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Full Length Research Paper

Roles of toll-like receptor 4 and immunomodulatory hormones in bovine endometritis

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The aim of the study is to explore the role of Toll-like receptor 4 (TLR4) and immunomodulatory hormones in bovine endometritis. The bovine endometrial epithelial cell model (BenEpC) was used in the study. After contamination by *Escherichia coli*, BenEpCs were randomly divided into several groups according to the different concentration of 17-β estradiol (E2) treatment and the different stimulation, lipopolysaccharide (LPS) or lipoteichoic acid (LTA). The expressions of TLR4 and COX-2 mRNA were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Prostaglandin F (PGF) and prostaglandin E (PGE) levels were evaluated by radioimmunoassay. A significant difference was observed in TLR4 mRNA expression of bacterial contaminated BEnEpCs among low, middle and high-dose E2 treatment group ($p < 0.05$). Meanwhile, there was also a statistical difference in the relative changes of the prostaglandin E_2 (PGE_2) and COX-2 mRNA expression in BEnEpCs among low, middle and high-dose E2 treatment group ($p < 0.05$). Furthermore, the most significant increase of PGE_2 and Cyclooxygenase-2 (COX-2) mRNA expression was achieved in the LPS-stimulated BEnEpCs compared with those stimulated with LTA. Our findings suggested that TLR4 was activated in bovine endometritis, and then led to more prostaglandin production as regulation of local inflammatory response. However, this response was dependent on and influenced by the immunomodulatory hormones.

Key words: Bovine endometritis, lipopolysaccharide, toll-like receptor 4, 17-β estradiol.

INTRODUCTION

Endometritis refers to inflammation of the endometrium, the lining of the uterus. The clinical and subclinical uterine diseases are associated with subfertility and infertility (Sheldon, 2008). Because of no systemic effects, the measurable effects of endometritis are indirect and mediated through effects on reproduction (LeBlanc et al., 2002). It is generally acknowledged that the uterus is resistant to infection in the period of estrus, when under the influence of estrogen. But some studies reported that intrauterine infusion of estrogen did not reduce the amount of bacteria in the uterus, but to bodies such as *Fusobacterium necrophorum* (*F. necrophorum*) and *Prevotella melaninogenicus* (*P. melaninogenicus*) to increased load bacteria (Sheldon et al., 2004). This apparent contradiction may be related to some other effects of estrogen, such as the increased production of mucus or uterine motility, or the low concentration of progesterone, so that the positive effects of estrogen are felt. There are also few studies that focus on the effects of estrogens on bacterial load, while most on the immune system. The intramuscular estradiol benzoate could be similar to that of prostaglandin F_2 (PGF) (PGF2) and oxytetracycline. However, the treatment-conception interval is higher than the other treatments (Sheldon and Noakes, 1998).

Toll-like receptor 4 (TLR4) has been identified as the much sought-receptor involved in immune response to lipopolysaccharide (LPS), cell wall component of Gram-negative bacteria responsible for septic shock (Akira et al., 2006). However, LPS alone is not able to interact with TLR4. This receptor initially forms a complex with MD2 on the cell surface that serves as the main binding component with LPS (Kim et al., 2007). There are also

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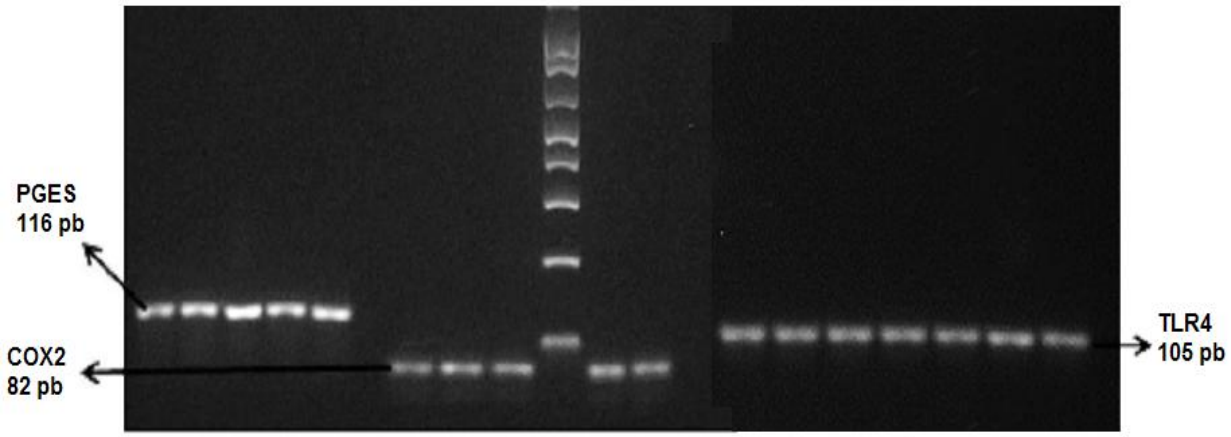

Figure 1. The PGEs, COX2 and TLR4 mRNA expressions.

other proteins that bind to LPS. CD14 is part of the LPS-binding protein (LBP), a soluble plasma protein. CD14 has the capacity of conjuncting with the LBP to bind to LPS as the complex TLR4-MD2 (Akashi-Takamura, 2008).

In cattle, it is known that TLR4 mRNA express in the glands of endocervix, endometrial glands and luminal epithelium of the uterine horns. Unlike TLR2, it did not express in the vagina and ectocervix. This distribution seems to have an important role in the modulation of immune tolerance in the female reproductive tract and plays a unique role of defense against ascending infections (Davies et al., 2008). Therefore, this study developed the roles of TLR4 and immunomodulatory hormones in bovine endometritis.

MATERIALS AND METHODS

Cell culture and challenge

The bovine endometrial epithelial cell line BEnEpC were cultured at 37°C, 5% CO_2 in air, in a humidified incubator. After being contaminated by Escherichia coli ($\times 10^6$ CFU/ml), BEnEpCs were challenged with different concentrations of 17-β estradiol (50 to 150 pmol/L, Sigma, St. Louis, USA), LPS (1 µg/ml, Sigma, St. Louis, USA) or lipoteichoic acid (LTA) (1 µg/ml, *E. coli* serotype 055:B5, Sigma, St. Louis, USA). Culture supernatants were harvested and frozen prior to cytokine and PG determination.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Briefly, genes were amplified by qRT-PCR using the same primers for RT-PCR, Each cDNA plasmid was obtained and identified by spectrophotometry and the standard number of plasmid copies of interest calculated according to Applied Biosystems Foster City. The reactions were performed in duplicate in 96-well plates Prism® 7300 SDS (Applied Biosystems, Foster City, CA, USA) by using universal temperature cycles: Pre-incubation of 10 min at 95°C, followed by 45 cycles at two temperatures (15 s at 95° C and 1 min at 60°C). Dissociation curves were acquired (15 s at 95°C, 30 s at 60°C and 15 s at 95°C) to confirm the expected amplification product only. The reactions were performed with 12.5 ml Power SYBR ® Green PCR Master Mix (Applied Biosystems, Warrington, UK), 1 µl cDNA diluted 1:5 and 80 nm of each primer in a total volume of 25 µl per reaction. After analyzing the dissociation curves, the PCR products were subjected to gel electrophoresis 2.5% agarose to confirm the expected size of them. To evaluate the TLR4 expression in the cell line, the following specific sets of primers were used: The forward and reverse were 5'AAC CAC CTC TCC ACC TTG ATA CTG 3' and 5'CCA GCC AGA CCT TGA ATA CAG G 3', respectively.

Radioimmunoassay

Culture supernatants were analysed for PGE_2 and PGF_2 by radioimmunoassay (RIA) as previously described (Cheng et al., 2001). The samples were diluted in 0.05 M Tris buffer (100 mm NaCl, 100 mm Tris, 0.1% gelatin, 0.01% sodium azide, pH 7.5). Standards and tracer tritium for PGs were provided by Sigma (St. Louis, MO, USA) and Amersham International PLC (Amersham, Bucks., UK), respectively. The detection limit of the assay for PGE_2 and PGF_2 was 2 pg/tube and 1 pg/tube, respectively.

Statistical analysis

The data are presented as means ± SD. Comparisons between groups of data were performed by using a Student's t-test. A p-value < 0.05 was considered to indicate statistical significance. Data were analyzed with the SPSS 18.0 statistical software package (SPSS Inc., Chicago, IL).

RESULTS

qRT-PCR results

The bacterial contaminated BEnEpC expressed mRNA of enzymes PGEs, PGFS, COX2 and TLR4. All amplified products had the expected molecular mass (Figures 1 and 2).

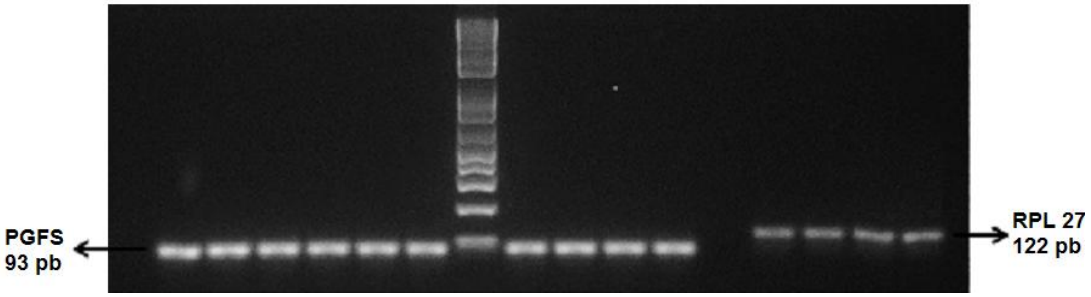

Figure 2. PGFs and RPL27 mRNA expressions.

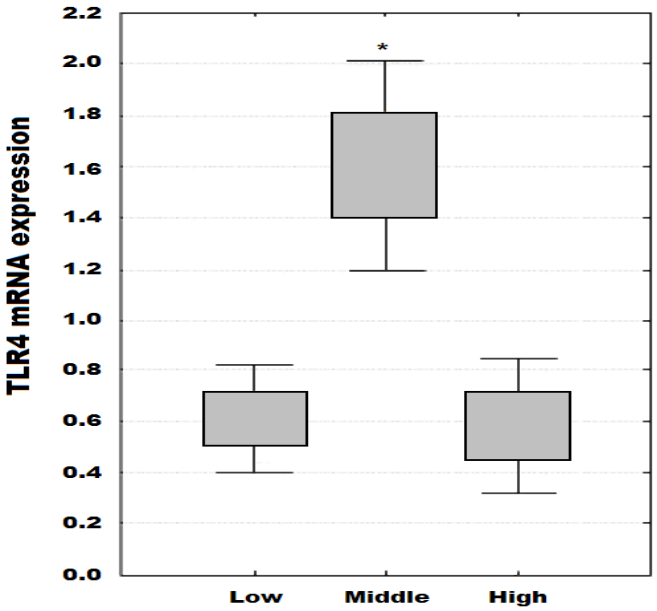

Figure 3. TLR4 mRNA expression in bacterial contaminated BEnEpCs treated with low, middle and high-dose E2.

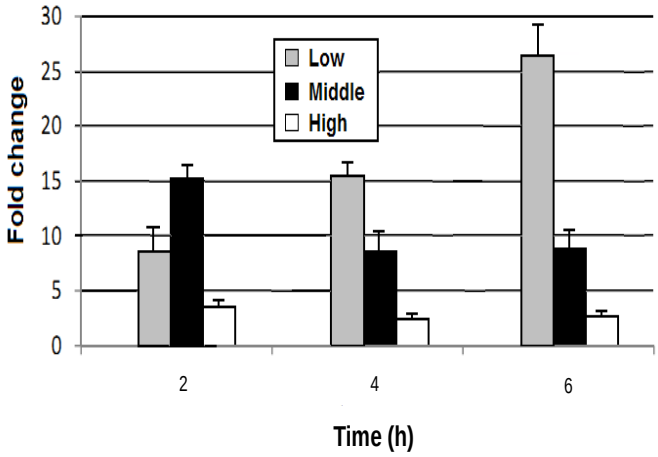

Figure 4. Relative changes of the PGE_2 mRNA expression in bacterial contaminated BEnEpCs treated with low, middle and high-dose E2.

TLR4 mRNA expression in bacterial contaminated BEnEpCs

TLR4 mRNA expressions in bacterial contaminated BEnEpCs are shown in Figure 3. The expressions of TLR4 mRNA increased when bacterial contaminated BenEpCs were treated with middle-dose E2. A significant difference was observed in TLR4 expression of bacterial contaminated BEnEpCs among low, middle and high-dose E2 treated group ($p < 0.05$). However, no significant difference in TLR4 expression was found between low and high-dose E2 treated group. For TLR4, we found no differences in mRNA expression BEnEpCs (not stimulated) after incubation (data not shown).

Prostaglandin E_2 (PGE_2) mRNA expression in bacterial contaminated BEnEpCs

PGE_2 mRNA expressions in bacterial contaminated BEnEpCs are shown in Figure 4. Two hours after incubation, an increased mRNA expression of PGE_2 in BEnEpCs (not stimulated it was observed). Depending on the sample, this increase was maintained for up to 24 h. And there was also a statistical difference in the relative changes of the PGE_2 mRNA expression in BEnEpCs among low, middle and high-dose E2 treated group ($p < 0.05$) (Figure 4).

After stimulation by LPS or LTA, an increase of PGE_2 mRNA expression was observed in bacterial contaminated BEnEpCs treated with different dose E2 during the stimulation compared with those non-stimulated (Figures 5 to 7). The most significant increase was achieved in cells stimulated with LPS compared with those stimulated with LTA ($p < 0.05$). The peak of PGE_2 mRNA expression was reached at 6 h in all samples.

Cyclooxygenase-2 (COX2) mRNA expression in bacterial contaminated BEnEpCs

COX2 mRNA expressions in bacterial contaminated BEnEpCs were shown in Figure 8. Two hours after

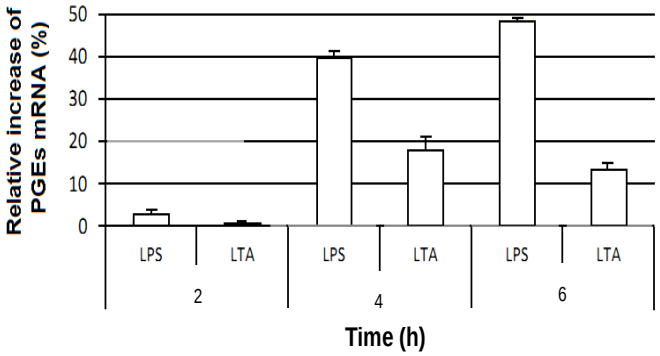

Figure 5. Relative change of the PGE_2 mRNA after stimulation with LPS and LTA in bacterial contaminated BEnEpCs treated with low-dose E2.

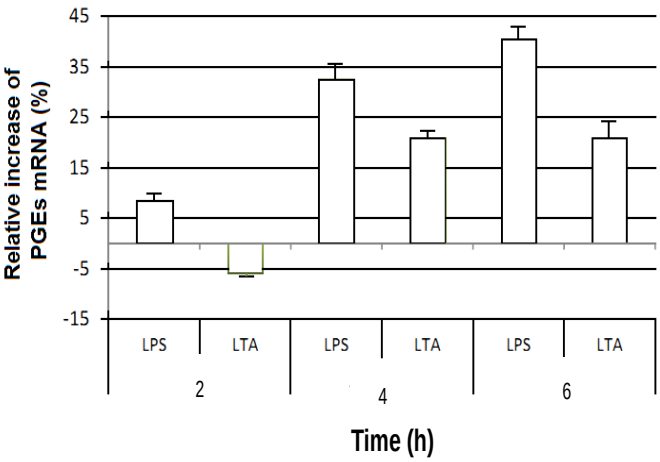

Figure 6. Relative change of the PGE_2 mRNA after stimulation with LPS and LTA in bacterial contaminated BEnEpCs treated with middle-dose E2.

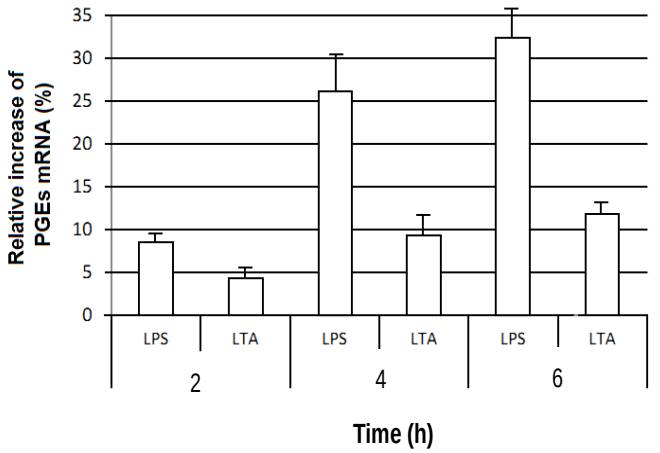

Figure 7. Relative change of the PGE_2 mRNA after stimulation with LPS and LTA in bacterial contaminated BEnEpCs treated with high-dose E2.

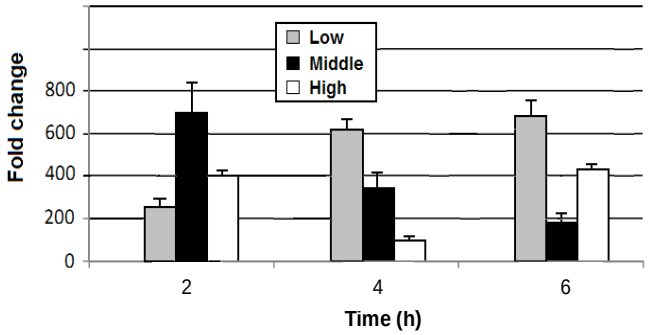

Figure 8. Relative changes of the COX-2 mRNA expression in bacterial contaminated BEnEpCs treated with low, middle and high-dose E2.

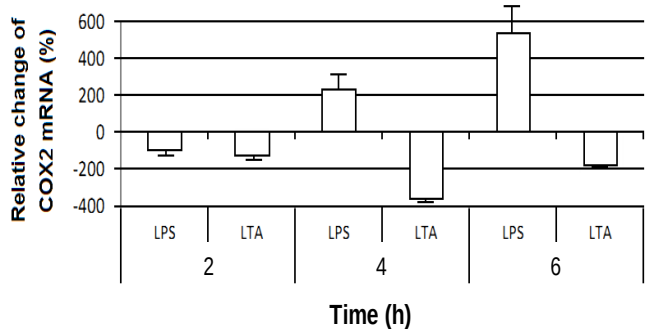

Figure 9. Relative change of the COX2 mRNA after stimulation with LPS and LTA in bacterial contaminated BEnEpCs treated with low-dose E2.

incubation, an increased in mRNA expression COX2 in BEnEpCs (not stimulated) was observed. Depending on the sample, this increase was maintained for up to 24 h And there was also a statistical difference in the relative changes of the COX-2 mRNA expression in BEnEpCs among low, middle and high-dose E2 treated group ($p < 0.05$) (Figure 8).

After stimulation by LPS or LTA, an increase of COX2 mRNA expression was observed in bacterial contaminated BEnEpCs treated with different dose E2 during the stimulation compared with those non-stimulated (Figures 9 to 11). The most significant increase was achieved in cells stimulated with LPS compared with those LTA-stimulated cells ($p < 0.05$). The peak of COX2 mRNA expression was reached at 6 h in bacterial contaminated BEnEpCs treated with low-dose E2, while peak of COX2 mRNA expression was reached at 4 h in those treated with middle- and high-dose E2.

DISCUSSION

This paper explored the roles of Toll-like receptor 4

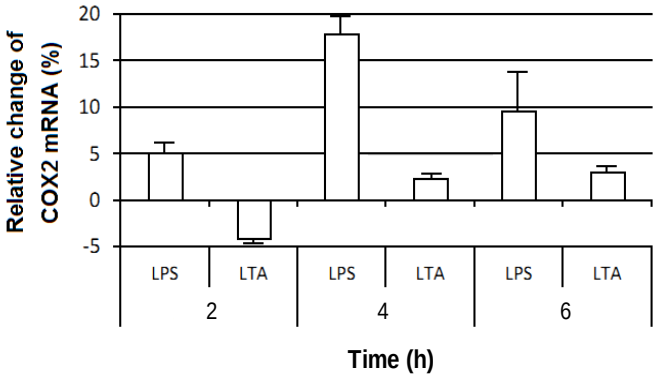

Figure 10. Relative change of the COX2 mRNA after stimulation with LPS and LTA in bacterial contaminated BEnEpCs treated with middle-dose E2.

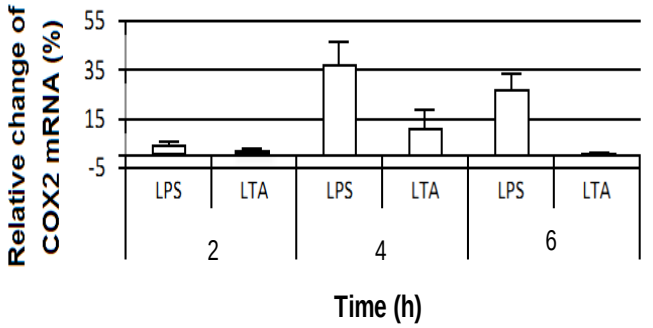

Figure 11. Relative change of the COX2 mRNA after stimulation with LPS and LTA in bacterial contaminated BEnEpCs treated with high-dose E2.

(TLR4) and immunomodulatory hormones in bovine endometritis. The results are consistent with previous studies in humans, both by qRT-PCR and radioimmunoassay, which was detected in mRNA expression of TLR4 in the endometrium (Fazeli et al., 2005; Aflatoonian et al., 2007). The mRNA expression of TLR4 was detected in pure lines of endometrial cells (ECC-1) and in primary cultures of uterine epithelial cells (Schaefer et al., 2004, 2005). Likewise, the transcriptions of TLRs genes were also demonstrated in bovine endometrium (Davies et al., 2008). These studies evaluated the mRNA expression of TLR1 to TLR10 in endometrial tissue while only TLR1-7 and TLR9 was in primary cultures of endometrial epithelial cells.

Similar to what happens in humans, the cattle endometrium has the capacity to recognize a wide variety of ligands through activation via TLRs, triggering an innate immune response against various pathogens. Such recognition becomes extremely important since the female reproductive tract of the cattle is subject to multiple contaminants during intercourse and during the phases of oestrus in which there is a predominance of estrogen hormone, which leads to relaxation and dilation of the cervix, providing ascending infections. The uterine environment is controlled by varying levels of steroid hormones, including *E2* and progesterone, during oestrus. Such variations cause not only anatomical and histological changes, but also change of the immune response. In the cattle, local immunity was reduced by inhibiting the proliferative capacity of lymphocytes during diestrus, in addition to stimulating the secretory activity of endometrial glands. On the other hand, the increase in the follicular phase of E2 promotes migration of immune cells into the uterine lumen, increasing uterine defenses.

Some studies have suggested that TLRs differentially expressed in various parts of the reproductive system in women, and TLR4 have higher level of expression in the endometrium of the uterine horns than in the cervix (Fazeli et al., 2005; Pioli et al., 2004). Growing studies focused on the transcription of TLRs during the menstrual cycle and revealed contradictory results. Hirata et al. (2007) demonstrated that the mRNA expression of TLR4 was also higher in the peri-menstrual phase. However, Aflatoonian et al. (2007) reported that the mRNA expression of TLR4 was higher in the secretory phase during the predominance of P4. Recently, King and Critchley (2010) verify an increased mRNA expression of TLR4 at mid-secretory phase throughout the human menstrual cycle.

During infection, PGE_2 may have special role for the duration of the cycle and is critical for the crosstalk between immunity and reproduction (Harris et al., 2002; Lewis, 2003, 2004; Poyser, 1995). Therefore, our results showed that bacterial contaminated BEnEpCs could produce more PGE_2 in response to LPS and *E. coli.* Furthermore, the LPS-induced immunomodulatory response promoted the production of prostaglandins. Above all, the more PGE_2 production determined the length of the reproductive cycle and showed a mechanism for the crosstalk between immunity and reproduction.

Conclusion

In summary, we found that TLR4 was activated in bovine endometritis, and then led to more prostaglandin production as regulation of local inflammatory response. However, this response was dependent on and influenced by the immunomodulatory hormones.

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REFERENCES

Aflatoonian R, Tuckerman E, Elliott SL, Bruce C, Aflatoonian A, Li TC,

Fazeli A (2007). Menstrual cycle-dependent changes of Toll-like receptors in endometrium. Human Reprod., 22(2): 586-593.
Akashi-Takamura S, Miyake K (2008). TLR accessory molecules. Curr. Opin. Immunol., 20(4): 420-425.
Akira S, Uematsu S, Takeuchi O (2006). Pathogen recognition and innate immunity. Cell, 124(4): 783-801.
Cheng Z, Robinson RS, Pushpakumara PG, Mansbridge RJ, Wathes DC (2001). Effect of dietary polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow. J. Endocrinol., 171: 463.
Davies D, Meade KG, Herath S (2008). Toll-like receptor and antimicrobial peptide expression in the bovine endometrium. Reprod. Biol. Endocrinol., 6: 53.
Fazeli A, Bruce C, Anumba DO (2005). Characterization of Toll-like receptors in the female reproductive tract in humans. Human Reprod., 20(5): 1372-1378.
Harris SG, Padilla J, Koumas L, Ray D, Phipps RP (2002). Prostaglandins as modulators of immunity. Trends Immunol., 23:144.
Hirata T, Osuga Y, Hamasaki K, Hirota Y, Nose E, Morimoto C, Harada M, Takemura Y, Koga K, Yoshino O, Tajima T, Hasegawa A, Yano T, Taketani Y. (2007). Expression of toll-like receptors 2, 3, 4, and 9 genes in the human endometrium during the menstrual cycle. J. Reprod. Immunol., 74(1-2): 53-60.
King AE, Critchley HO (2010). Oestrogen and progesterone regulation of inflammatory processes in the human endometrium. J. Steroid, Biochem. Mol. Biol., 120(2-3): 116-126.
Kim HM, Park BS, Kim JI, Kim SE, Lee J, Oh SC, Enkhbayar P, Matsushima N, Lee H, Yoo OJ, Lee JO (2007). Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran. Cell, 130(5): 906-917.
LeBlanc S, Duffield T, Leslie K, Bateman KG, Keefe GP, Walton JS, Johnson WH. (2002). Defining and Diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci., 85: 2223-2236.
Lewis GS (2003). Steroidal regulation of uterine resistance to bacterial infection in livestock. Reprod. Biol. Endocrinol., 1: 117.
Lewis GS (2004). Steroidal regulation of uterine immune defenses. Anim. Reprod. Sci., 281: 82-83.
Pioli PA, Amiel E, Schaefer TM, Connolly JE, Guyre PM (2004). Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. Infect. Immun., 72(10): 5799-5806.
Poyser NL (1995). The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. Prostaglandins Leukot. Essent. Fatty Acids, 53: 147.
Schaefer TM, Desouza K, Fahey JV, Beagley KW, Wira CR (2004). "Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. Immunology, 112(3): 428-436.
Schaefer TM, Fahey JV, Wright JA.Wira CR (2005). Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C). J. Immunol., 174(2): 992-1002.
Sheldon IM, Noakes DE (1998). Comparison of three treatments for bovine endometritis. Vet. Reco., 142(21): 575-579.
Sheldon IM, Noakes DE, Rycroft AN, Dobson H (2004). Effect of intrauterine administration of oestradiol on postpartum uterine bacterial infection in cattle. Anim. Reprod. Sci., 81: 13-23.
Sheldon IM (2008). The definition of metritis. In 21 Recomendations against metritis. Paris: Pfizer, pp. 8-16.