Theriogenology 89 (2017) 244–249

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

The acute effect of intravenous lipopolysaccharide injection on serum and intrafollicular HDL components and gene expression in granulosa cells of the bovine dominant follicle

Felipe Terres de Campos [a, 1], Joao Aveiro Alvarado Rincon [b, 1], Diego Andres Velasco Acosta [c], Pedro Augusto Silva Silveira [a], Jorgea Pradieé [d], Marcio Nunes Corrêa [b], Bernardo Garziera Gasperin [a], Luiz Francisco Machado Pfeifer [e], Carlos Castilho Barros [f], Ligia Margareth Cantareli Pegoraro [d], Augusto Schneider [f, *]

[a] *Department of Pathology, Veterinary College, Universidade Federal de Pelotas, Pelotas, RS, Brazil*
[b] *Department of Veterinary Clinics, Veterinary College, Universidade Federal de Pelotas, Pelotas, RS, Brazil*
[c] *Corporación Colombiana de Investigación Agropecuaria (CORPOICA), Bogota, Colombia*
[d] *Empresa Brasileira de Pesquisa Agropecuária (Embrapa) Clima Temperado, Pelotas, RS, Brazil*
[e] *Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Porto Velho, RO, Brazil*
[f] *Department of Nutrition, College of Nutrition, Universidade Federal de Pelotas, Pelotas, RS, Brazil*

A R T I C L E I N F O

Article history:
Received 7 September 2016
Received in revised form
7 November 2016
Accepted 17 November 2016
Available online 18 November 2016

Keywords:
Inflammation
PON1
Steroidogenesis
Cholesterol

A B S T R A C T

The aim of this study was to evaluate the effect of an acute systemic inflammatory response induced by lipopolysaccharide (LPS) in the serum and follicular fluid (FF) high-density lipoprotein (HDL) components, hormone concentrations and granulosa cell gene expression. For this purpose, twenty non-lactating Jersey dairy cows were submitted to a progesterone (P4) – estradiol (E2) based synchronization protocol. Cows received a single i.v. dose of LPS (2.5 μg/kg of body weight) or saline solution (CTL Group) 2 h after P4 insert removal. Blood, granulosa cells and FF samples were collected six hours after LPS injection. Five hours after LPS injection rectal temperature was increased in LPS ($P < 0.0001$, 40.4 ± 0.1 °C) compared to the CTL cows (38.8 ± 0.1 °C). Serum PON1 activity was reduced by LPS injection (130.2 ± 5.1 vs. 99.6 ± 3.3 U/mL; $P < 0.001$), as well as HDL-cholesterol concentrations (70.3 ± 5.3 vs. 50.1 ± 6.2 mg/dL; $P < 0.05$). The FF E2 and P4 concentrations were not different between groups ($P > 0.05$). The PON1 activity in the FF was also decreased by LPS injection ($P = 0.01$). In comparison to CTL group, cows injected with LPS had a ten fold reduction in STAR, TLR4 and TNF mRNA expression ($P < 0.05$). In conclusion, an intravenous LPS challenge in cows induced an acute systemic inflammatory response reducing HDL and its components in serum but not in the FF. Only PON1 activity serum reduction was reflected in the FF in the short term. Additionally, steroidogenic and inflammatory genes had reduced expression in the granulosa cells.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Uterine and mammary infections are common in postpartum dairy cattle and are associated to a subsequent reduction in fertility [1]. The most common bacteria causing postpartum uterine and mammary infections in dairy cows are *Escherichia coli* [2,3]. The external membrane of these gram-negative bacteria contains lipopolysaccharide (LPS), a potent stimulator of the inflammatory response [4]. Acute intravenous challenge with LPS in cows is followed by a systemic inflammatory response, characterized by increased body temperature and serum levels of pro-inflammatory cytokines [4]. Cows with postpartum uterine and mammary infections have reduced dominant follicle growth and estradiol (E2) output, which results in delayed postpartum ovulation [2,3]. Studies also have demonstrated that addition of high concentrations of LPS to granulosa cells culture media [5,6] or its increased presence in the follicular fluid [7] are associated with reduced

* Corresponding author. Rua Gomes Carneiro, 1 Sala 239, CEP 96010-610, Pelotas, RS, Brazil.
E-mail address: augusto.schneider@ufpel.edu.br (A. Schneider).
[1] Both authors contributed equally to this study.

http://dx.doi.org/10.1016/j.theriogenology.2016.11.013
0093-691X/© 2016 Elsevier Inc. All rights reserved.

expression of steroidogenic enzymes, followed by reduced E2 output.

Acute phase proteins are also associated with the postpartum infections [8,9]. The enzyme paraoxonase 1 (PON1) has antioxidant activity and is considered a negative acute phase protein, mainly synthesized in the liver [10]. In the circulation PON1 forms a complex along with high-density lipoproteins (HDL) and apoliprotein A I (ApoAI) [11,12], reducing its activity during an inflammatory response [13]. Therefore, the level of serum PON1 activity has been used in the early diagnosis of postpartum diseases in dairy cows [9,14]. Interestingly, systemic PON1 is transferred into the ovarian follicular fluid via HDL-mediated transport [15,16]. HDL is the only lipoprotein present in the follicular fluid due to the specific permeability of the ovarian follicle basement membrane [17] and its serum levels are also reduced during an inflammatory response in humans [18]. The role of HDL/ApoAI/PON1 complex in the preovulatory follicle fluid is pivotal since we recently demonstrated that increasing levels of PON1 activity during bovine oocyte *in vitro* maturation increase blastocyst rates [19].

Based on these considerations, the aim of this study was to evaluate the effect of an acute systemic inflammatory response induced by LPS in the follicular HDL components, hormone concentrations and granulosa cell gene expression.

2. Materials and methods

2.1. Animals and treatments

This study was approved by the Ethics Committee in Animal Experimentation from the Universidade Federal de Pelotas (Protocol 4006-2015). Twenty non-lactating Jersey dairy cows with body condition score (BCS) between 3 and 4 (on a scale of 1 to 5) and average body weight of 492.9 ± 18.9 kg were used for this study. Cows were given an intravaginal progesterone releasing device (CIDR, Zoetis, São Paulo, SP, Brazil) and an injection of 2 mg of estradiol benzoate (Hertape Calier, Juatuba, MG, Brasil) i.m. on Day 0 (D0) to synchronize ovarian follicular wave emergence. Twelve hours before the CIDR removal (D8) cows received an injection of 25 mg of dinoprost (Lutalyse, Zoetis) i.m. Two hours after CIDR removal, cows were randomly divided to receive a single i.v. dose of LPS (LPS Group; 2.5 mL solution of 2.5 μg/kg of body weight; Sigma-Aldrich, St. Louis, MO, USA) according to Carroll, Reuter [4] or saline solution (Control Group; 2 mL of 0.9% NaCl) (Fig. 1). The rectal temperature was measured with a thermometer five hours after LPS or saline injection in both groups.

2.2. Serum, follicular fluid and granulosa cell collection

Eight hours after CIDR removal cows received an epidural anesthesia (5 mL of 2% lidocaine) and the follicular fluid and granulosa cells were collected by transvaginal ultrasound-guided needle aspiration of the largest follicle according to the procedure previously described [15]. An ultrasound with a 5.0 MHz convex transducer (Aquila Pro, Esaote, Sao Paulo, SP, Brazil) fitted to a single lumen aspiration needle was used for aspirations. The follicular fluid was centrifuged at 1500×*g* for 10 minutes. The supernatant containing the clear follicular fluid was stored at -80 °C and the pellet of granulosa cells was added with Trizol (Life Technologies, Carlsbad, CA, USA), homogenized and stored in liquid nitrogen.

Just before follicle aspiration, blood samples were collected by coccygeal venipuncture. Serum was separated by centrifugation at 1000×*g* for 15 minutes and stored at -80 °C for further analysis.

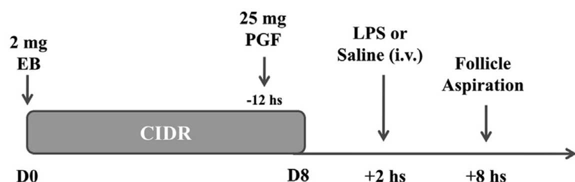

Fig. 1. Follicular wave synchronization protocol used for the study. Lipopolysaccharide (LPS; 2.5 μg/kg of body weight) or saline solution (CTL Group) were injected i.v. 2 h after progesterone insert (CIDR) removal.

2.3. Analysis of gene expression

RNA was extracted from granulosa cells using guanidine isothiocyanate (Trizol, Life Technologies), according to the manufacturer's protocol. RNA concentration was measured in a spectrophotometer and adjusted to 200 ng/μL. Reverse transcription was performed with 1 μg of total RNA using the iScript Synthesis Kit (BioRad, Hercules, CA, USA) in a 20 μL volume and incubated at 25 °C for 5 min, at 42 °C for 30 min and then 85 °C for 5 min (MyCycler ThermalCycler, BioRad). Real-time PCR was performed using the SYBR Green dye to evaluate the expression of the genes of interest. The H2A Histone Family Member Z (H2AFZ, Table 1) gene was used as internal control and the target genes evaluated are described in Table 1 [3beta-hydroxysteroid dehydrogenase (HSD-3B1), Cytochrome P450 Family 19 Subfamily A Member 1 (CYP19A1), Steroidogenic acute regulatory protein (STAR), Luteinizing Hormone/Choriogonadotropin Receptor (LHCGR), Cytochrome P450 Family 11 Subfamily A Member 1 (CYP11A1), Toll-like receptor 4 (TLR4), Nuclear Factor Kappa B Subunit 1 (NFKB1), The tumor necrosis factor alpha (TNF)]. PCR reactions were performed in duplicate in a volume of 20 μL, using 5 μL of SYBR Green Mastermix (Applied Biosystems, Foster City, CA, USA), 0.5 μL of each primer (10 μM), 2 μL of cDNA and 12 μL of ultrapure water. Fluorescence was measured in the ECO Real-Time PCR System (iLLumina, San Diego, CA, USA). For each assay 45 cycles were run (95 °C for 3 seconds and 60 °C for 30 s) and a dissociation

Table 1
Genes and respective primer sequences analyzed in this study.

Gene	Sequence	Reference
LHCGR	F: TGACTATGGTTTCTGCTTACCCAA R: CCATAATGTCTTCACAGGGATTGA	Spicer, Aad [39]
STAR	F: TCGCGGCTCTCTCCTAGGT R: CTGCCGGCTCTCCTTCTTC	Spicer, Aad [39]
CYP11A1	F: CTTCATCCCACTGCTGAATCC R: GGTGATGGACTCAAAGGCAAA	Tajima, Yoshii [40]
CYP19A1	F: TGCCAAGAATGTTCCTTACAGGTA R: CACCATGGCGATGTACTTTCC	Spicer, Aad [39]
CYP17A1	F: GAATGCCTTTGCCCTGTTCA R: CGCGTTTGAACACAACCCTT	Buratini, Teixeira [20]
HSD-3B1	F: CCAAGCAGAAAACCAAGGAG R: ATGTCCACGTTCCCATCATT	Nishimura, Sakumoto [41]
TLR4	F: CTTGCGTACAGGTTGTTCCTAA R: CTGGGAAGCTGGAGAAGTTATG	Price, Bromfield [6]
NFKB1	F: ATCTTTGACAACCGCGCC R: CGAGGCAGCTCCCAGAGTT	This study
TNF	F: AGCACAGAAAGCATGATCCG R: CTGATGAGAGGGAGGCCATT	Price, Bromfield [6]
H2AFZ	F: GAGGAGCTGAACAAGCTGTTG R: TTGTGGTGGCTCTCAGTCTTC	Portela, Machado [42]

3beta-hydroxysteroid dehydrogenase (HSD-3B1), Cytochrome P450 Family 19 Subfamily A Member 1 (CYP19A1), Steroidogenic acute regulatory protein (STAR), Luteinizing Hormone/Choriogonadotropin Receptor (LHCGR), Cytochrome P450 Family 11 Subfamily A Member 1 (CYP11A1), Cytochrome P450 Family 17 Subfamily A Member 1 (CYP17A1), Toll-like receptor 4 (TLR4), Nuclear Factor Kappa B Subunit 1 (NFKB1), The tumor necrosis factor alpha (TNF), H2A Histone Family Member Z (H2AFZ).

Table 2
–PON1 activity, cholesterol and protein concentrations in the total and high-density lipoprotein (HDL) fractions in serum and follicular fluid of cows six hours after a lipopolysaccharide (LPS) injection.

Parameter	Control	LPS	P	Control	LPS	P
	Serum			Intrafollicular		
PON1, U/mL	130.2 ± 5.1	99.6 ± 3.3	0.0004	47.2 ± 4.4	30.6 ± 3.1	0.01
Cholesterol, mg/dL	119.9 ± 5.9	107.3 ± 6.5	0.17	38.5 ± 4	36.5 ± 5.1	0.76
Protein, g/dL	8.1 ± 0.2	7.4 ± 0.2	0.02	4.7 ± 0.5	5.5 ± 0.1	0.23
PON1:HDL ratio	1.9 ± 0.1	2.2 ± 0.3	0.15	2.1 ± 0.4	1.8 ± 0.2	0.40
HDL-PON1, U/mL	44.8 ± 4.9	32.0 ± 1.9	0.03	16.5 ± 4.2	11.5 ± 2.3	0.37
HDL-Cholesterol, mg/dL	70.3 ± 5.3	50.1 ± 6.2	0.03	25.8 ± 3.4	19.4 ± 3.3	0.21
HDL-Protein, g/dL	3.9 ± 0.1	3.6 ± 0.1	0.07	2.5 ± 0.2	2.1 ± 0.3	0.27

curve was included at the end of the reaction to detect specificity of the amplification with a single PCR product. The analysis of the amplification plots was performed using the software EcoStudy (iLLumina). Each assay plate included a negative control using ultrapure water. The expression of the Cytochrome P450 Family 17 Subfamily A Member 1 (CYP17A1) gene (Table 1), exclusively expressed in the theca interna cells, was used as an indicator of contamination of granulosa cells with theca cells. Therefore, samples with Ct values for CYP17A1 lower than 30 were considered as contaminated and excluded from further gene expression analysis [20]. One sample from LPS and one sample from the Control group were excluded according to these criteria.

The coefficient of variation was less than 5% for all the pairs of primers used. Relative expression was calculated using the $2^{A-B}/2^{C-D}$ equation (where A is the cycle threshold [Ct] number for the gene of interest in the first control sample, B is the Ct number for the gene of interest in the analyzed sample, C is the Ct number for H2a in the first control sample, and D is the Ct number for H2a in the analyzed sample) [21]. The first control sample is a random reference sample from the control group and its value was 1.00 as expressed by this equation, and all other samples were calculated relative to this value. After that, all other results from the LPS group were divided by the mean relative expression of all the samples in the control group, which averaged 1.00 by this equation, to obtain the fold change expression of genes of interest for the LPS in comparison to the control group.

2.4. Biochemical and hormonal analysis in serum and follicular fluid

PON1 arylesterase activity was quantified in serum and follicular fluid according to a previously described protocol [22]. Spectrophotometer readings at 270 nm were recorded for 1 min after the addition of 3.3 μL of the sample to 500 μL of a 20 mM Tris/HCl solution containing 1 mM calcium chloride and 4 mM phenylacetate. The PON1 enzyme activity was determined by the formula ΔAbsorbance x 115 and expressed in U/mL.

Haptoglobin was measured in serum using an adapted colorimetric assay method by measuring absorbance at 450 nm according to Jones and Mould [23].

Concentrations of cholesterol were measured in serum and follicular fluid by an enzymatic method and total protein by a colorimetric method in an automated biochemical equipment (Labmax Plenno, Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil) using commercial kits (Labtest Diagnóstica S.A.).

The HDL fraction was isolated in serum and follicular fluid samples using a commercial kit (Colesterol HDL, Labtest Diagnostica S.A.). After the HDL phase separation, concentrations of cholesterol (HDL-cholesterol), total protein (HDL-protein) and PON1 activity (HDL-PON1) were measured in the HDL fraction recovered from serum and follicular fluid using the same

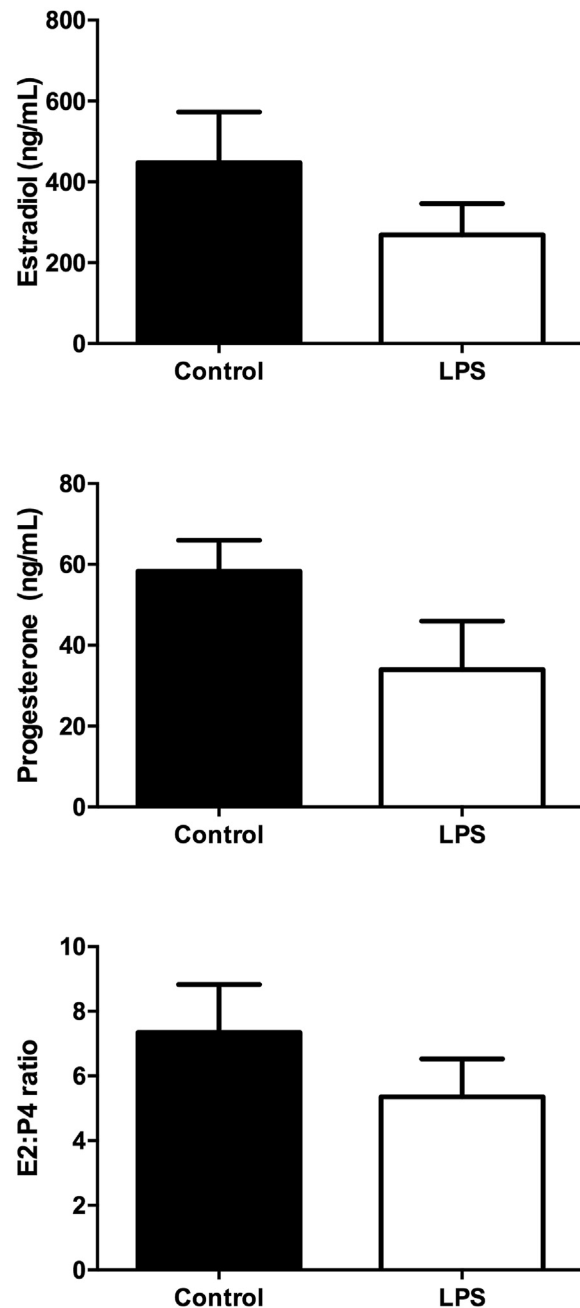

Fig. 2. Intrafollicular concentrations of estradiol (E2), progesterone (P4) and the E2:P4 ratio for cows injected with lipopolysaccharide (LPS) or saline solution (Control).

procedures described before.

Intrafollicular concentrations of E2 and progesterone (P4) were analyzed in a commercial laboratory by chemiluminescence methods (Laboratório Pasin, Santa Maria, RS, Brazil). Three samples (one from the LPS group and two from control group) were excluded from further analysis since intrafollicular E2:P4 ratio was lower than 1, indicating an atretic follicle [24].

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) using the Student's T test for temperature, diameter of the largest follicle, biochemical and hormonal analysis and the Mann-Whitney test for gene expression analysis. P values lower than 0.05 were considered as significant.

3. Results

Cows treated with LPS increased rectal temperature ($P < 0.0001$, 40.4 ± 0.1 °C) in comparison to the control group (38.8 ± 0.1 °C), indicating the effectiveness of the LPS challenge causing systemic inflammatory response. Serum PON1 activity was reduced by LPS injection ($P < 0.001$; Table 2). Cholesterol concentrations did not change between groups ($P = 0.17$), although protein concentration was reduced in LPS injected cows ($P = 0.02$; Table 2). Serum HDL-PON1 and HDL-cholesterol were also reduced by LPS injection ($P < 0.05$; Table 2). Serum haptoglobin concentrations were not changed by LPS injection (0.21 ± 0.05 g/L; $P = 0.18$).

Diameter of largest follicle was not different between Control and LPS groups ($P = 0.27$; 9.8 ± 0.8 mm). In addition, the intrafollicular E2 and P4 concentrations and the E2:P4 ratio were also not different between groups (Fig. 2; $P > 0.05$). Intrafollicular PON1 activity was decreased by LPS injection ($P = 0.01$; Table 2), although the HDL associated PON1 activity was not changed ($P = 0.37$; Table 2). The activity of PON1 was around 60% lower in the follicular fluid compared to serum ($P < 0.0001$; Table 2). Total and HDL-cholesterol concentrations in the follicular fluid did not change with LPS injection ($P > 0.05$; Table 2).

There was a ten fold reduction in STAR mRNA expression in the granulosa cells of cows injected with LPS in comparison to control group ($P < 0.05$, Fig. 3). Similarly, the expression of TLR4 and TNF mRNA was reduced by ten fold in cows injected with LPS ($P < 0.05$, Fig. 1). The target genes LHCGR, CYP11A1, CYP19A1, HSD-3B1, NFKB1 did not change between treatments ($P > 0.05$; Fig. 3).

4. Discussion

In the present study we were able to simulate a systemic inflammatory response with a single LPS injection, as evidenced by the increased rectal temperature and reduction of serum PON1 activity and HDL cholesterol concentrations. This systemic inflammatory response was followed by a reduction in STAR mRNA expression in the granulosa cells of the dominant follicle, an important regulator of steroidogenesis, in addition to decreased TNF and TLR4 mRNA expression, regulators of the inflammatory response. Intrafollicular PON1 activity was also reduced by LPS injection. These results indicate that even short-term inflammatory response can affect the expression of key enzymes for steroidogenesis in the follicle and may affect the final follicular development and ovulation rate if this insult persists for longer periods as suggested before by others [25].

We observed a strong decrease in STAR mRNA expression in the group challenged with LPS. STAR is responsible for cholesterol transport into mitochondria, an essential step in P4 production for conversion into E2 [26,27]. These results indicate that the inflammatory response caused by the acute challenge with LPS can adversely affect the steroidogenesis of the dominant follicle. Despite that, we did not observe a significant reduction in intrafollicular levels of E2 or P4 in LPS treated cows. The lack of difference can be associated to the short interval between LPS injection and follicle aspiration (i.e., only six hours). However, in a longer or chronic inflammatory response, such as observed in various postpartum disorders of cattle, more pronounced effects in steroidogenesis can be expected. Previous *in vitro* studies have shown a reduction of E2 secretion by bovine granulosa cells in response to LPS [6,28]. In addition, the presence of LPS was detected in the bovine follicular fluid and its increased levels were associated with reduced E2 production [7]. However, in the mentioned studies a reduction of CYP19A1 expression in granulosa cells was observed and attributed as a cause for reduced E2 secretion [6,28]. The different mechanism of action can be due the fact we used an *in vivo* model also accounting to the systemic inflammatory response. Nevertheless, we confirm previous *in vitro* investigations,

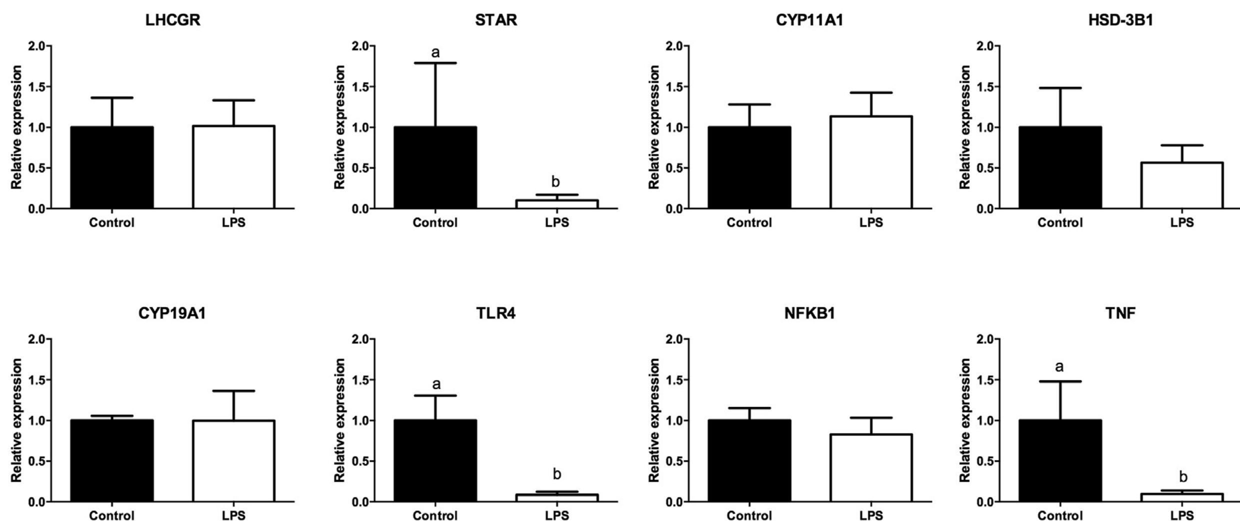

Fig. 3. Dominant follicle granulosa cell gene expression for cows injected with lipopolysaccharide (LPS) or saline solution (Control).

suggesting a harmful effect of LPS exposure on the granulosa cell steroidogenesis pathway.

We also observed a reduction in the expression of TLR4 and TNF in the granulosa cells of cows injected with LPS. Granulosa cells are free of infiltration by inflammatory cells but have the ability to respond to LPS, by increased production and release of pro-inflammatory cytokines [5]. Previous *in vitro* studies also observed a reduction in TLR4 expression in granulosa cells stimulated with LPS [6]. This evidence indicates that there is an inflammatory response of the granulosa cells to the presence of LPS, even when induced by a systemic challenge as in our current study. Although an inflammatory challenge is expected to induce the expression of various pro-inflammatory genes, TNF expression was reduced in granulosa cells in our study. *In vitro* studies had shown a strong stimulation of TNF expression in granulosa cells by LPS [6], although others observed no effect of LPS treatment on TNF expression [28], which can be related to the size and state of the follicles from which granulosa cells are obtained. It is hypothesized that ovulation is like an inflammatory reaction and involves many molecules that also regulate the inflammatory response (e.g. prostaglandins, leukotrienes, bradykinin, histamine and cytokines) [29,30]. TNF receptors are present in the granulosa cells of the bovine preovulatory follicle [31] and TNF treatment reduces steroidogenesis of bovine granulosa cells *in vitro* [31,32]. In addition, genetic polymorphisms in the TNF gene that lead to increased TNF mRNA expression are associated to earlier ovulation in postpartum dairy cows [33]. Therefore, although seems contradictory that TNF expression is reduced during an inflammatory challenge, this can be related to the paracrine role that TNF plays in granulosa cells during the ovulatory process of cows.

Previous studies suggest that the PON1 enzyme acts as a negative acute phase protein in dairy cows [9,14]. Despite that, there is no experimental evidence that PON1 activity is modulated during an acute inflammatory challenge. Therefore, in the present study we demonstrate that the acute inflammatory response to LPS is followed by a reduction of serum PON1 activity. Furthermore, previous studies suggest that serum PON1 is transferred to the follicular fluid bound to HDL in cattle [15] and humans [16]. In this sense, our model indicates that changes in serum PON1 in response to an inflammatory challenge are rapidly reflected in the composition of the follicular fluid of the pre-ovulatory follicle. Serum HDL-cholesterol concentrations were also reduced by the LPS injection, although its intrafollicular levels were not. HDL has been previously described as having anti-inflammatory properties in the basal state and pro-inflammatory properties during an acute-phase response [34]. Secretory phospholipase A_2 ($sPLA_2$) plasma levels increase dramatically during acute inflammatory response [35], which results in increased catabolism of HDL cholesteryl esters and ApoAI, resulting in decreased HDL plasma levels during the inflammatory response [36]. This is in agreement with our observations, were HDL-cholesterol serum levels were reduced by LPS injection. Interestingly, during the inflammatory response, increased sPLA2 induces a reduction in HDL particle size [18]. Since, HDL particles are selectively filtered through the follicle membrane, the follicular fluid is enriched in smaller size HDL particles [37]. This suggests that the serum reduction of large particles HDL-cholesterol does not affect the transfer of the smaller HDL-cholesterol particles to the follicle and can explain why no differences were observed for concentration of HDL-cholesterol in the follicular fluid.

The presence of the complex HDL-ApoAI-PON1 in the follicular fluid is important since serum and intra-follicular PON1 enzyme activity as well as ApoAI concentrations have been positively associated with embryo quality and blastomere number in women undergoing *in vitro* fertilization (IVF) procedures [16]. Studies from our group also demonstrate that the addition of PON1 during *in vitro* oocyte maturation is able to increase blastocyst development rates [19]. Furthermore, it has been reported that increased reactive oxygen species (ROS) and reduced serum levels of HDL and ApoAI are observed in clinically infertile woman [38]. Therefore, the fact that an acute LPS challenge can affect intrafolicular levels of PON1 but not HDL-cholesterol is interesting and can be a possible modulator of oocyte quality in cows suffering from postpartum bacterial contamination.

In conclusion, we could observe that an intravenous LPS challenge in cows induced an acute systemic inflammatory response reducing HDL and its components in serum but not in the follicular fluid. Only PON1 activity serum reduction was reflected in the follicular fluid in the short term, which can influence oocyte quality and subsequent fertility. Additionally, this short term inflammatory response reduced the expression of steroidogenic and inflammatory genes in the granulosa cells of the dominant follicle, which are essential for successful ovulation.

Competing interests

The authors declare no conflicts of interest.

Acknowledgements

Acknowledgements are given to Embrapa Clima Temperado, CAPES, CNPq and FAPERGS for financing the study. This research was also supported by Embrapa (MP1/PC3 Project; Grant 01.13.06.001.03.00).

References

[1] Ribeiro ES, Lima FS, Greco LF, Bisinotto RS, Monteiro AP, Favoreto M, et al. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. J Dairy Sci 2013;96:5682–97.
[2] Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth HJ. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod 2009;81:1025–32.
[3] Huszenicza G, Janosi S, Kulcsar M, Korodi P, Reiczigel J, Katai L, et al. Effects of clinical mastitis on ovarian function in post-partum dairy cows. Reprod Domest Anim = Zuchthygiene 2005;40:199–204.
[4] Carroll JA, Reuter RR, Chase Jr CC, Coleman SW, Riley DG, Spiers DE, et al. Profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge. Innate Immun 2009;15:81–9.
[5] Bromfield JJ, Sheldon IM. Lipopolysaccharide initiates inflammation in bovine granulosa cells via the TLR4 pathway and perturbs oocyte meiotic progression in vitro. Endocrinology 2011;152:5029–40.
[6] Price JC, Bromfield JJ, Sheldon IM. Pathogen-associated molecular patterns initiate inflammation and perturb the endocrine function of bovine granulosa cells from ovarian dominant follicles via TLR2 and TLR4 pathways. Endocrinology 2013;154:3377–86.
[7] Magata F, Horiuchi M, Echizenya R, Miura R, Chiba S, Matsui M, et al. Lipopolysaccharide in ovarian follicular fluid influences the steroid production in large follicles of dairy cows. Anim Reprod Sci 2014;144:6–13.
[8] Krause AR, Pfeifer LF, Montagner P, Weschenfelder MM, Schwegler E, Lima ME, et al. Associations between resumption of postpartum ovarian activity, uterine health and concentrations of metabolites and acute phase proteins during the transition period in Holstein cows. Anim Reprod Sci 2014;145:8–14.
[9] Schneider A, Correa MN, Butler WR. Short communication: acute phase proteins in Holstein cows diagnosed with uterine infection. Res Vet Sci 2013;95:269–71.
[10] Ferre N, Camps J, Prats E, Vilella E, Paul A, Figuera L, et al. Serum paraoxonase activity: a new additional test for the improved evaluation of chronic liver damage. Clin Chem 2002;48:261–8.
[11] Draganov DI, Stetson PL, Watson CE, Billecke SS, La Du BN. Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protects low density lipoprotein against oxidation. J Biol Chem 2000;275:33435–42.
[12] Sorenson RC, Primo-Parmo SL, Camper SA, La Du BN. The genetic mapping and gene structure of mouse paraoxonase/arylesterase. Genomics 1995;30:431–8.
[13] Feingold KR, Memon RA, Moser AH, Grunfeld C. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. Atherosclerosis 1998;139:307–15.
[14] Bionaz M, Trevisi E, Calamari L, Librandi F, Ferrari A, Bertoni G. Plasma

paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J Dairy Sci 2007;90:1740–50.
[15] Schneider A, Absalon-Medina V, Esposito G, Correa M, Butler W. Paraoxonase (PON) 1, 2 and 3 expression in granulosa cells and PON1 activity in follicular fluid of dairy cows. Reprod Domest Anim = Zuchthygiene 2013;48:989–94.
[16] Browne RW, Shelly WB, Bloom MS, Ocque AJ, Sandler JR, Huddleston HG, et al. Distributions of high-density lipoprotein particle components in human follicular fluid and sera and their associations with embryo morphology parameters during IVF. Hum Reprod 2008;23:1884–94.
[17] Jaspard B, Collet X, Barbaras R, Manent J, Vieu C, Parinaud J, et al. Biochemical characterization of pre-beta 1 high-density lipoprotein from human ovarian follicular fluid: evidence for the presence of a lipid core. Biochemistry 1996;35:1352–7.
[18] Tietge UJ, Maugeais C, Lund-Katz S, Grass D, deBeer FC, Rader DJ. Human secretory phospholipase A2 mediates decreased plasma levels of HDL cholesterol and apoA-I in response to inflammation in human apoA-I transgenic mice. Arteriosclerosis, Thromb Vasc Biol 2002;22:1213–8.
[19] Rincon J, Madeira EM, Campos FT, Mion B, Silva JF, Absalon-Medina VA, et al. Exogenous paraoxonase-1 during oocyte maturation improves bovine embryo development in vitro. Reprod Domest Anim = Zuchthygiene 2016;51:827–30.
[20] Buratini Jr J, Teixeira AB, Costa IB, Glapinski VF, Pinto MG, Giometti IC, et al. Expression of fibroblast growth factor-8 and regulation of cognate receptors, fibroblast growth factor receptor-3c and -4, in bovine antral follicles. Reproduction 2005;130:343–50.
[21] Masternak MM, Al-Regaiey KA, Del Rosario Lim MM, Bonkowski MS, Panici JA, Przybylski GK, et al. Caloric restriction results in decreased expression of peroxisome proliferator-activated receptor superfamily in muscle of normal and long-lived growth hormone receptor/binding protein knockout mice. Journals Gerontol Ser A, Biol Sci Med Sci 2005;60:1238–45.
[22] Browne RW, Koury ST, Marion S, Wilding G, Muti P, Trevisan M. Accuracy and biological variation of human serum paraoxonase 1 activity and polymorphism (Q192R) by kinetic enzyme assay. Clin Chem 2007;53:310–7.
[23] Jones GE, Mould DL. Adaptation of the guaiacol (peroxidase) test for haptoglobins to a microtritation plate system. Res Vet Sci 1984;37:87–92.
[24] Ireland JJ, Roche JF. Development of antral follicles in cattle after prostaglandin-induced luteolysis: changes in serum hormones, steroids in follicular fluid, and gonadotropin receptors. Endocrinology 1982;111:2077–86.
[25] Lavon Y, Leitner G, Goshen T, Braw-Tal R, Jacoby S, Wolfenson D. Exposure to endotoxin during estrus alters the timing of ovulation and hormonal concentrations in cows. Theriogenology 2008;70:956–67.
[26] Lin D, Sugawara T, Strauss 3rd JF, Clark BJ, Stocco DM, Saenger P, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 1995;267:1828–31.
[27] Chaffin CL, Dissen GA, Stouffer RL. Hormonal regulation of steroidogenic enzyme expression in granulosa cells during the peri-ovulatory interval in monkeys. Mol Hum Reprod 2000;6:11–8.
[28] Herath S, Williams EJ, Lilly ST, Gilbert RO, Dobson H, Bryant CE, et al. Ovarian follicular cells have innate immune capabilities that modulate their endocrine function. Reproduction 2007;134:683–93.
[29] Espey LL. Ovulation as an inflammatory reaction–a hypothesis. Biol Reprod 1980;22:73–106.
[30] Jabbour HN, Sales KJ, Catalano RD, Norman JE. Inflammatory pathways in female reproductive health and disease. Reproduction 2009;138:903–19.
[31] Sakumoto R, Shibaya M, Okuda K. Tumor necrosis factor-alpha (TNF alpha) inhibits progesterone and estradiol-17beta production from cultured granulosa cells: presence of TNFalpha receptors in bovine granulosa and theca cells. J Reprod Dev 2003;49:441–9.
[32] Spicer LJ. Tumor necrosis factor-alpha (TNF-alpha) inhibits steroidogenesis of bovine ovarian granulosa and thecal cells in vitro. Involvement of TNF-alpha receptors. Endocrine 1998;8:109–15.
[33] Shirasuna K, Kawashima C, Murayama C, Aoki Y, Masuda Y, Kida K, et al. Relationships between the first ovulation postpartum and polymorphism in genes relating to function of immunity, metabolism and reproduction in high-producing dairy cows. J Reprod Dev 2011;57:135–42.
[34] Navab M, Berliner JA, Subbanagounder G, Hama S, Lusis AJ, Castellani LW, et al. HDL and the inflammatory response induced by LDL-derived oxidized phospholipids. Arteriosclerosis, Thromb Vasc Biol 2001;21:481–8.
[35] Nevalainen TJ. Serum phospholipases A2 in inflammatory diseases. Clin Chem 1993;39:2453–9.
[36] Tietge UJ, Maugeais C, Cain W, Grass D, Glick JM, de Beer FC, et al. Overexpression of secretory phospholipase A(2) causes rapid catabolism and altered tissue uptake of high density lipoprotein cholesteryl ester and apolipoprotein A-I. J Biol Chem 2000;275:10077–84.
[37] Jaspard B, Fournier N, Vieitez G, Atger V, Barbaras R, Vieu C, et al. Structural and functional comparison of HDL from homologous human plasma and follicular fluid. A model for extravascular fluid. Arteriosclerosis, Thromb Vasc Biol 1997;17:1605–13.
[38] Marsillach J, Checa MA, Pedro-Botet J, Carreras R, Joven J, Camps J. Paraoxonase-1 in female infertility: a possible role against oxidative stress-induced inflammation. Fertil Sterility 2010;94:1132–4.
[39] Spicer LJ, Aad PY, Allen DT, Mazerbourg S, Payne AH, Hsueh AJ. Growth differentiation factor 9 (GDF9) stimulates proliferation and inhibits steroidogenesis by bovine theca cells: influence of follicle size on responses to GDF9. Biol Reprod 2008;78:243–53.
[40] Tajima K, Yoshii K, Fukuda S, Orisaka M, Miyamoto K, Amsterdam A, et al. Luteinizing hormone-induced extracellular-signal regulated kinase activation differently modulates progesterone and androstenedione production in bovine theca cells. Endocrinology 2005;146:2903–10.
[41] Nishimura R, Sakumoto R, Tatsukawa Y, Acosta TJ, Okuda K. Oxygen concentration is an important factor for modulating progesterone synthesis in bovine corpus luteum. Endocrinology 2006;147:4273–80.
[42] Portela VM, Machado M, Buratini Jr J, Zamberlam G, Amorim RL, Goncalves P, et al. Expression and function of fibroblast growth factor 18 in the ovarian follicle in cattle. Biol Reprod 2010;83:339–46.