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Repeated intrauterine infusions of lipopolysaccharide alter gene expression and lifespan of the bovine corpus luteum

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ABSTRACT

Inflammation of the uterus is associated with disturbed ovarian function and reduced reproductive performance in dairy cows. To investigate the influence of endometritis on the bovine corpus luteum, 8 heifers received intrauterine infusions with either phosphate-buffered saline (PBS; 9 mL) or *Escherichia coli* lipopolysaccharide (LPS; 3 μg/kg of body weight diluted in 9 mL of PBS) at 6-h intervals from 12 h before and until 9 d after ovulation during 2 cycles in a random order (ovulation = d 1). An untreated cycle was examined before and after PBS and LPS cycles, and the mean values from both untreated cycles were used as control. In all cycles, blood sampling and ultrasonography of the ovaries were performed on d 0, 1, 2, 4, 6, 8, 9, 10, 12, 15, 18, and then every 2 d until ovulation. Endometrial cells were collected for cytology and quantitative real-time reverse transcriptase PCR on d 0, 6, and 9, and on d 0 and 6, respectively, and luteal tissue was collected for quantitative real-time reverse transcriptase PCR on d 6 and 9. Both, PBS and LPS infusions induced subclinical endometritis, which was accompanied by increased endometrial mRNA abundance of proinflammatory cytokines *IL1β*, *IL8*, and tumor necrosis factor α. Additionally, LPS challenge induced premature luteolysis, which was characterized by increased plasma concentrations of $PGF_{2\alpha}$ metabolite, decreased plasma progesterone concentrations, and reduced luteal size and blood flow compared with the control. The luteal mRNA expression of the LPS receptor *TLR4*, PGE synthase, and the apoptosis-related factor *CASP3* were higher, and those of steroidogenic factors *STAR* and *HSD3B*, the PGF receptor, and the angiogenic factor $VEGFA_{121}$ were lower after LPS challenge compared with the control. In conclusion, repeated intrauterine LPS infusions during the first 9 d of the estrous cycle alter gene expression and shorten the lifespan of the bovine corpus luteum.

Key words: corpus luteum, endometritis, endotoxin, heifer

INTRODUCTION

Inflammatory diseases of the uterus have a negative effect on reproductive performance and over the decades they contribute to the deterioration of the fertility of high-yielding dairy cows (Lopez-Gatius, 2003; Walsh et al., 2011). The prevalence of clinical metritis and endometritis between 15 and 60 d postpartum was 25.9% (Gautam et al., 2009), and that of subclinical endometritis between 40 and 60 d postpartum ranged from 26% (Cheong et al., 2011) to 53% (Gilbert et al., 2005). Clinical and subclinical endometritis reduced pregnancy rates at 300 d postpartum by approximately 13% (70 vs. 83%; Deguillaume et al., 2012) and 26% (63 vs. 89%; Gilbert et al., 2005), respectively.

Apart from a direct impairment of the endometrium that reduces the pregnancy rates due to suboptimal conditions for the nidation of the embryo (Gabler et al., 2009; Gilbert, 2011), endometritis also seems to exert various indirect effects on ovarian function. Thus, an increased number of ovarian dysfunctions, such as prolonged anestrus and cystic ovarian follicles, were observed in cows with severe clinical metritis or endometritis compared with cows with an intact puerperium (Mateus et al., 2002; Tsousis et al., 2009). After intrauterine treatment with LPS, the endotoxin of gram-negative bacteria, a delayed or inhibited ovulation in cows was noticed and was associated with reduced plasma concentrations of LH and a lower LH peak (Peter et al., 1989). Therefore, systemic effects of intrauterine LPS were assumed, which interfere with the release of LH from the adenohypophysis (Battaglia et al., 2000;

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Suzuki et al., 2001). Furthermore, a delayed ovulation might be due to inhibition of estradiol production from the ovarian follicles (Suzuki et al., 2001; Shimizu et al., 2012). Consistently, the increased concentrations of LPS that were detected in the follicular fluid of cows with clinical and subclinical endometritis (Herath et al., 2007) reduced the transcription of steroidogenic enzymes in theca and granulosa cells (Herath et al., 2007; Magata et al., 2014).

In addition to the negative effects of an endometritis on ovulation and secretory function of ovarian follicles, evidence is increasing for a detrimental effect on the corpus luteum (**CL**). Prolonged luteal phases were observed in cows with metritis or endometritis (Opsomer et al., 2000), probably due to a reduced endometrial release of $PGF_{2\alpha}$. Furthermore, the lifespan of the CL tended to be shorter after an intrauterine application of living *Escherichia coli*, indicating premature luteolysis (Gilbert et al., 1990). However, in the study of Gilbert et al. (1990), an intrauterine application of LPS did not change the luteal lifespan nor the plasma concentrations of $PGF_{2\alpha}$ metabolites (**PGFM**) and progesterone ($\mathbf{P_4}$).

In contrast to the intrauterine LPS infusion, an intravenous treatment with LPS induced a 10-fold increase in PGFM and a transient decrease in P_4 concentrations but did not induce a complete premature luteolysis (Herzog et al., 2012). The different outcome in studies that used intrauterine or intravenous LPS challenge was likely due to the different concentrations of LPS that reached the bovine CL. However, it is widely accepted that intrauterine LPS in cattle is able to intrude into the peripheral circulation (Mateus et al., 2003; Williams et al., 2007; Magata et al., 2015), possibly by passing the oviduct, by moving transmurally into the peritoneal cavity and to the bloodstream (Peter et al., 1989), or by entering the uterine wall and following the utero-ovarian pathway, whose capabilities have been reviewed (Ginther, 1974).

In contrast to the single LPS peak that was induced by treatments in most previous studies, repeated applications of LPS into the uterus maintain high concentrations for several days and can simulate a naturally occurring endometritis more reliably. Therefore, we used repeated intrauterine LPS infusions to investigate the effects of endometritis on the morphology and function of the developing CL.

MATERIALS AND METHODS

Cattle

Eight nulliparous Red Holstein (n = 7) and Red Holstein × Limousin crossbred (n = 1) cows were used in the experiment. These heifers were (mean ± SEM) 20.8 ± 1.2 mo old, with an estimated BW of 448.5 ± 8.7 kg (Rondo measuring tape; Hoechstmass Balzer GmbH, Sulzbach, Germany), and a BCS of 3.75 (median; scale, 1–5). All heifers were clinically healthy with no apparent reproductive abnormalities. During the experiments, the heifers were housed in a tie stall barn and fed hay, minerals, and cattle salt, with ad libitum access to water.

The experimental procedures followed the Swiss Federal Law on Animal Protection and were approved by the Committee of Animal Experiments of the Canton Fribourg, Switzerland (application 25076).

Study Design

A modified ovulation synchronization (Ovsynch) protocol was initiated after normal cyclic activity had been confirmed by ultrasonography in all heifers. This protocol consisted of 10 μg of buserelin (GnRH analog, Receptal; MSD Animal Health GmbH, Luzern, Switzerland), 15 mg of luprostiol ($PGF_{2\alpha}$ analog, Prosolvin; Virbac AG, Glattbrugg, Switzerland) 7 d later, and finally 10 μg of buserelin 60 h after $PGF_{2\alpha}$ (all treatments were given intramuscularly). The time of the second GnRH application was defined as estrus (= d 0 h 0). Ovulation, which occurred in all heifers until 36 h after the second GnRH treatment, was defined as d 1 h 0.

Four estrous cycles were investigated in the heifers: one untreated cycle at the beginning, then 2 cycles in a randomized order with intrauterine infusions of either 9 mL of sterile PBS (Sigma-Aldrich, St. Louis, MO) or 3 μg/kg of BW *E. coli* LPS (serotype O26:B6; Sigma-Aldrich) diluted in 9 mL of sterile PBS, and a second untreated cycle at the end of the study. Additionally, the first 9 d of the next cycle after the LPS cycle (**NLPS**) were examined. Intrauterine treatments in the PBS and the LPS cycle were performed at 6-h intervals between d 0 h 24 and d 9 h 0.

In all cycles, blood sampling and ultrasonography of the uterus and ovaries were performed on d 0, 1, 2, 4, 6, 8, 9, 10, 12, 15, 18, and then every 2 d until ovulation. Furthermore, endometrial cells were collected for cytology and RNA extraction on d 0, 6, and 9 and on d 0 and 6, respectively, and luteal tissue was collected for RNA extraction on d 6 and 9.

To control clinical health of the heifers, their rectal temperature and habitus were examined at 6-h intervals between d 0 and 9, and once per day during the remaining cycle. Respiratory and cardiac frequencies were investigated immediately before each ultrasonographic examination.