Lipopolysaccharide and tumor necrosis factor-alpha alter gene expression of oocytes and cumulus cells during bovine in vitro maturation

Rachel L. Piersanti1, José E.P. Santos1, I. Martin Sheldon2, John J. Bromfield1*

1Department of Animal Sciences, University of Florida, Gainesville 32611.
2Institute of Life Science, Swansea University Medical School, Swansea, SA2 8PP, UK.

*Corresponding author: John J. Bromfield, PhD
Department of Animal Sciences, University of Florida
Gainesville, Florida, 32611, USA

E-mail: jbromfield@ufl.edu

Funding: Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number R01HD084316. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

ABSTRACT

Communication between the oocyte and cumulus facilitates oocyte growth, cell cycle regulation and metabolism. This communication is mediated by direct contact between oocytes and cumulus cells, and soluble secreted molecules. Secreted molecules involved in this process are known inflammatory mediators. Accumulation of inflammatory mediators in follicular fluid, including tumor necrosis factor-alpha (TNFa), is associated with female infertility, whereas lipopolysaccharide is detected in follicular fluid and is associated with reduced fertility. Maturation of oocytes in the presence of lipopolysaccharide or TNFa reduces meiotic maturation and the capacity to develop to the blastocyst. Here we evaluated the abundance of 92 candidate genes involved immune function, epigenetic modifications, embryo development, oocyte secreted factors, apoptosis, cell cycle and cell signaling in bovine cumulus cells or zona-free oocytes after exposure to lipopolysaccharide or TNFa during in vitro maturation. We hypothesize that lipopolysaccharide or TNFa will alter the abundance of transcripts in oocytes and cumulus cell in a cell type dependent manner. Exposure to lipopolysaccharide altered abundance of 31 transcripts in oocytes (including ACVR1V, BMP15, DNMT3A) and 12 transcripts in cumulus cells (including AREG, FGF4, PIK3IP1). Exposure to TNFa altered 1 transcript in oocytes (IGF2) and 4 transcripts in cumulus cells (GJA1, PLD2, PTGER4, STAT1). Cumulus expansion was reduced after exposure to lipopolysaccharide or TNFa. Exposing COCs to lipopolysaccharide had a marked effect on expression of targeted transcripts in oocytes. We propose that altered oocyte transcript abundance is associated with reduced meiotic maturation and embryo development observed in oocytes cultured in lipopolysaccharide or TNFa.

Keywords: gene expression, inflammation, oocyte competence.

INTRODUCTION

Although the sperm cell and the oocyte contribute equally to the genetic material of the newly formed embryo, the necessary molecular and organelle machinery for growth and regulation of early embryonic development are established during oocyte maturation (Coticchio et al., 2015). Oocyte growth involves the increase of cell mass, proliferation of organelles, accumulation of maternal transcripts and translational products that are required after fertilization, prior to activation of the embryonic genome (Li, Zheng, & Dean, 2010; Moussa, Shu, Zhang, & Zeng, 2015; Sirard et al., 1989; Sirard, Richard, Blondin, & Robert, 2006). Additionally, oocyte maturation requires changes to oocyte chromatin, repositioning of organelles, and facilitating meiotic resumption in response to the LH surge (Coticchio et al., 2015). Although infections cause infertility, little is known about how exposure of oocytes and cumulus cells to pathogen molecules alters their molecular signature, including exposure to lipopolysaccharide (LPS) or pro-inflammatory mediators such as tumor necrosis factor alpha (TNFa).

The follicular environment is critical to the developmental competence of the growing oocyte. Oocyte growth and maturation depends on close communication between the oocyte and the surrounding cumulus granulosa cells. Transzonal projections (TZPs) play an important role in facilitating oocyte and cumulus cell communication by direct cell to cell contact prior to retraction at the time of the LH surge (Albertini, Combelles, Benecchi, & Carabatsos, 2001), whereas oocyte secreted factors act to modify cumulus cell function (Albertini et al., 2001; Matzuk, Burns, Viveiros, & Eppig, 2002; McGinnis, Limback, & Albertini, 2013). The interaction between the oocyte and surrounding cumulus cells is bidirectional; the cumulus cells respond to oocyte signals and the follicular environment to supply the oocyte with essential

molecules for proper metabolism and regulation of meiotic maturation (Eppig, Freter, Ward63 Bailey, & Schultz, 1983; Gilchrist, Ritter, & Armstrong, 2004; Su et al., 2008).

A large part of the communication between oocytes and cumulus cells is conveyed by molecules that are also known for their role as immune mediators. Oocyte secreted factors act on the granulosa cells to control proliferation, development and expansion (Matzuk et al., 2002). Subsequently, cumulus cells mediate oocyte development by regulating availability of metabolites and signaling factors (Eppig, 1991; Sugiura & Eppig, 2005). In parallel, Toll-like receptors (TLRs) are involved in the immune response to pathogens, and when activated by bacterial LPS increase expression of cytokines such as interleukin (IL)-1ß, IL-6, IL-8 and TNFa. Granulosa cells of hens, pigs, cows, mice and humans express TLRs and respond to bacterial LPS to increase expression of pro-inflammatory cytokines (Alvarez et al., 2006; Bromfield & Sheldon, 2011; Ibrahim, Kramer, Williams, & Bromfield, 2016; Price, Bromfield, & Sheldon, 2013). Uterine infections are common in cows, and subsequently LPS accumulates in follicular fluid, altering the microenvironment of oocyte development (Herath et al., 2007; Piersanti et al., 2019). In vitro maturation (IVM) of bovine cumulus-oocyte complexes (COCs) in the presence of LPS increases failure of the meiotic cell cycle (Bromfield & Sheldon, 2011; Zhao et al., 2017), and subsequently decreases blastocyst development (Soto, Natzke, & Hansen, 2003b). Similarly in human in vitro production (IVP) of embryos, the presence of LPS culture medium reduces clinical pregnancy (Fishel, Jackson, Webster, & Faratian, 1988; Nagata & Shirakawa, 1996; Snyman & Van der Merwe, 1986). Interestingly, cumulus cells utilize endogenous ligands to TLR-4 to aid in final maturation of the oocyte and cumulus expansion (Shimada et al., 2008). Hyaluronic acid is a major component of the expanded COC that activates TLR-4 signaling to induce expression of IL-6 which acts as an autocrine regulator of cumulus cell function (Liu et

al., 2009; Liu, Shimada, & Richards, 2008). The proinflammatory mediator, TNFa, is upregulated in response to pathogenic components via TLR-4 signaling (Fock, Vinolo, de Moura Sa Rocha, de Sa Rocha, & Borelli, 2007). Infusion of TNFa into the ovarian bursa in rats inhibits ovulation (Yamamoto et al., 2015), and presence of TNFa during IVM increases meiotic failure in swine (Ma et al., 2010) and decreases blastocyst development in the cow (Soto, Natzke, & Hansen, 2003a). Elevated follicular fluid TNFa is associated with decreased oocyte quality in women undergoing IVP (Lee et al., 2000), and patients with polycystic ovarian syndrome (PCOS) have elevated follicular fluid TNFa concentrations compared with women not affected by PCOS (Amato et al., 2003).

Here we asked how LPS or TNFa exposure during IVM of bovine COCs affected specific mRNA abundance in oocytes and cumulus cells. We targeted the expression of genes involved in immune function, epigenetic modifications, embryo development, oocyte secreted factors, apoptosis, cell cycle and cell signaling. We hypothesized that exposure of COCs to lipopolysaccharide or TNFa during IVM alters the abundance of transcripts in oocytes and cumulus cell in a cell type dependent manner. These findings may aid in our understanding of how LPS or TNFa reduces the competence of oocytes to develop to the blastocyst.

MATERIALS AND METHODS

Cumulus Oocyte Complex Isolation and In Vitro Maturation

Ovaries were collected at a local abattoir and transported to the laboratory at 23°C in 0.9% saline solution containing 100 IU/mL penicillin and 100 µg/mL streptomycin (Caisson Labs, Smithfield, UT). Ovaries were processed within 4 h of collection, and COCs were collected by bisecting 3 to 8 mm follicles using a sterile scalpel blade and vigorously washing the ovary in oocyte collection medium (BoviPRO, MOFA Global, Verona, WI). The medium containing COCs and cells was then passed through a 100 µm filter (Corning Falcon, Tewksbury, MA) to collect the COCs, which were retrieved using a wiretrol pipette (Drummond Scientific Company, Broomall, PA) under a dissecting stereo microscope. An average of 10 to 15 COCs, having at least three layers of cumulus cells and an oocyte containing a homogeneous cytoplasm, were collected from each ovary and COCs from all ovaries were pooled as a single replicate. The COCs were washed three times in oocyte collection medium and groups of 10 to 15 COCs were matured in organ culture dishes (Corning Falcon) for 22 to 24 h in 1 mL of oocyte maturation medium (Medium 199, 0.25 mM pyruvate, 10% fetal calf serum, 2 µg/mL estradiol, 1% insulin/transferrin/sodium selenite solution, 50 IU/mL penicillin, 50 µg/mL streptomycin, and 0.4 mM L-glutamine [all Fisher Scientific, Hampton NH] and 20 µg/mL FSH [as Folltropin-V, Reproduction Resources, Walworth WI]) as previously described (Bromfield & Sheldon, 2011). Oocyte maturation was performed in humidified air containing 5% CO2 at 38.5°C, in control oocyte maturation medium, or medium containing 1, 10, 100, 1000 or 10,000 ng/mL ultrapure LPS from E. coli 0111:B4 (InvivoGen, San Diego, CA), or 1, 10 or 100 ng/mL recombinant bovine TNF. (R&D Systems, Minneapolis, MN). Every treatment replicate of COCs with either LPS or TNF. was always carried out with a corresponding vehicle control treatment. Following maturation, the COCs were washed three times in fresh Dulbecco's phosphate buffered saline (DPBS, Fisher Scientific) containing 0.1% polyvinylpyrrolidone (PVP; Kodak, Rochester, NY). The COCs were examined and considered fully expanded when all cumulus layers had progressed away from the oocyte. Cumulus cells were removed from oocytes using 1000 U/mL of hyaluronidase in HEPES-TALP. The zona pellucida of denuded oocytes were

removed with 0.1% protease from Streptococcus griseus (Sigma-Aldrich, St. Louis, MO) in DPBS, and zona-free oocytes were washed three times in fresh DPBS-PVP. Cumulus cells were obtained by centrifugation of the hyaluronidase medium following removal of denuded oocytes. Zona-free oocytes from a single replicate were pooled together for analysis, as were all cumulus cells from a single replicate. Pooled zona-free oocytes and pooled cumulus cell were separately suspended in 350 uL RLT buffer and stored at -80.C.

Each treatment was performed in 4 to 6 replicates per treatment. A replicate was defined as a single IVM procedure containing one dish per treatment. A total of 542 COCs were subjected to IVM in the presence of LPS, 378 in the presence of TNF. and 194 in control medium.

Multiplex Fluidigm Analysis of Oocyte and Cumulus Cell Gene Expression

Zona-free oocyte and cumulus cell RNA extraction was performed independently using the RNeasy Micro kit with DNase treatment (Qiagen) according to the manufacturer's instructions.

The Fluidigm qPCR microfluidic device Biomark HD system (Fluidigm Co., San Francisco, CA) was used for gene expression assays at the University of Miami Miller School Of Medicine, Center for AIDS Research (CFAR). Primers were designed by Fluidigm Delta Gene assays (Fluidigm). Primer validation was performed using cDNA obtained from bovine oocytes, endometrium, peripheral white blood cells, granulosa cells and ovarian cortex. All primers (Supplemental Table 1) were validated using the Fluidigm primer quality control criteria applied to serially diluted test cDNA: r2 = 0.97, efficiency of 80% to 130%.

A total of 96 primers for Fluidigm analysis (Supplemental Table 1 and 2) were validated for 4 housekeeping genes, 6 oocyte specific genes, 29 genes involved in cell growth and proliferation, 10 genes involved in the regulation of cell cycle, 19 genes related to immune response, 9 genes involved in control of gene expression and DNA modifications, 7 genes associated to apoptosis and cell death, and 12 other genes of interest.

Target specific pre-amplification after reverse transcription (RT-STA) was performed on all samples using the Preamp and Reverse Transcription Master Mix (Fluidigm) for 20 cycles. The procedure for real-time RT-PCR using the BioMark HD system (Fluidigm) (Dominguez et al., 2013) was as follows; primer sets and samples were loaded on an integrated fluidic circuit (IFC) plate and placed into a controller that prepares the nano-volume reactions. Real-time RT-PCR was carried out on the BioMark HD system. A total of 40 PCR cycles were performed using EvaGreen (Bio-Rad, Hercules CA) chemistry on the 96.96 dynamic array IFC developed by the manufacturer. Cycle threshold (Ct) values were calculated by the Fluidigm real-time PCR analysis software. The cutoff for undetectable genes was set at Ct > 29. The geometric mean of the four housekeeping genes was calculated and fold change relative to the geometric mean of the housekeepers was calculated for the 92 genes of interest using the 2-.Ct method.

Statistical Analysis

SPSS ver. 20.0 (IBM, New York NY) and SAS ver. 9.4 software package (SAS Institute Inc., Cary, NC) were used for statistical analyses. Data obtained from PCR were analyzed using the generalized linear model of SPSS using treatment or concentration as the fixed effect. When multiple concentrations of a single treatment were used, analysis was followed by a Dunnett's posthoc test comparing individual treatments to the control. Contrasts were performed grouping multiple concentrations of TNFa or LPS and compared to the medium alone control, specifically

increasing concentrations were combined and then compared to the control. Heatmaps and hierarchical clustering were generated using online ClustVis tools (Metsalu & Vilo, 2015). The generalized linear mixed models procedure of SAS (GLIMMIX) was used to evaluate the effects of treatment on the percent of COCs to expand. Each COC was considered as an individual observation and expansion was considered as a binary variable (0 = did not expand; 1 = expanded). Treatment was considered as a fixed effect and replicate was used as a random effect. A P-value = 0.05 was considered statistically significant.

RESULTS

The abundance of 92 cellular transcripts was independently evaluated in zona-free oocytes and cumulus cells following IVM for 24 h in the presence of LPS or TNFa. Heatmaps for the changes in gene expression compared with control medium are presented for oocytes (Fig. 1A-B) and cumulus cells (Fig. 1C-D) treated with LPS (Fig. 1A & C) or TNFa (Fig. 1B & D). The heatmaps show that LPS had a greater effect on transcript expression than TNFa, and that the effect of LPS and TNFa was greater for the oocytes than the cumulus cells.

Effect of LPS Exposure during IVM on Oocyte and Cumulus Cell Gene Expression

Collectively, a total of 31 genes in oocytes (Fig. 2 and 3), and 12 genes in cumulus cells (Fig. 4) were differentially abundant (P = 0.05) at a given concentration or using contrast analysis compared with those cultured in medium alone. Of the differentially abundant transcripts in oocytes, 24 genes increased and 7 decreased following exposure to LPS. The majority of differentially expressed genes in oocytes required contrast analysis to detect significant

difference from controls, with only 14 genes significantly altered at specific LPS concentrations. Abundance of ACVR1B, ACVR2B, ADMA10, AY192564, BMP15, CDC42EP4, CDK1, CREM, DNMT1, DNMT3A, ESPRP1, H2AFX, HAS2, HDAC1, ILF3, ITPR2, MADD, NEDD4, NF2, POLR2D, PTEN, S100A1, SOX2, and XIAP increased (P = 0.05) in oocytes following LPS exposure compared with control (Fig. 2 and 3); whilst the abundance of H2AFZ, HPSE, INHBA, MAB21L2, PLD2, POU5F1, and PRKAR2B decreased (P = 0.05) in LPS compared with control. A complete list of oocyte transcript abundance can be found in Supplemental Table 3.

Of the differentially abundant transcripts in cumulus cells, the expressions of 6 genes increased and those of another 6 genes decreased following exposure to LPS (Fig. 4). Half of the differentially abundant genes in cumulus cells required contrast analysis to detect significant difference from control, with only 6 genes significantly altered at specific LPS concentrations. Abundance of ACKR4, ACVR2B, ADAM17, NLRP5, POU5F1 and PTGER4 increased (P = 0.05) in cumulus cells following LPS exposure compared with control (Fig. 4); whilst the abundance of ACTA2, AREG, FGF4, HDAC8, IL6 and PIK3IP1 decreased (P = 0.05) following LPS exposure compared with control. A complete list of cumulus cell transcript abundance can be found in Supplemental Table 4.

Effect of TNFa Exposure during IVM on Oocyte and Cumulus Cell Gene Expression

Abundance of 92 cellular transcripts were independently evaluated in zona-free oocytes and cumulus cells following IVM for 24 h in the presence of 1, 10, or 100 ng/mL TNFa and compared with control. A total of only 4 genes in cumulus cells (Fig. 5A-D), and 1 gene in oocytes (Fig. 5E-H) were differentially abundant (P = 0.05) at a given concentration or using

contrast analysis compared with control. Abundance of GJA1 and PLD2 transcripts were decreased ($P = 0.05$) in cumulus cells following TNFa exposure, whereas PTGER4 and STAT1 transcripts were increased (Fig. 5A-D). Abundance of IGF2 transcript was increased ($P = 0.05$) in oocytes following exposure to 100 ng/mL of TNFa (Fig. 5G). A complete list of oocyte and cumulus cell transcript abundance can be found in Supplemental Tables 5 and 6.

Effect of LPS or TNFa Exposure during IVM on Cumulus Expansion

Exposure of COCs to LPS during IVM had little effect on complete cumulus expansion, except for an 8.3% reduction ($P < 0.05$) compared with medium alone following exposure to 1 ng/mL LPS (Fig. 6A). Exposure of COCs to TNFa reduced ($P < 0.05$) complete expansion by 22.1%, 21.0% and 9.2% compared with medium alone after treatment with 1, 10 or 100 ng/mL, respectively (Fig. 6B).

DISCUSSION

Previous work has demonstrated that maturation of oocytes in the presence of LPS or TNFa reduces meiotic competence and development to the blastocyst (Bromfield & Sheldon, 2011; Ma et al., 2010; Soto et al., 2003a, 2003b). The present experiment evaluated mRNA expression to assess how oocyte and cumulus cell transcript abundance was affected by exposure to LPS or TNFa. Here, we hypothesized that exposure of COCs to LPS or TNFa during IVM would alter the abundance of transcripts in oocytes and cumulus cell in a cell type dependent manner which may be responsible for reduce oocyte developmental competence. We observed an effect of LPS exposure on oocyte and cumulus cell transcript abundance, with minimal effects of TNFa exposure on the abundance of targeted genes.

Effect of LPS Treatment during IVM on Oocyte Gene Expression

Oocyte transcript availability is critical to bovine embryonic development prior to activation of the embryonic genome at the 8-cell stage and subsequent transcription of new mRNA (Graf et al., 2014). Exposure of COCs to LPS altered oocyte transcript abundance, with most transcripts being increased. Although the oocyte is considered to be transcriptional inactive during maturation, displaying a steady decline in transcript abundance over time, some transcripts do indeed increase in abundance between the germinal vesicle and MII stage of development (Reyes, Chitwood, & Ross, 2015), including those involved in oocyte maturation such as CCNB1, WEE2, FBXO43, and MELK. The changes in oocyte transcript abundance observed here may be associated with either increased oocyte transcription, decreased transcript degradation based on transcript polyadenylation (Su et al., 2007), or transcript transport from cumulus cells via transzonal projections (Macaulay et al., 2016). Exposure of COCs to LPS increased abundance of oocyte genes involved in oocyte maturation and embryonic development including BMP15 and SOX2 (Gilchrist, Lane, & Thompson, 2008; Masui et al., 2007). A member of the TGFß superfamily, BMP-15 is a paracrine factor secreted by the oocyte that regulates cumulus granulosa cell function, including proliferation. Along with GDF-9 (Dong et al., 1996; Spicer, Aad, Allen, Mazerbourg, & Hsueh, 2006), another oocyte secreted factor, BMP-15 regulates oocyte maturation, cumulus cell metabolism, apoptosis, and expansion (Coticchio et al., 2015; Gilchrist et al., 2008; Matzuk et al., 2002). The function of SOX2 remains to be fully elucidated in the bovine, but it is known to be involved in pluripotency of mouse embryonic stem cells

(Masui et al., 2007) and is essential for embryo development to the blastocyst stage in the rodent (Pan & Schultz, 2011). Similarly, in cattle, zygotes injected with SOX2 siRNA have a decline in development to the blastocyst stage (Goissis & Cibelli, 2014). However, the present experiment was unable to recapitulate the work of others that have demonstrated that exposure of bovine COCs to LPS reduces the proportion of oocytes to reach the blastocyst stage of development at day 8 from approximately 28% to 12% (Soto et al., 2003b). As such, it may be that LPS268 mediated alterations in oocyte transcript abundance have more subtle effects on the embryo than developmental progression, like epigenetic programming.

The abundance of transcripts for genes involved in transcription and epigenetic regulation were affected in oocytes after exposure to LPS. Transcripts for DNMT1, DNMT3A, ILF3, H2AFX, HDAC1 and POLR2D increased in oocytes after LPS exposure, whereas H2AFZ transcript was depleted compared with control oocytes. Epigenetic reprogramming of the oocyte and subsequent embryo is essential for embryonic development and heritable changes to the epigenome (Bromfield, Messamore, & Albertini, 2008). Epigenetic programming of the newly formed embryo genome and subsequent regulation of transcription include CpG DNA methylation and posttranslational acetylation, phosphorylation and methylation of histones (Beaujean, 2014). The process of embryonic genome activation involves the erasure and settlement of epigenetic marks, as well as non-erasure and preservation of methylation in some areas within the genome (Chong & Whitelaw, 2004). This process may lead to epigenetic inherence and potentially change the fate of the embryo and subsequent phenotype of the offspring. The differential abundance of oocyte transcripts involved in epigenetic programing identified here could potentially disrupt this process and change how the epigenome is established in the developing embryo. The DNA methyltransferase DNMT1 maintains CpG

methylation, whereas DNMT3A facilitates de novo CpG methylation. The process of DNA demethylation can either occur actively by ten-eleven translocation methylcytosine dioxygenase (TET)1 and TET2 which were not evaluated here, or passively by decreasing the expression of DNMT1 and DNMT3A (Hackett et al., 2013). Interestingly, both DNMT1 and DNMT3A transcript expression increased in oocytes following LPS exposure, suggesting a possible dysregulation of CpG demethylation during oocyte maturation. The extent of either oocyte or embryo CpG methylation needs to be quantified in response to LPS exposure to determine if changes in transcript abundance have any direct effect on the epigenetic status of the embryo. Among other genes involved in transcription regulation, LPS exposure increased abundance of oocyte POLR2D and ILF3 transcript. The gene POLR2D encodes for a subunit of RNA polymerase II responsible for mRNA synthesis, suggesting LPS exposure could increase the oocytes ability for gene transcription after LPS exposure. Additionally, ILF3 is a double-stranded RNA binding protein that regulates gene expression and stabilizes mRNAs, further suggesting that LPS mediated increased oocyte transcription. The abundance of oocyte H2AFX transcript was also increased after LPS exposure, which is an H2A histone family member involved in regulation of gene expression and the cellular response to DNA damage and stress (Cloutier et al., 2015). Interestingly, abundance of oocyte H2AFZ was decreased following exposure to LPS. H2AFZ is also a member of the H2A histone family, and depletion in mouse embryos results in a failure to develop, supporting the theory that appropriate chromatin structure is crucial for embryonic development (Faast et al., 2001). In addition, we observed an LPS-mediated increase in transcript abundance of the histone deacetylase HDAC1, suggesting possible dysregulation of histone post-translational modifications. Interestingly, it has been reported that oocytes matured in the presence of LPS have reduced DNA methylation (5-mC) and histone H3 lysine 9

dimethylation (H3K9me2), whereas histone H3 lysine 4 dimethylation (H3K4me2) is increased (Zhao et al., 2017). It is interesting to postulate if the altered transcripts involved in epigenetic programing observed here may affect enduring epigenetic marks that could affect the phenotype of subsequent offspring.

Exposure to LPS increased the abundance of transcripts for genes associated with apoptosis (MADD, NEDD4 and XIAP) in oocytes. Neural precursor cell expressed developmentally down314 regulated protein 4 (NEDD4) is a ubiquitin ligase that regulates membrane channels and receptors by controlling their density and availability on the cell surface (Cao et al., 2008). NEDD4 null mice present delayed embryonic development and lethality due to the role of NEDD4 in regulating insulin and insulin growth factor 1 (IGF-1) signaling (Cao et al., 2008). MADD is involved in the propagation of TNFa pro-apoptotic signals (Schievella, Chen, Graham, & Lin, 1997) and is present in human GV stage oocytes (P. Zhang et al., 2007). While increased expression of MADD is critical to cancer cell survival, this is due to protein phosphorylation, and

it is unclear what status MADD protein exists in the embryo (Jayarama et al., 2014). X-linked inhibitor of apoptosis protein (XIAP) is a protein that inhibits apoptosis and prevents cell death, even in response to TNFa signaling (Duckett et al., 1998). Collectively, it is unclear if the variable abundance of these apoptosis related transcripts in oocytes ultimately affects cell survival in the embryo. Analysis of blastomere apoptosis in response to oocyte LPS exposure is warranted to further describe any downstream effects of altered transcript abundance.

Exposure to LPS increased oocyte transcript abundance of genes encoding the activin receptors, ACVR1B and ACVR2B. Oocyte development and follicle integrity increased in the bovine in response to activin supplementation in vitro (McLaughlin, Bromfield, Albertini, & Telfer, 2010; McLaughlin & Telfer, 2010). However, these data are in conflict with our proposed hypothesis that LPS exposure would decrease oocyte competence, if we assume increased transcript abundance of activin receptor would mediate increased activin signaling.

Effect of LPS Treatment during IVM on Cumulus Cell Gene Expression

Of the 12 cumulus cell transcripts altered by LPS exposure only half increased, in contrast to oocyte transcript abundance in which 24 genes showed increased expression with exposure to LPS. Exposure to LPS decreased the abundance of cumulus cell transcript for the actin cytoskeleton gene, ACTA2, in a similar manner in which retinoic acid decreased expression in cumulus cells of the camel while simultaneously promoting maturation of oocytes (Saadeldin et al., 2019). Fibroblast growth factor-4 is involved in cell proliferation and developmental processes, and here transcript abundance of FGF4 decreased in cumulus cells following LPS exposure. It is unclear the exact role cumulus FGF signaling has on oocyte development, but inhibition of FGF receptor signaling decreases subsequent embryo development (K. Zhang & Ealy, 2012). Cumulus cell expression of Pik3ip1, a negative regulator of PI3K, is lower in cumulus cells of small antral follicles compared to large antral follicles of the mouse (Wigglesworth, Lee, Emori, Sugiura, & Eppig, 2015). Interestingly, we observed a decreased PIK3IP1 transcript abundance in cumulus cells following exposure to LPS, suggesting a developmental perturbation in these cells. Abundance of cumulus POU5F1 transcript increased following exposure to LPS, which encodes the OCT-4 transcription factor that regulates pluripotency of the embryo (Masui et al., 2007; Niwa, Miyazaki, & Smith, 2000). The transcript for the prostaglandin E2 receptor, PTGER4, has been previously reported to be expressed in bovine cumulus cells (Nuttinck et al., 2011), and its abundance increased in response to LPS exposure. Indeed, prostaglandin E2 signaling is vital to meiotic progression, cumulus expansion and embryonic development (Nuttinck et al., 2011), and inhibition of this signaling system results in failure of these various cellular progressions. Finally, we hypothesized that the abundance of cumulus cell IL6 transcript would be increased in response to LPS; however, the data suggests that only a mild expression of IL6 transcript was present in cumulus cells and that statistically this expression was decreased in response to LPS. We view this particular result with some skepticism as the data appears skewed due to a single high abundance outlier in the control samples. We have previously demonstrated a robust IL-6 response of bovine mural granulosa cells to LPS (Bromfield & Sheldon, 2011), and others have shown increased Il6 expression in murine COC in response to LPS and hyaluronan (Shimada et al., 2008).

Effect of TNFa Treatment during IVM on Gene Expression of Oocytes and Cumulus Cells

Tumor necrosis factor-alpha is a potent cytokine with contradictory roles: it is involved in the pro-inflammatory response and can further activate inflammation, but it can also exhibit a role in controlling the extent of the immune response and inflammation (Akdis et al., 2016). Exposure of COCs to TNFa increased abundance of only one gene in oocytes, IGF2. The imprinted IGF2 gene encodes for a growth factor important during embryo development in the bovine (Gebert et al., 2006). Tribulo (Tribulo, Siqueira, Oliveira, Scheffler, & Hansen, 2018) reported that IGF2 expression in the bovine endometrium is maximal on the day of estrus, suggesting potential effects on the oocyte, sperm or fertilization. In parallel, IVM of bovine oocytes in fatty acid-free medium expressed increased IGF2 transcript abundance in both oocytes and subsequent embryos compared to oocytes cultured in fatty-acid enriched maturation medium (Warzych, Wrenzycki, Peippo, & Lechniak, 2007). Interestingly, microglia increased IGF2 expression to prevent TNFa mediated apoptosis (Nicholas, Stevens, Wing, & Compston, 2002), which has also been reported

to occur in bovine embryos (Loureiro, Brad, & Hansen, 2007). It is possible that increased expression of IGF2, which is known to enhance cell proliferation and survival by signaling through the IGF1 and IGF2 receptors, in COCs exposed to TNFa occurred as a protective mechanism to prevent cell death. Exposure to TNFa decreased cumulus cell expression of GJA1 and PLD2. Connexin 43 (GJA1) is a component of cellular gap junctions found in abundance in granulosa-granulosa complexes (Johnson, Redmer, Reynolds, & Grazul-Bilska, 1999; Nuttinck et al., 2000). These granulosa gap junction complexes are fundamental for the developing oocyte in the avascular follicular environment, and mediate signal and metabolite transmission from the follicular fluid and granulosa to the growing oocyte (Albertini et al., 2001). Conversely, oocytes exposed to TNFa increased PTGER4 expression in cumulus cells in a similar fashion to exposure to LPS, as observed in this experiment. Interestingly, STAT1 expression increased in cumulus cells after exposure to TNFa which is involved in TNFa induced apoptosis signal transduction (Jiang et al., 2017), and may be involved in mediating cumulus cell apoptosis here, but this remains to be evaluated.

Speculations for LPS or TNFa Effects on Postnatal Development

Previous data suggest that exposure of COCs to either LPS or TNFa reduces progression to the blastocyst stage of development by 16 or 8 percentage points, respectively (Soto et al., 2003a, 2003b). Our work here and by others have demonstrated alterations in the machinery and status of the epigenome of the oocyte following exposure to LPS (Zhao et al., 2017). We hope that future experimentation will be able to evaluate the epigenetics and phenotype of cattle conceived following oocyte exposures. Indeed, moderate maternal protein restriction in mice for 3.5 days prior to conception significantly altered phenotype of offspring to 1 year of age (Watkins, Lucas, Wilkins, Cagampang, & Fleming, 2011). Previous reports suggest that the presence of serum in maturation medium could reduce embryo development rates (Del Collado et al., 2015; Sagirkaya et al.), as such it will be important to consider serum alternatives in future work. Phenotypic data from offspring conceived by dams with uterine infections may aid in elucidating long-term effects of oocyte exposure to LPS or TNFa. Alternatively, negative effects of LPS or TNFa on the oocyte may be resolved during embryo growth due to the plasticity of the early developing embryo and consequently have little to no impact to the resultant offspring.

Conclusions

We examined changes in transcript abundance in oocytes and cumulus cells when COCs were matured in the presence of LPS or TNFa. The results provide evidence that LPS had a greater effect on transcript abundance than TNFa, and the largest effect was that of LPS on oocyte transcripts. We assume that alterations to the abundance of maternal transcripts present in the oocyte will have some impact on pregnancy success or subsequent offspring phenotype. Further experimentation is required to define alterations to embryonic epigenetic programing, fetal development and offspring phenotype. These data highlight a need to understand the impact of bacterial components and inflammatory mediators on reproductive success in species where infection could have negative implications on reproductive performance.

ACKNOWLEDGEMENTS

Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number R01HD084316. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The authors would like to thank the laboratory of Dr Peter Hansen at the University of Florida, Department of Animal Sciences for access to slaughterhouse material. In addition, we would like to thank the staff of the University of Miami Miller School of Medicine, Center for AIDS

Research for assistance with execution of the Fluidigm platform.

REFERENCES

Akdis, M., Aab, A., Altunbulakli, C., Azkur, K., Costa, R. A., Crameri, R., . . . Akdis, C. A. (2016). Interleukins (from IL-1 to IL-38), interferons, transforming growth factor beta, and TNF-alpha: Receptors, functions, and roles in diseases. Journal of Allergy and Clinical Immunology, 138(4), 984-1010. doi:10.1016/j.jaci.2016.06.033

Albertini, D. F., Combelles, C. M., Benecchi, E., & Carabatsos, M. J. (2001). Cellular basis for paracrine regulation of ovarian follicle development. Reproduction, 121(5), 647-653. doi:10.1530/rep.0.1210647

Alvarez, B., Revilla, C., Chamorro, S., Lopez-Fraga, M., Alonso, F., Dominguez, J., & Ezquerra, A. (2006). Molecular cloning, characterization and tissue expression of porcine Toll-like receptor 4. Developmental & Comparative Immunology, 30(4), 345-355. doi:10.1016/j.dci.2005.06.020

Amato, G., Conte, M., Mazziotti, G., Lalli, E., Vitolo, G., Tucker, A. T., . . . Izzo, A. (2003). Serum and follicular fluid cytokines in polycystic ovary syndrome during stimulated cycles. Obstet Gynecol, 101(6), 1177-1182.

Beaujean, N. (2014). Epigenetics, embryo quality and developmental potential. Reproduction, Fertility and Development, 27(1), 53-62. doi:10.1071/RD14309

Bromfield, J., Messamore, W., & Albertini, D. F. (2008). Epigenetic regulation during mammalian oogenesis. Reproduction, Fertility and Development, 20(1), 74-80. doi:RD07181 [pii]

Bromfield, J. J., & Sheldon, I. M. (2011). Lipopolysaccharide initiates inflammation in bovine granulosa cells via the TLR4 pathway and perturbs oocyte meiotic progression in vitro. Endocrinology, 152(12), 5029-5040. doi:10.1210/en.2011-1124

Cao, X. R., Lill, N. L., Boase, N., Shi, P. P., Croucher, D. R., Shan, H., . . . Yang, B. (2008). Nedd4 controls animal growth by regulating IGF-1 signaling. Sci Signal, 1(38), ra5. doi:10.1126/scisignal.1160940

Chong, S., & Whitelaw, E. (2004). Epigenetic germline inheritance. Current Opinion in Genetics & Development, 14(6), 692-696. doi:10.1016/j.gde.2004.09.001

Cloutier, J. M., Mahadevaiah, S. K., ElInati, E., Nussenzweig, A., Toth, A., & Turner, J. M. (2015). Histone H2AFX Links Meiotic Chromosome Asynapsis to Prophase I Oocyte Loss in Mammals. PLoS Genetics, 11(10), e1005462. doi:10.1371/journal.pgen.1005462

Coticchio, G., Dal Canto, M., Mignini Renzini, M., Guglielmo, M. C., Brambillasca, F., Turchi, D., . . . Fadini, R. (2015). Oocyte maturation: gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization. Human Reproduction Update, 21(4), 427-454. doi:10.1093/humupd/dmv011

Del Collado, M., Saraiva, N. Z., Lopes, F. L., Gaspar, R. C., Padilha, L. C., Costa, R. R., . . . Garcia, J. M. (2015). Influence of bovine serum albumin and fetal bovine serum supplementation during in vitro maturation on lipid and mitochondrial behaviour in oocytes and lipid accumulation in bovine embryos. Reproduction, Fertility and Development. doi:10.1071/RD15067

Dominguez, M. H., Chattopadhyay, P. K., Ma, S., Lamoreaux, L., McDavid, A., Finak, G., . . . Roederer, M. (2013). Highly multiplexed quantitation of gene expression on single cells. Journal of Immunological Methods, 391(1-2), 133-145. doi:10.1016/j.jim.2013.03.002

Dong, J., Albertini, D. F., Nishimori, K., Kumar, T. R., Lu, N., & Matzuk, M. M. (1996). Growth differentiation factor-9 is required during early ovarian folliculogenesis. Nature, 383(6600), 531-535.

Duckett, C. S., Li, F., Wang, Y., Tomaselli, K. J., Thompson, C. B., & Armstrong, R. C. (1998). Human IAP-like protein regulates programmed cell death downstream of Bcl-xL and cytochrome c. Molecular and Cellular Biology, 18(1), 608-615.

Eppig, J. J. (1991). Intercommunication between mammalian oocytes and companion somatic cells. Bioessays, 13(11), 569-574.

Eppig, J. J., Freter, R. R., Ward-Bailey, P. F., & Schultz, R. M. (1983). Inhibition of oocyte maturation in the mouse: participation of cAMP, steroid hormones, and a putative maturation-inhibitory factor. Developmental Biology, 100(1), 39-49.

Faast, R., Thonglairoam, V., Schulz, T. C., Beall, J., Wells, J. R., Taylor, H., . . . Lyons, I. (2001). Histone variant H2A.Z is required for early mammalian development. Current Biology, 11(15), 1183-1187.

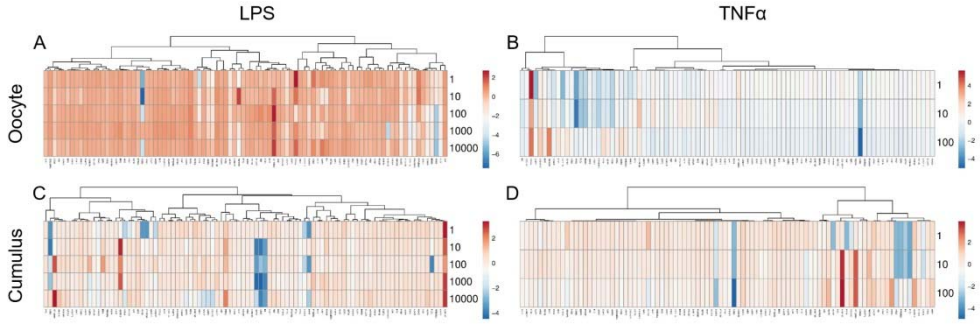
Fishel, S., Jackson, P., Webster, J., & Faratian, B. (1988). Endotoxins in culture medium for

human in vitro fertilization. Fertil Steril, 49(1), 108-111.
Fock, R. A., Vinolo, M. A., de Moura Sa Rocha, V., de Sa Rocha, L. C., & Borelli, P. (2007). Protein-energy malnutrition decreases the expression of TLR-4/MD-2 and CD14 receptors in peritoneal macrophages and reduces the synthesis of TNF-alpha in response to lipopolysaccharide (LPS) in mice. Cytokine, 40(2), 105-114. doi:10.1016/j.cyto.2007.08.007
Gebert, C., Wrenzycki, C., Herrmann, D., Groger, D., Reinhardt, R., Hajkova, P., . . . Niemann, H. (2006). The bovine IGF2 gene is differentially methylated in oocyte and sperm DNA. Genomics, 88(2), 222-229. doi:10.1016/j.ygeno.2006.03.011
Gilchrist, R. B., Lane, M., & Thompson, J. G. (2008). Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Human Reproduction Update, 14(2), 159-177. doi:10.1093/humupd/dmm040
Gilchrist, R. B., Ritter, L. J., & Armstrong, D. T. (2004). Oocyte-somatic cell interactions during follicle development in mammals. Animal Reproduction Science, 82-83, 431-446.
Goissis, M. D., & Cibelli, J. B. (2014). Functional characterization of SOX2 in bovine preimplantation embryos. Biology of Reproduction, 90(2), 30. doi:10.1095/biolreprod.113.111526
Graf, A., Krebs, S., Heininen-Brown, M., Zakhartchenko, V., Blum, H., & Wolf, E. (2014). Genome activation in bovine embryos: review of the literature and new insights from RNA sequencing experiments. Animal Reproduction Science, 149(1-2), 46-58. doi:10.1016/j.anireprosci.2014.05.016
Hackett, J. A., Sengupta, R., Zylicz, J. J., Murakami, K., Lee, C., Down, T. A., & Surani, M. A. (2013). Germline DNA demethylation dynamics and imprint erasure through 5 hydroxymethylcytosine. Science, 339(6118), 448-452. doi:10.1126/science.1229277
Herath, S., Williams, E. J., Lilly, S. T., Gilbert, R. O., Dobson, H., Bryant, C. E., & Sheldon, I. M. (2007). Ovarian follicular cells have innate immune capabilities that modulate their endocrine function. Reproduction, 134(5), 683-693.
Ibrahim, L. A., Kramer, J. M., Williams, R. S., & Bromfield, J. J. (2016). Human granulosa515 luteal cells initiate an innate immune response to pathogen-associated molecules. Reproduction, 152(4), 261-270. doi:10.1530/REP-15-0573
Jayarama, S., Li, L. C., Ganesh, L., Mardi, D., Kanteti, P., Hay, N., . . . Prabhakar, B. S. (2014). MADD is a downstream target of PTEN in triggering apoptosis. Journal of Cellular Biochemistry, 115(2), 261-270. doi:10.1002/jcb.24657
Jiang, Y., Yu, M., Hu, X., Han, L., Yang, K., Ba, H., . . . Wang, J. (2017). STAT1 mediates transmembrane TNF-alpha-induced formation of death-inducing signaling complex and apoptotic signaling via TNFR1. Cell Death and Differentiation, 24(4), 660-671. doi:10.1038/cdd.2016.162
Johnson, M. L., Redmer, D. A., Reynolds, L. P., & Grazul-Bilska, A. T. (1999). Expression of gap junctional proteins connexin 43, 32, and 26 throughout follicular development and atresia in cows. Endocrine, 10(1), 43-51. doi:10.1385/ENDO:10:1:43
Lee, K. S., Joo, B. S., Na, Y. J., Yoon, M. S., Choi, O. H., & Kim, W. W. (2000). Relationships between concentrations of tumor necrosis factor-alpha and nitric oxide in follicular fluid and oocyte quality. J Assist Reprod Genet, 17(4), 222-228.
Li, L., Zheng, P., & Dean, J. (2010). Maternal control of early mouse development. Development, 137(6), 859-870. doi:10.1242/dev.039487
Liu, Z., de Matos, D. G., Fan, H. Y., Shimada, M., Palmer, S., & Richards, J. S. (2009). Interleukin-6: an autocrine regulator of the mouse cumulus cell-oocyte complex expansion process. Endocrinology, 150(7), 3360-3368.
Liu, Z., Shimada, M., & Richards, J. S. (2008). The involvement of the Toll-like receptor family in ovulation. J Assist Reprod Genet, 25(6), 223-228.
Loureiro, B., Brad, A. M., & Hansen, P. J. (2007). Heat shock and tumor necrosis factor-alpha induce apoptosis in bovine preimplantation embryos through a caspase-9-dependent mechanism. Reproduction, 133(6), 1129-1137. doi:10.1530/REP-06-0307
Ma, C. H., Yan, L. Y., Qiao, J., Sha, W., Li, L., Chen, Y., & Sun, Q. Y. (2010). Effects of tumor necrosis factor-alpha on porcine oocyte meiosis progression, spindle organization, and chromosome alignment. Fertil Steril, 93(3), 920-926. doi:10.1016/j.fertnstert.2009.01.131
Macaulay, A. D., Gilbert, I., Scantland, S., Fournier, E., Ashkar, F., Bastien, A., . . . Robert, C. (2016). Cumulus Cell Transcripts Transit to the Bovine Oocyte in Preparation for Maturation. Biology of Reproduction, 94(1), 16. doi:10.1095/biolreprod.114.127571
Masui, S., Nakatake, Y., Toyooka, Y., Shimosato, D., Yagi, R., Takahashi, K., . . . Niwa, H. (2007). Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nature Cell Biology, 9(6), 625-635. doi:10.1038/ncb1589

550 Matzuk, M. M., Burns, K. H., Viveiros, M. M., & Eppig, J. J. (2002). Intercellular
551 communication in the mammalian ovary: oocytes carry the conversation. Science,
552 296(5576), 2178-2180.
553 McGinnis, L. K., Limback, S. D., & Albertini, D. F. (2013). Signaling modalities during
554 oogenesis in mammals. Curr Top Dev Biol, 102, 227-242. doi:10.1016/B978-0-12555
416024-8.00008-8
556 McLaughlin, M., Bromfield, J. J., Albertini, D. F., & Telfer, E. E. (2010). Activin promotes
557 follicular integrity and oogenesis in cultured pre-antral bovine follicles. Molecular
558 Human Reproduction, 16(9), 644-653. doi:10.1093/molehr/gaq021
559 McLaughlin, M., & Telfer, E. E. (2010). Oocyte development in bovine primordial follicles is
560 promoted by activin and FSH within a two-step serum-free culture system. Reproduction,
561 139(6), 971-978. doi:10.1530/REP-10-0025
562 Metsalu, T., & Vilo, J. (2015). ClustVis: a web tool for visualizing clustering of multivariate data
563 using Principal Component Analysis and heatmap. Nucleic Acids Research, 43(W1),
564 W566-570. doi:10.1093/nar/gkv468
565 Moussa, M., Shu, J., Zhang, X. H., & Zeng, F. (2015). Maternal control of oocyte quality in
566 cattle "a review". Animal Reproduction Science, 155, 11-27.
567 doi:10.1016/j.anireprosci.2015.01.011
568 Nagata, Y., & Shirakawa, K. (1996). Setting standards for the levels of endotoxin in the embryo
569 culture media of human in vitro fertilization and embryo transfer. Fertil Steril, 65(3),
570 614-619.
571 Nicholas, R. S., Stevens, S., Wing, M. G., & Compston, D. A. (2002). Microglia-derived IGF-2
572 prevents TNFalpha induced death of mature oligodendrocytes in vitro. Journal of
573 Neuroimmunology, 124(1-2), 36-44.
574 Niwa, H., Miyazaki, J., & Smith, A. G. (2000). Quantitative expression of Oct-3/4 defines
575 differentiation, dedifferentiation or self-renewal of ES cells. Nature Genetics, 24(4), 372
576 376. doi:10.1038/74199
577 Nuttinck, F., Gall, L., Ruffini, S., Laffont, L., Clement, L., Reinaud, P., . . . Marquant-Le
578 Guienne, B. (2011). PTGS2-related PGE2 affects oocyte MAPK phosphorylation and
579 meiosis progression in cattle: late effects on early embryonic development. Biology of
580 Reproduction, 84(6), 1248-1257. doi:10.1095/biolreprod.110.088211
581 Nuttinck, F., Peynot, N., Humblot, P., Massip, A., Dessy, F., & Flechon, J. E. (2000).
582 Comparative immunohistochemical distribution of connexin 37 and connexin 43
583 throughout folliculogenesis in the bovine ovary. Molecular Reproduction and
584 Development, 57(1), 60-66. doi:10.1002/1098-2795(200009)57:1<60::AID585
MRD9>3.0.CO;2-6
586 Pan, H., & Schultz, R. M. (2011). Sox2 modulates reprogramming of gene expression in two-cell
587 mouse embryos. Biology of Reproduction, 85(2), 409-416.
588 doi:10.1095/biolreprod.111.090886
589 Piersanti, R. L., Horlock, A. D., Block, J., Santos, J. E. P., Sheldon, I. M., & Bromfield, J. J.
590 (2019). Persistent effects on bovine granulosa cell transcriptome after resolution of
591 uterine disease. Reproduction, 158(1), 35-46. doi:10.1530/REP-19-0037
592 Price, J. C., Bromfield, J. J., & Sheldon, I. M. (2013). Pathogen-associated molecular patterns
593 initiate inflammation and perturb the endocrine function of bovine granulosa cells from
594 ovarian dominant follicles via TLR2 and TLR4 pathways. Endocrinology, 154(9), 3377
595 3386. doi:10.1210/en.2013-1102
596 Reyes, J. M., Chitwood, J. L., & Ross, P. J. (2015). RNA-Seq profiling of single bovine oocyte
597 transcript abundance and its modulation by cytoplasmic polyadenylation. Molecular
598 Reproduction and Development, 82(2), 103-114. doi:10.1002/mrd.22445
599 Saadeldin, I. M., Swelum, A. A., Elsafadi, M., Mahmood, A., Yaqoob, S. H., Alfayez, M., &
600 Alowaimer, A. N. (2019). Effects of all-trans retinoic acid on the in vitro maturation of
601 camel (Camelus dromedarius) cumulus-oocyte complexes. J Reprod Dev.
602 doi:10.1262/jrd.2018-073
603 Sagirkaya, H., Misirlioglu, M., Kaya, A., First, N. L., Parrish, J. J., & Memili, E. (2007).
604 Developmental potential of bovine oocytes cultured in different maturation and culture
605 conditions. Animal Reproduction Science, 101(3-4), 225-240.
606 Schievella, A. R., Chen, J. H., Graham, J. R., & Lin, L. L. (1997). MADD, a novel death domain
607 protein that interacts with the type 1 tumor necrosis factor receptor and activates
608 mitogen-activated protein kinase. Journal of Biological Chemistry, 272(18), 12069609
12075.
610 Shimada, M., Yanai, Y., Okazaki, T., Noma, N., Kawashima, I., Mori, T., & Richards, J. S.
611 (2008). Hyaluronan fragments generated by sperm-secreted hyaluronidase stimulate
612 cytokine/chemokine production via the TLR2 and TLR4 pathway in cumulus cells of

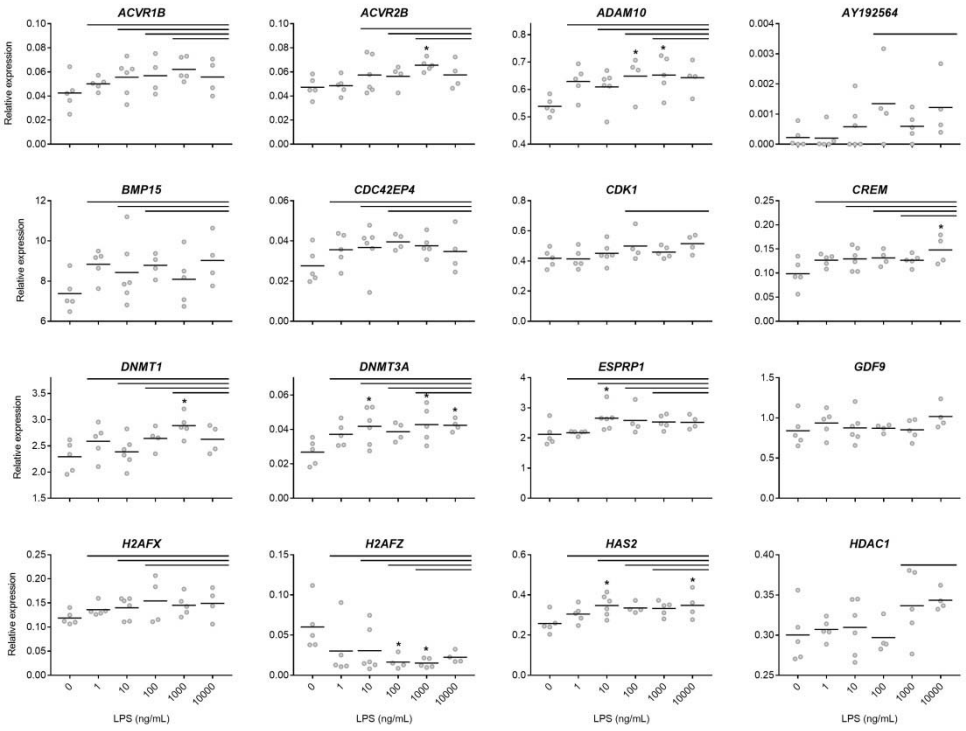
ovulated COCs, which may enhance fertilization. Development, 135(11), 2001-2011.
Sirard, M. A., Florman, H. M., Leibfried-Rutledge, M. L., Barnes, F. L., Sims, M. L., & First, N. L. (1989). Timing of nuclear progression and protein synthesis necessary for meiotic maturation of bovine oocytes. Biology of Reproduction, 40(6), 1257-1263. doi:10.1095/biolreprod40.6.1257
Sirard, M. A., Richard, F., Blondin, P., & Robert, C. (2006). Contribution of the oocyte to embryo quality. Theriogenology, 65(1), 126-136. doi:10.1016/j.theriogenology.2005.09.020
Snyman, E., & Van der Merwe, J. V. (1986). Endotoxin-polluted medium in a human in vitro fertilization program. Fertil Steril, 46(2), 273-276.
Soto, P., Natzke, R. P., & Hansen, P. J. (2003a). Actions of tumor necrosis factor-alpha on oocyte maturation and embryonic development in cattle. American Journal of Reproductive Immunology, 50(5), 380-388.
Soto, P., Natzke, R. P., & Hansen, P. J. (2003b). Identification of possible mediators of embryonic mortality caused by mastitis: actions of lipopolysaccharide, prostaglandin F2alpha, and the nitric oxide generator, sodium nitroprusside dihydrate, on oocyte maturation and embryonic development in cattle. American Journal of Reproductive Immunology, 50(3), 263-272.
Spicer, L. J., Aad, P. Y., Allen, D., Mazerbourg, S., & Hsueh, A. J. (2006). Growth differentiation factor-9 has divergent effects on proliferation and steroidogenesis of bovine granulosa cells. Journal of Endocrinology, 189(2), 329-339. doi:10.1677/joe.1.06503
Su, Y. Q., Sugiura, K., Wigglesworth, K., O'Brien, M. J., Affourtit, J. P., Pangas, S. A., . . . Eppig, J. J. (2008). Oocyte regulation of metabolic cooperativity between mouse cumulus cells and oocytes: BMP15 and GDF9 control cholesterol biosynthesis in cumulus cells. Development, 135(1), 111-121. doi:10.1242/dev.009068
Su, Y. Q., Sugiura, K., Woo, Y., Wigglesworth, K., Kamdar, S., Affourtit, J., & Eppig, J. J. (2007). Selective degradation of transcripts during meiotic maturation of mouse oocytes. Developmental Biology, 302(1), 104.
Sugiura, K., & Eppig, J. J. (2005). Society for Reproductive Biology Founders' Lecture 2005. Control of metabolic cooperativity between oocytes and their companion granulosa cells by mouse oocytes. Reproduction, Fertility and Development, 17(7), 667-674.
Tribulo, P., Siqueira, L. G. B., Oliveira, L. J., Scheffler, T., & Hansen, P. J. (2018). Identification of potential embryokines in the bovine reproductive tract. Journal of Dairy Science, 101(1), 690-704. doi:10.3168/jds.2017-13221
Warzych, E., Wrenzycki, C., Peippo, J., & Lechniak, D. (2007). Maturation medium supplements affect transcript level of apoptosis and cell survival related genes in bovine blastocysts produced in vitro. Molecular Reproduction and Development, 74(3), 280-289. doi:10.1002/mrd.20610
Watkins, A. J., Lucas, E. S., Wilkins, A., Cagampang, F. R., & Fleming, T. P. (2011). Maternal periconceptional and gestational low protein diet affects mouse offspring growth, cardiovascular and adipose phenotype at 1 year of age. PLoS One, 6(12), e28745. doi:10.1371/journal.pone.0028745
Wigglesworth, K., Lee, K. B., Emori, C., Sugiura, K., & Eppig, J. J. (2015). Transcriptomic diversification of developing cumulus and mural granulosa cells in mouse ovarian follicles. Biology of Reproduction, 92(1), 23. doi:10.1095/biolreprod.114.121756
Yamamoto, Y., Kuwahara, A., Taniguchi, Y., Yamasaki, M., Tanaka, Y., Mukai, Y., . . . Irahara, M. (2015). Tumor necrosis factor alpha inhibits ovulation and induces granulosa cell death in rat ovaries. Reprod Med Biol, 14(3), 107-115. doi:10.1007/s12522-014-0201-5
Zhang, K., & Ealy, A. D. (2012). Disruption of fibroblast growth factor receptor signaling in bovine cumulus-oocyte complexes during in vitro maturation reduces subsequent embryonic development. Domestic Animal Endocrinology, 42(4), 230-238. doi:10.1016/j.domaniend.2011.12.006
Zhang, P., Kerkela, E., Skottman, H., Levkov, L., Kivinen, K., Lahesmaa, R., . . . Kere, J. (2007). Distinct sets of developmentally regulated genes that are expressed by human oocytes and human embryonic stem cells. Fertil Steril, 87(3), 677-690. doi:10.1016/j.fertnstert.2006.07.1509
Zhao, S. J., Pang, Y. W., Zhao, X. M., Du, W. H., Hao, H. S., & Zhu, H. B. (2017). Effects of lipopolysaccharide on maturation of bovine oocyte in vitro and its possible mechanisms. Oncotarget, 8(3), 4656-4667. doi:10.18632/oncotarget.13965

674 **FIGURES**



675 Figure 1. Comparative effect of LPS or TNF exposure during IVM on gene expression.
676 Cumulus oocyte complexes were cultured in the presence of ultrapure LPS (A, C; ng/mL), or
677 recombinant bovine TNFa (C, D; ng/mL) for 24 h. Expression of 92 genes was evaluated in
678 oocytes (A-B) and cumulus cells (C-D) independently. Heatmaps were generated using the log2
679 fold change of each gene compared with control cultures.

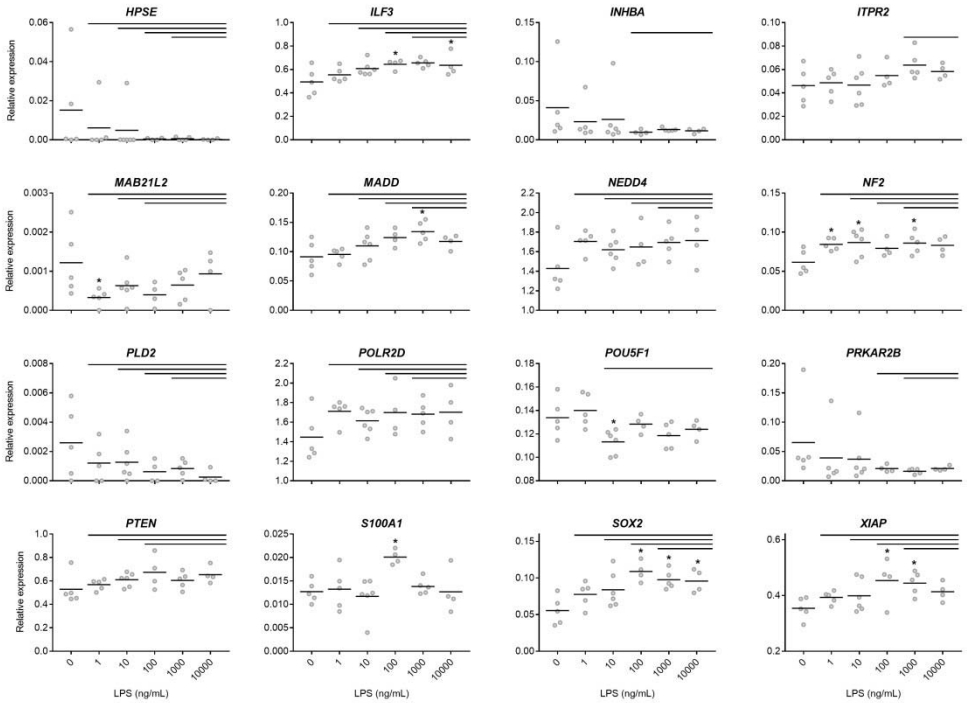
680



681 Figure 2. Effect of LPS during IVM on oocyte gene expression. Cumulus oocyte complexes
682 were cultured in the presence of 1, 10, 100, 1000 or 10000 ng/mL of ultrapure LPS or control
683 medium alone for 24 h. Zona free oocytes were subjected to gene expression analysis. Data are

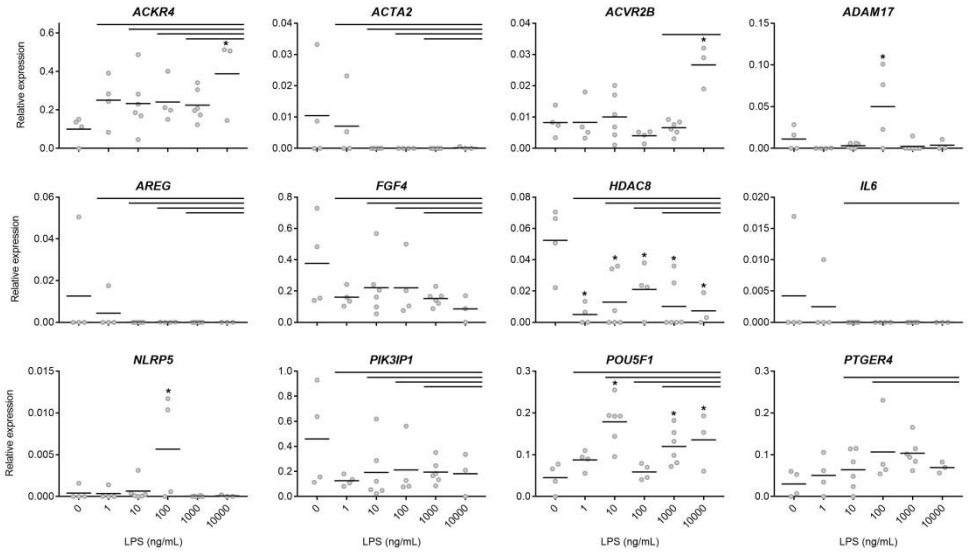
684 presented as relative expression to the arithmetic mean of housekeeping genes (SDHA, GAPDH,
685 ACTB and YWHAZ). Each replicate is represented by a single circle, the mean for each treatment
686 is represented by the horizontal line. Data were analyzed by generalized linear model followed
687 by a Dunnett's posthoc test comparing individual treatments with the control. Contrasts were
688 performed grouping multiple concentrations of LPS and compared with the control. Significant
689 (P = 0.05) contrasts are indicated by the horizontal lines above the combined concentrations. *
690 indicates a significant effect for the specific dose of LPS (P = 0.05) compared with the control.

691



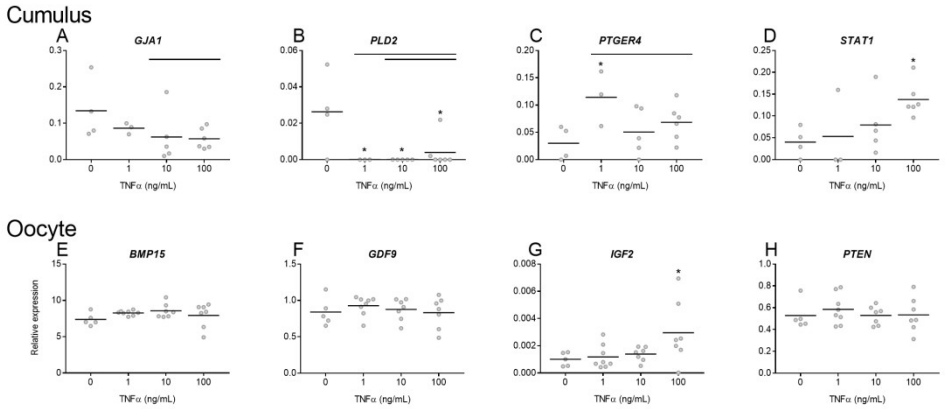
692 Figure 3. Effect of LPS during IVM on oocyte gene expression. Cumulus oocyte complexes
693 were cultured in the presence of 1, 10, 100, 1000 or 10000 ng/mL of ultrapure LPS or control
694 medium alone for 24 h. Zona free oocytes were subjected to gene expression analysis. Data are
695 presented as relative expression to the arithmetic mean of housekeeping genes (SDHA, GAPDH,
696 ACTB and YWHAZ). Each replicate is represented by a single circle, the mean for each treatment
697 is represented by the horizontal line. Data were analyzed by generalized linear model followed
698 by a Dunnett's posthoc test comparing individual treatments with the control. Contrasts were
699 performed grouping multiple concentrations of LPS and compared with control. Significant (P =
700 0.05) contrasts are indicated by the horizontal lines above the combined concentrations. *
701 indicates a significant effect for the specific dose of LPS (P = 0.05) compared with the control.

702



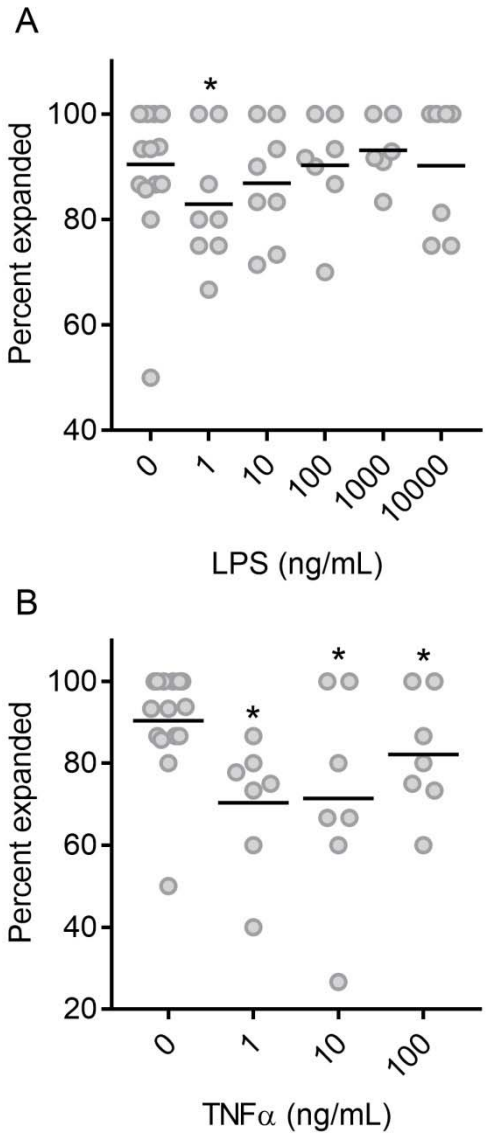
703 Figure 4. Effect of LPS during IVM on cumulus cell gene expression. Cumulus oocyte
704 complexes were cultured in the presence of 1, 10, 100, 1000 or 10000 ng/mL of ultrapure LPS or
705 control medium alone for 24 h. Cumulus cells were subjected to gene expression analysis. Data
706 are presented as relative expression to the arithmetic mean of housekeeping genes (SDHA,
707 GAPDH, ACTB and YWHAZ). Each replicate is represented by a single circle, the mean for each
708 treatment is represented by the horizontal line. Data were analyzed by generalized linear model
709 followed by a Dunnett's posthoc test comparing individual treatments with the control. Contrasts
710 were performed grouping multiple concentrations of LPS and compared with the control.
711 Significant (P = 0.05) contrasts are indicated by the horizontal lines above the combined
712 concentrations. * indicates a significant effect for the specific dose of LPS (P = 0.05) compared
713 with the control.

714



715 Figure 5. Effect of TNFa during IVM on oocyte or cumulus cell gene expression. Cumulus
716 oocyte complexes were cultured in the presence of 1, 10, or 100 ng/mL of recombinant bovine
717 TNFa or control medium alone for 24 h. Cumulus cells (A-D) and zona free oocytes (E-H) were
718 subjected to gene expression analysis. Data are presented as relative expression to the arithmetic
719 mean of housekeeping genes (SDHA, GAPDH, ACTB and YWHAZ). Each replicate is
720 represented by a single circle, the mean for each treatment is represented by the horizontal line.
721 Data were analyzed by generalized linear model followed by a Dunnett's posthoc test comparing
722 individual treatments with the control. Contrasts were performed grouping multiple
723 concentrations of TNFa and compared with the control. Significant (P = 0.05) contrasts are
724 indicated by the horizontal lines above the combined concentrations. * indicates a significant
725 effect for the specific dose of TNFa (P = 0.05) compared with the control.

726



727 Figure 6. Effect of LPS or TNFa exposure during IVM on COC expansion. Cumulus oocyte
728 complex expansion was evaluated following culture in the presence of (A) ultrapure LPS (1, 10,
729 100, 1000 or 10000 ng/mL), (B) recombinant bovine TNFa (1, 10, or 100 ng/mL), or control
730 medium alone for 24 h. Each replicate is represented by a single circle, the mean for each
731 treatment is represented by the horizontal line. The percentage of COCs to fully expand was
732 analyzed using the generalized linear mixed models procedure. * indicates a significant effect for
733 the specific dose (P = 0.05) compared with the control.

734

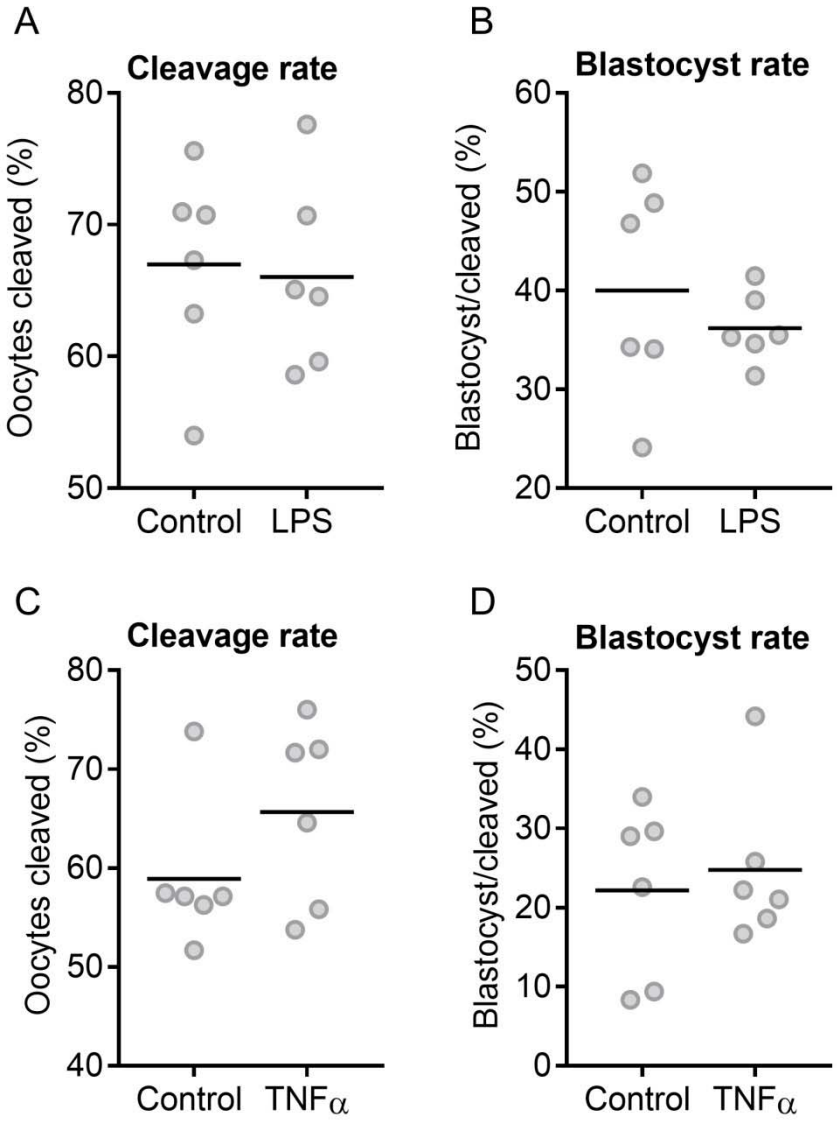


Figure 7. Effect of LPS or TNFα exposure during IVM on blastocyst development. Cumulus oocyte complexes were exposed to (A-B) ultrapure LPS (1000 ng/mL), (C-D) recombinant bovine TNFα (100 ng/mL), or control medium alone for 24 h prior to fertilization and embryo culture. The proportion of oocytes to undergo cleavage at 3 d following insemination (A, C) and the proportion of cleaved oocytes that became blastocysts 7.5 d following insemination (B, D) are shown. Each replicate is represented by a single circle, the mean for each treatment is represented by the horizontal line. The proportion of oocytes to cleave, and proportion of cleaved oocytes to develop to blastocyst was analyzed using the generalized linear mixed models procedure.

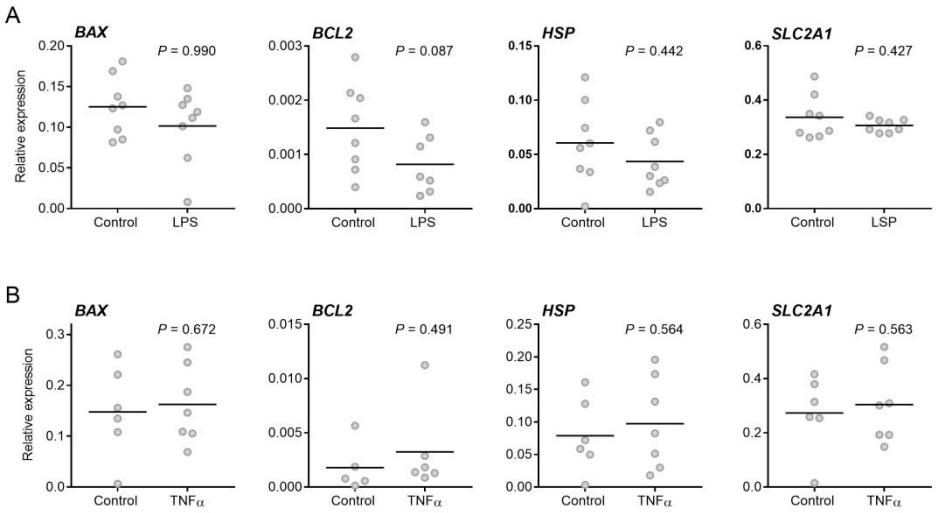


Figure 8. Effect of LPS or TNFα exposure during IVM on blastocyst gene expression. Cumulus oocyte complexes were exposed to (A) ultrapure LPS (1000 ng/mL), (B) recombinant bovine TNFα (100 ng/mL), or control medium alone for 24 h prior to fertilization and embryo culture. Blastocyst stage embryos were subjected to gene expression analysis. Data are presented as relative expression to the arithmetic mean of housekeeping genes (*SDHA* and *RLP19*). Each replicate is represented by a single circle, the mean for each treatment is represented by the horizontal line. Data were analyzed using the general linear model.