, reduced milk yield, and costs associated with treatment (Sheldon et al., 2009). The term PVD has been proposed for use in place of clinical endometritis because endometrial inflammation is not always present in cows with PVD (Dubuc et al., 2010). Occurrence of dystocia, twinning, retained fetal membranes, stillbirth, abortion, and metritis predispose cows to PVD (Galvão, 2012). Treatment of cows that were at high risk of de-

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[1]Current address: Division of Animal Sciences, University of Missouri, Columbia, MO.
[2]Corresponding author: rcchebel@ufl.edu

veloping uterine disease at 24 h after parturition with ceftiofur crystalline free acid reduced the probability of PVD at 35 ± 3 d postpartum (Dubuc et al., 2011). Treatment of cows at high risk for uterine diseases with systemic antimicrobials, however, does not necessarily improve their reproductive performance (Dubuc et al., 2011; McLaughlin et al., 2013). Intrauterine treatment of cows with PVD using cephapirin benzathine is approved in Canada, Europe, and New Zealand, and it is reported to increase reproductive performance (LeBlanc et al., 2002a; Kasimanickam et al., 2005). In the United States, no products for intrauterine treatment of lactating dairy cows containing cephapirin benzathine are available. Other intrauterine antimicrobial therapies may inhibit phagocytic activity of neutrophils in the uterus (Masera et al., 1980), compromising resolution of uterine infections. Additionally, the increased scrutiny of the use of antimicrobials relevant for human medicine in food-producing animals may require the development of non-antimicrobial strategies for the treatment of diseases.

Lipopolysaccharide is an important bacterial molecule that stimulates pathogen recognition receptors and triggers inflammatory response (Mogensen, 2009). In mammals, transcription factors such as nuclear factor-κB and IFN regulatory factor family are activated (O'Neill and Bowie, 2007; Lu et al., 2008) upon recognition of LPS by the toll-like receptor 4 and myeloid differentiation factor 2 complex (Park et al., 2009), causing the secretion of cytokines and chemokines that regulate innate and adaptive immunity (Li and Verma, 2002; Schroder et al., 2004). Additionally, LPS stimulates NADPH oxidase activation, leading to production of large amounts of superoxide, a key product associated with the killing ability of PMNL (Huang et al., 2009). Intrauterine infusion of endometritic cows with 100 μg of *Escherichia coli* LPS resulted in elimination of uterine bacterial infection in 75% of treated cows and increased intrauterine total leukocyte count, the proportion of PMNL in uterine cytology, and the proportion of live PMNL in the uterus (Singh et al., 2000). Additionally, 8 of the 12 cows treated with LPS conceived, whereas only 1 of the 12 untreated control cows conceived (Singh et al., 2000).

The hypothesis of the current experiment was that intrauterine treatment of cows with PVD with *E. coli* LPS would increase the number of PMNL in the uterine cytology, resolve PVD, improve pregnancy per AI (**P/AI**) after first and second postpartum AI, and improve pregnancy rate up to 200 DIM. The objectives of the present experiment were to evaluate the effects of intrauterine infusion of LPS in cows with PVD on intrauterine cell population, uterine health, and reproductive performance of lactating dairy cows.

MATERIALS AND METHODS

Animals, Treatments, and BCS

Lactating Jersey cows (n = 3,084) from a freestall dairy located in Nicollet (MN) were examined for PVD at 35 ± 6 DIM using the Metricheck device (Simcro, Hamilton, New Zealand; McDougall et al., 2007) from December 2010 to May 2012. Four hundred twenty (14%) cows had ≥ 50% pus in the vaginal exudate and were considered positive for PVD. All cows with PVD were examined by palpation per rectum, and 110 cows were determined to have large amounts of fluid in the uterus. These cows were considered to have a different and more severe uterine pathology (i.e., pyometra) compared with cows with PVD without a fluid-filled uterus and were, therefore, not included in the present experiment.

At PVD diagnosis, the retrieved exudate was placed on a black plastic plate to improve the visualization of the discharge. The purulent discharge was scored based on the amount of pus present in the exudate retrieved by the Metricheck device. Scores were given according to the following classification: mild (retrieved exudate contained approximately 50–60% of pus), moderate (retrieved exudate contained approximately 60–90% of pus), and severe (retrieved exudate contained approximately 90–100% pus). Of the 310 cows positive for PVD without large amounts of uterine fluid, 267 were used in the present experiment. To ensure proper and timely treatment of cows and collection and processing of samples, only 9 cows were enrolled per day, with 3 cows per treatment enrolled per day. Only 43 cows with PVD were not enrolled (10% of cows positive for PVD).

Enrolled cows were balanced for lactation number, BCS (1 = emaciated, 5 = obese; Ferguson et al., 1994), PVD score (mild, moderate, severe), and weekly average milk yield up to 28 DIM. Blocks of 3 cows were created, and treatments were randomly assigned to the cows within blocks using a sealed envelope. Cows assigned to the PBS treatment (n = 87) received an intrauterine infusion of 20 mL of PBS, cows assigned to the LPS150 treatment (n = 91) received an infusion of 20 mL of PBS containing 150 μg of *E. coli* LPS (*E. coli* serotype 026:B6, containing 10,000 endotoxin units per milligram of LPS; Sigma-Aldrich, St. Louis, MO), and cows assigned to the LPS300 treatment (n = 89) received an intrauterine infusion of 20 mL of PBS containing 300 μg of LPS. Lyophilized LPS from *E. coli* serotype 026:B6 containing 10,000 endotoxin units per milligram of LPS (cat. no. L8274; Sigma-Aldrich) was reconstituted by adding 1× sterile PBS (pH 7.4, 137 m*M* sodium chloride, 2.7 m*M* potassium chloride, 12 m*M* phosphate buffer; cat. no. E504; Amresco LLC,