

Lipopolysaccharide modulation of ovarian hormonal profile¹

Emily M. Ferranti,* Bahaa H. Aloqaily,* Craig A. Gifford,[†] Clint A. Löest,*
John C. Wenzel,[†] and Jennifer A. Hernandez Gifford*²

*Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003; [†]Department of Extension Animal Sciences and Natural Resources, Extension Animal Sciences and Natural Resources, New Mexico State University, Las Cruces, NM 88003

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INTRODUCTION

Circulating estrogen (E_2) produced by ovarian granulosa cells contributes to numerous physiological events, including female sexual behavior (Simpson et al., 2005) and folliculogenesis (Drummond and Findlay, 1999). Biosynthesis of estrogen depends on regulated expression of key steroidogenic enzymes by FSH as well as local ovarian signaling molecules. In primary cultures of granulosa cells, the transcriptional coactivator beta-catenin is required for FSH induction of aromatase expression (Parakh et al., 2006) and subsequent E_2 production (Hernandez Gifford et al., 2009). Furthermore, FSH regulates beta-catenin accumulation in E_2 active follicles of cattle (Castanon et al., 2012).

Beta-catenin is a diverse molecule that regulates biological processes in the developing and adult animal. Studies also implicate beta-catenin as a factor capable of modulating inflammatory responses in disease states (Ma and Hottiger, 2016). In inflammatory responses caused by lipopolysaccharide (LPS) induction of inflammatory cytokines, an associated increase in beta-catenin protein is reported (Jang et al., 2014). Several diseases affecting cattle result from

exposure to Gram-negative bacteria of which the glycolipid LPS makes up a majority of the outer membrane (Raetz and Whitfield, 2002). These inflammatory diseases can negatively impact reproductive performance and impair fertility.

Magata et al. (2014) showed that follicles with high amounts of LPS in the follicular fluid had reduced aromatase expression and E_2 production. Likewise, animals with subclinical mastitis showed decreased E_2 in follicular fluid and plasma (Lavan et al., 2011). Long-term intramammary infusions of LPS induce subclinical immune response shown to decrease follicular E_2 concentrations (Furman et al., 2014). Collectively, results in the literature suggest a role of LPS in impaired fertility in cattle, though the mechanism remains unclear.

Therefore, we hypothesize that LPS may cause alterations in the levels of beta-catenin, resulting in modulation of aromatase activity and ultimately changes in E_2 output. Our objectives were to determine the effect of LPS infusion on follicular fluid and serum hormone levels and evaluate the response within the beta-catenin pathway.

MATERIALS AND METHODS

Animal Procedures

All procedures were approved by the New Mexico State University Institutional Animal Care

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²Corresponding author: jgifford@nmsu.edu

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and Use Committee (2017–030). Fourteen cross-bred heifers (BW average = 416 kg) were housed and maintained at New Mexico State University (Las Cruces, NM).

Heifers were stratified by BW and assigned to vehicle-treated control (CON, $n = 7$) or LPS ($n = 7$) treatment groups. LPS (*Escherichia coli* O55:B5; Sigma–Aldrich, Inc., Milwaukee, WI) was prepared by dissolving 1 mg in 5 mL of sterile saline, then subcutaneously injected at a rate of 2.0 $\mu\text{g}/\text{kg}$ of BW. Each animal received approximately 4 mL of solution on days 2, 5, and 8. Control animals were injected with sterile saline. Baseline rectal temperatures were taken 1 d before the start of the experiment to preclude any potential febrile response prior to administering treatment.

Heifers were synchronized to estrus using the Select Synch plus controlled internal drug release (CIDR) protocol. On day 0, a GnRH (2 mL, i.m., Factrel, Zoetis Inc. US) injection was administered, and a CIDR (Eazi-Breed, Zoetis Inc., Kalamazoo, MI) device was inserted. On day 7, the CIDR was removed, and heifers received PGF 2α (Lutalyse, 25 mg, i.m., Zoetis Inc. US).

Rectal temperatures were taken just prior to treatment (30-Second Digital Thermometer, Target Corporation, Minneapolis, MN) and at 2, 3, 4, 8, and 10 h after injection.

Sample Collection

Blood samples were collected via coccygeal or jugular venipuncture into Corvac Integrated Serum Separator Tubes (Covidien, Mansfield, MA), once daily from days 0 to 7. Beginning on day 7, samples were taken every 8 h until tissue harvest. Blood samples

were centrifuged at $1,500 \times g$ at 4°C for 15 min. Serum was decanted and stored at -20°C until analysis.

Tissues were collected 50 h after CIDR withdrawal following administration of anesthesia. A left paralumbar celiotomy was performed to externalize the reproductive tract, and ovaries were removed. Ovaries were handled and processed as described previously (Castanon et al., 2012), with aspirations of the dominant follicle of each ovary pair for collection of follicular fluid and granulosa cell lysate.

Radioimmunoassay

Follicular fluid and serum were analyzed for E_2 and progesterone (P_4) by solid-phase RIA using components of commercial kits manufactured by MP Biomedicals (Santa Ana, CA). Detection limit (95% of maximum binding) of the assay was 2 pg/mL. Serum E_2 had an interassay CV of 10.3% with an intra-assay CV of 14.6%. Follicular fluid E_2 CV was 4.7%, and P_4 CV was 7.1%.

Statistical Analysis

All statistical analysis was performed using SAS (version 9.3; SAS Institute, Inc., Cary, NC). Follicular fluid E_2 and P_4 concentrations and $E_2:P_4$ ratios were analyzed using the GLM procedure in SAS. Serum estradiol and rectal temperatures were analyzed using the MIXED procedure in SAS. Analysis by ANOVA and least square means comparisons between treatments were performed when significant differences ($P < 0.1$) were detected.

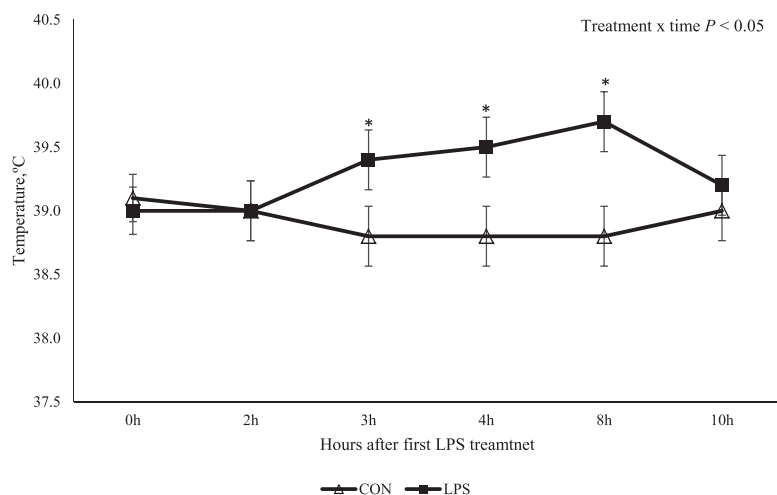


Figure 1. Rectal temperatures in heifers administered a subcutaneous injection of saline (CON) or LPS (2.0 $\mu\text{g}/\text{kg}$ of BW) on day 2 of the study. LPS-treated heifers demonstrated elevated rectal temperatures compared with controls at 3, 4, and 8 h ($P < 0.1$) after treatment. *Denotes level of significance when comparing CON vs. LPS within a time point ($P < 0.05$).

RESULTS AND DISCUSSION

LPS Treatment Induced an Immune Response

LPS purified from Gram-negative *E. coli* invokes an inflammatory response similar to that experienced by animals infected with Gram-negative bacteria (Steiger et al., 1999; Waggoner et al., 2009). Inflammatory responses can be characterized by a rise in body temperature (Steiger et al., 1999) and were used to indicate LPS response following treatment. A treatment × time interaction ($P < 0.01$) for rectal temperature was detected following the first treatment of LPS on day 2 (Figure 1), confirming an immune response. Rectal temperatures did not differ on days 5 or 8 ($P > 0.05$)

between treatment groups. This lack of hyperthermic response on subsequent treatment days is consistent with O’Reilly et al. (1988) noting that body temperature response to LPS decreases with infusion over time.

LPS Treatment on Serum and Follicular Fluid Hormone Concentrations

LPS derived from infections caused by Gram-negative bacteria have been detected in ovarian follicular fluid, and increased amounts of LPS are correlated with decreased E_2 synthesis and aromatase mRNA expression in granulosa cells (Magata et al., 2014). However, the low dose of LPS administered in the present study did not alter

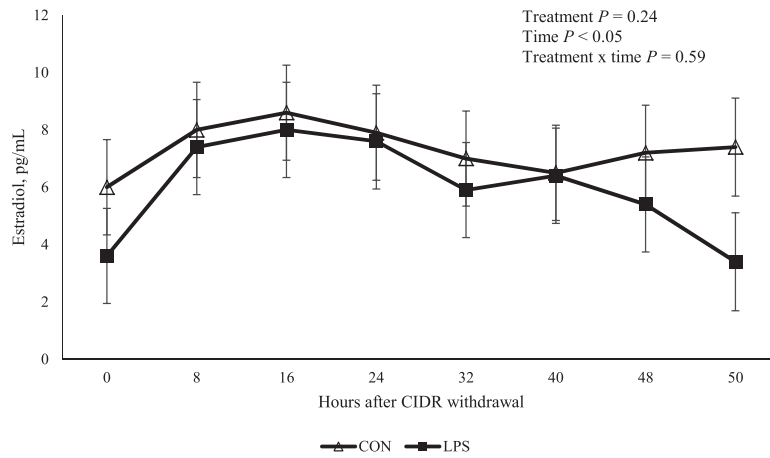


Figure 2. Serum estradiol (E_2) concentration in heifers administered a subcutaneous injection of saline (CON) or LPS (2.0 $\mu\text{g}/\text{kg}$ of BW) after CIDR withdrawal. There was no effect of treatment or a treatment × time interaction ($P > 0.1$), but there was an effect of time ($P < 0.1$).

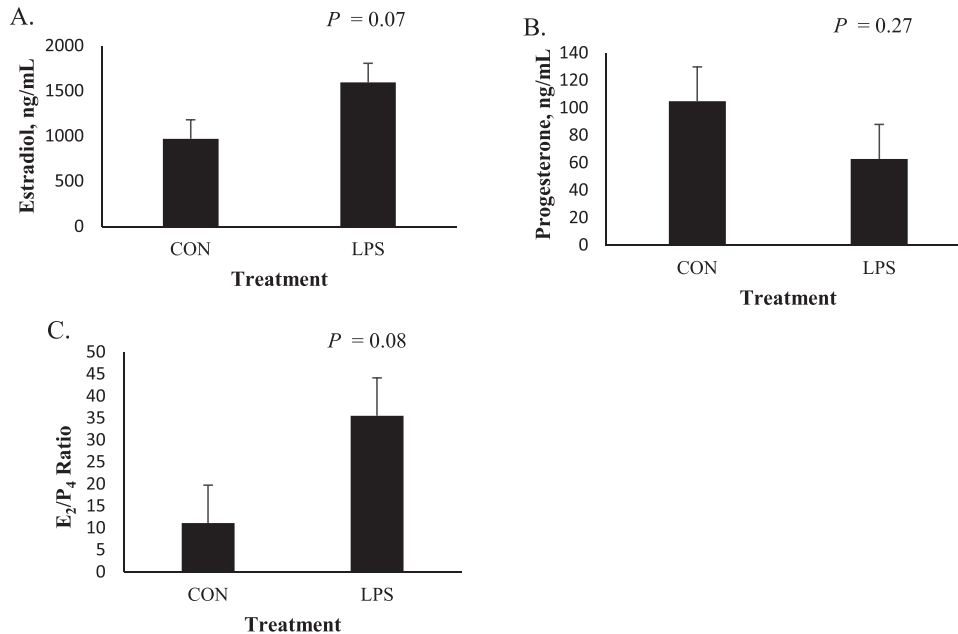


Figure 3. (A) Follicular fluid estradiol (E_2) concentration in heifers administered a subcutaneous injection of saline (CON) or LPS (2.0 $\mu\text{g}/\text{kg}$ of BW). There was a significant increase ($P < 0.10$) in E_2 concentration in LPS-treated heifers compared with controls. (B) Follicular fluid progesterone (P_4) concentration in CON vs. LPS heifers. There was no change in P_4 concentration in LPS-treated heifers compared with controls ($P > 0.1$). (C) Follicular fluid $E_2:P_4$ ratios in CON vs. LPS heifers. There was a significant decrease ($P < 0.10$) in $E_2:P_4$ observed in CON vs. LPS-treated heifers.

serum E_2 concentrations in LPS-treated or control heifers (treatment \times time; $P > 0.10$) (Figure 2). Though no significant changes in E_2 were detected, it appears that E_2 was beginning to decline more rapidly in the LPS group at the last sampling point. Future studies will sample beyond 50 h to evaluate long-term E_2 production in response to LPS treatment.

There was a significant ($P = 0.07$) increase in E_2 concentration in ovarian follicular fluid of LPS heifers compared with CON (Figure 3A). Follicular fluid concentrations of P_4 did not differ ($P > 0.10$) between treatment groups (Figure 3B). However, $E_2:P_4$ ratios were significantly lower ($P < 0.10$) in control animals compared with LPS-treated heifers (Figure 3C). Ovarian production of estradiol is regulated by numerous signaling pathways including beta-catenin which is also recognized to regulate inflammation. Alterations in beta-catenin levels within the follicles will be necessary to elucidate contributions of this molecule to regulation of intrafollicular hormone production. It is possible that the low-dose treatment of LPS may be modulating beta-catenin in such a manner that allows for E_2 synthesis and secretion to increase. These data demonstrate a clear modulation of the ovarian follicular hormonal milieu with LPS treatment. Increasing both number of animals and sampling time points after 50 h may allow for detection of changes to E_2 in the serum.

Conclusions

Factors affecting female fertility could have major implications for producer profit. Diseases common to cattle can affect fertility by disrupting normal E_2 production. Results indicate that low-level exposure to LPS can modulate the hormonal profile within ovarian follicles, which can subsequently affect fertility. Numerous signaling pathways converge to regulate E_2 production and understanding mechanisms that may contribute to E_2 dysregulation remains to be determined.

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