Short communication: Characterizing ovine serum stress biomarkers during endotoxemia

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ABSTRACT

Breeding stress-resilient livestock is a potential strategy to help mitigate the negative effect of environmental and pathogenic stressors. The hypothalamic-pituitary-adrenal axis and immune system are activated during stress events and release mediators into the circulation that help restore physiological homeostasis. The purpose of this study was to assess a comprehensive set of circulatory mediators released in response to an acute immune stress challenge to identify candidate biomarkers that can be used for the selection of stress-resilient animals. Fifteen female lambs were stress challenged with an intravenous bolus of lipopolysaccharide (LPS; 400 ng/kg), and blood was collected from the jugular vein at 0, 2, 4, and 6 h after LPS challenge to identify and monitor candidate stress biomarkers; temperature was also recorded over time. Biomarker responses were evaluated with a repeated-measures model to compare time points with baseline values. As expected, all sheep had a monophasic febrile response to LPS challenge, and cortisol increased and returned to baseline by 6 h. The cytokines tumor necrosis factor- α , IL-6, IFN- γ (proinflammatory), and IL-10 (anti-inflammatory) increased, but only tumor necrosis factor- α returned to baseline during the monitoring period. The cytokines IL-1 α , IL-1 β , IL-17 α (proinflammatory), and IL-4 (anti-inflammatory) did not respond to LPS challenge. All chemokines (CCL2, CCL3, CCL4, CXCL10, and IL-8) responded to LPS challenge; however, only CCL2, CCL3, CCL4, and CXCL10 increased over time, and only CCL3, CCL4, and CXCL10 returned to baseline during the monitoring period. MicroRNA (miR-145, miR-233, and miR-1246) also increased and remained

elevated during the study. In summary, the LPS challenge induced a strong stress response in Rideau-Dorset sheep that could be monitored with a distinct profile of circulatory biomarkers.

Key words: sheep, lipopolysaccharide, stress, biomarker

Short Communication

Breeding stress-resilient livestock is a potential strategy to help mitigate the negative effect of environmental and pathogenic stressors. Contributing to these concerns, climate change is expected to exacerbate the effect of environmental and microbial stressors that have known negative consequences on health, production, and welfare in animals (Rojas-Downing et al., 2017). Current concerns over the sustainability of livestock production and increasing antimicrobial resistance can potentially be addressed through genetic selection programs that focus on animal resiliency. Incorporating stress resiliency into selection criteria could help mitigate negative effects of increased stress exposure and reduce reliance on antimicrobials.

The neuroendocrine and immune systems are activated by stressors, including physical (Sevi and Caroprese, 2012), microbial (Kabaroff et al., 2006), and psychological stressors (Caroprese et al., 2010). These systems work in concert to assist in overcoming stress challenges by restoring homeostasis. Recognition of pathogen-associated molecular patterns and host alarmins by innate immune cells triggers the release of proinflammatory cytokines and chemokines into the circulation to initiate an innate immune response, which manifests as inflammation. Proinflammatory cytokines such as tumor necrosis factor (TNF)- α and IL-6 activate the hypothalamic-pituitary-adrenal axis, the major neuroendocrine stress system whose end product is the stress hormone cortisol. Cortisol functions to control the inflammatory response and has modulatory functions in energy metabolism and physiology (Karrow, 2006).

Received October 8, 2019.

Accepted January 13, 2020.

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Lipopolysaccharide is a pathogen-associated molecular pattern derived from the cell wall of gram-negative bacteria and contributes to several livestock illnesses, including mastitis (Herry et al., 2017), endometritis (Bilal et al., 2016), acidosis (Abaker et al., 2017), and heat stress due to gut leakage (Vargas and Marino, 2016). The relevance of LPS to livestock health has contributed to its widespread use in modeling the host response and pathologies related to bacterial infection. Moreover, LPS-induced fever, proinflammatory cytokine (IL-6 and TNF- α), and cortisol responses have been well characterized in ruminants (Kabaroff et al., 2006; Hadfield et al., 2018). The endogenous mediators that are activated during the response to LPS, however, are far more extensive and may also serve as stress biomarkers.

Circulating microRNA (**miRNA**) have more recently been discovered, and their roles in regulating gene expression, including influencing immune cell differentiation and inflammation, are being explored (Dai and Ahmed, 2011). Furthermore, LPS challenge results in the rapid upregulation of miRNA by human monocytes (Taganov et al., 2006). A similar response would be expected in sheep; however, although the miRNA transcriptome of sheep has been characterized (Zhang et al., 2013), little has been done to monitor ovine miRNA responses to systemic LPS challenge.

The collective mediators released into the circulation during stress fine-tune the host response to overcome a stressor and help restore homeostasis; therefore, stress resiliency may be determined by the profile of circulatory protein and miRNA biomarkers during stress challenge. The purpose of this study was to perform a comprehensive assessment of the ovine stress response to a microbial stressor by measuring circulating cortisol, pro- and anti-inflammatory cytokines and chemokines, miRNA, and fever responses to an acute systemic LPS immune stress challenge.

Outbred Rideau-Dorset ewe lambs at 70 d of age were used in this study and housed at the University of Guelph Ponsonby sheep research station (Guelph, ON, Canada). The day before the experiment, 15 randomly selected sheep were weighed and housed in individual pens with ad libitum access to hay and water. Sheep were challenged intravenously with *Escherichia coli* O111:B4 LPS endotoxin (400 ng/kg of BW; Sigma Chemical Co., St. Louis, MO; Kabaroff et al., 2006). No animals showed signs of clinical disease at the beginning of the experiment, and the average BW was 31.4 ± 5.6 kg (mean $\pm$ SD). Blood samples were collected from the jugular vein in 10-mL BD Vacutainer serum tubes (Becton Dickinson, Franklin Lakes, NJ) just before LPS challenge (0 h; baseline) and at 2, 4,

and 6 h following LPS challenge. Serum was isolated and aliquoted into microcentrifuge tubes and stored at -80°C until further analysis. Rectal temperatures were monitored hourly during the experiment. All experimental procedures were approved by the University of Guelph Animal Care Committee (Institutional Animal Utilization Protocol No. 3436).

Total serum cortisol concentrations were measured with a competitive chemiluminescent enzyme immunoassay using the Immulite/Immulite 1000 Cortisol kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) and an Immulite 1000 analyzer (Inter Medico, Markham, ON, Canada) according to the manufacturers' instructions. The antibody is highly specific for cortisol, and the analytical sensitivity is 5.5 nmol/L.

Serum cytokine concentrations were measured in duplicate using a commercially available multiplex immunoassay kit (Milliplex MAP Custom 13-Plex Bovine Magnetic Bead Panel; EMD Millipore Corp., Burlington, MA) according to the manufacturer's instructions. Serum samples were diluted 1:2 with the provided assay buffer before being added to the wells. The median fluorescence intensity of each well was obtained using a Luminex 200 (Bio-Rad, Mississauga, ON, Canada), and data were processed using Milliplex Analyst software (MilliporeSigma, Burlington, MA). Concentrations were determined using the 4PL fitting model. The average intra-assay coefficient of variation for the analytes was 4.96%, and all were below 10%.

The miRNA were isolated from serum using an miRNeasy Serum/Plasma Advanced Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A spike-in control comprising known concentrations of CE-MIR-39-1, included in the kit, was added as reference miRNA.

The candidate miRNA (miR-145, miR-223, and miR-1246) were selected based on previous human and bovine literature involving LPS; the forward primer sequences used were 5'-GTCCAGTTTTCCAGGAATCCCT-3', 5'-CCTGTCAGTTTGTCAAATACCCCA-3', and 5'-GAATGGATTTTGGAGCAGGAA-3' (Li et al., 2015), respectively. The universal reverse primer (Qiagen) was used for these miRNA assays. The spike-in control was used as a reference miRNA to normalize the expression data. The $2^{-\Delta\Delta C_t}$ method was used to determine change in miRNA expression (Pfaffl, 2001).

For cytokine and chemokine analysis, samples below the minimum limit of detection (**LOD**) were replaced with $\text{LOD}/\sqrt{2}$ as suggested by Croghan and Egeghy (2003). For IL-1 β , IL-17 α , and CCL4, more than 30% of the values were below the minimum LOD, and for IL-8 and CXCL10, more than 30% of the values were above the maximum LOD, so median fluorescence in-

tensity values of these analytes were used for analysis (Breen et al., 2016).

For all analyses, SAS software version 9.4 was used (SAS Institute Inc., Cary, NC). Cytokines and chemokines, cortisol, temperature, and miRNA expression were analyzed over time using a general linear mixed model with repeated measures. Time was modeled as a fixed effect with plate (plate relevant only for cytokine and chemokine analysis) and sheep ID as random effects. Repeated measures were accounted for by fitting autocorrelation structures. The Akaike information criterion was used as a guide for the best-fitting model. The assumptions of the model were checked by examining the residuals for normality after passing through the model with a Shapiro-Wilk test, and data were log-transformed when necessary to meet the assumptions of normality. Specifically, the data that required a log transformation were IL-1 α , IL-6, IFN- γ , TNF- α , IL-10, CCL2, CCL3, cortisol, miR-145, miR-223, and miR-1246. Post hoc tests were used on a Dunnett adjustment, and significance was set at $P \leq 0.05$. Logged data were back-transformed showing geometric means, and untransformed data were represented as the arithmetic mean, with both upper and lower 95% confidence limits.

The present study demonstrated that a low systemic dose of LPS elicited a strong stress response as indicated by the induction of numerous circulatory pro- and anti-inflammatory cytokines and chemokines, cortisol, and miRNA. The LPS challenge also elicited a monophasic fever response as reported previously (Kabaroff et al., 2006) that peaked at 3 h after LPS challenge, with temperature approaching baseline levels by 6 h (Figure 1A).

Fever is considered a cardinal response to infection (Evans et al., 2015) and is thought to be driven by some proinflammatory cytokines, particularly IL-1, IL-6, and TNF- α . Given this, it is not surprising that TNF- α displayed a significant response over time ($P < 0.01$), peaking at 2 h and then returning to baseline by 6 h (Figure 1C), and an early increase in IL-6 was observed at 2 h and plateaued around 4 h before beginning to decrease (Figure 1D). Early induction of TNF- α is associated with increased blood flow to sites of infection, signaling endothelial cells to produce chemokines, and the recruitment of leukocytes (mainly neutrophils) to potentiate the innate immune response (Waters et al., 2013). Though generally considered proinflammatory, IL-6 can have anti-inflammatory functions depending on which IL-6 receptor is involved (Kaplanski et al., 2003).

Although the IL-1 family of cytokines is thought to have a primary role in innate immunity and inflamma-

tion, it was interesting to observe that IL-1 α (Figure 1E) and IL-1 β (data not shown) concentrations did not change over time ($P > 0.05$). As a soluble mediator, IL-1 α can be classified as an alarmin that is released from necrotic cells (Di Paolo and Shayakhmetov, 2016); it is possible that the low LPS dose used in this study did not induce necroptosis. Although other studies have detected IL-1 β responses in sheep to either a high-dose LPS infusion or a lethal dose of live *E. coli*, large variation was observed in these studies, and it was determined that TNF- α and IL-6 had more consistent responses (Peake et al., 2002; Byrne et al., 2018). In addition to the IL-1, IL-17 α (data not shown) and IL-4 (Figure 1H) did not have an observable response over time ($P > 0.05$).

Interferon- γ , known for driving T-helper 1 cell and inhibiting T-helper 2 cell differentiation (Deng et al., 2012), also responded to LPS challenge ($P < 0.01$) and approached baseline by 6 h (Figure 1F). It is well known that LPS induces IFN- γ production (Varma et al., 2002). Interferon- γ also promotes the differentiation of monocytes to macrophages rather than dendritic cells (Delneste et al., 2003) and enhances macrophage production of TNF- α and IL-6 (Chen and Ivashkiv, 2010).

Interleukin-10 is a major anti-inflammatory cytokine that controls immune cell differentiation and the production of proinflammatory cytokines by activated macrophages and antigen-presenting cells (Murray, 2005). We observed an IL-10 response ($P < 0.01$) that peaked later than most proinflammatory cytokines, around 4 h, and approached baseline by 6 h (Figure 1G).

Chemokines mediate immune cell trafficking and interactions during the immune response (Sokol and Luster, 2015), and we found that all chemokines measured (CCL2, CCL3, CCL4, IL-8, and CXCL10) displayed a response over time ($P < 0.01$) to LPS challenge. Chemokines CCL3, CCL4, and CCL2 peaked around 2 h (Figure 2A–C), and CXCL10 had a relatively more gradual increase over time, peaking around 4 h (Figure 2D). Last, IL-8 (CXCL8) was different from baseline only at 6 h, where levels decreased ($P < 0.01$; Figure 2E). Also known as monocyte chemoattractant protein 1, CCL2 is known for promoting infiltration of macrophages and monocytes by acting through the CCR2 receptor with high affinity (Lu et al., 1998). However, CCL3 can also bind this receptor; CCL3 has been shown to mediate neutrophil migration during inflammation (Ramos et al., 2005), and its contribution to alveolar macrophage activation infection has been previously demonstrated (Lindell et al., 2001). Although CCL4 acts on the same CC receptors as CCL3 (Jing et al., 2003), one study

found that activated T cells and natural killer cells were preferentially recruited by this chemokine (Megjugorac et al., 2004). In a canine endotoxemia study, CXCL10 was found to be upregulated in the heart, lung, kidney, liver, and spleen and was associated with T lymphocyte infiltration (Frangogiannis et al., 2000). The chemokine IL-8 has been shown to selectively recruit neutrophils during *E. coli*-induced mastitis in sheep (Gangur et al., 2002). The decreased IL-8 levels at 6 h in our study could indicate a transition from an initial neutrophil influx to a mononuclear leukocyte population.

Expression of miR-145, miR-1246, and miR-223 was upregulated ($P < 0.05$) during the 6-h period after LPS

challenge and displayed high variation during peak expression. The increase in expression of MiR-145 was delayed and began at 6 h ($P < 0.05$; Figure 3A); MiR-1246 expression increased from 4 to 6 h (Figure 3B), and miR-223 expression increased at 4 h before plateauing (Figure 3C). Given that miRNA target mRNA, it is not surprising that they have important regulatory roles in immune cell development and immune responses. For example, MiR-223 has been found to be upregulated during granulopoiesis, leading to enhanced granulocyte differentiation (Fazi et al., 2005). However, another study involving miR-223 knockout mice found miR-223 to be a negative regulator of granulocyte differentiation

Figure 1. (A) Rectal temperature, (B) serum cortisol, (C) tumor necrosis factor (TNF)- α , (D) IL-6, (E) IL-1 α , (F) IFN- γ , (G) IL-10, and (H) IL-4 responses in female sheep ($n = 15$) following intravenous LPS (400 ng/kg) challenge. Results are reported as geometric means if back transformed or the mean, with an upper and lower 95% confidence limit. An asterisk (*) represents $P < 0.05$ and a double asterisk (**) represents $P \leq 0.01$ compared with the time-zero baseline.

and activation; knockout mice had increased numbers of hyperactive granulocytes, causing excessive tissue damage (Johnnidis et al., 2008). In addition to being upregulated in the serum of heat-stressed Holstein cows, miR-1246 has been described as having a role during LPS-induced inflammation (Wu et al., 2017), and further enrichment analysis identified target genes related to the stress response and immune system development and response (Zheng et al., 2014). Another study assessing miRNA expression in Holstein mammary tissue during heat stress found miR-145 to be among the highest differentially expressed miRNA (Li et al., 2018a). Overexpression of miR-145 has also been found to promote the expression of TNF- α and CCL4 (Li et al., 2018b). However, we observed opposite profiles between these factors; the miRNA we measured were upregulated toward the end of the monitoring period, suggesting that they could be involved in resolution of acute inflammation.

As anticipated, cortisol levels increased in response to LPS ($P < 0.01$) and peaked around 2 h before returning to baseline by the end of the sampling period; we observed the highest variation at 2 and 4 h after LPS challenge (Figure 1B). The induction of cortisol

is consistent with the strong proinflammatory cytokine response, particularly IL-6, because this cytokine can stimulate cortisol release at the level of the adrenal gland (Bethin et al., 2000) and the brain (Girotti et al., 2013). Cortisol helps control the inflammatory response by preventing transcription of proinflammatory cytokines and promoting the production of anti-inflammatory cytokines (Tian et al., 2014).

To date, this is the most comprehensive assessment of the ovine immune, stress, and miRNA responses to systemic LPS challenge. Collectively, a low dose of LPS induced a strong inflammatory response that almost resolved within the 6-h monitoring period based on the recovery of fever. The inflammatory response can be detected through the measurement of immune and stress biomarkers IL-6, TNF- α , IFN- γ , IL-10, CCL2, CCL3, CCL4, CXCL10, miR-145, miR-1246, miR-223, and cortisol. However, IL-1 β , IL-1 α , IL-17 α , and IL-4 did not have an observable response over time. Cytokine profiles and, more recently, miRNA are associated with disease susceptibility, and the high variation we observed in the responses of some of these measured variables could be investigated further to determine what contributes to this variation and whether it is

Figure 2. Serum (A) CCL3, (B) CCL4, (C) CCL2, (D) CXCL10, and (E) IL-8 chemokine responses in female sheep ($n = 15$) following intravenous LPS (400 ng/kg) challenge. Results are reported as geometric means if back transformed or the mean, with an upper and lower 95% confidence limit. An asterisk (*) represents $P < 0.05$ and a double asterisk (**) represents $P \leq 0.01$ compared with the time-zero baseline. MFI = median fluorescence intensity.

Figure 3. Fold-change expression of (A) microRNA (miR)-145, (B) miR-1246, and (C) miR-223 in female sheep ($n = 15$) following intravenous LPS (400 ng/kg) challenge. Results are reported as geometric means with an upper and lower 95% confidence limit. Fold-change expression is reported relative to baseline levels and normalized to the spike-in control CE-MIR-39-1. An asterisk (*) represents $P < 0.05$ and a double asterisk (**) represents $P \leq 0.01$ compared with the time-zero baseline.

associated with stress resiliency. Considering that the miRNA transcriptome of sheep contains thousands of miRNA, future work should evaluate a larger panel of miRNA. Additionally, immune cell populations that are involved in LPS recognition and the initiation of the inflammatory response should be evaluated, which could help verify some functional capabilities of the measured chemokines. Last, cortisol responsiveness could be evaluated further as it is critical in the resolution of the inflammatory response, which is necessary for stress resiliency.

ACKNOWLEDGMENTS

We thank Pam Hasson and the staff at the Ponsonby Research Station (Ponsonby, ON, Canada) for animal handling assistance and Gabrielle Monteith (University of Guelph, Guelph, ON, Canada) for statistical analysis support. This research was financially supported by the University of Guelph's Food from Thought research program (Guelph, ON, Canada), funded by the Canada First Research Excellence Fund (Ottawa, ON, Canada), the Ontario Ministry of Agriculture and Food (Guelph, ON, Canada), and the MITACS Elevate program (Toronto, ON, Canada). The authors have not stated any conflicts of interest.

REFERENCES

- Abaker, J. A., T. L. Xu, D. Jin, G. J. Chang, K. Zhang, and X. Z. Shen. 2017. Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. *J. Dairy Sci.* 100:666–678. <https://doi.org/10.3168/jds.2016-10871>.
- Bethin, K. E., S. K. Vogt, and L. J. Muglia. 2000. Interleukin-6 is an essential, corticotropin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. *Proc. Natl. Acad. Sci. USA* 97:9317–9322. <https://doi.org/10.1073/pnas.97.16.9317>.
- Bilal, M. S., J. A. Abaker, Z. ul Aabdin, T. Xu, H. Dai, K. Zhang, X. Liu, and X. Shen. 2016. Lipopolysaccharide derived from the digestive tract triggers an inflammatory response in the uterus of mid-lactating dairy cows during SARA. *BMC Vet. Res.* 12:284. <https://doi.org/10.1186/s12917-016-0907-1>.
- Breen, E. J., W. Tan, and A. Khan. 2016. The statistical value of raw fluorescence signal in luminex xMAP based multiplex immunoassays. *Sci. Rep.* 6:26996. <https://doi.org/10.1038/srep26996>.
- Byrne, L., N. G. Obonyo, S. Diab, K. Dunster, M. Passmore, A. C. Boon, L. S. Hoe, K. Hay, F. Van Haren, L. Tung, L. Cullen, K. Shekar, K. Maitland, and J. F. Fraser. 2018. An ovine model of hyperdynamic endotoxemia and vital organ metabolism. *Shock* 49:99–107. <https://doi.org/10.1097/SHK.0000000000000904>.
- Caroprese, M., M. Albenzio, A. Marzano, L. Schena, G. Annicchiarico, and A. Sevi. 2010. Relationship between cortisol response to stress and behavior, immune profile, and production performance of dairy ewes. *J. Dairy Sci.* 93:2395–2403. <https://doi.org/10.3168/jds.2009-2604>.
- Chen, J., and L. B. Ivashkiv. 2010. IFN- γ abrogates endotoxin tolerance by facilitating Toll-like receptor-induced chromatin remodeling. *Proc. Natl. Acad. Sci. USA* 107:19438–19443. <https://doi.org/10.1073/pnas.1007816107>.

- Croghan, C., and P. P. Egeghy. 2003. Methods of dealing with values below the limit of detection using SAS. Presented at Southeastern SAS User Group, St. Petersburg, FL.
- Dai, R., and S. A. Ahmed. 2011. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. *Transl. Res.* 157:163–179. <https://doi.org/10.1016/j.trsl.2011.01.007>.
- Delneste, Y., P. Charbonnier, N. Herbault, G. Magistrelli, G. Caron, J. Y. Bonnefoy, and P. Jeannin. 2003. Interferon- γ switches monocyte differentiation from dendritic cells to macrophages. *Blood* 101:143–150. <https://doi.org/10.1182/blood-2002-04-1164>.
- Deng, S., Q. Wu, K. Yu, Y. Zhang, Y. Yao, W. Li, Z. Deng, G. Liu, W. Li, and Z. Lian. 2012. Changes in the relative inflammatory responses in sheep cells overexpressing of Toll-like receptor 4 when stimulated with LPS. *PLoS One* 7:e47118. <https://doi.org/10.1371/journal.pone.0047118>.
- Di Paolo, N. C., and D. M. Shayakhmetov. 2016. Interleukin 1 α and the inflammatory process. *Nat. Immunol.* 17:906–913. <https://doi.org/10.1038/ni.3503>.
- Evans, S. S., E. A. Repasky, and D. T. Fisher. 2015. Fever and the thermal regulation of immunity: The immune system feels the heat. *Nat. Rev. Immunol.* 15:335–349. <https://doi.org/10.1038/nri3843>.
- Fazi, F., A. Rosa, A. Fatica, V. Gelmetti, M. L. De Marchis, C. Nervi, and I. Bozzoni. 2005. A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBP α regulates human granulopoiesis. *Cell* 123:819–831. <https://doi.org/10.1016/j.cell.2005.09.023>.
- Frangogiannis, N. G., L. H. Mendoza, C. W. Smith, L. H. Michael, and M. L. Entman. 2000. Induction of the synthesis of the C-X-C chemokine interferon-gamma-inducible protein-10 in experimental canine endotoxemia. *Cell Tissue Res.* 302:365–376. <https://doi.org/10.1007/s004410000274>.
- Gangur, V., N. P. Birmingham, and S. Thanavorakul. 2002. Chemokines in health and disease. *Vet. Immunol. Immunopathol.* 86:127–136. [https://doi.org/10.1016/s0165-2427\(02\)00018-1](https://doi.org/10.1016/s0165-2427(02)00018-1).
- Girotti, M., J. J. Donegan, and D. A. Morilak. 2013. Influence of hypothalamic IL-6/gp130 receptor signaling on the HPA axis response to chronic stress. *Psychoneuroendocrinology* 38:1158–1169. <https://doi.org/10.1016/j.psyneuen.2012.11.004>.
- Hadfield, J. M., E. C. Bowdridge, I. Holásková, T. H. Elsasser, and R. A. Dailey. 2018. Breed-specific differences in the immune response to lipopolysaccharide in ewes. *J. Anim. Sci.* 96:4220–4228. <https://doi.org/10.1093/jas/sky288>.
- Herry, V., C. Gitton, G. Tabouret, M. Répérant, L. Forge, C. Tasca, F. B. Gilbert, E. Guitton, C. Barc, C. Staub, D. G. E. Smith, P. Germon, G. Foucras, and P. Rainard. 2017. Local immunization impacts the response of dairy cows to *Escherichia coli* mastitis. *Sci. Rep.* 7:3441. <https://doi.org/10.1038/s41598-017-03724-7>.
- Jing, H., E. Vassiliou, and D. Ganea. 2003. Prostaglandin E2 inhibits production of the inflammatory chemokines CCL3 and CCL4 in dendritic cells. *J. Leukoc. Biol.* 74:868–879. <https://doi.org/10.1189/jlb.0303116>.
- Johnnidis, J. B., M. H. Harris, R. T. Wheeler, S. Stehling-Sun, M. H. Lam, O. Kirak, T. R. Brummelkamp, M. D. Fleming, and F. D. Camargo. 2008. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature* 451:1125–1129. <https://doi.org/10.1038/nature06607>.
- Kabaroff, L. C., A. Rodriguez, M. Quinton, H. Boermans, and N. A. Karrow. 2006. Assessment of the ovine acute phase response and hepatic gene expression in response to *Escherichia coli* endotoxin. *Vet. Immunol. Immunopathol.* 113:113–124. <https://doi.org/10.1016/j.vetimm.2006.04.003>.
- Kaplanski, G., V. Marin, F. Montero-Julian, A. Mantovani, and C. Farnarier. 2003. IL-6: A regulator of the transition from neutrophil to monocyte recruitment during inflammation. *Trends Immunol.* 24:25–29. [https://doi.org/10.1016/s1471-4906\(02\)00013-3](https://doi.org/10.1016/s1471-4906(02)00013-3).
- Karrow, N. A. 2006. Activation of the hypothalamic–pituitary–adrenal axis and autonomic nervous system during inflammation and altered programming of the neuroendocrine–immune axis during fetal and neonatal development: Lessons learned from the model inflammation, lipopolysaccharide. *Brain Behav. Immun.* 20:144–158. <https://doi.org/10.1016/j.bbi.2005.05.003>.
- Li, Q., C. Yang, J. Du, B. Zhang, Y. He, Q. Hu, M. Li, Y. Zhang, C. Wang, and J. Zhong. 2018a. Characterization of miRNA profiles in the mammary tissue of dairy cattle in response to heat stress. *BMC Genomics* 19:975. <https://doi.org/10.1186/s12864-018-5298-1>.
- Li, R., C.-L. Zhang, X.-X. Liao, D. Chen, W.-Q. Wang, Y.-H. Zhu, X.-H. Geng, D.-J. Ji, Y.-J. Mao, Y.-C. Gong, and Z.-P. Yang. 2015. Transcriptome microRNA profiling of bovine mammary glands infected with *Staphylococcus aureus*. *Int. J. Mol. Sci.* 16:4997–5013. <https://doi.org/10.3390/ijms16034997>.
- Li, S., W. Sun, H. Zheng, and F. Tian. 2018b. MicroRNA-145 accelerates the inflammatory reaction through activation of NF- κ B signaling in atherosclerosis cells and mice. *Biomed. Pharmacother.* 103:851–857. <https://doi.org/10.1016/j.biopha.2018.03.173>.
- Lindell, D. M., T. J. Standiford, P. Mancuso, Z. J. Leshen, and G. B. Huffnagle. 2001. Macrophage inflammatory protein 1 α /CCL3 is required for clearance of an acute *Klebsiella pneumoniae* pulmonary infection. *Infect. Immun.* 69:6364–6369. <https://doi.org/10.1128/IAI.69.10.6364-6369.2001>.
- Lu, B., B. J. Rutledge, L. Gu, J. Fiorillo, N. W. Lukacs, S. L. Kunkel, R. North, C. Gerard, and B. J. Rollins. 1998. Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *J. Exp. Med.* 187:601–608. <https://doi.org/10.1084/jem.187.4.601>.
- Megjugorac, N. J., H. A. Young, S. B. Amrute, S. L. Olshalsky, and P. Fitzgerald-Bocarsly. 2004. Virally stimulated plasmacytoid dendritic cells produce chemokines and induce migration of T and NK cells. *J. Leukoc. Biol.* 75:504–514. <https://doi.org/10.1189/jlb.0603291>.
- Murray, P. J. 2005. The primary mechanism of the IL-10-regulated anti-inflammatory response is to selectively inhibit transcription. *Proc. Natl. Acad. Sci. USA* 102:8686–8691. <https://doi.org/10.1073/pnas.0500419102>.
- Peake, S. L., J. Pierides, P. I. Leppard, and G. R. Russ. 2002. Anti-ovine interleukin-1 β monoclonal antibody immunotherapy in an ovine model of gram-negative septic shock. *Crit. Care Med.* 30:171–181. <https://doi.org/10.1097/00003246-200201000-00025>.
- Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 29:e45. <https://doi.org/10.1093/nar/29.9.e45>.
- Ramos, C. D. L., C. Canetti, J. T. Souto, J. S. Silva, C. M. Hogaboam, S. H. Ferreira, and F. Q. Cunha. 2005. MIP-1 α [CCL3] acting on the CCR1 receptor mediates neutrophil migration in immune inflammation via sequential release of TNF- α and LTB $_4$. *J. Leukoc. Biol.* 78:167–177. <https://doi.org/10.1189/jlb.0404237>.
- Rojas-Downing, M. M., A. P. Nejadhashemi, T. Harrigan, and S. A. Woznicki. 2017. Climate change and livestock: Impacts, adaptation, and mitigation. *Clim. Risk Manage.* 16:145–163. <https://doi.org/10.1016/j.crm.2017.02.001>.
- Sevi, A., and M. Caroprese. 2012. Impact of heat stress on milk production, immunity and udder health in sheep: A critical review. *Small Rumin. Res.* 107:1–7. <https://doi.org/10.1016/j.smallrumres.2012.07.012>.
- Sokol, C. L., and A. D. Luster. 2015. The chemokine system in innate immunity. *Cold Spring Harb. Perspect. Biol.* 7:a016303. <https://doi.org/10.1101/cshperspect.a016303>.
- Taganov, K. D., M. P. Boldin, K. J. Chang, and D. Baltimore. 2006. NF- κ B-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc. Natl. Acad. Sci. USA* 103:12481–12486. <https://doi.org/10.1073/pnas.0605298103>.
- Tian, R., G. Hou, D. Li, and T. F. Yuan. 2014. A possible change process of inflammatory cytokines in the prolonged chronic stress and its ultimate implications for health. *ScientificWorldJournal* 2014:780616. <https://doi.org/10.1155/2014/780616>.
- Vargas, N., and F. Marino. 2016. Heat stress, gastrointestinal permeability and interleukin-6 signaling—Implications for exercise per-

- formance and fatigue. *Temperature (Austin)* 3:240–251. <https://doi.org/10.1080/23328940.2016.1179380>.
- Varma, T. K., C. Y. Lin, T. E. Toliver-Kinsky, and E. R. Sherwood. 2002. Endotoxin-induced gamma interferon production: Contributing cell types and key regulatory factors. *Clin. Diagn. Lab. Immunol.* 9:530–543. <https://doi.org/10.1128/cdli.9.3.530-543.2002>.
- Waters, J. P., J. S. Pober, and J. R. Bradley. 2013. Tumour necrosis factor in infectious disease. *J. Pathol.* 230:132–147. <https://doi.org/10.1002/path.4187>.
- Wu, D. P., J. L. Zhang, J. Y. Wang, M. X. Cui, J. L. Jia, X. H. Liu, and Q. D. Liang. 2017. MiR-1246 promotes LPS-induced inflammatory injury in chondrogenic cells ATDC5 by targeting HNF4 γ . *Cell. Physiol. Biochem.* 43:2010–2021. <https://doi.org/10.1159/000484162>.
- Zhang, S., F. Zhao, C. Wei, X. Sheng, H. Ren, L. Xu, J. Lu, J. Liu, L. Zhang, and L. Du. 2013. Identification and characterization of the miRNA transcriptome of *Ovis aries*. *PLoS One* 8:e58905. <https://doi.org/10.1371/journal.pone.0058905>.
- Zheng, Y., K. Chen, X. Zheng, H. Li, and G. Wang. 2014. Identification and bioinformatics analysis of microRNAs associated with stress and immune response in serum of heat-stressed and normal Holstein cows. *Cell Stress Chaperones* 19:973–981. <https://doi.org/10.1007/s12192-014-0521-8>.

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