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Identification of Possible Mediators of Embryonic Mortality Caused by Mastitis: Actions of Lipopolysaccharide, Prostaglandin $F_{2\alpha}$, and the Nitric Oxide Generator, Sodium Nitroprusside Dihydrate, on Oocyte Maturation and Embryonic Development in Cattle

Soto P, Natzke RP, Hansen PJ. Identification of Possible Mediators of Embryonic Mortality Caused by Mastitis: Actions of Lipopolysaccharide, Prostaglandin $F_{2\alpha}$, and the Nitric Oxide Generator, Sodium Nitroprusside Dihydrate, on Oocyte Maturation and Embryonic Development in Cattle. AJRI 2003; 50:263–272 © Blackwell Munksgaard, 2003

PROBLEM: Mastitis and immunization against constituents of organisms causing mastitis can reduce fertility of cattle and sheep, respectively. For the current experiments, it was hypothesized that these effects are mediated via actions of lipopolysaccharide (LPS), prostaglandin $F_{2\alpha}$ (PGF_2), and nitric oxide on oocyte maturation and embryonic development.
METHOD OF STUDY: To evaluate effects on oocyte maturation, oocytes were matured with various concentrations of LPS, $PGF_{2\alpha}$, or the nitric oxide (NO) generator, sodium nitroprusside (SNP). Following maturation, oocytes were fertilized and cultured until day 8 after fertilization. To test effects on embryo growth, oocytes were matured and fertilized and cultured after fertilization with LPS, $PGF_{2\alpha}$, or SNP.
RESULTS: Addition of 100 and 1000 ng/mL LPS and 50 and 100 ng/mL $PGF_{2\alpha}$ to oocyte maturation medium reduced the proportion of oocytes that became blastocysts at day 8 after fertilization. When added after fertilization, in contrast, neither LPS nor $PGF_{2\alpha}$ reduced development to the blastocyst stage. Unlike for LPS and $PGF_{2\alpha}$, addition of SNP during oocyte maturation was without effect on the proportion of oocytes that became blastocysts at day 8 after fertilization. However, addition of 10 μM SNP to culture medium after fertilization completely prevented development to the blastocyst stage while 0.1 and 1 μM SNP did not affect development.
CONCLUSIONS: Results indicate that increased local concentrations of LPS, $PGF_{2\alpha}$, and NO can have deleterious consequences on oocyte function (LPS, $PGF_{2\alpha}$) and embryonic development (NO). Thus, these molecules are putative mediators of effects of infectious disease or inflammation, including mastitis, on fertility of cattle.

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Key words: Embryo, lipopolysaccharide, prostaglandin $F_{2\alpha}$, nitric oxide, mastitis, cows

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INTRODUCTION

Mastitis, or infection of the mammary gland, causes a variety of clinical changes in cattle including fever, increased local vascular permeability, heart rate, and respiratory rate, and reduced milk production, feed intake and rumen motility.[1,2] Moreover, mastitis is associated with disruption of reproductive processes in dairy cattle. Cows diagnosed with clinical mastitis between first insemination after calving and pregnancy diagnosis required more inseminations to achieve a confirmed pregnancy and had a longer interval between calving and insemination leading to a confirmed pregnancy compared with cows that had clinical mastitis after confirmed pregnancy or with cows without clinical mastitis.[3] In another study,[4] cows with mastitis between first insemination and pregnancy diagnosis had an increase in days from calving to subsequent pregnancy and an increase in the number of inseminations required to achieve pregnancy compared with either cows having mastitis after pregnancy confirmation or uninfected animals. Early pregnancy loss was increased in ewes immunized with either peptidoglycan–polysacharide complexes isolated from *Streptococcus pyogenes* or killed streptococcal cells.[5] Thus, the immune or inflammatory responses associated with bacterial challenge are likely to compromise establishment or maintenance of pregnancy.

The mechanisms by which mastitis is associated with poor reproductive function are unknown. Animals with mastitis have increased cortisol concentrations in the blood.[6,7] Mastitis has been reported to cause a decrease in gonadotropin releasing hormone secretion in sheep,[6] but no relationship was established between occurrence of mastitis and changes in circulating concentrations of luteinizing hormone or estradiol in cows.[7] It is also possible that mastitis interferes with maintenance of the corpus luteum during pregnancy because the magnitude of uterine prostaglandin release triggered by oxytocin injection was greater for cows with mastitis as compared with control cows.[7] The increase in body temperature experienced by animals with mastitis could also directly disrupt the oocyte or embryo: elevated temperature during oocyte maturation or early cleavage stages of embryonic development reduces subsequent development.[8]

One possibility is that the processes of oocyte maturation, fertilization, and early embryonic development are disrupted by products of the microorganisms that colonize the mammary gland or by cytokines and other bioactive molecules produced by the cow in response to infection. Among the molecules that could potentially disrupt oocyte and embryonic function are endotoxins that consist of lipopolysaccharides (LPS) present in the cell wall of gram-negative bacteria. Effects of LPS on the oocyte and embryo have not been clarified. In some experiments, LPS reduced embryonic development of cultured mouse embryos.[9,10] In another experiment, LPS only reduced development if mouse embryos also were exposed to tumor necrosis factor-α (TNF-α).[11] Dumoulin et al.[12] found no effect of endotoxin on viability of cultured mouse oocytes or zygotes. Also, contamination of bovine serum albumin (BSA) with endotoxin did not affect embryonic development although it did induce polyspermy.[13]

Other molecules produced during inflammation that might compromise oocyte and embryo function are prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and nitric oxide (NO). In several tissues, including the endometrium, synthesis of $PGF_{2\alpha}$ is increased by LPS, inflammatory cytokines such as TNF-α and interleukin-1β (IL-1β), and NO.[14–16] Culture of frozen and thawed bovine morulae with 10 ng/mL $PGF_{2\alpha}$ reduced development to the expanded and hatched blastocyst stage.[17] NO synthesis is increased by LPS, TNF-α and interferon-γ in 2-cell mouse embryos[10] and by $PGF_{2\alpha}$ in rat oviduct.[18] Treatment of bovine embryos grown in co-culture with granulosa cells with sodium nitroprusside dihydrate (SNP), a molecule that releases NO upon metabolism, decreased the proportion of embryos that developed to the blastocyst stage.[19]

For the present study, it was hypothesized that LPS, $PGF_{2\alpha}$, and NO act during oocyte maturation to reduce the ability of oocytes to cleave and develop following fertilization. In addition, it was hypothesized that addition of these molecules after fertilization will also inhibit embryonic development.

MATERIALS AND METHODS

Materials

Escherichia coli LPS, serotype O55:B5, was purchased from Sigma Chemical (St Louis, MO, USA). The activity of the LPS was verified by confirming that it stimulated [^{3}H]-thymidine incorporation in bovine peripheral blood lymphocytes as determined using procedures described elsewhere.[20] Concentrations of LPS at 0.1 ng/mL and above stimulated [^{3}H]-thymidine incorporation (results not shown). $PGF_{2\alpha}$ was obtained from Cayman Chemical (Ann Arbor, MI, USA). SNP was purchased from Sigma.

Modified Tyrode's solutions were obtained from Cell and Molecular Technologies (Lavallette, NJ, USA) to prepare HEPES-Tyrode's albumin lactate pyruvate solution (TALP), *in vitro* fertilization (IVF)-TALP and Sperm-TALP (SP-TALP) as described elsewhere.[21] Essentially fatty-acid free-BSA

(EFAF-BSA) and BSA were purchased from Sigma. Pituitary-derived follicle stimulating hormone (FSH) (Folltropin-V) was obtained from Vetrepharm Canada, Inc. (London, ON). Percoll was from Amersham Pharmacia Biotech (Uppsala, Sweden). Frozen semen from various bulls was obtained from Select Sires, Inc. (Rocky Mount, VA, USA) and Southeastern Semen Services (Wellborn, FL, USA). Oocyte collection medium was Tissue Culture Medium (TCM)-199 with Hank's salts without phenol red (Sigma or Atlanta Biologicals, Norcross, GA, USA) with the addition of 2% (v/v) bovine steer serum, 0.04 U heparin/mL, 100 U/mL penicillin-G, 0.1 mg/mL streptomycin, and 1 mM glutamine. Oocyte maturation medium was TCM-199 with Earle's salts (Cell and Molecular Technologies) supplemented with 10% (v/v) steer serum, 2 μg/mL estradiol 17-β, 20 μg/mL FSH, 22 μg/mL sodium pyruvate, 50 μg/mL gentamicin and an additional 1 mM glutamine. Two modifications of Potassium Simplex Optimized Medium (KSOM) called KSOM-Bovine Embryo 1 (BE1) and KSOM-BE2, respectively, were prepared (Table I) using basal KSOM media from Cell and Molecular Technologies and amino acid solutions from Sigma.

In Vitro Production of Embryos

Ovaries were obtained from a local abattoir and transported at ambient temperature in saline solution [0.9% (w/v) NaCl] containing 100 U/mL of penicillin-G and 100 μg/mL of streptomycin. In the laboratory, ovaries were washed with pre-warmed saline solution to remove debris and blood. Cumulus oocyte complexes (COCs) were collected by slicing 2–10 mm follicles on the surface of each ovary into a beaker containing oocyte collection medium. The COCs were washed two times in oocyte collection medium and transferred in groups of 10 to pre-equilibrated 50 μL drops of oocyte maturation medium covered with mineral oil. The COCs were matured for 22 hr at 38.5°C in an atmosphere of 5% (v/v) CO_2 in humidified air. After maturation, COCs were washed once in HEPES-TALP and transferred to 600 μL of IVF-TALP in groups of ~30 per well. A pool of frozen–thawed sperm from three bulls was purified by Percoll gradient centrifugation; a separate pool was used for each replicate. The pellet was collected, transferred to a 15-mL centrifuge tube containing 10 mL of SP-TALP, and centrifuged. The supernatant was removed and the sperm pellet was re-suspended in IVF-TALP to give an approximate concentration of 25 million spermatozoa per milliliter. Oocytes were fertilized by adding 25 μL sperm suspension and 25 μL of a solution containing 0.5 mM penicillamine, 0.25 mM hypotaurine, and 25 μM epinephrine in 0.9% (w/v) NaCl to each 600 μL well. After 8–10 hr at 38.5°C and 5% (v/v) CO_2 in humidified air, presumptive zygotes were removed from the fertilization wells. Zygotes were denuded of cumulus cells by vortexing for 5 min in a 2-mL microcentrifuge tube with ~40 μL of HEPES-TALP, washed two to three times in HEPES-TALP to remove remaining cumulus cells and cultured in pre-equilibrated 50 μL drops of modified KSOM-BE1 or KSOM-BE2 overlaid with mineral oil. For all experiments, cleavage rate was determined on day 3 after fertilization and the proportion of oocytes and cleaved embryos that developed to the blastocyst stage was recorded on day 8 after fertilization.

Oocyte and Embryo Experiments

Effects of LPS. To test the effect of LPS on oocyte maturation, COCs were matured in groups of 10 in oocyte maturation medium containing 0.01–1000 ng/mL LPS or an equivalent amount of Dulbecco's phosphate buffered saline (DPBS) [diluent for LPS; used at 1% (v/v) final concentration in oocyte maturation medium]. The COCs were washed after maturation and fertilized in medium without LPS. After fertilization, presumptive zygotes for each treatment were placed in one or more groups of up to 30 in 50 μL drops of embryo culture medium (KSOM-BE1) without LPS. For each replicate, the number of presumptive zygotes per drop was made as constant as possible for all treatments. Variation in the number of embryos recovered after fertilization resulted in within-replicate variation in embryo density per drop. The experiment was replicated four times using 80–136 oocytes per group.

In a second experiment, oocytes were matured and fertilized in medium without LPS. Following fertilization, presumptive zygotes were cultured in one or more groups of up to 30 in 50 μL drops of embryo culture medium (KSOM-BE1) containing 0.01 to 1000 ng/mL LPS or, for control wells, an equivalent amount of vehicle for LPS [1% (v/v) DPBS]. For each replicate, the number of presumptive zygotes was distributed approximately equally across treatments. The experiment was replicated 12 times using 254–272 presumptive embryos per group.

Effects of $PGF_{2\alpha}$. The action of $PGF_{2\alpha}$ on oocyte maturation was determined by culturing COCs in microdrops of oocyte maturation medium containing 0, 10, 50, or 100 ng/mL $PGF_{2\alpha}$ (10 COCs per drop). The concentration of ethanol in oocyte maturation medium, which was used to prepare $PGF_{2\alpha}$ stock solutions, was 0.1% (v/v) for all treatments. After maturation, COCs were fertilized in medium without $PGF_{2\alpha}$ and presumptive zygotes were cultured in groups of up to 30 in 50 μL microdrops of KSOM-BE2. For each replicate, the number of presumptive

TABLE I. Formulations of KSOM-BE1 and KSOM-BE2

Component	KSOM-BE1[a]	KSOM-BE2[b]
Inorganic salts (mg/L)		
$CaCl_2{\cdot}2H_2O$	251.00	250.00
KCl	186.00	186.38
KH_2 PO_4	47.60	47.99
$MgSO_4$ (anhyd.)	24.10	–
$MgSO_4{\cdot}7H_2O$	–	49.30
NaCl	5550.00	5551.80
$NaHCO_3$	2100.00	2100.25
Other components		
BSA (mg/mL)[c]	1.00	1.00
BSA-EFAF (mg/mL)[d]	3.00	3.00
EDTA (mg/L)[e]	3.00	3.72
D-glucose (mg/L)	36.00	36.03
Sodium lactate (mg/L)	–	1121.00
Sodium lactate 60% (mL/L)	1.86	–
Sodium pyruvate (mg/L)	22.00	22.00
Amino acids (mg/L)		
L-alanine	8.90	8.90
L-arginine	–	63.20
L-arginine·HCl	21.00	–
L-asparagine·H_2O	15.00	15.00
L-aspartic acid	13.30	13.20
L-cystine	–	12.02
L-cystine·2HCl	12.00	–
L-glutamine	73.10	146.15
L-glutamic acid	14.70	14.71
Glycine	7.50	7.50
L-histidine	8.00	–
L-histidine·HCl·H_2O	–	20.96
L-isoleucine	26.00	26.23
L-leucine	26.00	26.24
L-lysine·HCl	37.00	36.52
L-methionine	7.50	7.46
L-phenylalanine	16.50	16.52
L-proline	11.50	11.51
L-serine	10.50	10.51
L-threonine	24.00	23.82
L-tryptophan	4.00	5.11
L-tyrosine	18.00	18.12
L-valine	23.50	23.42
Antibiotics		
Gentamicin (g/L)	2.50	2.50
Penicillin G, Na salt (U/L)	100 000.00	100 000.00
Streptomycin sulfate (mg/L)	50.00	50.00

[a] *Prepared by addition of 100 μL essential amino acids (basal medium Eagle) and 50 μL non-essential amino acids (minimum essential medium) to 5 mL KSOM (Cell and Molecular Technologies, Lavalette, NJ, USA; catalog number MR-020). The medium was further modified on the day of use by addition of 3 mg/mL EFAF-BSA and 2.5 μg/mL gentamicin.*

[b] *Prepared by addition of 25 μL non-essential amino acids (minimum essential medium) to 5 mL KSOM with 1/2 amino acids (catalog number MR-106). The medium was further modified on the day of use by addition of 3 mg/mL EFAF-BSA and 2.5 μg/mL gentamicin.*

[c] *Bovine serum albumin.*

[d] *Essentially fatty acid free bovine serum albumin.*

[e] *Ethylenediaminetetraacetic acid.*

zygotes per group was made as constant as possible for all treatments. The experiment was replicated five times using 159–208 oocytes per group.

To test the effect of PGF$_{2\alpha}$ during embryonic development, COCs were subjected to maturation and fertilization without PGF$_{2\alpha}$ and presumptive zygotes were cultured in groups of up to 30 in 50 µL microdrops of embryo culture medium (KSOM-BE2) containing 0.1% (v/v) ethanol and 0, 10, 50, or 100 ng/mL PGF$_{2\alpha}$. For each replicate, the number of presumptive zygotes were distributed approximately equally across treatments. The experiment was replicated six times using 163–172 presumptive embryos per group.

Effects of SNP. Effects of SNP on oocyte maturation and embryonic development were evaluated in two experiments using procedures similar to those described for LPS and PGF$_{2\alpha}$ except that SNP was dissolved directly in medium at concentrations of 0, 0.1, 1, and 10 µM (i.e. control embryos did not receive a vehicle). To test effects of SNP on oocyte maturation, the experiment was replicated five times using a total of 177–251 oocytes per group. To test effects of SNP on embryonic development, the experiment was replicated five times using 144–151 presumptive embryos per group.

Statistical Analysis

The percent of oocytes that cleaved and the percent of oocytes and cleaved embryos that developed to the blastocyst stage was calculated for all embryos treated alike within each replicate. Data (with and without arcsin transformation) were analyzed by least-squares analysis of variance using the General Linear Models procedure of the Statistical Analysis System.[22] Data are reported as values of least-squares mean ± SEM of the untransformed data while probability values are derived from analyses of transformed data. Differences between various levels of a main effect were determined in two ways. First, differences between control oocytes or embryos and embryos treated with various concentrations of LPS, PGF$_{2\alpha}$ or SNP were determined by the pdiff mean separation test of SAS. In addition, variance associated with treatment effects was partitioned into individual orthogonal contrasts. For example, the orthogonal contrasts for LPS experiments were 0, 0.01, and 0.1 versus other concentrations; 0 versus 0.01 and 0.1 ng/mL; 0.01 ng/mL versus 0.1 ng/mL; 100 and 1000 ng/mL versus 1 and 10 ng/mL; 1 ng/mL versus 10 ng/mL; and 10 ng/mL versus 100 ng/mL. For PGF$_{2\alpha}$ experiments, contrasts were 0 and 10 ng/mL versus 50 and 100 ng/mL, control versus 10 ng/mL, and 50 ng/mL versus 100 ng/mL. For SNP experiments, contrasts were 0 and 0.1 µM versus 1 and 10 µM; 0 versus 0.1 µM; and 1.0 µM versus 10 µM.

RESULTS

Effects of LPS on Oocytes and Embryos

Addition of LPS to oocyte maturation medium had no effect on the proportion of oocytes that cleaved (Fig. 1, top panel). As compared with control oocytes, however, the proportion of oocytes that developed to the blastocyst stage at day 8 was reduced ($P < 0.05$) at the two highest concentrations of LPS tested (100 and 1000 ng/mL) (Fig. 1, bottom panel). Similar conclusions were derived from separation of the treatment variance by orthogonal contrasts (control, 0.01 and 0.1 ng/mL versus other concentrations, $P < 0.05$; 100 and 1000 ng/mL versus 1 and 10 ng/mL, $P = 0.07$).

As shown in Fig. 2 (top panel), addition of LPS after fertilization at concentrations of 10 and 1000 ng/mL caused a small but significant ($P < 0.05$) reduction in cleavage rate compared with controls cultured without LPS. A similar conclusion was apparent from analysis of orthogonal contrasts (0, 0.01 and 0.1 versus others; $P < 0.05$; 1 ng/mL versus 10 ng/mL; $P = 0.09$). There was, however, no consistent reduction in the

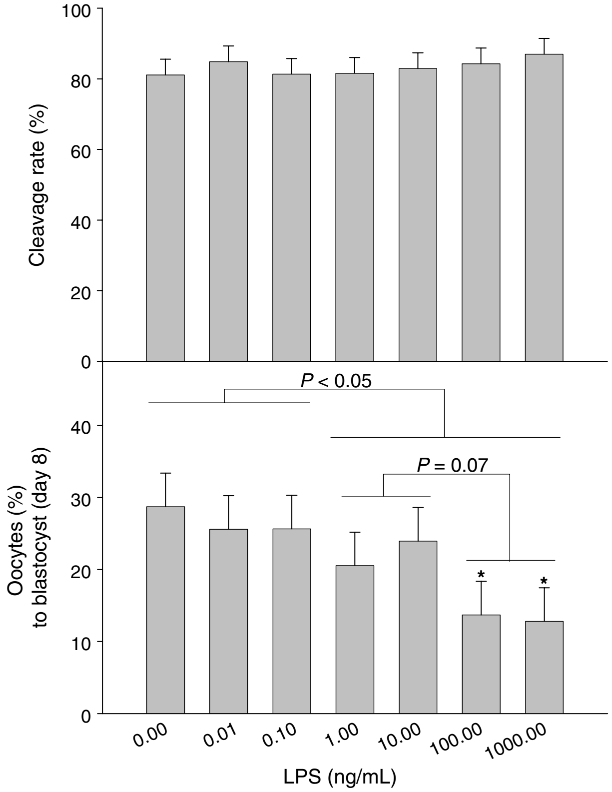

Fig. 1. Effect of lipopolysaccharide (LPS) during oocyte maturation on the percentage of oocytes that cleaved (top panel) and developed into blastocysts on day 8 after insemination (bottom panel). Mean values that differ ($P < 0.05$) from control oocytes (0 ng/mL) are indicated by asterisks. Orthogonal contrasts that are significantly different or approach significance are indicated by horizontal lines over the bars.

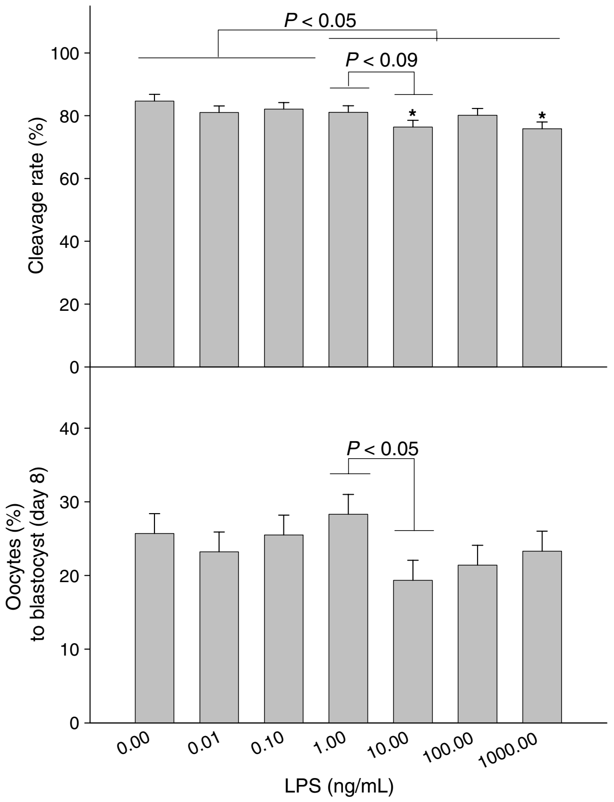

Fig. 2. Effect of lipopolysaccharide (LPS) during embryo culture on the percentage of oocytes that cleaved (top panel) and developed into blastocysts on day 8 after insemination (bottom panel). Mean values that differ ($P < 0.05$) from control oocytes (0 ng/mL) are indicated by asterisks. Orthogonal contrasts that are statistically different or approach significance are indicated by horizontal lines over the bars.

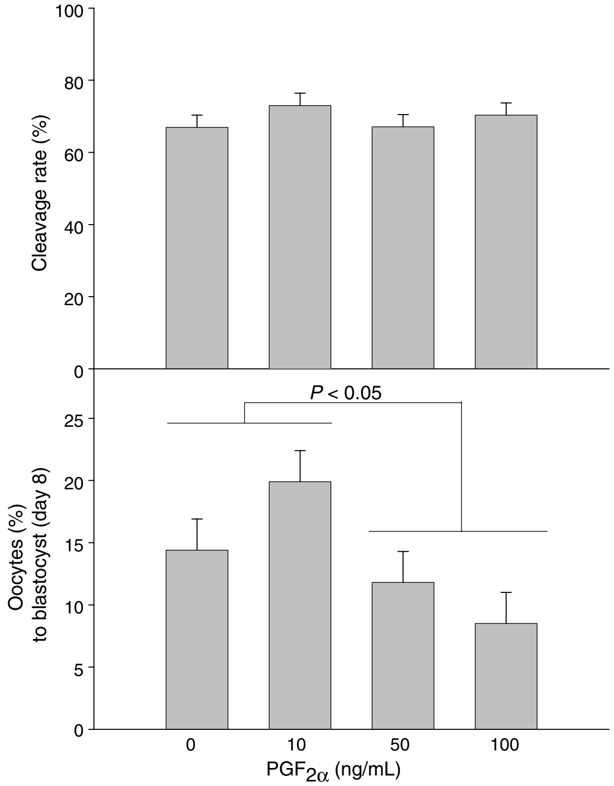

Fig. 3. Effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during oocyte maturation on the percentage of oocytes that cleaved (top panel) and developed into blastocysts on day 8 after insemination (bottom panel). Orthogonal contrasts that are statistically different or approach significance are indicated by horizontal lines over the bars.

percentage of oocytes developing to the blastocyst stage at day 8 after fertilization. The only significant effect was the orthogonal contrast comparing 1 ng/mL versus 10 ng/mL (Fig. 2, bottom panel).

Effects of $PGF_{2\alpha}$ on Oocytes and Embryos

Addition of $PGF_{2\alpha}$ to oocytes during maturation did not alter cleavage rate (Fig. 3, top panel) but development to blastocyst at day 8 was lower ($P < 0.05$) for oocytes cultured during maturation with 50 and 100 ng/mL $PGF_{2\alpha}$ than for oocytes cultured without $PGF_{2\alpha}$ or with 10 ng/mL $PGF_{2\alpha}$ (Fig. 3, bottom panel). In contrast, addition of $PGF_{2\alpha}$ after fertilization had no effect on cleavage rate or percentage of oocytes that developed to the blastocyst stage at day 8 after fertilization (Fig. 4).

Effects of SNP on Oocytes and Embryos

Addition of SNP during oocyte maturation was without effect on cleavage rate or the proportion of oocytes that developed to the blastocyst stage at day 8 after fertilization (Fig. 5). As shown in Fig. 6, addition of 10 M SNP to culture medium after fertilization completely prevented development to the blastocyst stage (control versus 10 μM; $P < 0.001$) while other concentrations of SNP did not affect development (orthogonal contrasts: 0 and 0.1 μM SNP versus 1 and 10 μM SNP, $P < 0.05$).

DISCUSSION

The present results indicate that oocyte maturation and embryonic development in cattle can be compromised by various molecules associated with inflammation including LPS, $PGF_{2\alpha}$, and NO. In particular, LPS and $PGF_{2\alpha}$ disrupted events during oocyte maturation while SNP, which is metabolized to NO in culture,[23] disrupted embryonic development after fertilization. Thus, these molecules, which are either synthesized by bacteria (LPS) or produced in response to inflammatory cytokines ($PGF_{2\alpha}$ and NO), may mediate some of the deleterious effects of mastitis,[3,4] peripheral immunization,[5] and other inflammatory processes on fertility.

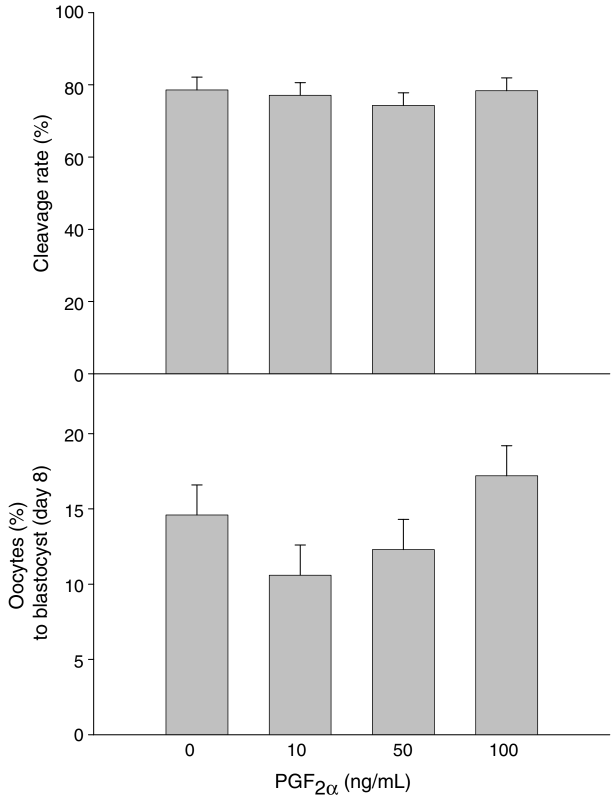

Fig. 4. Effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during embryo culture on the percentage of oocytes that cleaved (top panel) and developed into blastocysts on day 8 after insemination (bottom panel).

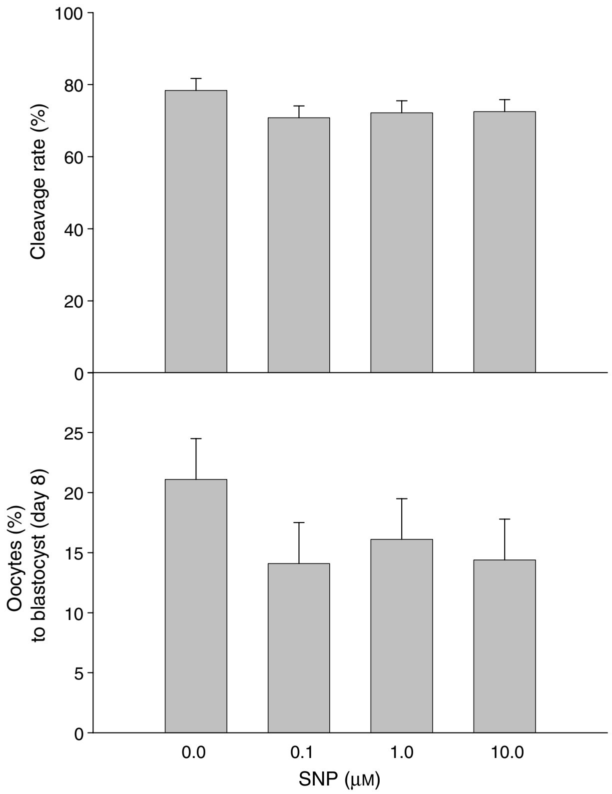

Fig. 5. Effect of sodium nitroprusside dihydrate (SNP) during oocyte maturation on the percentage of oocytes that cleaved (top panel) and developed into blastocysts on day 8 after insemination (bottom panel).

Lipopolysaccharide at concentrations of 1 ng/mL or higher inhibited events associated with oocyte maturation in a way that compromised the ability of embryos formed after fertilization to continue development to the blastocyst stage. Relatively high concentrations of LPS (1 ng/mL or more) were required to elicit an effect on oocyte maturation. These concentrations are higher than reported in plasma of cows during induced experimental mastitis with *E. coli* (55–134 pg/mL)[24] or naturally occurring gangrenous *E. coli* mastitis (85 pg/mL).[25] Thus, while concentrations of LPS may be high enough to disrupt oocyte maturation when ovarian or oviductal production of LPS occurs as a result of local infection, it is doubtful whether LPS concentrations become sufficiently high in the reproductive tract during mastitis to compromise the oocyte.

While LPS affected oocyte function, there was no consistent inhibitory effect of LPS during embryo culture. In other studies in mice, addition of LPS did not affect development of cultured embryos[12] or only reduced development when TNF-α was also present.[11]

The effect of LPS on oocyte maturation could reflect direct actions of the molecule on the oocyte, on the cumulus cells associated with the oocyte or, as they have been found resident in cumulus,[26] on T cells or macrophages located within the COC. The cumulus layer is important for oocyte maturation to take place.[27] Lipopolysaccharide can bind granulosa-luteal cells recovered from human follicular aspirates.[28] In vivo, systemic immune challenge with LPS increased apoptosis in rat granulosa cells.[29] Perhaps LPS acts to effect oocyte maturation indirectly by altering secretion of one or more cytokines from the COC. Indeed, LPS can increase TNF-α secretion from human granulosa-luteal cells.[28] Actions of LPS at extra-ovarian sites could also lead to the production of cytokines or other regulatory molecules that could in turn inhibit oocyte or embryonic function.

One molecule released that may be involved in disruption of oocyte maturation during inflammation is $PGF_{2\alpha}$. When added during oocyte maturation, $PGF_{2\alpha}$ at concentrations of 50 or 100 ng/mL decreased the proportion of oocytes to blastocyst. The synthesis of $PGF_{2\alpha}$ is increased by LPS in many tissues including uterine arteries,[30] endometrium,[16] and placenta.[31] Other inflammatory cytokines can also increase $PGF_{2\alpha}$ secretion including TNF-α and interleukin-1 (IL-1) in endometrium[14,32,33] and IL-1 in granulosa cells.[34]

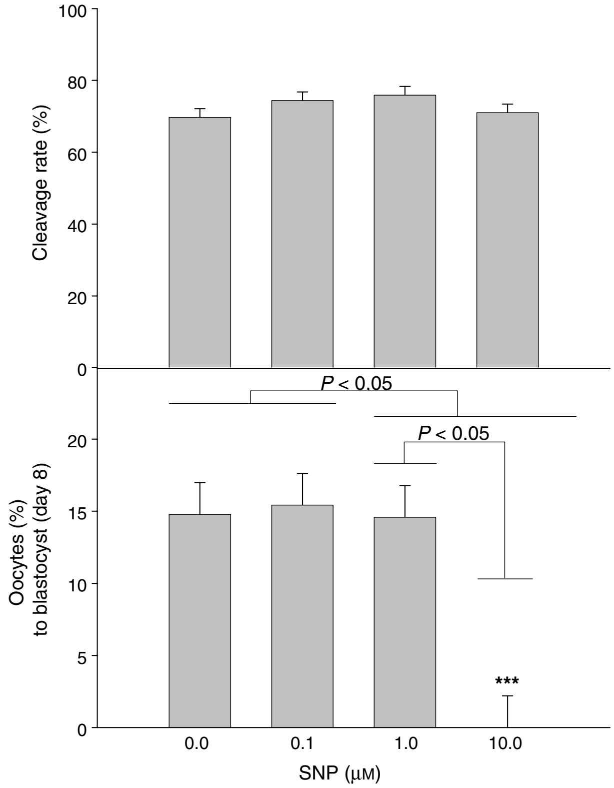

Fig. 6. Effect of sodium nitroprusside dihydrate (SNP) added after insemination on the percentage of oocytes that cleaved (top panel) and developed into blastocysts on day 8 after insemination (bottom panel). Mean values that differ ($P < 0.05$) from control oocytes (0 ng/mL) are indicated by asterisks. Orthogonal contrasts that are statistically different or approach significance are indicated by horizontal lines over the bars.

Like for LPS, addition of $PGF_{2\alpha}$ to cultured embryos beginning after fertilization did not affect development to the blastocyst stage. The absence of an effect of $PGF_{2\alpha}$ on development of embryos is in contrast to a report that $PGF_{2\alpha}$ decreased bovine embryonic development when added at the morula stage.[17] One possibility is that embryos do not acquire susceptibility to the disrupting actions of $PGF_{2\alpha}$ until the morula stage and that, in the current experiments, the $PGF_{2\alpha}$ added immediately after fertilization lost its biological activity by the morula stage. The half-life of $PGF_{2\alpha}$ in cultures of decidual stromal cells was reported as 15 ± 8.2 hr.[35]

Nitric oxide is not a mediator of the direct effects of LPS on the oocyte as addition of SNP had no effect on oocyte maturation. Previous reports demonstrated that low concentrations of SNP (10^{-7} M) promoted maturation of mouse cumulus-enclosed oocytes to metaphase II while addition of an inhibitor of NO synthase blocked maturation.[36] In contrast, addition of 10 μM SNP after fertilization was catastrophic for embryonic development. Similar results with bovine embryos were seen earlier[19] except that development was inhibited by concentrations of SNP as low as 1.6 μM. In the present study, there was no inhibitory effect of 1 μM SNP. The discrepancy in effective dose of SNP between the present study (inhibitory effects at 10 μM but not at 1 μM) and that of Lim and Hansel[18] could reflect greater metabolism of SNP to NO in the study by Lim and Hansel[18] as, in that study, embryos were co-cultured with granulosa cells. In mouse embryos, inhibitory effects of SNP on embryonic development or apoptosis were seen at concentrations of SNP ranging from 0.1 to 1000 μM.[36] One possible mechanism by which NO blocks embryonic development is by activating programmed cell death or apoptosis. Chen et al. (2001) reported that SNP at concentrations of 10 μM or higher increased apoptosis and expression of p53 and Bax in mouse embryos.[37]

While SNP was inhibitory to embryonic development in this and other studies,[19,36] it is likely that low concentrations of NO might be beneficial for embryonic development. Inhibition of NO synthase with L-NAME reduced development of mouse embryos to the blastocyst stage and this effect could be reversed by addition of 0.1 μM SNP.[37] Perhaps low concentrations of NO regulate embryonic growth through receptor-mediated regulation of embryonic development while higher concentrations exert an oxidative stress that compromises development. Hydrogen peroxide can also block preimplantation embryonic development.[38]

In conclusion, results of this study indicate that increased local concentrations of LPS, $PGF_{2\alpha}$, and NO can have deleterious consequences on oocyte function (LPS, $PGF_{2\alpha}$) and embryonic development (NO). Thus, these molecules are putative mediators of effects of infectious disease or inflammation, including mastitis, on fertility of cattle. Identification of the mechanism through which infectious diseases affect reproductive efficiency may lead to new pharmaceutical approaches for improving fertility in animals experiencing infectious disease or inflammation.

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