Journal of Animal and Feed Sciences, 25, 2016, 283–291 https://doi.org/10.22358/jafs/67366/2016
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

How does bacterial endotoxin influence gonadoliberin/gonadotropins secretion and action?

D. Tomaszewska-Zaremba[1], A.P. Herman and K. Haziak

The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences
Instytucka 3, 05–110 Jabłonna, Poland

KEY WORDS: lipopolysaccharide, gonadoliberin, luteinizing hormone, cytokines, TLR4/TLR2 receptors, reproduction

Received: 8 December 2015
Revised: 29 August 2016
Accepted: 25 November 2016

[3] Corresponding author:
e-mail: d.tomaszewska@ifzz.pl

ABSTRACT. This review summarizes data concerning the mechanisms by which bacterial endotoxin, lipopolysaccharide (LPS), inhibits gonadoliberin (GnRH)/luteinizing hormone (LH) secretion in mammals. LPS is a major component of Gram-negative bacteria cell walls and is released from the surface of replicated and dying Gram-negative bacteria into circulation. LPS is commonly used to induce immune/inflammatory challenge in animals. In this article the site of endotoxin action as well as LPS induced mediators are discussed. Hypothalamus seems to be a place where majority of the immune-neuroendocrine interactions occur, however the results of many research suggest that LPS may interfere with reproductive system at the pituitary level as well. Endotoxin may affect GnRH/LH secretion directly *via* toll-like receptors (TLR)4/TLR2 located both in the hypothalamus and pituitary or indirectly through the intermediates such as cytokines, catecholamines, prostaglandins or opioids.

Introduction

The development and survival of an organism depends on many external and internal factors. Among them are: environmental conditions, the availability of food, the ability to prevent invasion of other potentially pathogenic organisms, and well-functioning reproductive system, which is possible only with perfect interaction with the immune system. There is an evidence that impaired reproductive functioning may accompany bacterial and viral infections or autoimmune diseases. The ongoing inflammation in the body affects the activity of the hypothalamic-pituitary-gonadal (HPG) axis, causing disturbances in female reproductive health ranging from disruption of cyclicity (Peter et al., 1989; Battaglia et al., 2000) to loss of pregnancy (Schlafer et al., 1994).

Most experimental systems studying the impact of an immune challenge on reproductive functions commonly use bacterial endotoxin: lipopolysaccharide (LPS), as a model of immune stress without necessity of infecting the animal with active pathogen. LPS is a major component of Gram-negative bacteria cell walls and is released from the surface of replicating and dying Gram-negative bacteria into the circulation (Rietschel et al., 1994). It is known that LPS is responsible for many clinical symptoms of sepsis. It stimulates monocytes and macrophages to produce large amounts of proinflammatory mediators like tumor necrosis factor alpha (TNFα), interleukin (IL)-1β and IL-6 (Givalois et al., 1994). Bacterial endotoxin action occurs *via* toll-like receptor (TLR) type 2 and 4. TLRs are part of a group of innate immunity receptors, known as 'pattern-recognition receptors' (PRR) that recognize

structural motifs characteristic of bacteria, viruses and fungi, called pathogen-associated molecular patterns (PAMPs) (Kumar et al., 2009). A transmembrane TLR4 is one of the recognized receptors from the TLRs family, and it is considered to be pivotal LPS-interacting receptor. An exogenous ligand of TLR4 is not only LPS, but is also a protein of a respiratory syncytial virus (RSV) (Majewska and Szczepanik, 2006). Apart from this, TLR4 recognizes some endogenous ligands such as heat shock proteins (HSP)60 and (HSP)70, fibrinogen, fibronectin, surfactant protein-A (SP-A), resistin and endoplazmin (Yu et al., 2010). TLR4 is present on the immune cells, such as monocytes, macrophages, neutrophils, mast cells and lymphocytes B (Majewska and Szczepanik, 2006). The presence of TLR4 was also found on non-immune cells like epithelial cells of the gut and respiratory tract, adipocytes, endothelial cells, decidual cells at the blastocyst implantation site of mice and hamsters (Janeway et al., 2001), microglial cells and even neurons (Chakravarty and Herkenham, 2005). Endotoxin stimulation of cells occurs through signalling cascades with several proteins including cluster of differentiation (CD)14 protein, lymphocyte antigen 96 MD-2 protein, and soluble acute-phase protein – LPS-binding protein (LBP). In the blood stream, circulating LBP binds LPS and, LPS-LBP complex binds to the CD14 protein, which is necessary for the activation of TLR4. However, to function as an LPS receptor, TLR4 requires a helper molecule MD-2 that is associated with TLR4 on the cell surface and enables TLR4 to respond to LPS (Dziarski et al., 2001; Fitzgerald et al., 2004). It is worth mentioning that events following the formation of this receptor complex depend on different sets of adapters. An early response, which is dependent on myeloid differentiation primary response gene 88 (MyD88) and MyD88-like adapter (Mal), leads to the activation of nuclear factor-κB (NF-κB). A later response to LPS makes use of Toll/IL-1 receptor (TIR) domain-containing adapter protein inducing interferon β (TRIF) and TRIF-related adapter molecule (TRAM), and leads to the late activation of NF-κB and interferon regulatory factor 3 (IRF3), and to the induction of cytokines, chemokines and other transcription factors (Pålsson-McDermott and O'Neill, 2004). However, not only TLR4 but also TLR2 is involved in the mediation of LPS-induced cellular signalling. TLR2 is activated by LPS in a response that depends on LBP and is enhanced by CD14 (Yang et al., 1998). A unique feature of TLR2 is its ability to interact with other TLRs (TLR1 and TLR6) to form heterodimers which discriminate between different bacterial lipoproteins. These cooperative interactions expand the structural array of pathogen-derived components recognized by TLR2 and enhance ligand-induced TLR2 cell activation (Good et al., 2012). In the studies using an enzyme complementation assay and co-immunoprecipitation to examine TLR dimerization in transfected cells it was found that TLR2 was also capable to interact with TLR4 (Lee et al., 2004). However, the possible biological and functional significance of a TLR2-TLR4 association is poorly understood. The studies on rats show that TLR4 associates with TLR2 in the renal medullary thick ascending limb (MTAL) and these two receptors interact functionally in the basolateral membrane of MTAL cells to mediate LPS-induced extracellular signal-regulated kinase (ERK) activation. It is suggested that TLR2 may play a dual role in bacterial recognition and the induction of cell signals both through cooperative interaction with TLR4 to mediate ERK activation by Gram-negative LPS and through a TLR4-independent signalling pathway activated by Gram-positive cell wall molecules (Good et al., 2012). It should be mentioned that TLR2, in contrast to TLR4, is not essential for LPS signalling because TLR2-deficient cells that expressed TLR4 exhibit normal responses to enterobacterial LPS (Heine et al., 1999). On the other hand, regulation of TLR2 expression and its interaction with TLR4 may provide new mechanisms for controlling TLR4-mediated LPS responses (Good et al., 2012). However, the role of TLR2 in mediating actions of LPS is still controversial.

The results of our earlier experimental works indicate the existence of multiple mechanisms that mediate the inhibitory effect of immune stress induced by bacterial endotoxin on reproductive processes. The network of interactions between reproductive and immune systems is complicated and specific for each level of the HPG axis, i.e. hypothalamus, pituitary and gonads (Tomaszewska-Zaremba and Herman, 2009). In this article we will focus on the data concerning the pathways of LPS action on the GnRH/LH secretion at the level of the hypothalamus and the pituitary gland.

Indirect effect of LPS on GnRH/LH secretion

The results of previous studies have shown the existence of several mechanisms that mediate the immune inhibitory effect of inflammation on repro-

ductive processes. The most probable hypothesis assumes that this effect could be mediated by proinflammatory cytokines (Tomaszewska-Zaremba and Herman, 2009). It has been shown that the inflammation associated with the administration of LPS caused an increase in the concentration of proinflammatory cytokines such as TNFα, IL-1β and IL-6 in the peripheral blood (Givalois et al., 1994). These cytokines are also present in the human and rabbit cerebrospinal fluid (CSF) during inflammatory condition (Waage et al., 1989; Fassbender et al., 1996). This may suggest that proinflammatory cytokines can affect the neuroendocrine system at the level of the brain. The hypothalamus is a structure believed to be a critical site in the suppression of reproductive axis during immune challenge. The most of brain's responses to LPS have been attributed to the action of cytokines. The results of our previous study carried out on sheep showed that these three cytokines are synthesized directly in the hypothalamic area not only during inflammation but also during homeostasis milieu (Herman et al., 2014). This suggests that these proinflammatory cytokines may play a role of important regulators of neuroendocrine system also during physiological condition. Especially IL-1β is considered as an essential negative regulator of the HPG axis activity at the level of the hypothalamus in rat (Kang et al., 2000). Also, in our previous studies on anoestrous ewes we have demonstrated that IL-1β is produced in the hypothalamic structures and its synthesis is stimulated by an intravenous administration of LPS (Herman et al., 2010). Moreover, the administration of IL-1β into the third ventricle of the brain modulated secretory activity of GnRH neurons in anoestrous ewes (Herman et al., 2012). The potency of IL-1β to affect the neuroendocrine system may result from their widespread corresponding receptors in the brain tissue.

In the study on anoestrous ewes we demonstrated that the IL-1 receptor (IL-1R) mRNA is expressed in the hypothalamic structures involved in the GnRH-ergic activity and this expression is enhanced during an inflammation (Herman et al., 2010) that further suggests that IL-1β may be an important modulator of GnRH secretion. Moreover, *in vitro* studies showed that IL-1 receptors were present in the membrane of immortalized GnRH neurons – the Gnv-4 cell line (Igaz et al., 2006). This finding suggests that GnRH neurons may possess the cellular machinery to respond directly to IL-1β stimulation, which may directly affect the GnRH secretion in these cells. On the other hand, it was revealed that proinflammatory cytokines may influence the hypothalamic GnRH synthesis and release by promoting other central processes, which inhibit GnRH secretion. Indirect effects of IL-1β on GnRH neurons can be achieved with the participation of endogenous opioids (EOP). In experiments conducted on rats it was shown that central administration of IL-1β inhibited the release of GnRH/LH by the stimulation of the activity of the endogenous opioid system (Kalra et al., 1990). It has been shown that IL-1β produced centrally in response to inflammation induced synthesis of EOP in the hypothalamus of rats (Kalra et al., 1998). IL-1β could suppress GnRH secretion indirectly by inducing changes in the level of catecholamines. The changes in noradrenaline and dopamine release could be one of the indirect mechanisms modulating GnRH secretion *via* central IL-1β. It is known that catecholamines play an important role in the regulation of GnRH secretion (Kochman et al., 2007). It can be assumed that IL-1β could suppress gonadoliberin secretion by inducing changes in the level of the noradrenaline and dopamine. In the study performed on cyclic rats, intraperitoneal IL-1β treatment blocked the LH surge and the rise in noradrenaline release in medial preoptic area (Sirivelu et al., 2009). On the other hand, in our studies on ewes no effects of intracerebroventricular (icv) IL-1β administration on catecholamine level in hypothalamic structures was observed (Tomaszewska et al., 2013). The other pathway *via* IL-1β may affect GnRH secretion is its influence on neuropeptide Y (NPY) synthesis in the hypothalamus. In our earlier experiment on anoestrous ewes it was demonstrated that, this cytokine decreased the gene expression of NPY in the hypothalamus (Tomaszewska et al., 2013). Peripherally given LPS can induce an answer at the central level also by a mechanism associated with prostaglandins (PGs). Harris et al. (2000) showed that PGs mediated the inhibitory effect of immune stress on the pulsatile secretion of GnRH/LH in sheep. Moreover, the inhibition of PGs synthesis by administration of cyclooxygenase 2 (COX-2) selective inhibitor completely abolished suppression of GnRH/LH secretion caused by prolonged LPS administration in ewes (Herman et al., 2016). It is postulated that one of the mechanisms *via* IL-1β suppresses GnRH secretion could be the induction of inflammatory mediators such as PGs (Tomaszewska-Zaremba and Herman, 2009). *Ex vivo* experiments carried out on medial basal hypothalamus (MBH) explants collected from rats have shown that suppression of the prostaglandin E_2 (PGE_2) release stimulated by IL-1 inhibited the release of GnRH/LH (Rettori et al., 1991).

Despite that most studies support the key role of the hypothalamus in the suppression of reproductive processes during an immune/inflammatory challenge, it couldn't be excluded that observed effect of LPS treatment on the reproductive system could partially result from the action of proinflammatory cytokines at the level of pituitary. The presence of IL-type I receptor (IL-1R1) in the pituitary (Parnet et al., 1993) supports this hypothesis. It was shown that folliculostellate cells in the pituitary gland are the source as well as the target of numerous cytokines such as IL-1, IL-6 and TNFα (Bilezikjian et al., 2003; Meilleur et al., 2007). It was reported that LPS stimulated the folliculostellate cells to release cytokines (Lohrer et al., 2000). It is possible that these cytokines could affect in the paracrine way the secretion of LH from the pituitary gonadotropes. On the other hand, there is an evidence that IL-1 profoundly alters the secretion of several pituitary hormones – IL-1β stimulated LH release *in vitro* from dispersed mice anterior pituitary fragments (Bernton et al., 1987). In other experiments conducted on cultured rat anterior pituitary cells IL-1β inhibited GnRH-stimulated secretion of follicle-stimulating hormone (FSH) but enhanced LH release (Murata and Ying, 1991). In *in vitro* study on the cells from ovine pituitary gland, Braden et al. (1998) showed that IL-1β and IL-1α stimulated the release of LH. However, the results of our *ex vivo* study on the ovine pituitary explants showed that IL-1β rather down-regulated LH secretion from the pituitary gland (Herman et al., 2013b). This suggests that circulating proinflammatory cytokines, particularly IL-1β released during an inflammatory condition, may directly affect LH secretion from the anterior pituitary cells and this direct mechanism may accompany the processes occurring at the level of hypothalamus.

Direct action of LPS on the GnRH/LH secretion

The blood-brain barrier and the blood-cerebrospinal fluid barrier

In general, brain parenchyma is protected from the penetration of pathogens and uncontrolled influx of inflammatory mediators due to the presence of brain barriers. Two barrier layers limit and regulate the exchange of molecules at the interface between the blood and brain tissue and its fluid spaces: the blood-brain barrier (BBB) between blood and interstitial fluid surrounding the neural tissue, and the blood-cerebrospinal fluid (CSF) barrier (BCSFB) that consists the internally situated choroid plexus and the externally located arachnoid membrane (Skipor and Thiery, 2008). The BBB effectively protects the brain from bacterial infections that occur in the brain very rarely. However, there are areas of the brain deprived of the BBB. It does not occur in the region of the pituitary gland and around the choroid plexus and vascular organ of *lamina terminalis*. It is suspected that circulating LPS interferes with the TLRs localized on the cells forming these barriers and that activated TLRs play an important role in transferring an inflammatory signal from periphery to the brain (Mallard, 2012). Laflamme and Rivest (2001) using *in situ* hybridization technique provided evidence that mRNA for TLR4 is present in the circumventricular organs, choroid plexus and leptomeninges. Moreover, based on Real-Time polymerase chain reaction (PCR) assay Skipor et al. (2015) determined that not only mRNA encoding TLR4 is expressed in the choroid plexus but also transcript for other TLRs, except TLR8, is expressed in this structure, which suggests that the BCSFB may play a pivotal role in the immune-neuroendocrine interaction. The choroid plexus is easily reached by molecules present in the bloodstream and therefore in systemic infection is early exposed to pathogens or their derived molecules (Mishra et al., 2006). Disturbance of the BCSFB in association with infectious and endogenous, non-infectious stimuli has been demonstrated (Marques et al., 2009a,b; Schwerk et al., 2010). Marques et al. (2009a,b) found that the mouse choroid plexus displays a sustained response to the repeated inflammatory stimuli by altering the expression profile of several genes. The pathways displaying the most significant changes include those facilitating entry of cells into the CSF, and those participating in the innate immune response to infection. The integrity, paracellular permeability and polarization of epithelial cells, in which the BCSFB is localized, depend on the expression of tight junction (TJ) proteins (Wolburg and Lippoldt, 2002). Studies on mice demonstrated that LPS can affect the integrity of the BCSFB by down-regulation of TJ protein expression which could alter interactions between circulating and central mediators (Marques et al., 2009a). In ewes, the amount of TJ proteins in the choroid plexus is modulated by photoperiod – higher during short than long days (Lagaraine et al., 2011), which suggests a potential photoperiodic differentiation of LPS effect on TJ protein expression in the choroid plexus of ewes.

Penetration of LPS across the brain barriers

However, LPS may affect functioning of the central nervous system (CNS) not only by induction of the synthesis of proinflammatory mediators by the cells of the BBB and BCSFB, but also, as it was suggested, some amounts of LPS may cross brain barriers and penetrate the brain parenchyma. In the study conducted on mice (Banks and Robinson, 2010), which evaluated the ability of LPS labelled with radioactive iodine (I-LPS) to cross the BBB, it was found that the majority of I-LPS was bound by the endothelial cells of the brain. However, a small but measurable amount of I-LPS (0.025% of the dose given intravenously), crossed the BBB and penetrated the brain. It was also shown that the I-LPS given intraventricularly got into circulation by reabsorption of the cerebrospinal fluid (Banks and Robinson, 2010). This confirmed the previous results of experiment carried out on rats, in which the I-LPS was used to determine the pharmacodynamics of endotoxin transition from the brain to the blood (Chen et al., 2000). Studies conducted on mice (Banks and Robinson, 2010) and rats (Chen et al., 2000) have demonstrated that LPS penetrated in measured quantities across the BBB, which may indicate the possibility of passing endotoxin from peripheral blood to the cerebrospinal fluid. However, it should be noted that different results were obtained by Singh and Jiang (2004). They showed that intravenously given LPS did not penetrate the BBB in rat, but was accumulated in endothelial cells of the brain vessels, resulting in increased permeability of the BBB. Also in the experiment carried out on cats (Dascombe and Milton, 1979) no infiltration of endotoxin labelled with radioactive chromium from the blood to the brain was found.

Central action of bacterial endotoxin

However, the results of the study carried out on chimeric mice with low sensitivity to LPS (TLR4 mutant) caused by the lack of TLR4 expression in the CNS seems to support the thesis about the central action of LPS molecule during the inflammation (Chakravarty and Herkenham, 2005). This study confirmed that inflammation influences the CNS, not only by proinflammatory cytokines, but in order to develop a full pathophysiological response the expression of TLR4 on cells of this system is required (Chakravarty and Herkenham, 2005). The results of our studies on ewes, in which the expression of TLR4 transcript was determined in the hypothalamic structures associated with GnRH-ergic activity, such as preoptic area (POA), anterior hypothalamus (AHA), MBH and medial eminence (ME), and anterior pituitary as well (Herman et al., 2013a; Haziak et al., 2014), support hypothesis that LPS can acts directly at the hypothalamic and pituitary level. In addition, the expression of TLR4 gene in those structures was up-regulated by intravenous administration of endotoxin that indicates that under inflammatory condition these regions of the hypothalamus may be characterized by increased sensitivity on the action of LPS molecules (Herman et al., 2013a). An important role of the centrally acting LPS and the complexity of its receptors expressed in the hypothalamus in the regulation of the GnRH/LH secretion during an immune/inflammatory challenge was suggested in our studies performed on anoestrous ewes (Haziak et al., 2014). It was found that icv administration of anti-LPS antibody, as well as blockade of receptors components: LBP and MD-2 in the hypothalamus, significantly reduced inflammatory dependent reduction of GnRH mRNA content in the ME and LHβ gene expression in the anterior pituitary. The fact that TLR4 blockade in the hypothalamus, as well as administration of an anti-LPS antibody to the region of the hypothalamus, restored GnRH mRNA content in the ME but not in the POA indicate that the administration of these compounds rather prevented decreasing the stability of the GnRH mRNA induced by immune stimulation than decreasing the GnRH gene transcription. It could be suggested that inhibition of TLR4 in the hypothalamus led to unlock the inhibition of GnRH secretion, which is the most important modulator of LHβ gene expression (Kaiser et al., 1995). For the direct involvement of LPS in the suppression of the reproductive processes at the level of hypothalamus also indicate the results of the studies performed on rats (Ebisui et al., 1992). There was showed a significant decrease in the level of LH one hour after ventricular injection of endotoxin in doses of 5 and 25 μg. Also, the importance of TLR4 signalling in modulation of the centrally regulated processes was also proved in the study on rats. It was found that the blockade of TLR4 in the paraventricular nucleus of the hypothalamus delayed the progression of hypertension, reduced cardiac hypertrophy, attenuated proinflammatory cytokines synthesis, inducible nitric oxide (NO) synthase and norepinephrine levels as well as NFκB activity (Dange et al., 2015).

Antigonadotropic effect of LPS at the level of pituitary

The antigonadotropic effect of LPS may not only result from its central action. It cannot be excluded that LPS influences the LH secretion

directly at the level of the pituitary, which is located beyond the BBB. LPS effect on LHβ gene expression *ex vivo* may be dependent on the immunological status of the animal (control sheep vs sheep treated with LPS) and that the protein LBP enhances the response to endotoxin (Haziak et al., 2013). It has been found that in the pituitary explants derived from intact ewes the influence of LPS on the LHβ gene expression was observed only when it was added to culture medium together with LBP. In the case of tissue taken from sheep treated with endotoxin response to LPS was independent upon the LBP (Haziak et al., 2013). This antigonadotropic action of LPS is possible due to the expression of both LPS-interacting receptors: TLR4 and TLR2 in the pituitary gland (Breuel et al., 2004; Haziak et al., 2013). However, the exact mechanism *via* LPS inhibits LH secretion from the pituitary is not known. Because the expression of functional TLR4 and TLR2 have not been found in the gonadotropes yet, it could be supposed that endotoxin interacts with pituitary folliculostellate cells which express functional TLR4 (Tichomirowa et al., 2005). Activated folliculostellate cells may express number of factors including proinflammatory cytokines, growth factors and diverse bioactive peptides which may affect the activity of the pituitary gonadotropes in the paracrine manner (Inoue et al., 1999).

Gonadotropins LH and FSH released from the anterior pituitary exert their effects on gonads to enhance production of steroids and other hormones, which promotes the growth and development of gonadal germ cells and other sex-related organs. It was reported that LPS suppresses gonadotropin release and in consequence perturbs follicle growth and functioning in the ovary of sheep and cattle. Moreover, LPS is assumed to exert a direct effect on the ovary, including follicular components, such as theca and granulosa cells or oocyte. In bovine follicles, granulosa cells and theca cells express TLR4, indicating that follicular cells are capable of responding to LPS (Magata et al., 2014).

The role of LBP in the central action of endotoxin

Our results (Haziak et al., 2013) as well as earlier results of Knapp et al. (2006) suggest an important role of the protein LBP in the mechanism of the LPS action in the organism both in its peripheral and central action. LBP facilitates the interaction of the Gram-negative cell wall component LPS with CD14, thereby enhancing the immune response to LPS. The spontaneous diffusion of LPS monomers to the cellular-binding site is very slow and transfer by LBP enhances the immune response to LPS up to 1000-fold *in vitro* (Martin et al., 1997). LBP is constitutively produced by hepatocytes under normal conditions but during an acute phase reaction IL-1β and IL-6 synergise in inducing strongly enhanced LBP synthesis (Schumann et al., 1996).

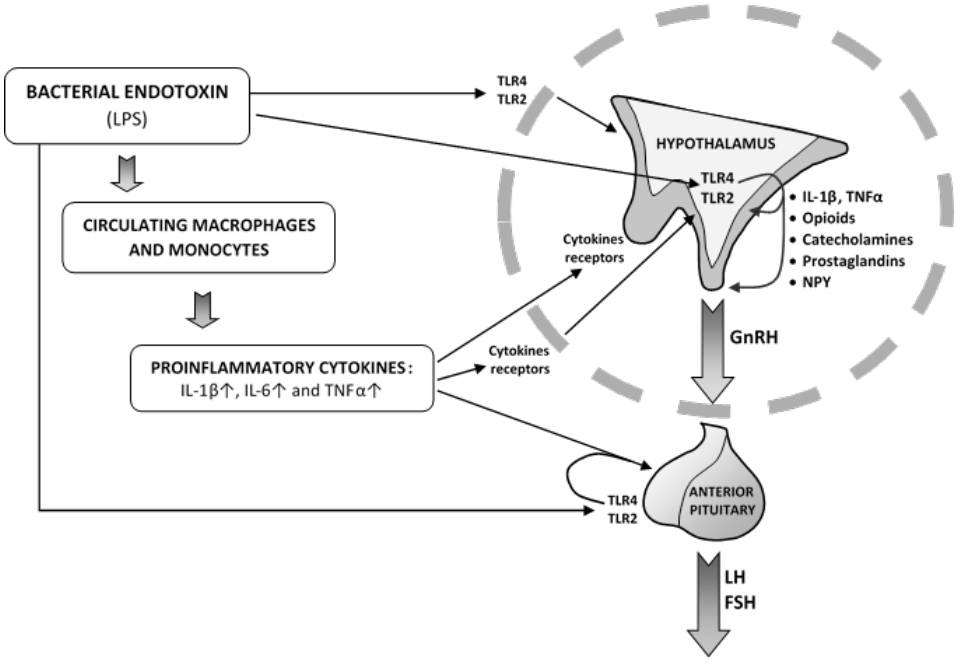

Figure 1. Scheme illustrating possible pathways involved in the bacterial endotoxin (LPS) regulation of GnRH/LH secretion during immune stress. LPS – lipopolysaccharide, GnRH – gonadoliberin, LH – luteinizing hormone, FSH – follicle stimulating hormone, TLR4, TLR2 – toll-like receptors 4 and 2, IL-1β – interleukin 1β , IL-6 – interleukin 6, TNFα – tumor necrosis factor α, NPY – neuropeptide Y

Increased LBP serum levels were found upon systemic infection, during infectious and allergic lung inflammation (Martin et al., 1997). Whereas the low concentration of LBP enhances response to LPS, the high can inhibit LPS bioactivity *in vitro* and *in vivo* (Kitchens and Thompson, 2005). Although hepatocytes are the main source of LBP, this protein may be synthesized also by other tissue (Dentener et al., 2000). LBP is present in both plasma and the CSF even during homeostatic milieu of the organism and its mean concentration in normal CSF is about 1/10 of the concentration measured in normal plasma (Heumann et al., 1995). The constant presence of this acute phase reactant in the CSF, as well as the expression of TLR2 and TLR4 in the brain, suggests the existence of permanently active mechanism enabling induction of LPS action in the CNS.

Conclusions

Immune stress caused by circulating bacterial endotoxin results in disturbances of reproduction processes in mammals. The intravenous injection of LPS results in inhibition of the GnRH/LH secretion. Different mechanisms could be involved in the suppression of GnRH/LH secretion by endotoxin (Figure 1). Firstly, LPS could act indirectly *via* proinflammatory cytokines, such as IL-1β, Il-6 and TNF-α, which release is likely to play a central role in the inhibition of the reproductive axis during immune challenge. Also, the different neural mechanisms dependent on other mediators, such as opiods, catecholamines, γ-aminobutyric acid (GABA), PGs or NO, could be involved in the suppression of GnRH/LH secretion. On the other hand, LPS may directly modulate the activity of the HPG axis at the level of CNS. In the mechanism through which endotoxin inhibits GnRH/LH secretion participate LPS-interacting receptors, particularly TLR4, which expression was found both in the hypothalamus and in the pituitary in numerous mammalian species.

Understanding the exact mechanism of the interaction between immune and reproductive systems is very important because various immune stress-induced infections can cause significant reduction in reproduction rates in humans and animals.

References

Banks W.A., Robinson S.M., 2010. Minimal penetration of lipopolysaccharide across the murine blood-brain barrier. Brain Behav. Immun. 24, 102–109, https://doi.org/10.1016/j.bbi.2009.09.001

Battaglia D.F., Krasa H.B., Padmanabhan V., Viguié C., Karsch F.J., 2000. Endocrine alterations that underlie endotoxin-induced disruption of the follicular phase in ewes. Biol. Reprod. 62, 45–53, https://doi.org/10.1095/biolreprod62.1.45

Bernton E.W., Beach J.E., Holaday J.W., Smallridge R.C., Fein H.G., 1987. Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. Science 238, 519–521, https://doi.org/10.1126/science.2821620

Bilezikjian L.M., Leal A.M.O., Blount A.L., Corrigan A.Z., Turnbull A.V., Vale W.W., 2003. Rat anterior pituitary folliculostellate cells are targets of interleukin-1β and a major source of intrapituitary follistatin. Endocrinology 144, 732–740, https://doi.org/10.1210/en.2002-220703

Braden T.D., Fry C., Sartin J.L., 1998. Effects of interleukins on secretion of luteinizing hormone from ovine pituitary cells. Am. J. Vet. Res. 59, 1488–1493

Breuel K.F., Kougias P., Rice P.J., Wei D., De Ponti K., Wang J., Laffan J.J., Li C., Kalbfleisch J., Williams D.L., 2004. Anterior pituitary cells express pattern recognition receptors for fungal glucans: implications for neuroendocrine immune involvement in response to fungal infections. Neuroimmunomodulation 11, 1–9, https://doi.org/10.1159/000072963

Chakravarty S., Herkenham M., 2005. Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. J. Neurosci. 25, 1788–1796, https://doi.org/10.1523/JNEUROSCI.4268-04.2005

Chen G., McCuskey R.S., Reichlin S., 2000. Blood interleukin-6 and tumor necrosis factor-α elevation after intracerebroventricular injection of *Escherichia coli* endotoxin in the rat is determined by two opposing factors: peripheral induction by LPS transferred from brain to blood and inhibition of peripheral response by a brain-mediated mechanism. Neuroimmunomodulation 8, 59–69, https://doi.org/10.1159/000026454

Dange R.B., Agarwal D., Teruyama R., Francis J., 2015. Toll-like receptor 4 inhibition within the paraventricular nucleus attenuates blood pressure and inflammatory response in a genetic model of hypertension. J. Neuroinflamm. 12, 31, https://doi.org/10.1186/s12974-015-0242-7

Dascombe M.J., Milton A.S., 1979. Study on the possible entry of bacterial endotoxin and prostaglandin E_2 into the central nervous system from the blood. Brit. J. Pharmacol. 66, 565–572, https://doi.org/10.1111/j.1476-5381.1979.tb13695.x

Dentener M.A., Vreugdenhil A.C.E., Hoet P.H.M., Vernooy J.H.J., Nieman F.H.M., Heumann D., Janssen Y.M.W., Buurman W.A., Wouters E.F.M., 2000. Production of the acute-phase protein lipopolysaccharide-binding protein by respiratory type II epithelial cells: implications for local defense to bacterial endotoxins. Am. J. Respir. Cell Mol. Biol. 23, 146–153, https://doi.org/10.1165/ajrcmb.23.2.3855

Dziarski R., Wang Q., Miyake K., Kirschning C.J., Gupta D., 2001. MD-2 enables toll-like receptor 2 (TLR2)-mediated responses to lipopolysaccharide and enhances TLR2-mediated responses to Gram-positive and Gram-negative bacteria and their cell wall components. J. Immunol. 166, 1938–1944, https://doi.org/10.4049/jimmunol.166.3.1938

Ebisui O., Fukata J., Tominaga T., Murakami N., Kobayashi H., Segawa H., Muro S., Naito Y., Nakai Y. Masui Y., 1992. Roles of interleukin-1 alpha and -1 beta in endotoxin-induced suppression of plasma gonadotropin levels in rats. Endocrinology 130, 3307–3313, https://doi.org/10.1210/endo.130.6.1597143

Fassbender K., Ries S., Schminke U., Schneider S., Hennerici M., 1996. Inflammatory cytokines in CSF in bacterial meningitis: association with altered blood flow velocities in basal cerebral arteries. J. Neurol. Neurosurg. Psychiatr. 61, 57–61, https://doi.org/10.1136/jnnp.61.1.57

Fitzgerald K.A., Rowe D.C., Golenbock D.T., 2004. Endotoxin recognition and signal transduction by the TLR4/MD-2 complex. Microbes Infect. 6, 1361–1367, https://doi.org/10.1016/j.micinf.2004.08.015

Givalois L., Dornand J., Mekaouche M., Solier M.D., Bristow A.F., Ixart G., Siaud P., Assenmacher I., Barbanel G., 1994. Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 267, R164–R170

Good D.W., George T., Watts B.A. 3rd, 2012. Toll-like receptor 2 is required for LPS-induced toll-like receptor 4 signaling and inhibition of ion transport in renal thick ascending limb. J. Biol. Chem. 287, 20208–20220, https://doi.org/10.1074/jbc.M111.336255

Harris T.G., Battaglia D.F., Brown M.E., Brown M.B., Carlson N.E., Viguié C., Williams C.Y., Karsch F.J., 2000. Prostaglandins mediate the endotoxin-induced suppression of pulsatile gonadotropin-releasing hormone and luteinizing hormone secretion in the ewe. Endocrinology 141, 1050–1058, https://doi.org/10.1210/endo.141.3.7393

Haziak K., Herman A.P., Tomaszewska-Zaremba D., 2013. The effect of LPS on LH release and gene expression of *LH-β, GnRH-R* and *TLR4* in the anterior pituitary of follicular phase ewes – an *in vitro* study. J. Anim. Feed Sci. 22, 97–105 , https://doi.org/10.22358/jafs/65999/2013

Haziak K., Herman A.P., Tomaszewska-Zaremba D., 2014. Effects of central injection of anti-LPS antibody and blockade of TLR4 on GnRH/LH secretion during immunological stress in anestrous ewes. Mediat. Inflamm. 2014, 867170, https://doi.org/10.1155/2014/867170

Heine H., Kirschning C.J., Lien E., Monks B.G., Rothe M., Golenbock D.T., 1999. Cutting edge: cells that carry a null allele for toll-like receptor 2 are capable of responding to endotoxin. J. Immunol. 162, 6971–6975

Herman A.P., Herman A., Haziak K., Tomaszewska-Zaremba D., 2013a. Immune stress up regulates TLR4 and Tollip gene expression in the hypothalamus of ewes. J. Anim. Feed Sci. 22, 13–18 , https://doi.org/10.22358/jafs/66011/2013

Herman A.P., Krawczyńska A., Bochenek J., Antushevich H., Herman A., Tomaszewska-Zaremba D., 2014. Peripheral injection of SB203580 inhibits the inflammatory-dependent synthesis of proinflammatory cytokines in the hypothalamus. BioMed Res. Int. 2014, 475152, https://doi.org/10.1155/2014/475152

Herman A.P., Krawczyńska A., Bochenek J., Antushevich H., Herman A., Tomaszewska-Zaremba D., 2016. Involvement of prolactin in the meloxicam-dependent inflammatory response of the gonadotropic axis to prolonged lipopolisacharide treatment in anoestrous ewes. Reprod. Fert. Develop. 28, 914–923, https://doi.org/10.1071/RD13435

Herman A.P., Krawczyńska A., Bochenek J., Dobek E., Herman A., Tomaszewska-Zaremba D., 2013b. LPS-induced inflammation potentiates the IL-1β-mediated reduction of LH secretion from the anterior pituitary explants. Clin. Dev. Immunol. 2013, 926937, https://doi.org/10.1155/2013/926937

Herman A.P., Misztal T., Herman A., Tomaszewska-Zaremba D., 2010. Expression of interleukin (IL)-1β and IL-1 receptors genes in the hypothalamus of anoestrous ewes after lipopolysaccharide treatment. Reprod. Domest. Anim. 45, e426–e433, https://doi.org/10.1111/j.1439-0531.2010.01595.x

Herman A.P., Misztal T., Romanowicz K., Tomaszewska-Zaremba D., 2012. Central injection of exogenous IL-1b in the control activities of hypothalamic pituitary gonadal axis in anestrous ewes. Reprod. Domest. Anim. 47, 44–52, https://doi.org/10.1111/j.1439-0531.2011.01800.x

Heumann D., Gallay P., Barras C., Albrecht H., de Flaugergues J.C., Steck A., Glauser M.P., 1995. Lipopolysaccharide binding protein (LBP) as a marker of protein leakage in cerebrospinal fluid. Innate Immun. 2, 1–7, https://doi.org/10.1177/096805199500200101

Inoue K., Couch E.F., Takano K., Ogawa S., 1999. The structure and function of folliculo-stellate cells in the anterior pituitary gland. Arch. Histol. Cytol. 62, 205–218, https://doi.org/10.1679/aohc.62.205

Igaz P., Salvi R., Rey J.-P., Glauser M., Pralong F.P., Gaillard R.C., 2006. Effects of cytokines on gonadotropin-releasing hormone (GnRH) gene expression in primary hypothalamic neurons and in GnRH neurons immortalized conditionally. Endocrinology 147, 1037–1043, https://doi.org/10.1210/en.2005-0729

Janeway C.A. Jr., Travers P., Walport M., Shlomchik M.J., 2001. Immunobiology. 5th Edition. Garland Publishing. New York (NY), USA

Kaiser U.B., Sabbagh E., Katzenellenbogen R.A., Conn P.M., Chin W.W., 1995. a mechanism for the differential regulation of gonadotropin subunit gene expression by gonadotropin-releasing factor. Proc. Natl. Acad. Sci. USA 92, 12280–12284, https://doi.org/10.1073/pnas.92.26.12280

Kalra P.S., Edwards T.G., Xu B., Jain M., Kalra S.P., 1998. The antigonadotropic effects of cytokines: the role of neuropeptides. Domest. Anim. Endocrinol. 15, 321–332, https://doi.org/10.1016/S0739-7240(98)00030-7

Kalra P.S., Fuentes M., Sahu A., Kalra S.P., 1990. Endogenous opioid peptides mediate the interleukin-1-induced inhibition of the release of luteinizing hormone (LH)-releasing hormone and LH. Endocrinology 127, 2381–2386, https://doi.org/10.1210/endo-127-5-2381

Kang S.S., Kim S.R., Leonhardt S., Jarry H., Wuttke W., Kim K., 2000. Effect of interleukin-1β on gonadotropin-releasing hormone (GnRH) and GnRH receptor gene expression in castrated male rats. J. Neuroendocrinol. 12, 421–429, https://doi.org/10.1046/j.1365-2826.2000.00466.x

Kitchens R.L., Thompson P.A., 2005. Modulatory effects of sCD14 and LBP on LPS-host cell interactions. J. Endotoxin Res. 11, 225–229, https://doi.org/ 10.1177/09680519050110040701

Knapp S., Florquin S., Golenbock D.T., van der Poll T., 2006. Pulmonary lipopolysaccharide (LPS)-binding protein inhibits the LPS-induced lung inflammation in vivo. J. Immunol. 176, 3189–3195, https://doi.org/10.4049/jimmunol.176.5.3189

Kumar H., Kawai T., Akira S., 2009. Toll-like receptors and innate immunity. Biochem. Biophys. Res. Commun. 388, 621–615, https://doi.org/10.1016/j.bbrc.2009.08.062

Kochman K., Przekop F., Okrasa S., 2007. Neural regulation of the reproductive processes at the central level (in Polish) In: T. Krzymowski (Editor). Physiological Regulation of Reproduction in Female. UWM, Olsztyn (Poland)

Laflamme N., Rivest S., 2001. Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components. FASEB J. 15, 155–163, https://doi.org/10.1096/fj.00-0339com

Lagaraine C., Skipor J., Szczepkowska A., Dufourny L., Thiery J.-C., 2011. Tight junction proteins vary in the choroid plexus of ewes according to photoperiod. Brain Res. 1393, 44–51, https://doi.org/10.1016/j.brainres.2011.04.009

Lee H.-K., Dunzendorfer S., Tobias P.S., 2004. Cytoplasmic domain-mediated dimerizations of toll-like receptor 4 observed by β-lactamase enzyme fragment complementation. J. Biol. Chem. 279, 10564–10574, https://doi.org/10.1074/jbc.M311564200

Lohrer P., Gloddek J., Nagashima A.C., Korali Z., Hopfner U., Pereda M.P., Arzt E., Stalla G.K., Renner U., 2000. Lipopolysaccharide directly stimulates the intrapituitary interleukin-6 production by folliculostellate cells via specific receptors and the p38α mitogen-activated protein kinase/nuclear factor-κB pathway. Endocrinology 141, 4457–4465

Magata F., Horiuchi M., Echizenya R., Miura R., Chiba S., Matsui M., Miyamoto A., Kobayashi Y., Shimizu T., 2014. Lipopolysaccharide in ovarian follicular fluid influences the steroid production in large follicles of dairy cows. Anim. Reprod. Sci. 144, 6–13, https://doi.org/10.1016/j.anireprosci.2013.11.005

Majewska M., Szczepanik M., 2006. The role of toll-like receptors (TLR) in innate and adaptive immune responses and their function in immune response regulation (in Polish). Postep. Hig. Med. Dosw. 60, 52–63

Mallard C., 2012. Innate immune regulation by toll-like receptors in the brain. ISRN Neurol. 2012, 701950. doi:10.5402/2012/701950, https://doi.org/10.5402/2012/701950

Marques F., Sousa J.C., Coppola G., Falcao A.M., Rodrigues A.J., Geschwind D.H., Sousa N., Correia-Neves M., Palha J.A., 2009a. Kinetic profile of the transcriptome changes induced in the choroid plexus by peripheral inflammation. J. Cereb. Blood Flow Metab. 29, 921–932, https://doi.org/10.1038/jcbfm.2009.15

Marques F., Sousa J.C., Coppola G., Geschwind D.H., Sousa N., Palha J.A., Correia-Neves M., 2009b. The choroid plexus response to a repeated peripheral inflammatory stimulus. BMC Neurosci. 10, 135, https://doi.org/10.1186/1471-2202-10-135

Martin T.R., Rubenfeld G.D., Ruzinski J.T., Goodman R.B., Steinberg K.P., Leturcq D.J., Moriarty A.M., Raghu G., Baughman R.P., Hudson L.D., 1997. Relationship between soluble CD14, lipopolysaccharide binding protein, and the alveolar inflammatory response in patients with acute respiratory distress syndrome. Am. J. Respir. Crit. Care Med. 155, 937–944, https://doi.org/10.1164/ajrccm.155.3.9117029

Meilleur M.-A., Akpovi C.D., Pelletier R.-M., Vitale M.L., 2007. Tumor necrosis factor-α-induced anterior pituitary folliculostellate TtT/GF cell uncoupling is mediated by connexion 43 dephosphorylation. Endocrinology 148, 5913–5924, https://doi.org/10.1210/en.2007-0767

Mishra B.B., Mishra P.K., Teale J.M., 2006. Expression and distribution of toll like receptors in the brain during murine neurocysticercosis. J. Neuroimmunol. 181, 46–56, https://doi.org/10.1016/j.jneuroim.2006.07.019

Murata T., Ying S.-Y., 1991. Effects of interleukin-1β on secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by cultured rat anterior pituitary cells. Life Sci. 49, 447–453, https://doi.org/10.1016/0024-3205(91)90587-2

Pålsson-McDermott E.M., O'Neill L.A., 2004. Signal transduction by the lipopolysaccharide receptor, toll-like receptor-4. Immunology 113, 153–162, https://doi.org/10.1111/j.1365-2567.2004.01976.x

Parnet P., Brunke D.L., Goujon E., Mainard J.D., Biragyn A., Arkins S., Dantzer R., Kelley K.W., 1993. Molecular identification of two types of interleukin-1 receptors in the murine pituitary gland. J. Neuroendocrinol. 5, 213–219, https://doi.org/10.1111/j.1365-2826.1993.tb00384.x

Peter A.T., Bosu W.T.K., DeDecker R.J., 1989. Suppression of preovulatory luteinizing hormone surges in heifers after intrauterine infusions of Escherichia coli endotoxin. Am. J. Vet. Res. 50, 368–373

Rettori V., Gimeno M.F., Karara A., Gonzalez M.C., McCann S.M., 1991 Interleukin 1α inhibits prostaglandin E_2 release to suppress pulsatile release of luteinizing hormone but not follicle-stimulating hormone. Proc. Natl. Acad. Sci. USA 88, 2763–2767, https://doi.org/10.1073/pnas.88.7.2763

Rietschel E.T., Kirikae T., Schade F.U., Mamat U., Schmidt G., Loppnow H., Ulmer A.J., Zähringer U., Seydel U., Di Padova F., 1994. Bacterial endotoxin: molecular relationships of structure to activity and function. FASEB J. 8, 217–225

Schlafer D.H., Yuh B., Foley G.L., Elssaser T.H., Sadowsky D., Nathanielsz P.W., 1994. Effect of Salmonella endotoxin to the pregnant sheep at 133-142 days gestation on fetal oxygenation, maternal and fetal adsrenocorticotropic hormone and cortisol, and maternal plasma tumor necrosis factor a concentrations. Biol. Reprod. 50, 1297–1302, https://doi.org/10.1095/biolreprod50.6.1297

Schumann R.R., Kirschning C.J., Unbehaun A., Aberle H., Knope H.-P., Lamping N., Ulevitch R.J., Herrmann F., 1996. The lipopolysaccharide-binding protein is a secretory class 1 acute-phase protein whose gene is transcriptionally activated by APRF/STAT-3 and other cytokine-inducible nuclear proteins. Mol. Cell. Biol. 16, 3490–3503, https://doi.org/10.1128/MCB.16.7.3490

Schwerk C., Rybarczyk K., Essmann F., Seibt A., Mölleken M.-L., Zeni P., Schroten H., Tenenbaum T., 2010. TNFα induces choroid plexus epithelial cell barrier alterations by apoptotic and nonapoptotic mechanisms. J. Biomed. Biotech. 2010, 307231, https://doi.org/10.1155/2010/307231

Singh A.K., Jiang Y., 2004. How does peripheral lipopolysaccharide induce gene expression in the brain of rats? Toxicology 201, 197–207, https://doi.org/10.1016/j.tox.2004.04.015

Sirivelu M.P., Burnett R., Shin A.C., Kim C., MohanKumar P.S., MohanKumar S.M.J., 2009. Interaction between GABA and norepinephrine in interleukin-1β-induced suppression of the luteinizing hormone surge. Brain Res. 1248, 107–114, https://doi.org/10.1016/j.brainres.2008.10.057

Skipor J., Szczepkowska A., Kowalewska M., Herman A.P., Lisiewski P., 2015. Profile of toll-like receptor mRNA expression in the choroid plexus in adult ewes. Acta Vet. Hung. 63, 69–78, https://doi.org/10.1556/AVet.2014.027

Skipor J., Thiery J.-C., 2008. The choroid plexus - cerebrospinal fluid system: undervaluated pathway of neuroendocrine signaling into the brain. Acta Neurobiol. Exp. 68, 414–428

Tichomirowa M., Theodoropoulou M., Lohrer P., Schaaf L., Losa M., Uhl E., Lange M., Arzt E., Stalla G.K., Renner U., 2005. Bacterial endotoxin (lipopolysaccharide) stimulates interleukin-6 production and inhibits growth of pituitary tumour cells expressing the toll-like receptor 4. J. Neuroendocrinol. 17, 152–160, https://doi.org/10.1111/j.1365-2826.2005.01286.x

Tomaszewska-Zaremba D., Herman A., 2009. The role of immunological system in the regulation of gonadoliberin and gonadotropin secretion. Reprod. Biol. 9, 11–23, https://doi.org/10.1016/S1642-431X(12)60091-6

Tomaszewska-Zaremba D., Herman A.P., Misztal T., 2013. Does central IL-1β affect GnRH secretion in the hypothalamus of anoestrous ewes *via* different regulatory pathways? J. Anim. Feed Sci. 22, 5–12, https://doi.org/10.22358/jafs/66010/2013

Waage A., Halstensen A., Shalaby R., Brandtzaeg P., Kierulf P., Espevik T., 1989. Local production of tumor necrosis factor α, interleukin 1, and interleukin 6 in meningococcal meningitis. Relation to the inflammatory response. J. Exp. Med. 170, 1859–1867, https://doi.org/10.1084/jem.170.6.1859

Wolburg H., Lippoldt A., 2002. Tight junctions of the blood-brain barrier: development, composition and regulation. Vasc. Pharmacol. 38, 323–337, https://doi.org/10.1016/S1537-1891(02)00200-8

Yang R.-B., Mark M.R., Gray A., Huang A., Xie M.H., Zhang M., Goddard A., Wood W.I., Gurney A.L., Godowski P.J., 1998. Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. Nature 395, 284–288, https://doi.org/10.1038/26239

Yu L., Wang L., Chen S., 2010. Endogenous toll-like receptor ligands and their biological significance. J. Cell. Mol. Med. 14, 2592–2603, https://doi.org/10.1111/j.1582-4934.2010.01127.x