DOI: 10.1002/vms3.115

Original Article

Rifaximin anti-inflammatory activity on bovine endometrium primary cell cultures: a preliminary study

Lisa Flammini*, Leonardo Mantelli†, Alessandra Volpe*, Giuseppe Domenichini*, Rosanna Di Lecce†, Maurizio Dondi†, Anna Maria Cantoni†, Elisabetta Barocelli* and Fausto Quintavalla†

*Food and Drug Department, University of Parma, Parma, Italy and †Department of Medical Veterinary Sciences, University of Parma, Parma, Italy

Abstract

Rifaximin is an unabsorbed oral antibiotic showing anti-inflammatory properties in human pathologies like irritable bowel syndrome and inflammatory bowel disease. In veterinary medicine, rifaximin is primarily used in the treatment of dermatological diseases in all animal species, in therapy and prophylaxis of mastitis in cows and in the treatment of endometritis in cattle and horses. The aim of this preliminary study was to evaluate the anti-inflammatory properties of rifaximin on primary cell cultures from bovine endometrium in which inflammatory response was induced by Lipopolysaccaride (LPS) treatment. Epithelial and stromal cells were isolated from bovine endometrium and separately incubated for 24 h with 1 μg mL^{-1} LPS after rifaximin (10, 50 and 100 μmol L^{-1}) or dexamethasone (10 μmol L^{-1}) pre-treatment for 24 h. Supernatants were collected 24 h after LPS treatment and interleukin (IL)-6 and IL-8 accumulation was measured by ELISA. Rifaximin (10, 50 and 100 μmol L^{-1}) dose dependently inhibited the LPS-induced increase in IL-6 and IL-8 in stromal cells, whereas in epithelial cells it was not possible to detect any accumulation of these interleukins. Rifaximin reduced IL-6 and IL-8 production, showing a potential anti-inflammatory effect that opens up to new possibilities for the use of this drug in uterine inflammatory diseases.

Keywords: Bovine, cell cultures, endometrium, inflammation, rifaximin.

Corresponding author: Lisa Flammini, Department of Food and Drug, University of Parma, Parco Area delle Scienze 27/A, Parma 43124, Italy.
E-mail: lisa.flammini@unipr.it

Introduction

Rifaximin is a broad-spectrum antibiotic of the rifamycin class, synthesized in Alfa Wassermann laboratories in 1980. Rifaximin acts by blocking the beginning of bacterial messenger RNA chain formation, permanently binding β-subunit-DNA-dependent RNA-polimerase, in a 1:1 ratio (Wehrli & Staehelin 1971). Another property of rifaximin, is the capability to bind the human nuclear receptor PXR (pregnane-x-receptor), which is frequently activated by xenobiotics and is involved in the production of inflammatory mediators (Ma *et al.* 2007; Mencarelli *et al.* 2010, 2011; Cheng *et al.* 2012; Calanni *et al.* 2014). Activated PXR forms a heterodimer with RXR (retinoid-X-receptor). Afterwards this complex binds nuclear transcriptor factors such as NF-kB (Nuclear Factor-kappa B) e CBP (CREB-binding protein), suppressing the expression of NF-kB and CBP target genes resulting in anti-inflammatory activity (Cheng *et al.* 2012).

Rifaximin is not absorbed by the intestinal mucosa either in healthy or in pathological conditions (Calanni *et al.* 2014) therefore the particularly low bioavailability (<0.01%) allows rifaximin to act locally in the gut after oral administration (DuPont 2015).

In 1987 rifaximin was approved in Italy for the treatment of acute and chronic bacterial infection of the gut, bacterial diarrhoea and hepatic encephalopathy. In 2004 it was approved in USA for the treatment of traveller's diarrhoea caused by non-

enteroinvasive *Escherichia coli* and in 2015 it was approved for the therapy of irritable bowel syndrome associated with diarrhoea. The high efficacy of rifaximin in these gastro-intestinal pathologies was accounted for by its antibiotic and anti-inflammatory properties. A study of Ma and collaborators (Ma *et al.* 2007) demonstrated that rifaximin prevents an inflammatory response reducing the production of cytokines and chemokines.

In veterinary medicine, rifaximin is primarily used in the treatment of diseases affecting the skin in all animal species, in the therapy and prophylaxis of mastitis in cows (Malinowski *et al.* 2008), in the topical treatment of female reproductive system disorders in cattle and horses (Parmigiani & Truszkowska 1990) and in the treatment of dogs with chronic enteropathy (Menozzi *et al.* 2016).

Rifaximin inability to cross the epithelia makes this drug particularly advantageous for the local treatment of endometritis avoiding any withdrawal period before milk or meat usage (Truszkowska *et al.* 1990; Manjarrez *et al.* 2012). Since endometritis is a pathology often characterized by both infective and inflammatory aetiology, a compound combining antibiotic and anti-inflammatory activities could be particularly effective. However, to the best of our knowledge, no evidence so far has demonstrated the anti-inflammatory activity of rifaximin on bovine tissues. Therefore, the aim of this preliminary study was to investigate the possible anti-inflammatory properties of rifaximin on a primary culture of bovine endometrial cells treated with *Escherichia coli* Lipopolysaccaride (LPS).

Materials and methods

Organs isolation

Bovine uteri were collected at a local abattoir from a total of 10 post-pubertal non-pregnant cattle in luteinic phase, with no evidence of genital disease or microbial infection. Post-partum animals were not used for the possibility of current inflammation and epithelium damage (Cronin *et al.* 2012). The stage of the reproductive cycle was determined by examination of ovarian morphology: only uteri with an ovarian stage I corpus luteum were selected for endometrial cell culture. The endometrium from the horn ipsilateral to the corpus luteum was cut into strips and placed into serum-free RPMI 1640 medium (Euroclone Spa) supplemented with 50 IU/ml penicillin (Euroclone Spa), 50 μg mL^{-1} streptomycin (Euroclone Spa), 2.5 μg mL^{-1} amphotericin B (Euroclone Spa) and 240 IU mL^{-1} nystatin (Sigma-Aldrich, SRL, Milano, Italy) (Herath *et al.* 2009).

Tissue digestion and isolation of endometrial cells

The following procedures were performed aseptically under a laminar flow hood. The broad ligament, vascular and muscular tissues were removed from endometrial strips (Fortier *et al.* 1988). The small strips of endometrium were placed into a digestive solution: Hank solution (HBSS) (Sigma-Aldrich) with trypsin (Euroclone) (0.5 mg mL^{-1}), collagenase II (Sigma-Aldrich) (0.5 mg mL^{-1}), BSA (Sigma-Aldrich) (1 mg mL^{-1}) and deoxyribonuclease I (Sigma-Aldrich) (0.1 mg mL^{-1}). After 90 min of incubation in a shaking water bath at 37°C, the cell suspension was filtered through a 40 μm mesh, to remove undigested material, and the filtrate is resuspended in a washing medium consisting of HBSS supplemented with 10% foetal bovine serum (FCS) (Euroclone). The suspension was centrifuged at 1500 rpm for 10 min and after two further washes in the washing medium, the cells were resuspended in RPMI 1640 with L-glutamine (Euroclone) (2 mmol L^{-1}), FCS (10%), penicillin (50 UI mL^{-1}), streptomycin (50 μg mL^{-1}) and amphotericin B (2.5 μg mL^{-1}). The cells were then plated in 25 cm^2 flasks and maintained at 37°C with 5% CO_2. To obtain separate stromal and epithelial cell populations, the supernatant containing epithelial cells suspension was removed 18 h after plating. Epithelial cells in suspension were transferred to another flask and incubated to allow epithelial cells to adhere. Stromal and epithelial cell populations were distinguished based on morphology and immuno-histochemical staining. Cell cultures were maintained at 37°C, 5% CO_2 in air, in a humidified incubator (Euroclone) (Herath *et al.* 2009).

Histological and Immunoistochemical staining

Uterus samples were fixed in buffered formalin (4%) and embedded in paraffin, sectioned (5 μm) and stained with Haematoxylin–Eosin (H&E).

For immuno-histochemical staining was used anti-vimentin marker for stromal cells and anti-cytocheratin for epithelial cells (Leong *et al.* 2003). Antigen retrieval was carried out by microware (3 cycles of 5 min at 400 W) in EDTA buffer (pH = 9) for anti-vimentin and with trypsin for 12 min at 37°C for anti-cytokeratin. Enzymatic reaction was blocked immersing tissue sections in PBS (pH = 7.4) at 4°C. Endogenous peroxidase was inhibited by incubation of tissue sections with 3% hydrogen peroxide for 15 min at room temperature.

The sections were incubated for 1 h at room temperature with primary antibodies (Monoclonal Mouse Anti-Vimentin Clone V9, M0725, Dako; Monoclonal Mouse Anti-Human Cytokeratin Clones AE1/AE3, M3515, Dako) at a dilution 1:50 with BSA 1%. Tissue sections were incubated with biotinylated anti mouse secondary antibody (BA 9200, Vector) (1:200) for 30 min. Later an avidin-biotin-peroxidase complex (ABC, elite standard PK6100, Vector) was incubated for 30 min. Reactivity was revealed with 3,3′-diaminobenzidine in chromogen solution kit (DAB, Sigma-Aldrich) and stopped with rinses in distilled water. Between each step the sections were washed with PBS. Slides were counterstained with haematoxylin, dehydrated with graded ethanol, cleared in xylene and then cover slipped.

Cell cultures immuno-phenotyping

Epithelial and stromal cells were transferred in chamber slide and incubated for 24 h. Then they were fixed in acetone for 10 min and washed in PBS. Endogenous peroxidase was inhibited by incubation of tissue sections with 0,3% hydrogen peroxide for 15 min at room temperature (RT). After other washes with PBS (pH 7.4), the cells were incubated for 1 h at room temperature with primary antibodies (vimentin or cytokeratin AE1/AE3) at a diluition 1:50; later the cells were incubated with mouse biotin-conjugated secondary antibody (1:200) for 30 min. Cells were then processed according to the avidin/biotin peroxidase complex method and reactivity was revealed with 3,3′ diaminobenzidine in chromogen solution kit and stopped with rinses in distilled water. Between each step the sections were washed with PBS. Slides were counterstained with haematoxylin, dehydrated with graded ethanol, cleared in xylene and then cover slipped. Immunostained uterus samples and cell cultures were examined with microscopy (Nikon Eclipse E800) and scanned with Digital Sight DS-Fil camera.

Cell viability assay

Cell viability was evaluated using MTT colorimetric assay (Denizot & Lang 1986). Epithelial cells and stromal cells (10^5 cells mL^{-1}) were seeded in 96-well plates. After 24 h cells were treated with rifaximin (Fatro) (10, 50 and 100 μmol L^{-1}), dexamethasone (Sigma Aldrich) (10 μmol L^{-1}) or vehicle (DMSO 0.05% in RPMI) and were cultured at 37°C, CO_2% in air for 48 h. Then MTT (Sigma Aldrich) was added at the final concentration of 1 mg mL^{-1} and incubated for 2 h. Formazan crystals were dissolved with 100 μL of DMSO. The absorbance was measured at 550 nm using an ELISA plate reader (Sunrise, TECAN) and then results were expressed as a percent ratio between absorbance of treated and untreated cells.

Anti-inflammatory activity

Cells were cultured at 37°C in 5% CO_2 until >80% confluence was achieved. Afterwards, cells were seeded in 12 wells plates (3×10^5 cells mL^{-1}). Cells were pre-treated with rifaximin (10, 50 and 100 μmol L^{-1}), dexamethasone (10 μmol L^{-1}) or vehicle (DMSO 0.05% in RPMI) and 24 h later they were treated with LPS (*Escherichia coli* O111:B4, Sigma Aldrich) (1 μg mL^{-1}). After an additional 24 h, supernatants were centrifuged at 1000*g* for 20 min and IL-6 and IL-8 concentrations were measured by ELISA according manufacturer's instruction (Cloud-Clone Corp.). These cytokines were chosen among the set of cytokines generally

considered in inflammation since they represent the most sensitive markers of inflammatory response in endometrial cells. (Cronin *et al.* 2012).

Proteins dosage

Protein concentration of each sample, collected for IL-6 and IL-8 evaluation, was determined by bicinconcinic assay (BCA) (Thermoscientific). In particular, cells were lysed after 24 h LPS stimulation with IC12 buffer. 200 μL of BCA was added to 25 μL of sample and after 30 min incubation at 37°C protein concentration was measured spectrophotometrically at 550 nm.

Statistical analysis

Data were presented as mean ± standard error (SEM). Cytokine values were normalized for protein concentration of each sample. Significant differences between groups were evaluated by one-way ANOVA, followed by Dunnett's post-test. $P < 0.05$ was designed as significance.

Results

Uterus samples staining

The endometrial samples used for the study were subjected to histological examination and showed normal histological patterns without the presence of inflammatory or degenerative processes (Fig. S1).

The endometrial samples were stained with immunohistochemical marker anti-cytokeratin AE1/AE3 and anti-vimentin to identify, respectively, the epithelial and stromal cells of endometrial mucous membrane (Fig. S2, S3).

Cell cultures immuno-phenotyping

Immunohistochemical analysis was also applied to primary cell cultures with the goal of distinguishing and phenotyping mesenchymal and epithelial cells.

As expected, epithelial cells were positive for anti-cytokeratin but negative for anti-vimentin (Fig. S4), whereas stromal cells staining was the opposite, being positive to anti-vimentin, but negative to anti-cytokeratin (Fig. S5).

Immunohistochemical staining also highlighted morphologic difference between the two cell types: epithelial cells had polygonal shape and were organized in clusters, whereas stromal cells had irregular starry shape and were dispersed in the plate.

Viability assay

Rifaximin was not cytotoxic for both epithelial and stromal cells at all concentrations tested, with cell viability consistently over 80% (Fig. 1).

Anti-inflammatory activity

Cultured epithelial and stromal cells in 96 well plates were pre-treated for 24 h with rifaximin (10, 50 and 100 μmol L^{-1}), dexamethasone (10 μmol L^{-1}) or vehicle (DMSO 0.05% in RPMI) and 24 h later they were treated with LPS (1 μg mL^{-1}). LPS binds the toll-like receptors 4 (TLR4), primarily involved in the innate immune response towards many pathogens such as numerous viruses and bacteria. TLR4 activation by LPS induces a signalling pathway that leads to the activation of NF-kB and determines

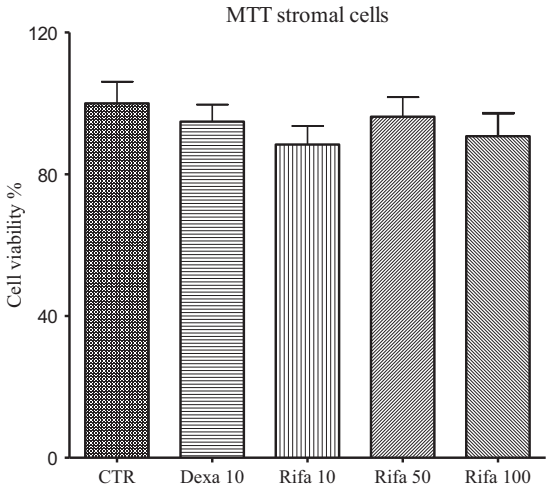

Fig. 1 Stromal cell viability measured by MTT assay after 48 h exposure to different rifaximin (Rifa) concentrations (10, 50 and 100 μmol L^{-1}) or with dexamethasone (Dexa)10 μmol L^{-1} compared to vehicle (CTR). $N = 12$ independent experiments

cytokine and chemokine production (Mencarelli *et al.* 2011; Cronin *et al.* 2012). In this study IL-6 and IL-8 production by epithelial and stromal cells was evaluated using an ELISA kit.

Inflammatory response was observed only in stromal cells whereas epithelial cells did not respond to LPS stimulation. In stromal cells after 24 h of LPS stimulation, IL-6 levels were tripled compared to basal condition, as observed by Cronin and co-workers in stromal cells isolated from bovine endometrium and cultured in the same conditions (Cronin *et al.* 2012). In these cells dexamethasone (10 μmol L^{-1}) and rifaximin (100 μmol L^{-1}) pre-treatment significantly prevented IL-6 increment induced by LPS, keeping values comparable to basal conditions and reducing IL-6 concentration, respectively, of 77% and 70% compared to LPS stimulation. Also rifaximin lower concentrations (10 e 50 μmol L^{-1}) determined an anti-inflammatory effect reducing IL-6 levels although without reaching statistical significance (Fig. 2).

Rifaximin was more effective than dexamethasone in reducing LPS-induced IL-8 release producing a significant drop (about 80%) of IL-8 concentration when tested at 100 μmol L^{-1} compared to LPS challenge (Fig. 3).

Discussion

Currently, in dairy farms, peri- and post-calving uterine diseases represent a very important problem. In particular, endometritis results in significant loss in reproductive performance with subsequent economic loss to the farmer. Unfortunately, today there is not a uniform scientific opinion on both diagnostic and therapeutic strategies to be applied to endometritis, a frequent bovine disease characterized by infection and local inflammation. Consequently, a drug endowed with both antibiotic and anti-inflammatory activity seems to be of great interest.

Rifaximin is a poorly absorbed molecule already used in uterine diseases for its antibacterial activity without risks of drug traces in animal products. In this work we wanted to explore its anti-inflammatory activity on a primary bovine endometrium cell culture.

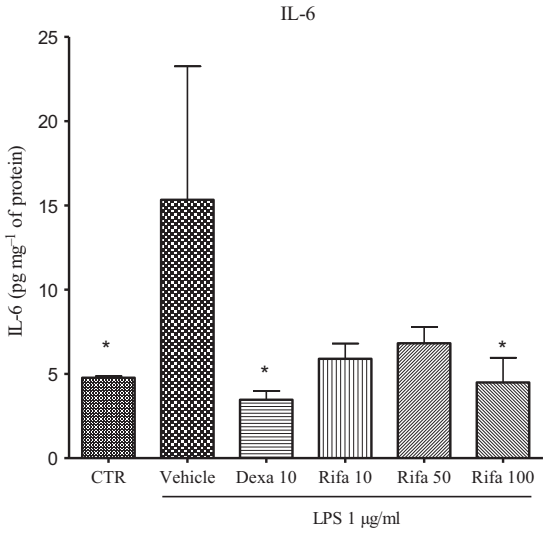

Fig. 2 IL-6 production in stromal cells stimulated with LPS 1 μg mL^{-1}, pre-treated with dexamethasone (Dexa) 10 μmol L^{-1}, rifaximin (Rifa) 10, 50, 100 μmol L^{-1} or vehicle (RPMI with 0.5% DMSO). CTR refers to cells without any treatments *=$P < 0.05$, one-way ANOVA, Dunnett's post-test versus vehicle. $N = 8$ independent experiments

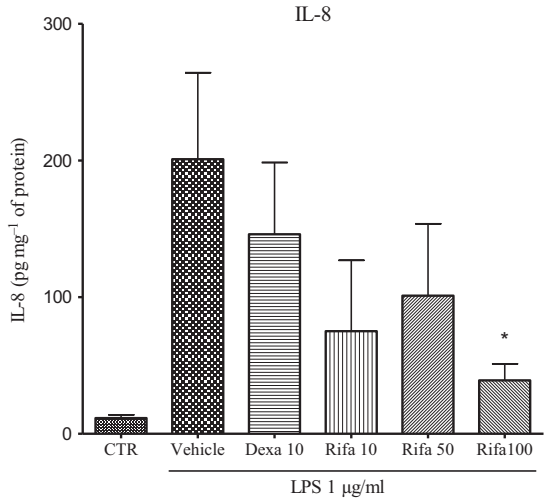

Fig. 3 IL-8 production in stromal cells stimulated with LPS 1 μg mL^{-1}, pre-treated with dexamethasone (Dexa) 10 μmol L^{-1}, rifaximin (Rifa) 10, 50, 100 μmol L^{-1} or vehicle (RPMI with 0.5% DMSO). CTR refers to cells without any treatments. *=$P < 0.05$, one-way ANOVA, Dunnett's post-test versus vehicle. $N = 8$ independent experiments

Rifaximin showed an anti-inflammatory effect on stromal endometrial cells reducing IL-6 and IL-8 production after LPS stimulation opening a new possibility for the use of this antibiotic for uterine diseases. In the experimental conditions used in our study, epithelial cells did not respond to LPS (1 μg mL^{-1}) stimulation. The absence of interleukin in epithelial supernatant could be due both to a diminished response to LPS stimulation of epithelial cells and to a low sensitivity of immune-enzymatic technique. Hirata and collaborators observed that in human endometrium both epithelial and stromal cells express toll-like receptors 4 (TLR4), but LPS when used alone, was able to stimulate IL-8 secretion only in stromal cells (Hirata *et al.* 2005).

Cronin and co-workers, in a study carried out on primary epithelial and stromal cells (Cronin *et al.* 2012), were able to detect by ELISA IL-6 and IL-8 accumulation after LPS incubation only in stromal cells. They could observe an inflammatory response in epithelial cells by evaluating IL-1B, IL-6 and IL-8 mRNA expression.

Endometrial epithelial cells are the first cells involved in inflammatory-infectious events, whereas stromal cells play an important role in endometritis when, after calving, the epithelial barrier is damaged and stromal cells are exposed to bacteria in the uterine lumen. Moreover, stromal cells are more abundant than epithelial cells and they are adjacent to vessels so their production of cytokines and chemokines can have more effects on immune cells.

Knowing the greater presence of mesenchymal cells in endometrium compared to epithelial cells and their proximity to blood vessels and immune cells, it is clear that these cells play a fundamental role in the innate immune response and in developing an inflammatory response in the presence of pathogens. (Cronin *et al.* 2012).

Rifaximin has already shown anti-inflammatory properties on the human gut by the activation of Pregnane-X-Receptor (PXR), resulting in inhibition of NF-kB and reduction in the production of cytokines and chemokines. PXR receptors have a conserved N-terminal DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD). The most studied are the human and murine PXRs, showing an identity in the amino acid sequence of 80% in the LBD and a 96% homology in the DBD, but present marked differences in the interaction with specific ligands. In particular, rifaximin is a specific agonist of human PXR, while it does not activate murine PXR. The ability to stimulate PXRs also varies according expression of the receptor in the different tissues. For example rifaximin activates the human intestinal PXR, but it is not able to induce the transcription of target genes of human hepatic PXR (Cheng *et al.* 2012).

In the literature there is little data about bovine PXRs, but in an *in vitro* study on C3A hepatoma transfected cells this receptor lacked the activation by rifampicin (Küblbeck *et al.* 2016). More studies are needed to investigate the possible mechanism that underlies rifaximin anti-inflammatory activity.

Conclusions

This study showed for the first time that rifaximin is able to reverse LPS-induced IL-6 and IL-8 production in bovine stromal endometrium cells, suggesting a potential anti-inflammatory activity of rifaximin in endometritis. This leads us to speculate about its beneficial applications in inflammatory uterine diseases. The possible mechanism underlying this effect and the potential involvement of PXR receptors remain to be investigated.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. Rifaximin used in this study was kindly supplied by Fatro.

Conflict of Interest

None declared.

References

Calanni F., Renzulli C., Barbanti M. & Viscomi G.C. (2014) Rifaximin: beyond the traditional antibiotic activity. *The Journal of Antibiotics* **67**, 667–670.

Cheng J., Shah Y.M. & Gonzalez F.J. (2012) Pregnane X receptor as a target for treatment of inflammatory bowel disorders. *Trends in Pharmacological Sciences* **33**, 323–330.

Cronin J. G., Turner M.L., Goetze L., Bryant C.E., Sheldon I.M. (2012). Toll-like receptor 4 and MyD88 dependent signaling mechanisms of the innate immune system are essential for the response to lipopolysaccharide by epithelial and stromal cells of the bovine endometrium. *Biology of Reproduction* **86**, (article 51) 1–9.

Denizot F. & Lang R. (1986) Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *Journal of Immunological Methods* **89**, 271–277.

DuPont H.L. (2015) Review article: the antimicrobial effects of rifaximin on the gut microbiota. *Alimentary Pharmacology and Therapeutics* **43**(Suppl. 1), 3–10.

Fortier M.A., Guilbault L.A. & Grasso F. (1988) Specific properties of epithelial and stromal cells from the endometrium of cows. *Journal of Reproduction and Fertility* **83**, 239–248.

Herath S., Lilly S.T., Fischer D.P., Williams E.J., Dobson H., Bryant C.E. & Sheldon I.M. (2009) Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2α to prostaglandin E2 in bovine endometrium. *Endocrinology* **150**, 1912–1920.

Hirata T., Osuga Y., Hirota Y., Koga K., Yoshino O., Harada M. *et al.* (2005) Evidence for the presence of toll-like receptor 4 system in the human endometrium. *The Journal of Clinical Endocrinology and Metabolism* **90**, 548–556.

Küblbeck J., Zancanella V., Prantner V., Molnár F., Squires E.J., Dacasto M. *et al.* (2016) Characterization of ligand-dependent activation of bovine and pig constitutive androstane (CAR) and pregnane X receptors (PXR) with interspecies comparisons. *Xenobiotica* **46**, 200–210.

Leong A.S.Y., Cooper K., Leong F.J.W.M. (2003). *Manual of Diagnostic Antibodies for Immunohistology* (2nd ed.). pp. 179–180, pp. 439-441. Greenwich Medical Media Ltd.: London.

Ma X., Shah Y.M., Guo G.L., Wang T., Krausz K.W., Idle J.R. & Gonzalez F.J. (2007) Rifaximin is a gut-specific human pregnane X receptor activator. *The Journal of Pharmacology and Experimental Therapeutics* **322**, 391–398.

Malinowski E., Lassa H., Smulski S., Klossowska A. & Kaczmarowski M. (2008) Antimicrobial susceptibility of bacteria isolated from cows with mastitis in 2006-2007. *Bulletin of the Veterinary Institute in Pulawy* **52**, 565–572.

Manjarrez J.L.A., Téllez S.A., Martínez R.R. & Hernández V.O.F. (2012) Determination of rifaximine in milk of dairy cows using high pressure liquid chromatography (HPLC). *FCV-LUZ* **22**, 112–119.

Mencarelli A., Migliorati M., Barbanti M., Cipriani S., Palladino G., Distrutti E. *et al.* (2010) Pregnane-X-receptor mediates the anti-inflammatory activities of rifaximin on detoxification pathways in intestinal epithelial cells. *Biochemical Pharmacology* **80**, 1700–1707.

Mencarelli A., Renga B., Palladino G., D'Amore C., Ricci P., Distrutti E. *et al.* (2011) Inhibition of NF-κB by a PXR-dependent pathway mediates counter-regulatory activities of rifaximin on innate immunity in intestinal epithelial cells. *European Journal of Pharmacology* **668**, 317–324.

Menozzi A., Dall' Aglio M., Quintavalla F., Dallavalle L., Meucci V. & Bertini S. (2016) Rifaximin is an effective and safe alternative to metronidazole for the treatment of chronic enteropathy in dogs: a randomised trial. *BMC Veterinary Research* **12**, 217.

Parmigiani E. & Truszkowska B. (1990) Terapia dell'endometrite nella bovina e nella cavalla. *Summa* **7**, 43–46.

Truszkowska B., Paggi J., Belluzzi S. & Masi M. (1990) Comportamento della rifaximina in formulazione schiumosa dopo somministrazione endouterina. *Summa* **7**, 98–102.

Wehrli W. & Staehelin M. (1971) Actions of the Rifamycins. *Bacteriological Reviews* **35**, 290–309.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Endometrium histological section. The endometrium appears normal without the presence of inflammatory cells (H&E., 4X).

Figure S2. Endometrium histological section. The epithelial cells of endometrial lining are immunopositive to cytokeratin AE1/AE3 and the stromal cells are negative (CK AE1/AE3, 20X)

Figure S3. Endometrium histological section. The stromal cells appear positive to vimentin and epithelial cells are negative (Vimentin, 10X).

Figure S4. Cell cultures.

Figure S5. Cell cultures. Stromal cells are positive to vimentin (Vimentin, 10X).