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Research Article

Inflammation during oocyte maturation reduces developmental competence and increases apoptosis in blastocysts†

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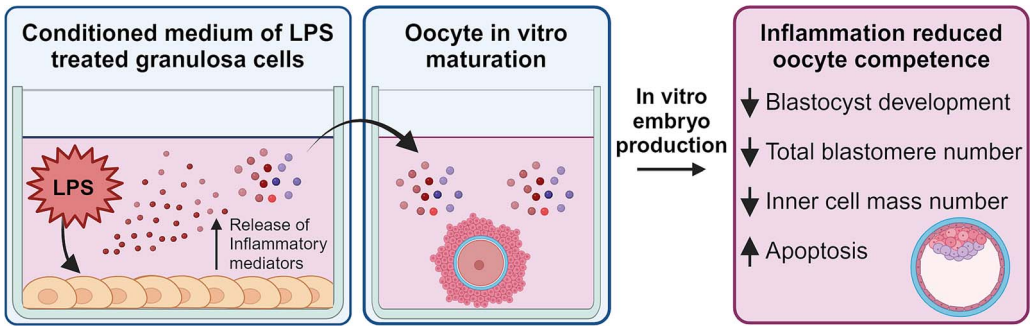
Abstract

Uterine infections cause ovarian dysfunction and infertility. The bacterial endotoxin, lipopolysaccharide, accumulates in the follicular fluid of dominant follicles of cows with uterine infections. Granulosa cells produce an innate inflammatory response to lipopolysaccharide, altering the follicular microenvironment of the oocyte. We hypothesized that developmental competence and embryo quality would be reduced when oocytes are matured in an inflammatory environment. Bovine mural granulosa cells were exposed to either 1 μg/mL of lipopolysaccharide or medium alone for 24 h to produce a conditioned medium. Inflammatory responses of mural granulosa cells were confirmed by increased expression of *CXCL8*, *IL1B*, *IL6*, and *TNF*. Bovine cumulus–oocyte complexes were matured for 22 ± 1 h in a medium supplemented with either 1 μg/mL of lipopolysaccharide, 10% v/v conditioned medium of granulosa cells treated with either lipopolysaccharide (LCM) or medium alone, or no supplementation. In addition, polymyxin B (20 μg/mL) was added to the maturation medium to sequester LPS. Following maturation, cumulus–oocyte complexes were fertilized and cultured for 7.5 days with no further treatment. Oocyte maturation using lipopolysaccharide or LCM impaired development to the blastocysts stage, reduced the number of total and CDX2-negative blastomeres, and increased TUNEL-positive cells in blastocysts. Polymyxin B could rescue these effects in the lipopolysaccharide group but not in the LCM group, indicating factors produced by granulosa cells and not lipopolysaccharide alone compromised oocyte development. These findings suggest that the inflammatory milieu produced by granulosa cells in response to lipopolysaccharide impairs oocyte competence and the quality of resultant blastocyst-stage embryos.

Summary Sentence

In vitro maturation of bovine oocytes under inflammatory conditions reduces developmental competence and alters embryo quality by increasing apoptosis and decreasing blastomere cell number.

Graphical Abstract



Key words: inflammation, oocyte maturation, development competence, bovine embryo

Introduction

Postpartum bacterial contamination of the uterus is prevalent in dairy cows [1]. While many cows remain healthy, 40% of cows fail to clear the bacterial contamination of the uterus, which causes uterine diseases such as metritis and endometritis [1, 2]. These uterine diseases are associated with lower conception rates, increased pregnancy losses, and increased culling, which compromise the profitability and sustainability of the dairy sector [2–5]. Generally, disease-associated subfertility occurs after the resolution of the uterine disease, leaving a significant number of cows unable to conceive [6, 7]. The causes of subfertility in cows after the

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uterine disease is unclear, but are likely to include effects on the uterus, ovary, and hypothalamic–pituitary–gonadal axis. Uterine diseases cause tissue damage of the endometrium due to inflammation, delayed uterine involution, decreased follicle growth, and disrupted ovulation—all of which could impact fertility [1, 4, 8–12].

Oocyte quality is a key determinant of female fertility [13]. Lipopolysaccharide (LPS) of Gram-negative bacteria including *Escherichia coli* accumulates in the follicular fluid of cows with uterine diseases [12, 14, 15]. The concentration of LPS in follicular fluid is positively correlated with the severity of uterine inflammation observed in cows and ranges from 1 μg/mL in cows with metritis to 0.8 μg/mL in cows with endometritis [12, 16]. In parallel, granulosa cells express the molecular machinery required to recognize pathogen-associated patterns such as LPS, including Toll-like receptor 4, cluster of differentiation 14 (CD14), and myeloid differentiation factor-2 (MD2) [12, 17]. Activation of this molecular machinery by LPS initiates an innate inflammatory response that increases the synthesis of proinflammatory cytokines including interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNFα) and chemokines like IL-8 [12, 17–20]. The innate inflammatory response of granulosa cells to LPS alters the intrafollicular environment, which is crucial for oocyte development [21]. Perturbations to the intrafollicular environment are likely to reduce oocyte developmental competence, subsequent embryo quality, and pregnancy establishment [20, 22, 23]. Intriguingly, oocytes collected from cows after induced uterine infection have a reduced capacity to develop morula-stage embryos after in vitro fertilization [24]. Also, the maturation of oocytes in the presence of LPS increases oocyte meiotic failure [19] and reduces the development of blastocyst-stage embryos after in vitro fertilization [25]. While the direct exposure of oocytes to LPS has been previously investigated, the consequence of granulosa cell responses to LPS and an altered intrafollicular environment on oocyte quality remain unexplored.

The present study aimed to investigate the impact of inflammatory conditions, induced by LPS from granulosa cells, on oocyte competence and embryo quality during in vitro maturation. We hypothesized that developmental competence and embryo quality would be reduced when oocytes are matured in an inflammatory environment produced by granulosa cells.

Materials and methods

All procedures described here are in accordance with the University of Florida Institutional Animal Care and Use Committee and did not use any live animals.

Granulosa cell isolation and culture

Granulosa cells were isolated and cultured as previously described by Dickson et al. [26], which produces cultures free of contaminating theca (CYP17A1) and immune (CD45) cells. Briefly, fresh bovine ovaries were obtained from a commercial abattoir and transported to the laboratory at 22°C in 0.9% saline supplemented with 10 000 units/mL of penicillin and 10 000 μg/mL of streptomycin (Thermo Fisher Scientific; Walton, MA). Granulosa cells and cumulus–oocyte complexes (COCs) were collected from small- to medium-diameter follicles (2–8 mm) by repeatedly slashing the ovarian surface using a scalpel blade and releasing the follicle contents into commercial oocyte collection medium (BoviPlus; Minitube, Verona, WI). Large-diameter follicles (>8 mm) were punctured outside of the collection beaker to avoid mixing granulosa cells from large follicles with our targeted cells of small to medium-diameter follicles. To reduce cow-to-cow variation and potential impacts of prior infection, the collection medium was pooled from a group of 10–15 ovaries and filtered through a sterile 100 μm cell strainer (Corning; Corning, NY) to capture tissue debris and COCs (used below for embryo production). The resultant supernatant was then passed through a sterile 40 μm cell strainer (Thermo Fisher Scientific) to capture mural granulosa cells. Granulosa cells were recovered from the cell strainer and rinsed with warm complete culture medium (Medium 199 (Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal calf serum (Corning), 10 mg/L human recombinant insulin, 5.5 mg/L human recombinant transferrin, 6.7 μg/L selenious acid (Corning), 50 IU/mL penicillin, 50 μg/mL streptomycin (Thermo Fisher Scientific), and 2 mM l-alanyl-l-glutamine dipeptide in 0.85% NaCl (GlutaMAX; Thermo Fisher Scientific) [19]. The cell suspension was centrifuged at $500 \times g$ for 10 min, and the supernatant was discarded. The resultant cell pellet was subjected to red blood cell lysis by the addition of 900 μL of cell culture–grade H_2O (HyClone; Chicago, IL), immediately followed by the addition of 100 μL of 10× phosphate buffer saline (PBS). Cells were washed with Dulbecco PBS (DPBS) by centrifugation at $500 \times g$ for 10 min. The resultant cell pellet was resuspended in 1 mL of the complete culture medium containing hyaluronidase (100 U/mL; Millipore Sigma; Burlington, MA) and vortexed for 10 s every 3 min for a total of 9 min. Granulosa cells were washed again by centrifugation at $500 \times g$ for 10 min in a complete cell culture medium. Cell concentration was adjusted to 1.5×10^5 cells per milliliter and plated (TPP; Trasadingen, Switzerland) in 2 mL (6-well plates), 500 μL (24-well plates), or 200 μL (96-well plates) aliquots and cultured at 35.8°C in humidified air with 5% CO_2.

Preparation of granulosa cell–conditioned media

Granulosa cells were equilibrated in culture plates for 12–14 h. Adherent cells were washed with warm DPBS and treated with complete cell culture medium containing 1 μg/mL ultrapure LPS (*E. coli* 0111-B4; tlrl3pelps, Invivogen, San Diego, CA) or complete cell culture medium alone for 24 h [19]. After the 24-h treatment, supernatants were collected as a conditioned medium from LPS-treated granulosa cells (LCM) and medium alone–treated granulosa cells (CCM) and stored at −20°C. The conditioned medium from seven biological replicates was pooled and used as a treatment during in vitro maturation of oocytes, and an additional six biological replicates were performed to evaluate the secretion of inflammatory mediators into the supernatant. Cells were stored in RLT RNA lysis buffer (Qiagen, Hilden, Germany) at −80°C for RNA isolation.

Inhibition of lipopolysaccharide response using polymyxin B

Cultured granulosa cells were treated with a complete cell culture medium containing increasing concentrations of polymyxin B (0, 10, 20, 30, 50, 80, and 100 μg/mL; Invivogen) in the presence or absence of 1 μg/mL ultrapure LPS (Invivogen) for 24 h. Polymyxin B was incubated in a complete medium with LPS for 10 min before being added to

the cultured cells. After 24 h of treatment, cells were either stored in RLT buffer (Qiagen) at −80°C for RNA isolation or immediately assessed for cell viability (below).

Cell viability assay

Granulosa cell viability was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Merck, Rahway, NJ) assay [17]. After a 24-h treatment with polymyxin B and/or ultrapure LPS, cells were cultured in a complete cell culture medium containing 5 mg/mL of MTT for 4 h. Subsequently, the supernatant was removed, and cells were washed with warm DPBS prior to lysis in 100 μL of dimethyl sulfoxide followed by a 15-min incubation in the dark at room temperature. The optical density (OD_{540}) of each well was measured using a microplate reader (BioTek Instruments, Winooski, VT). The background OD_{540} of a blank well containing no cells was subtracted from the OD_{540} of each sample well. Each treatment of an experimental replicate was evaluated in triplicate and the average blank-corrected OD_{540} was calculated for each treatment. Data were normalized to a percent change in viability of cells treated with complete cell culture medium alone.

Quantification of inflammatory mediators in granulosa cell–conditioned medium

Supernatants of cultured granulosa cells were subjected to protein analysis using the Milliplex Bovine Cytokine/Chemokine 15-plex array (BCYT1–33 K; MilliporeSigma) following the manufacturer's instruction. The multiplex array was designed to quantify interferon gamma (IFNγ), IL-1α, IL-1β, IL-4, IL-6, IL-8 (CXCL8), IL-10, IL-17A, interleukin-36 receptor antagonist IL-36RA (IL-1F5), interferon gamma–induced protein 10 (IP-10, CXCL10), monocyte chemoattractant protein-1 (MCP-1, CCL2), macrophage inflammatory protein-1 alpha (MIP-1α, CCL3), MIP-1β (CCL4), TNFα, and vascular endothelial growth factor A (VEGFA) using a FlexMAP 3D instrument with xPONENT software (Luminex Corporation, Austin, TX). The minimum detectable concentrations for the assay were 0.08 pg/mL (IFN-γ), 0.36 pg/mL (IL-1α), 4.93 pg/mL (IL-1β), 16.57 pg/mL (IL-4), 11.23 pg/mL (IL-6), 22.6 pg/mL (IL-8), 1.05 pg/mL (IL-10), 0.67 pg/mL (IL-17A), 98.54 pg/mL (MIP-1α), 1.23 pg/mL (IL-36RA), 0.91 pg/mL (IP-10), 2.74 pg/mL (MCP-1), 2.32 pg/mL (MIP-1β), 22.62 pg/mL (TNFα), and 1.18 pg/mL (VEGFA).

Treatment of cumulus–oocyte complexes during in vitro maturation and subsequent in vitro embryo production

Each replicate consisted of processing 10–15 ovaries together that yielded 250–350 COCs. A total of 1770 COCs were obtained from six replicates and 30–40 COCs were used per treatment for each replicate. Cumulus–oocyte complexes with multiple layers of cumulus cells and homogenous oocyte cytoplasm were selected and washed three times in an oocyte collection medium containing bovine serum albumin (BoviPlus; Minitube). Cumulus–oocyte complexes were matured for 22 h at 38.5°C in a humidified atmosphere with 5% CO_2 in 500 μL of commercial in vitro maturation medium (IVF Bioscience; Falmouth, UK). In vitro maturation medium was supplemented with either 1 μg/mL ultrapure LPS, 10% v/v CCM, 10% v/v LCM, or not supplemented as a control. The final concentration of LPS in the maturation medium for the LCM group was 100 ng/mL. Additionally, each treatment group was expanded to include a subset of COCs treated with 20 μg/mL of polymyxin B to inhibit LPS signaling (concentration of polymyxin B was determined to inhibit LPS signaling and maximize cell viability). Following maturation, COCs were subjected to in vitro fertilization and embryo culture in a medium without any further treatment and maintained separately according to treatment groups. Briefly, matured COCs were washed three times in a wash medium (IVF Bioscience) and transferred to 4-well plates (Thermo Fisher Scientific) containing a BO-IVF medium (IVF Bioscience). A uniform sperm suspension was prepared at a concentration of 2×10^6/mL spermatozoa from a pool of conventional frozen–thawed semen of three different bulls. After a 16–18-h co-incubation, putative zygotes were removed from the fertilization wells and denuded of cumulus cells by vortexing in a wash medium. Denuded, putative zygotes were washed three times in a wash medium and cultured in 500 μL of BO-IVC (IVF Bioscience) under oil (IVF Bioscience). Zygotes were cultured at 38.5°C in a humidified atmosphere with 5% CO_2, 5% O_2, and 90% N_2 in a benchtop embryo incubator (Planer; Sunbury-On-Thames, UK). Zygote cleavage was determined at d 3.5 post-fertilization, and blastocyst development was assessed on d 7.5 post-fertilization. At d 7.5 blastocysts stage, embryos were collected and either fixed in 4% (w/v) paraformaldehyde for 15 min and stored at 4°C in PBS with 0.2% (w/v) polyvinylpyrrolidone or stored in RLT buffer at −80°C for isolation of RNA.

RNA isolation and real-time reverse transcription-PCR

Total RNA was extracted from pools of 6–15 blastocysts from each treatment group using Qiashredder columns and the RNeasy micro extraction kit with DNAse according to the manufacturer's instruction (Qiagen). Reverse transcription of RNA was performed using the Verso cDNA synthesis kit (Thermo Fisher Scientific). Resultant cDNA was subjected to selective pre-amplification utilizing SsoAdvanced PreAmp Supermix (Bio-Rad; Hercules, CA) in combination with a pool of target gene primers for a total of 12 preamplification cycles. Total RNA of granulosa cells was extracted using the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. Quality and quantity of extracted RNA was assessed by an ultraviolet-visible spectrophotometer, Nanodrop 2000 (Thermo Fisher Scientific), and reverse transcription was performed using the Verso cDNA synthesis kit (Thermo Fisher Scientific). A no reverse transcriptase (no-RT) control was included for granulosa cells and embryos. Primers for each target gene (Table 1) were designed using the NCBI database to span exon–exon boundaries and were validated according to Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines ($R^2 > 0.98$ and efficiency of 90%–110%) [27]. Real-time RT-PCR for each sample including granulosa cells and blastocysts was performed in triplicate using a three-step protocol that included a 20 μL of reaction consisting of 300 nM of each forward and reverse primer, iTaq Universal SYBR Green Supermix (Bio-Rad), and cDNA. Amplification was carried out using a Bio-Rad CFX Connect system with polymerase activation at 95°C for 30 s followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 10 s (62°C for *PTGS2* and *TNF*), and extension at 60°C for 30 s. A no-template pre-amplification

Table 1. Primer sequence and annealing conditions used for real-time RT-PCR

Gene Symbol	Primer sequence	Annealing temperature (°C)	Accession number
BAX	5′ – CAGGGTGGTTGGGACGG 3′ – CTTCCAGATGGTGAGCGAGG	60	NM_173894.1
BCL2	5′ – GAGTTCGGAGGGGTCATGTG 3′ – ACAAAGGCGTCCCAGCC	60	NM_001166486.1
CXCL8	5′ – GCAGGTATTTGTGAAGAGAGCTG 3′ – CACAGAACATGAGGCACTGAA	60	NM_173925.2
DNMT3A	5′ – CCATGTACCGCAAGGCTATCTA 3′ – CCTGTCATGGCACATTGGAA	60	XM_024998.68.1
GAPDH	5′ – AGGTCGGAGTGAACGGATTC 3′ – ATGGCGACGATGTCCACTTT	60	NM_001034034.2
HSPA1A	5′ – GACAAGTGCCAGGAGGTGATTT 3′ – CAGTCTGCTGATGATGGGGTTA	60	NM_203322.3
IFNT2	5′ – TCCATGAGATGCTCCAGCAGT 3′ – TGTTGGAGCCCAGTGCAGA	60	NM_001015511.4
IGF2R	5′ – CAGGTCTTGCAACTGGTGTATGA 3′ – TTGTCCAGGGAGATCAGCATG	60	NM_174352.2
IL1B	5′ – CTTCATTGCCCAGGTTTCTG 3′ – CAGGTGTTGGATGCAGCTCT	60	NM_174093.1
IL6	5′ – ATGACTTCTGCTTTCCCTACCC 3′ – GCTGCTTTCACACTCATCATTC	60	NM_173923.2
PTGS2	5′ – CGTGAAAGGCTGTCCCTTTA 3′—ATCTAGTCCAGAGTGGGAAGAG	62	NM_001105323.1
RPL19	5′ – ATGCCAACTCCCGCCAGCAGAT 3′ – TGTTTTTCCGGCATCGAGCCCG	60	NM_001040516.2
SDHA	5′ – GGAACACTGACCTGGTGGAG 3′ – GGAACACTGACCTGGTGGAG	60	NM_174178.2
SLC2A1	5′ – AGCGTCATCTTCATCCCAGC 3′ – AGCTTCTTCAGCACGCTCTT	60	NM_174602.2
TNF	5′ – CACATACCCTGCCACAAGGC 3′ – CTGGGGACTGCTCTTCCCTCT	62	NM_173966.3
TLR4	5′ – AGCCACGGCCATCCTCTCCT 3′ – AGCTCAGGTCCAGCATCTTGGT	60	NM_174198.6

negative control and a no-template negative control were used to confirm specific amplification for each primer pair. Relative expression for target genes was calculated using the $2^{-\Delta Ct}$ method relative to selected reference genes (Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) for granulosa cells data; geometric mean of *GAPDH*, ribosomal protein L19 (*RPL19*), and succinate dehydrogenase complex flavoprotein subunit A (*SDHA*) for blastocysts data.

TUNEL and immuno-labeling of blastocysts

Fixed d 7.5 blastocysts were permeabilized with 0.3% v/v Triton X-100 (Thermo Fisher Scientific) in DPBS containing 0.2% w/v polyvinylpyrrolidone (PVP) for 30 min. Blastomere apoptosis was evaluated using the fluorescein in situ Cell Death Detection Kit (Millipore Sigma). Briefly, 25–30 blastocysts per replicate were incubated in 25 μL of TUNEL solution containing the deoxynucleotidyl terminal transferase enzyme and 2′-deoxyuridine 5′-triphosphate-dUTP-fluorescein isothiocyanate labeling solution for 1 h at 37°C in a humidified chamber in the dark. A subset of blastocysts was treated with 50 U/mL of DNAse (Promega Co., Madison, WI, USA) at 37°C for 1 h in the dark to induce DNA damage prior to TUNEL labeling as a positive control. The deoxynucleotidyl terminal transferase enzyme was omitted from the assay in a separate subset of blastocysts as a negative control. After washing three times with DPBS/PVP, all samples were incubated in a blocking buffer (5% w/v BSA Fraction V in DPBS/PVP) for 1 h at room temperature. Blastocysts were incubated with a mouse monoclonal anti-CDX2 IgG1 antibody at a final concentration of 0.4 μg/mL (#CDX2–88 AM392-5 M; BioGenex Laboratories, Fremont, CA) for 1 h at room temperature, washed with 0.2% w/v DPBS/PVP containing 0.05% v/v Tween-20 and incubated with an anti-mouse IgG-Alexa 594 antibody (1:1000 dilution; Thermo Fisher Scientific) for 1 h at room temperature. Blastocysts were washed three times with 0.2% w/v DPBS/PVP and incubated with 1 μg/mL Hoechst 33342 for 15 min and mounted on slides using 50% (v/v) glycerol/PBS mounting medium. Blastocysts were imaged using an Axio Observer 7 and Plan-Apochromat 40× objective lens (Zeiss; Jena, Germany) fitted with an Andor DSD2 confocal unit and Zyla Plus 4.2-megapixel camera (Oxford Instruments, Abingdon, UK). Optical sections of each entire blastocyst were acquired and cell counting was performed using ImageJ software [28]. Total blastomere number was determined by counting nuclei stained with Hoechst 33342, and trophectoderm cell number was determined by counting cells immunoreactive for CDX2. The number of apoptotic blastomeres was determined by counting the number of TUNEL-positive blastomeres. The number of inner cell mass cells was determined by subtracting the number of CDX2 cells from the total number of blastomeres.

Statistical analysis

All data were analyzed using the SAS statistical package (version 9.4; SAS/STAT. SAS Inst. Inc., Cary, NC, USA). Gene

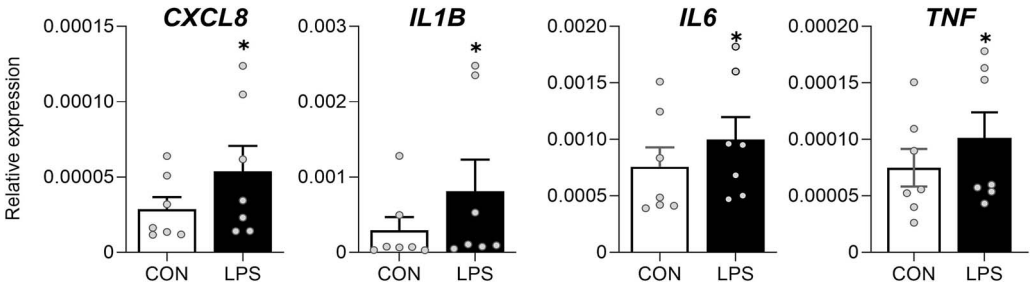

Figure 1. Lipopolysaccharide induces an inflammatory response in granulosa cells. Granulosa cells were exposed to either 1 μg/mL of LPS or medium alone as a control (CON) for 24 h. (A) Gene expression of *CXCL8*, *IL1B*, *IL6*, and *TNF* were evaluated by real-time RT-PCR in seven biological replicates. Data are presented as the mean ± SEM relative expression normalized to the geometric mean of *GAPDH*, *RPL19*, and *SDHA*. Each dot represents an individual replicate, and bars represent the mean ± SEM relative expression normalized to the geometric mean of *GAPDH*, *RPL19*, and *SDHA*.

expression (as the $2^{-\Delta Ct}$) and protein accumulation response in granulosa cells were analyzed using the MIXED procedure in SAS. Data were analyzed for the fixed effect of LPS treatment (LPS vs. CON) including the random effect of replicate. The efficacy of the polymyxin B treatment was analyzed for the fixed effect of treatment (LPS vs. CON), polymyxin B (PMB vs. CON), and interaction between LPS and polymyxin B, including the random effect of replicate. The GLIMMIX procedure in SAS was applied to analyze oocyte cleavage and blastocyst development response variables with the fixed effect of treatment including the random effect of replicate. When a significant effect of treatment was observed ($P < 0.05$), data were further analyzed using a Dunnett post hoc test comparing each treatment group to the no supplementation control group. Variables that failed to meet the assumption of normality were log-transformed prior to analysis. All analyses were considered statistically significant if $P \leq 0.05$.

Results

Lipopolysaccharide increases the expression of inflammatory mediators in granulosa cells that is inhibited by polymyxin B

To generate a conditioned medium that would be used during in vitro maturation of oocytes, granulosa cells were treated with either 1 μg/mL of ultrapure LPS or medium alone for 24 h. Treatment of granulosa cells with LPS increased ($P \leq 0.05$) the transcript expression of *CXCL8*, *IL1B*, *IL6*, and *TNF* compared to medium alone (Figure 1). The presence of IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-17A, IL-36RA, IFNγ, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα, and VEGF-A were also evaluated in the supernatants of cultured cells (Table 2). Treatment with LPS increased ($P \leq 0.05$) the 24-h accumulation of IL-36RA, MIP-1α, and MIP-1β in supernatant compared to the medium alone control. Moreover, there was a tendency ($P < 0.1$) for LPS to increase the accumulation of IFNγ, IL-1α, IP-10, and MCP-1 in the supernatant. Treatment with LPS for 24 h had no effect on the accumulation of IL-1β, IL-4, IL-6, IL-8, IL-10, IL-17A, TNFα, or VEGF-A.

Inclusion of 20–100 μg/mL of polymyxin B inhibited ($P \leq 0.05$) the LPS-induced expression of *CXCL8* and *IL6* in granulosa cells (Figure 2A and B). Moreover, polymyxin B alone at concentrations ≥30 μg/mL decreased ($P \leq 0.05$) granulosa cell viability compared to control cells (Figure 2C). It was therefore determined that a polymyxin B concentration of 20 μg/mL was sufficient to inhibit LPS signaling and maintain cell viability in experiments described below.

Inclusion of inflammatory conditioned medium during in vitro maturation reduces developmental competence of oocytes to develop blastocyst-stage embryos

Cumulus–oocyte complexes were matured in the presence of either 1 μg/mL of LPS, 10% v/v conditioned medium of granulosa cells treated with either LPS (LCM) or medium alone (CCM), or medium alone (CON). Additionally, each treatment group was expanded to include a subset of COCs treated with 20 μg/mL of polymyxin B to inhibit LPS signaling present in the LPS alone treatment or LPS present in the LCM treatment. The final concentration of LPS in maturation medium was 1 μg/mL for the LPS group (500 ng total) and 100 ng/mL for the LCM group (50 ng total). The inclusion of the CCM control group was to ascertain any negative effects of the granulosa cell culture medium on oocyte competence. Oocytes were subsequently fertilized, and presumptive embryos were cultured in the absence of any further treatment. The proportion of oocytes that cleaved at d 3.5 post-fertilization was not affected by any treatment ($P > 0.05$) when compared to the medium alone group (CON = 67.9 ± 3.5, LPS = 58.5 ± 3.6, CCM = 62.4 ± 3.5, and LCM = 61.09 ± 3.5%; Figure 3A). In the medium alone group, the proportion of oocytes to develop to the blastocyst stage was 36.9 ± 3.6%, which was not different in the polymyxin B alone group (32.1 ± 3.4%; Figure 3B and D). Treatment of oocytes with LPS reduced ($P \leq 0.05$) the proportion of oocytes to develop to the blastocyst stage to 16.5 ± 2.6% (Figure 3B and E), which was rescued when polymyxin B was included in the treatment (31.1 ± 3.4%). The addition of CCM had no effect on the proportion of oocytes to develop to the blastocyst stage (33.7 ± 3.4%; Figure 3B and F) compared to the medium alone group. Conversely, the addition of LCM decreased ($P \leq 0.05$) the proportion of oocytes to develop to the blastocyst stage (17.3 ± 2.6%; Figure 3B and G) compared to the medium alone group. Interestingly, the addition of polymyxin B to the LCM treatment did not rescue ($P \leq 0.05$) the proportion of oocytes to develop to the blastocyst stage to the rates observed in the medium alone group (27.8 ± 3.1%).

Furthermore, exposure of COCs to LPS or LCM reduced ($P \leq 0.05$) the proportion of cleaved zygotes to develop to the blastocyst stage compared to the medium alone group (CON = 54.7 ± 5.3% *vs.* LPS = 28.1 ± 4.7% *vs.*

Table 2. Accumulation of inflammatory mediators after 24-h exposure of granulosa cells to LPS.

Analyte	Control medium (pool)	LPS (pool)	*P*-value	Intra-assay CV (%)
IFNγ	284.7 ± 22.4 (280.5)	324.4 ± 22.4 (334.2)	0.06	6.1
IL-1α	184.3 ± 14.6 (190.5)	210.0 ± 14.6 (223.5)	0.07	5.0
IL-1β	192.7 ± 10.9 (173.0)	212.0 ± 10.9 (218.2)	0.13	7.4
IL-4	104.8 ± 1.98 (101.5)	103.0 ± 1.93 (102.0)	0.52	6.6
IL-6	663.9 ± 51.2 (668.5)	727.0 ± 51.2 (769.5)	0.11	4.2
IL-8 (CXCL8)	5614.6 ± 488.6 (5047.8)	6209.0 ± 488.6 (6623.8)	0.12	3.4
IL-10	266.0 ± 25.7 (239.5)	297.5 ± 25.7 (293.8)	0.16	4.4
IL-17A	173.6 ± 9.7 (160.8)	181.9 ± 9.6 (200.8)	0.24	6.0
IL-36RA (IL-1F5)	658.5 ± 32.9 (647.0)	722.8 ± 32.9 (768.3)	0.02	5.4
IP-10 (CXCL10)	1483.3 ± 103.7 (1430.0)	1647.3 ± 103.7 (1909.5)	0.07	4.4
MCP-1 (CCL2)	264.2 ± 19.6 (247.3)	293.9 ± 19.6 (293.8)	0.06	3.9
MIP-1α (CCL3)	273.7 ± 30.7 (261.3)	349.7 ± 30.7 (314.5)	0.01	3.8
MIP-1β (CCL4)	431.5 ± 79.4 (460.5)	730.13 ± 79.4 (489.0)	0.01	3.1
TNFα	193.0 ± 16.4 (181.5)	211.0 ± 16.4 (229.3)	0.15	6.3
VEGF-A	1626.3 ± 122.6 (1352.8)	1742.6 ± 122.6 (1715.0)	0.19	2.5

Granulosa cells were exposed to either 1 μg/mL of LPS or medium alone as a control for 24 h. Accumulation of inflammatory mediators were evaluated using a multiplex assay in six biological replicates. In addition, the pooled conditioned medium (pool) used to supplement in vitro maturation was also evaluated and shown in parentheses. All data are presented least square mean of pg/mL ± SEM.

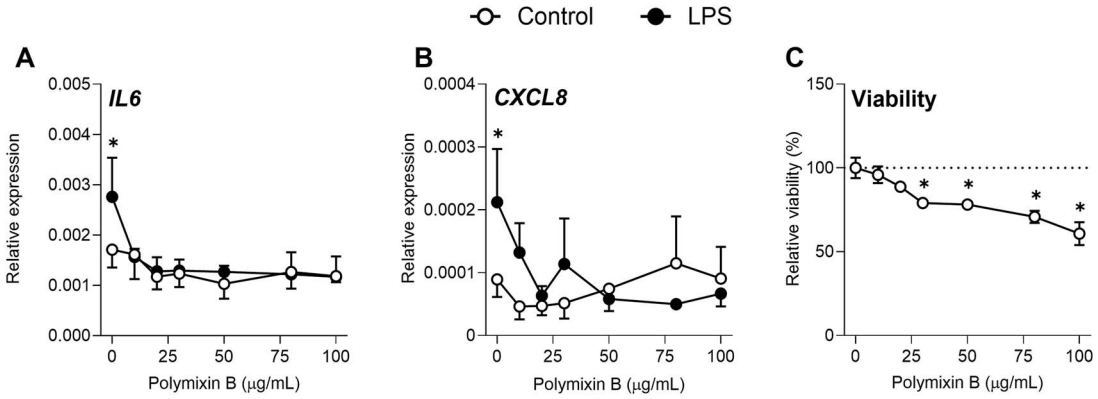

Figure 2. Efficacy of polymyxin B to inhibit LPS response in granulosa cells. Granulosa cells were cultured ($n = 5$) with increasing concentrations of polymyxin B (0–100 μg/mL) in the presence or absence of 1 μg/mL of ultrapure LPS for 24 h. Expression of *IL6* (A) and *CXCL8* (B) was evaluated by real-time RT-PCR, and data are presented as the mean ± SEM relative expression normalized to the geometric mean of *GAPDH*, *RPL19*, and *SDHA*. * represents differences ($P \leq 0.05$) between the LPS and medium alone group within a single concentration of polymyxin B. Viability of granulosa cells (C) was evaluated using the MTT assay in cells cultured for 24 h in increasing concentrations of polymyxin B (0 to 100 μg/mL) in the absence of LPS. Data are presented as the mean ± SEM percent change from cells cultured in medium alone (normalized to 100%). * indicates statistical significance ($P \leq 0.05$) compared to the medium alone group.

LCM = 28.4 ± 4.7%; Figure 3C). While the addition of polymyxin B to the LPS treatment rescued the proportion of cleaved zygotes to develop to the blastocyst stage (46.4 ± 5.3%), the addition of polymyxin B to the LCM group did not rescue ($P \leq 0.05$) the proportion of cleaved zygotes to develop to the blastocyst stage compared to the medium alone group (38.3 ± 4.9%). Exposure of COCs to CCM or polymyxin B alone did not affect the proportion of cleaved embryos to develop to blastocysts compared to the control group (54.6 ± 5.4% and 44.8 ± 5.2%, respectively; Figure 3C).

Inclusion of inflammatory conditioned medium during in vitro maturation increased apoptosis and reduced the number of total and trophectoderm cells in blastocyst stage embryos

Following treatment of COCs, in vitro fertilization, and embryo culture, the number of total, CDX2-positive, and CDX2-negative blastomeres was evaluated in 106 blastocyst-stage embryos of four replicates (Figure 4), while a subset of 92 blastocyst-stage embryos from three replicates were assessed for the number of TUNEL-positive blastomeres (Figure 5). The total number of blastomeres (Figure 4A) in the medium alone group was 202.8 ± 17.2, while treatment with LPS reduced ($P \leq 0.05$) the total number of blastomeres to 93.5 ± 10.4, which was rescued by the addition of polymyxin B (179.6 ± 14.8). The addition of CCM had no effect on the total number of blastomeres (187.3 ± 6.6), while the addition of LCM decreased ($P \leq 0.05$) the total number of blastomeres (134.4 ± 28.9) compared to the medium alone group. Interestingly, the addition of polymyxin B to the LCM treatment did not rescue ($P \leq 0.05$) the total number of blastomeres (118.7 ± 16.3).

In the medium alone group, the number of CDX2-negative blastomeres (Figure 4B), which reflect the inner cell mass, was 61.2 ± 4.3. Treatment with LPS reduced ($P \leq 0.05$) the number of CDX2 negative blastomeres to 28.3 ± 2.5, which was rescued by the addition of polymyxin B (57.4 ± 9.4). The addition of CCM had no effect on the number of CDX2-negative blastomeres (61.1 ± 9.4) compared to the medium alone group, while the addition of LCM decreased ($P \leq 0.05$)

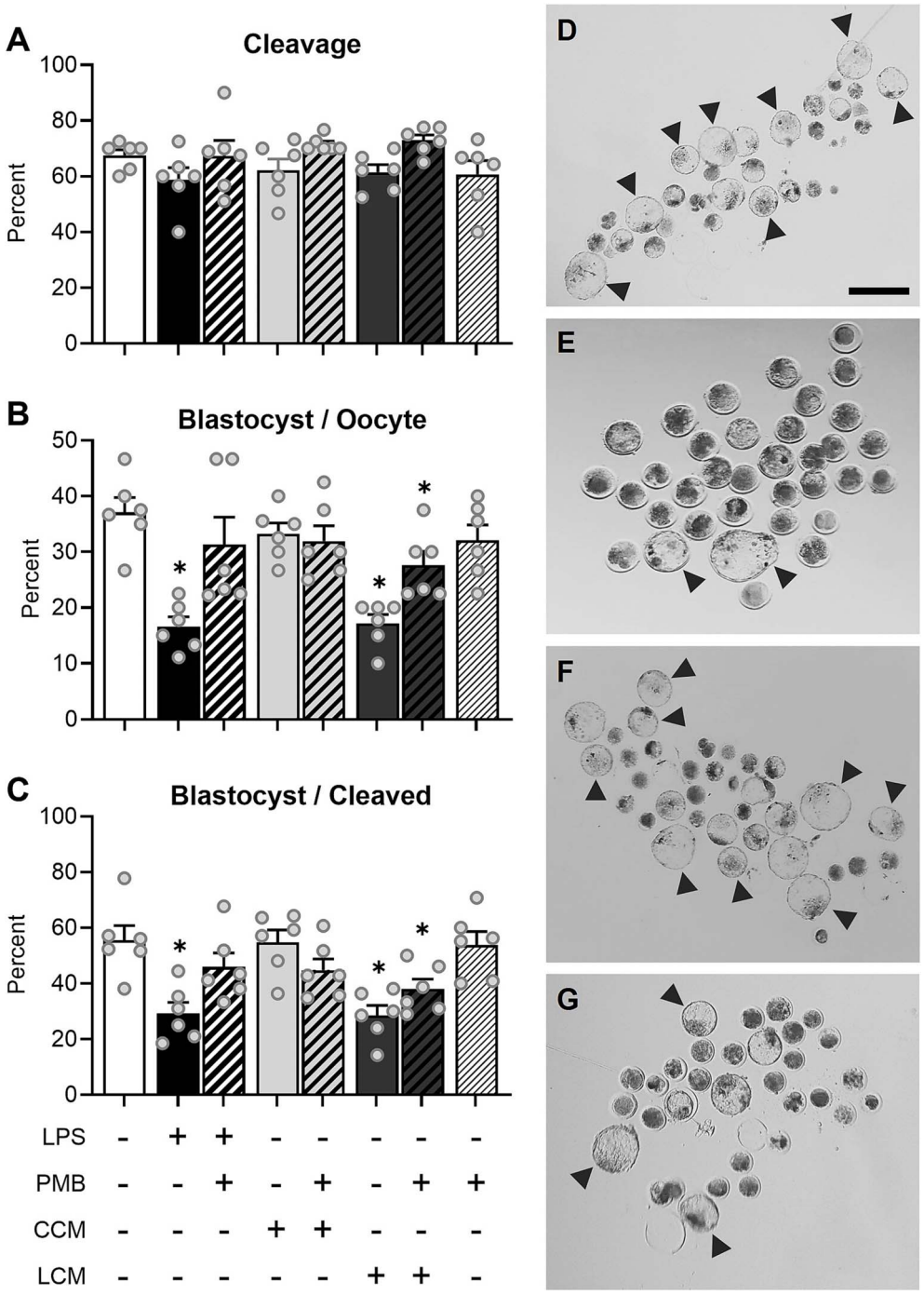

Figure 3. Inclusion of an inflammatory conditioned medium during in vitro maturation reduces the developmental competence of oocytes. A total of six experimental replicates were performed with 30–40 COCs per treatment per replicate. Cumulus–oocyte complexes were matured with LPS, CCM, LCM, or medium alone. Additionally, each treatment group was supplemented with polymyxin B (PMB) to inhibit LPS signaling. Oocytes were subjected to in vitro fertilization and embryo culture in a medium without any further treatment. The proportion of oocytes that cleaved by d 3.5 (A), the proportion of oocytes that developed to blastocyst-stage embryos by d 7.5 (B), and the proportion of cleaved oocytes that developed to blastocyst-stage embryos by d 7.5 (C) are reported. Each dot represents an individual replicate, and bars represent the mean ± SEM. * indicates statistical significance ($P \leq 0.05$) compared to the medium alone group. Representative photomicrographs of embryo cultures on d 7.5 from medium alone control (D), LPS (E), CCM (F), and LCM (G) groups are shown with arrowheads indicating expanded blastocysts. Scale bar equals 50 μm.

the number of CDX2-negative blastomeres (34.1 ± 5.9) compared to the medium alone group. Interestingly, the addition of polymyxin B to the LCM treatment did not rescue ($P \leq 0.05$) the number of CDX2-negative blastomeres (28.4 ± 5.0).

In the medium alone group, the number of CDX2-positive blastomeres (Figure 4C), which reflects trophectoderm cells, was 141.0 ± 15.7. Treatment with LPS reduced ($P \leq 0.05$) the number of CDX2-positive blastomeres to 65.2 ± 10.8, which was rescued by polymyxin B (122.6 ± 14.1). Treatment with

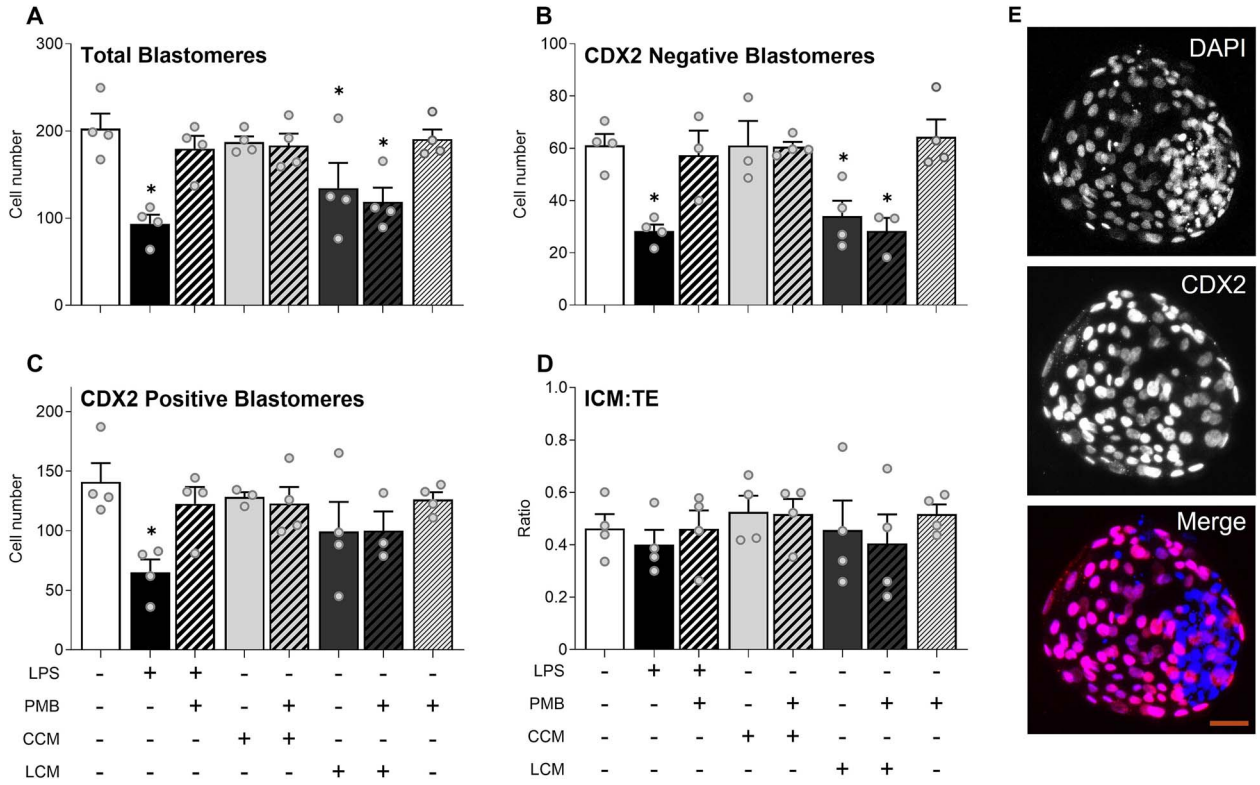

Figure 4. Cellular allocation of blastocysts is altered after oocyte maturation in an inflammatory conditioned medium. Cumulus–oocyte complexes were matured with LPS, CCM, LCM, or medium alone. Additionally, each treatment group was supplemented with PMB to inhibit LPS signaling. Oocytes were subjected to in vitro fertilization and embryo culture in a medium without any further treatment. A total of 106 blastocysts at d 7.5 from four biological replicates were stained to determine the number of total blastomeres (A), and a total of 92 blastocysts at d 7.5 in three biological replicates were labeled with the trophectoderm marker CDX2 to determine the number of CDX2-negative blastomeres (B), CDX2-positive blastomeres (C), and the ratio of CDX2-positive to CDX2-negative blastomeres (D). Each dot represents an individual replicate, and the bar represents the mean ± SEM. * indicates statistical significance ($P \leq 0.05$) compared to the medium alone group. Representative photomicrographs (E) of Hoechst 33342 (blue) and CDX2 (red) staining are shown highlighting the inner cell mass (CDX2-negative) and trophectoderm (CDX2-positive).

CCM or LCM had no effect on the number of CDX2-positive blastomeres compared to the medium alone group (128.3 ± 4.1 and 99.4 ± 24.9, respectively). The ratio of inner cell mass to trophectoderm was calculated for each embryo and was not affected in any treatment group (Figure 4D).

In the medium alone group, the proportion of blastomeres that were TUNEL positive (Figure 5A) was 4.4 ± 0.7%. Treatment with LPS increased ($P \leq 0.05$) the proportion of blastomeres that were TUNEL positive to 23.0 ± 4.9%, which was rescued by the addition of polymyxin B (6.8 ± 1.0%). The addition of CCM had no effect the proportion of blastomeres that were TUNEL positive (5.6 ± 1.2%) compared to the medium alone group, while the addition of LCM increased ($P \leq 0.05$) the proportion of blastomeres that were TUNEL positive (17.8 ± 2.6%) compared to the medium alone group. Again, the addition of polymyxin B to the LCM treatment did not rescue ($P \leq 0.05$) the proportion of blastomeres that were TUNEL positive (19.9 ± 1.8%). In parallel, the total number of TUNEL-positive blastomeres (Figure 5B) was 9.5 ± 2.9 in the medium alone group, which increased ($P \leq 0.05$) to 23.3 ± 4.7 in the LPS group. The addition of polymyxin B to the LPS treatment rescued the total number of TUNEL-positive blastomeres (7.9 ± 1.5). The addition of CCM did not affect the total number of TUNEL-positive blastomeres (10.6 ± 2.9) as compared to the medium alone group; however, the total number of TUNEL-positive blastomeres was increased ($P \leq 0.05$) in the LCM group (25.8 ± 2.4) but was not rescued by the addition of polymyxin B (24.4 ± 2.7).

Inclusion of inflammatory conditioned medium during in vitro maturation did not affect gene expression of blastocyst-stage embryos

Expression of transcripts associated with blastocyst quality was evaluated in pools of d 7.5 blastocyst-stage embryos (Figure 6). The expressions of DNA methyltransferase 3 alpha (*DNMT3A*), insulin-like growth factor 2 receptor (*IGF2R*), solute carrier family 2-member 1 (*SLC2A1*), interferon tau (*IFNT2*), BCL2-associated X, apoptosis regulator (*BAX*), BCL2 apoptosis regulator (*BCL2*), heat shock protein family A (Hsp70) member 1A (*HSPA1A*), and prostaglandin-endoperoxide synthase 2 (*PTGS2*) were detected in all pools of blastocysts. There was no effect ($P \geq 0.05$) of any treatment on the expression of any target transcripts compared to blastocysts in the medium alone group. The transcripts for *IL6*, *TNF*, and *TLR4* were also detected in the blastocysts with no effect of any treatment on the expression compared to the medium alone group. The mean ± SEM for *IL6*, *TNF*, and *TLR4* in the medium alone group were 0.02 ± 0.002, 0.0007 ± 0.0001, and 0.00018 ± 0.0001, respectively. The expression of all reference transcripts was stable across all experimental treatments ($P > 0.05$).

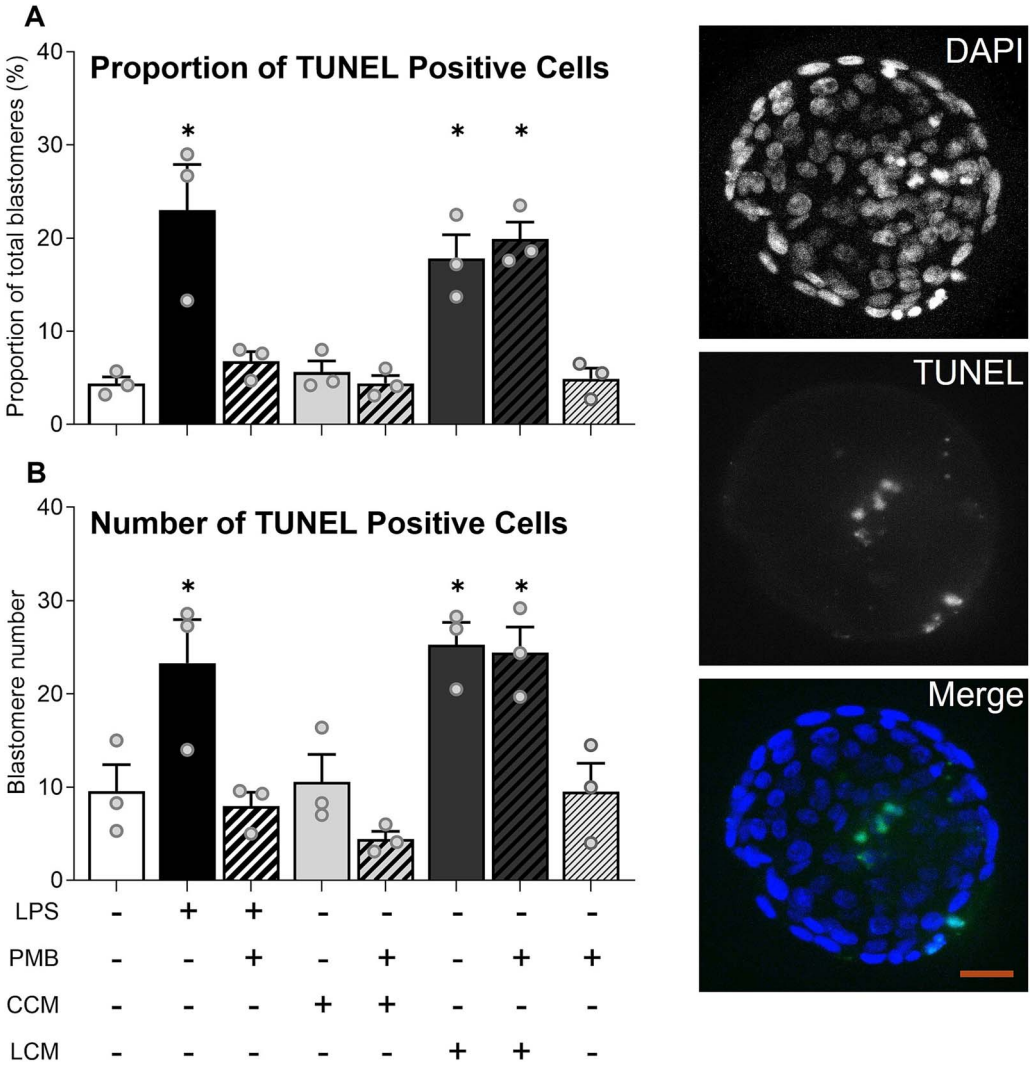

Figure 5. Apoptosis of blastocysts is increased after oocyte maturation in an inflammatory conditioned medium. Cumulus–oocyte complexes were matured with LPS, CCM, LCM, or medium alone. Additionally, each treatment group was supplemented with PMB to inhibit LPS signaling. Oocytes were subjected to in vitro fertilization and embryo culture in a medium without any further treatment. A total of 98 blastocysts at d 7.5 in three biological replicates were stained with Hoechst 33342 and labeled using the TUNEL assay. Data represent the proportion of total blastomeres that were TUNEL positive (A) and the absolute number of blastomeres that were TUNEL positive (B). Each dot represents an individual replicate, and the bar represents the mean ± SEM. * indicates statistical significance ($P \leq 0.05$) compared to the medium alone group. Representative photomicrographs of Hoechst 33342 (blue) and TUNEL (green) staining are shown.

Discussion

Uterine infections and subsequent inflammatory diseases lead to ovarian dysfunction and reduced pregnancy rates in postpartum dairy cows, with the decline in fertility persisting after disease resolution [1, 29]. Uterine infections disrupt ovarian physiology including follicular growth, hormone production, and oocyte quality [4, 12, 18, 19, 24, 30]. However, the exact mechanism by which these diseases disrupt oocyte competence is not clear. This study utilized an in vitro embryo production model to investigate the effects of an inflammatory environment during in vitro maturation on oocyte developmental competence and subsequent embryo quality. Here, in vitro maturation of bovine oocytes in the presence of LPS or conditioned medium obtained from mural granulosa cells challenged with LPS significantly impaired the developmental potential of oocytes and subsequent blastocyst quality. Interestingly, sequestering LPS in the inflammatory medium could not rescue the developmental competence of oocytes to blastocyst-stage embryos and resulted in lower-quality embryos characterized by fewer total and inner cell mass blastomere numbers and increased apoptosis. These data indicate that maturation of oocytes in the presence of LPS, or also inflammatory products of granulosa cells, significantly compromises oocyte competence and embryo quality.

A healthy follicular environment is essential for oocyte growth and maturation, ultimately resulting in the development of a competent metaphase II (M-II)–stage oocyte [21]. During follicle growth, granulosa cells contribute to the follicular microenvironment that is in close contact with the developing COC [31]. These granulosa cells provide metabolites, nutrients, and ATP to the oocyte while also regulating oocyte and follicle development by the release of cytokines and hormones [32–35]. The processes of follicle growth, oocyte maturation, and ovulation all depend on signaling pathways that involve cytokines and chemokines,

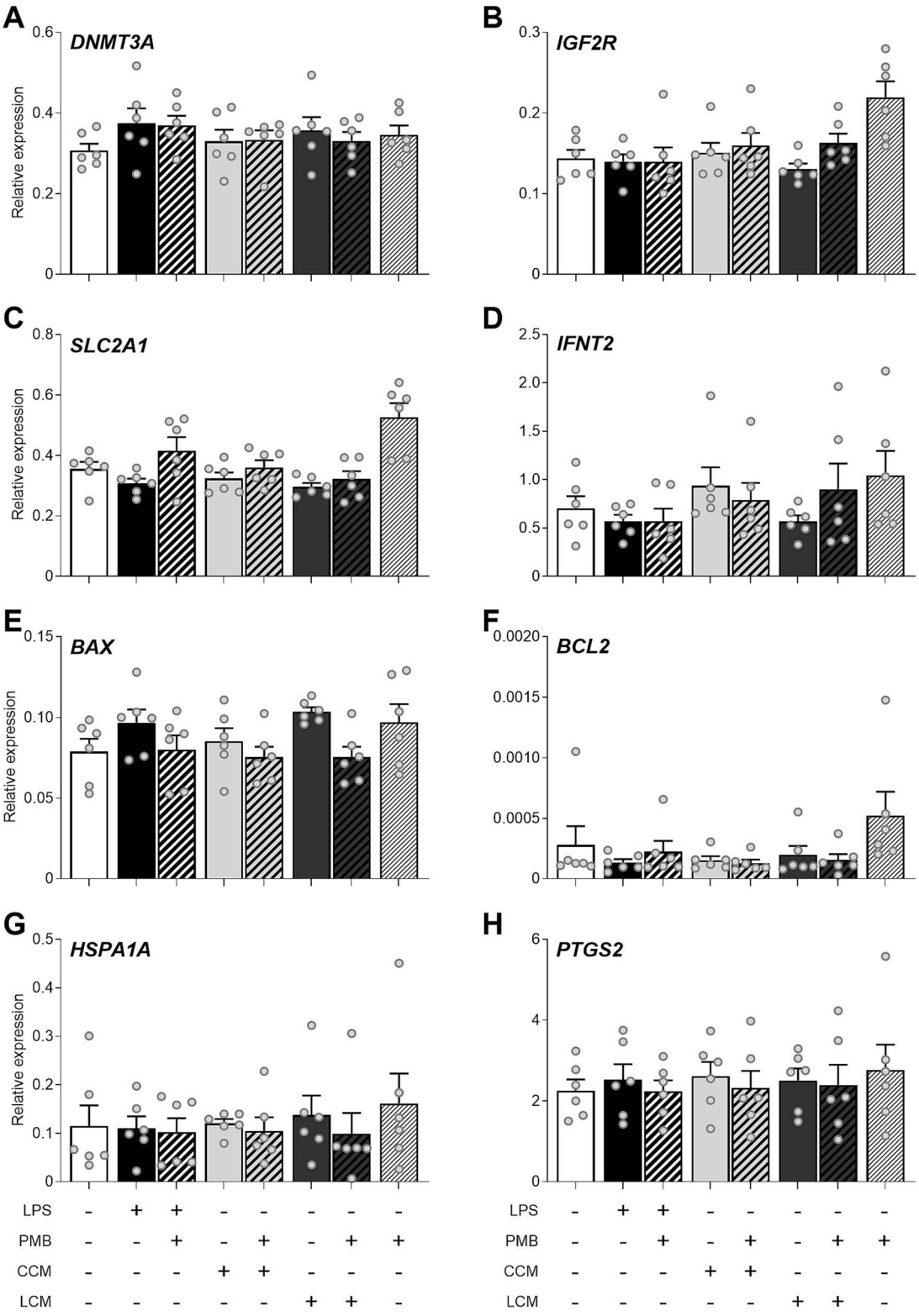

Figure 6. Transcript expression of blastocysts after oocyte maturation in inflammatory conditioned medium. Cumulus–oocyte complexes were matured with LPS, CCM, LCM, or medium alone. Additionally, each treatment group was supplemented with PMB to inhibit LPS signaling. Oocytes were subjected to in vitro fertilization and embryo culture in a medium without any further treatment in six biological replicates. Relative transcript expression of *DNMT3A* (A), *IGF2R* (B), *SLC2A1* (C), *IFNT2* (D), *BAX* (E), *BCL2* (F), *HSPA1A* (G), and *PTGS2* (H) was evaluated in pools of d 7.5 blastocyst-stage embryos. Each dot represents an individual replicate, and the bar represents the mean ± SEM.

similar to those observed during inflammation [36, 37]. Interestingly, postpartum uterine infection in cows results in the accumulation of LPS in the follicular fluid of antral follicles which results in an increased production of inflammatory mediators including cytokines and chemokines from granulosa cells [12, 38, 39]. While endometrial inflammation is positively correlated to follicular fluid LPS concentration, the follicular fluid concentration of LPS is also positively correlated to follicular fluid IL-8 at day 63 postpartum [12, 16], suggesting that uterine inflammation can alter the follicular microenvironment after the resolution of the disease. The concentration of LPS in follicular fluid of cows with postpartum uterine infections resulting in metritis or endometritis has been reported to be as high as 0.8–1 μg/mL, which corresponds to concentrations applied to oocytes or granulosa cells here [12, 16]. In parallel, intrafollicular IL-1β, IL-6, IL-8, and TNFα of dominant follicles are increased in buffalo with uterine infection [40]. These observations of an altered follicular microenvironment are likely the result of granulosa cells responding to intrafollicular LPS via Toll-like receptor 4 and associated coreceptors to increase the synthesis and secretion of inflammatory mediators into the follicular fluid [19]. Increased concentrations of inflammatory mediators in follicular fluid have been linked to compromised oocyte quality [41, 42]. Specifically, follicular fluid TNFα is positively associated with poor-quality oocytes in humans [43] and fewer fertilized oocytes in bovine [44]. Similarly, in vitro maturation of bovine oocytes in the presence of TNFα reduces cumulus expansion [45], blastocyst development [46], and increases blastomere apoptosis [46, 47]. Additional non-cytokine products of LPS-mediated inflammation including nitric oxide and prostaglandins have also been shown to reduce oocyte competence [48–51]. It is important to consider that clinical uterine diseases exist on a spectrum with some cases of disease being worse than others, while infections at sites distant from the reproductive tract may not develop the same ovarian phenotype. The culture systems employed here merely lend themselves to help explain a potential component of disease-associated infertility and may not be the causative mechanism in all cows with all diseases. In vitro culture of granulosa cells is also known to result in spontaneous luteinization within 48 h [52]; here, we restricted our entire culture period to approximately 36 h in an attempt to avoid to potential confounding effects of luteinizing; however, future experiments should consider this phenomenon and evaluate luteinization as part of the experimental system. As in previous studies, here, mural granulosa cells mounted an inflammatory response to LPS by increasing the expression of *CXCL8*, *IL1B*, *IL-6*, and *TNF* while increasing the accumulation of the inflammatory mediators IL-36RA, MIP-1α, and MIP-1β [4, 44]. Although we did not observe a significant increase in the protein accumulation of IL-8, IL-1β, IL-6, or TNFα, such discrepancies are likely due to post-transcriptional or post-translational regulatory mechanisms, including variation in mRNA stability, translation efficiency, or protein degradation. We suggest that these inflammatory mediators and other products of granulosa cells produced in response to LPS may contribute to the negative effects on oocyte competence observed here. Alternatively, the altered expression profile of expression for inflammatory mediators may be perturbing the normal expression of these factors that play a physiological role in the processes of follicle growth, oocyte development, and ovulation [36, 37].

We demonstrate that oocyte maturation supplemented with LPS alone or inflammatory conditioned medium in the absence of LPS (after polymyxin B treatment) reduced the proportion of oocytes to develop blastocyst-stage embryos to a similar extent in both groups. This observation agrees with previous work that describes the direct effect of oocyte exposure to LPS on developmental competence, increased meiotic failure, delayed cell cycle progression, and perturbed cytoplasmic maturation [19, 53]. However, the specific nature of the inflammatory components responsible for reduced oocyte competence observed here is unknown and could include not only gene products like cytokines but also cellular metabolites, reactive oxygen, extracellular vesicles, or other undetermined moieties. Additional mechanisms that cannot be studied using in vitro cultures system also warrant further investigation, as it is known that LPS can alter hypothalamic–pituitary signaling that impacts GnRH and LH pulsatility in ruminants that could compromise ovarian function and subsequent fertility of animals after infection [54, 55].

In addition to the negative effects of LPS or inflammatory conditioned medium on embryo development, we also observed a reduction in the number of total or inner cell mass blastomeres in embryos. Previously, Hill and Gilbert reported a reduction in the number of total blastomeres when bovine embryos were cultured in a conditioned medium prepared by culturing an endometrium in inflamed conditions [56], suggesting that uterine inflammation may also exert a negative impact on embryo development after fertilization. Furthermore, we observed a reduction in the number of trophoblast blastomeres only in the LPS group, which agrees with previous reports where exposure of oocyte to LPS reduced the number of trophectoderm blastomeres in d 8 blastocysts [57]. Interestingly, the ratio of inner cell mass to trophoblast blastomeres was consistent among all treatment groups here, suggesting that the distribution of cell lineages was not disrupted by the LPS or inflammatory conditioned medium and simply resulted in smaller blastocysts. In addition, we evaluated a panel of previously reported genes that are associated with embryo quality; however, the expression of these transcripts was not affected by any of our treatments [58–60]. Future analysis should include a new panel of transcripts that have been validated by machine learning to predict pregnancy success in cattle, known as the embryo competence index [61].

In the current study, maturation of oocytes in the presence of LPS or inflammatory conditioned medium in the absence of LPS (after polymyxin B treatment) increased apoptosis in blastocyst-stage embryos. Previous reports indicate that exposure of oocytes to the proinflammatory cytokine TNFα increases blastomere apoptosis in bovine embryos [46]. Inflammation is a major driver of genomic instability associated with cellular apoptosis [62]; however, LPS can also mediate cellular production of superoxide radicals and other reactive oxygen species that result in oxidative stress that causes DNA damage, DNA strand breaks, and chromosome alignment errors associated with apoptosis [63–65]. As suggested by Hardy [66], apoptosis observed in d 7 embryos may be related to the removal of defective blastomeres from the embryo and may be related to the decreased total number of blastomeres observed here when oocytes are exposed to LPS or inflammatory conditioned medium. Increased embryo apoptosis has been associated with disrupted developmental competence after fertilization [67], which may help to explain the reduced development of embryos observed here when

oocytes were exposed to LPS or inflammatory conditioned medium resulting in embryos with increased apoptosis. While here LPS or inflammatory conditioned medium increased blastomere apoptosis, there was no effect of treatment on the blastocyst expression of *BAX* or *BCL2*, key regulators of apoptosis. It is possible that the apoptosis observed here may have originated earlier in development, so that gene expression was no longer altered at d 7.5, or more likely that the translation and post-translational regulation of BAX and BCL2 is more important to functional apoptosis than their transcriptional regulation [68].

Cytokines play a crucial role in regulating ovarian physiology, including follicle growth and oocyte development [69]. It could be speculated that an alteration in the synthesis or abundance of key cytokines could significantly impact follicle or oocyte development. For instance, the cytokine IL-6 is primarily known for its role in triggering the acute-phase response in the innate immune system [70]. However, IL-6 also has a role in oocyte development and is produced by granulosa cells in response to Toll-like receptor 4 activation by the exogenous ligand hyaluronan, which is a major matrix component of the expanding COC [71]. Conversely, the presence of LPS further increases IL-6 synthesis by the granulosa cells [19], potentially altering the intrafollicular environment and altering signaling required for oocyte development. While our model of conditioned medium utilizes the entire inflammatory milieu produced by granulosa cells, it is imperative to explore which key inflammatory mediators are responsible for the observed negative impacts on oocyte competence and embryo quality. Future studies could be designed to investigate the effects of potent inflammatory molecules by supplementing them individually or in combinations during in vitro maturation or blocking target molecules in a conditioned medium using neutralizing antibodies. Moreover, embryos produced using this approach could be transferred to produce calves for the evaluation of their growth and performance, mimicking animals born from dams with uterine disease.

In conclusion, this study demonstrated that oocyte maturation in the presence of LPS or, more importantly, under inflammatory conditions in the absence of LPS compromises developmental competence and subsequent embryo quality. The inhibition of LPS in an inflammatory conditioned medium suggests that granulosa cell products of unknown identity can also reduce the developmental competence of oocytes and subsequent embryo quality. Understanding the precise mechanisms by which follicle inflammation reduces oocyte quality will allow for the development of tools to minimize or mitigate these effects and help preserve fertility in animals who encounter uterine infections.

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Conflict of interest: The authors have declared that no conflict of interest exists.

Author contributions

AT performed the experimentation, data analysis, and writing. ZKS assisted in experimentation and proofreading. JJB acquired funding and performed data analysis, data interpretation, and writing.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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