

Unconventional effects of anti-microbial agents in bovine reproduction



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SUMMARY

This review discusses the unconventional properties of some anti-microbial agents, with a particular focus on those administered in dairy cattle reproduction.

Several antibiotics also possess inherent anti-inflammatory and immunomodulatory properties and could act synergistically with other anti-inflammatory drugs.

For example, amoxicillin exerts a powerful anti-inflammatory action, modifying the transcriptional profile of specific cytokines, and it induces a relaxing effect on basal and oxytocin-induced myometrium contractility, in bovine. Macrolides act as anti-inflammatory agents by inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and exert a relaxing effect on myometrium. Aminoglycosides are believed to increase the phagocytic activity of leukocytes and inhibit the induction of spontaneous contractions of the myometrium in non-pregnant and pregnant cows. Some fluoroquinolones modulate the leukocytes production and the synthesis of a wide spectrum of cytokines and chemokines with specific anti-inflammatory effects. Enrofloxacin was shown to increase basal uterine contractions in strips collected from bovine uterus in a concentration-dependent manner. Rifaximin exhibits anti-inflammatory and immunomodulatory properties and exerts a contractile effect during the follicular phase of cow. The association of Trimethoprim-sulfamethoxazole shows an anti-inflammatory effect but data regarding contractility are unavailable. The choice of antibiotics that may be used during pregnancy should also take into account the aforementioned unconventional properties and, therefore, favor the active principles that reduce uterine contractility and promote immune quiescence (such as amoxicillin, erythromycin, clarithromycin, gentamicin, rifaximin).

Understanding these unconventional properties is mandatory to improve the use of antibiotics in a manner that is advantageous to both human and animal health and to enhance their beneficial effects.

Therefore, a thorough understanding of the effects of antibiotics on the immune system and uterine smooth muscle cells may be useful in clinical practice, helping design efficient therapies, reduce the indiscriminate use of antibiotics, and prevent the antibiotic resistance. Moreover, this may help improve animal welfare and reduce the significant economic losses resulting from the impact of reproduction diseases on livestock.

KEY WORDS

Antibiotics, anti-inflammatory, cow, immunomodulatory, muscle contractility.

INTRODUCTION

The continuous discovery, development, and use of antibiotics in animal and human healthcare has immensely aided the fight against infectious diseases caused by bacteria and contributed to individual and social well-being. However, the persistent emergence of bacterial strains resistant to nearly all known antibiotics is a matter of serious concern for public health. The unwise and widespread use of antibiotics has contributed to the spread of these bacterial strains in the environment, thereby increasing the risk of antibiotic resistance ¹.

In addition to anti-microbial effects, several antibiotics show other properties, such as anti-inflammatory effects ²⁻⁵ and the ability to affect smooth muscle contractility in some organs,

such as the intestine ^{6,7} and the uterus ^{8,9}. These unconventional properties are important to understand and standardise the use of antibiotics in a manner that is advantageous to both human and animal health.

In this review, we discuss the unconventional properties of some anti-microbial agents, with a particular focus on those administered in dairy cattle reproduction (Table 1; Figure 1).

Classification of antibiotics

Antibiotics are anti-microbial substances produced by bacteria and fungi. Apart of these, molecules of synthesis, such as sulphonamides and quinolones, are also included ¹⁰. Antibiotics can be classified according to different characteristics, such as on the type (bactericidal or bacteriostatic) ¹¹ and spectrum of action (broad, medium, or narrow) ¹², origin (natural, semi-synthetic, or synthetic) ¹³, and resistance (access, watch, or reserve) ^{14,15}. They can also be classified according to their mechanisms of action, including inhibition of cell wall synthesis, breakdown of cell membrane structure or function, prevention

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Table 1 - Summary of the effects of antibiotics on inflammation.

Mechanisms of action	Antibiotic class	Molecules	Effects on inflammation
Inhibition of cell wall synthesis	Penicillins	Amoxicillin	<ul style="list-style-type: none"> • Edits the transcriptional profile of IL-6, IL-10, TNF-α, TNF-β, and TNF-γ and increase production of IL-4^{26,27} • Has pro-oxidant effect²⁹
	Cephalosporins	Ceftiofur	<ul style="list-style-type: none"> • Downregulates TNF-α, IL-1β, and IL-6 synthesis³⁵
Inhibition protein synthesis	Macrolides	Erythromycin	<ul style="list-style-type: none"> • Inhibits NF-κB synthesis and gene expression of IL-2, IL-6, IL-8, TNF-α⁵⁵ • Decreases neutrophil accumulation⁵⁶
	Tetracyclines	Doxycyclin Tetracyclines in general	<ul style="list-style-type: none"> • Inhibits metalloproteinases activity^{60,61} • Decrease neutrophil chemotaxis⁶² • Scavenge ROS⁶³
	Aminoglycosides	Gentamicin	<ul style="list-style-type: none"> • Induces production of IL-1b and IL-6⁶⁶
Inhibition of the structure and function of nucleic acids	Quinolones	Enrofloxacin Norfloxacin and Enofloxacin	<ul style="list-style-type: none"> • Modulates a wide spectrum of cytokines and chemokines, with specific anti-inflammatory effects^{78,79} and induces an increase in ROS production⁸⁰ • Induce the release of PGF2α⁸⁸
	Rifamycins	Rifampicin	<ul style="list-style-type: none"> • Has anti-inflammatory and immunomodulatory properties that exert through binding to Pregnane X receptor¹⁰³
Blockage of key metabolic pathway	Sulfonamides	trimethoprim-sulfamethoxazole	<ul style="list-style-type: none"> • Decrease production of toxic metabolites by neutrophils⁵⁵ • Scavenge ROS⁵

IL: interleukin; TNF: tumour necrosis factor; NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; ROS: reactive oxygen species; PGF2 α : prostaglandin F2 α .

of bacterial protein synthesis by inhibition of ribosomal subunits (30S or 50S), disruption of the structure and function of nucleic acids, and blocking of key metabolic pathways¹⁴. Here, the classification of antibiotics based on mechanism of action has been reviewed to discuss their unconventional properties.

Cell wall-formation inhibitors

This class includes β -lactam antibiotics (penicillins, cephalosporins, cephamycins, β -lactamase inhibitors, monobactams, and carbapenems)¹⁶, cycloserine, vancomycin, and bacitracin, all used in veterinary medicine¹⁷.

Penicillins

Amoxicillin is widely used in cattle medicine for the prevention and treatment of respiratory (e.g., pneumonia, shipping fever)¹⁸, gastrointestinal (e.g., bacterial enteritis in calves),¹⁹ and urinary²⁰ infections, as well as for the treatment of metritis and mastitis^{21,22}.

In addition to the classical anti-microbial effect, amoxicillin also exerts a powerful anti-inflammatory action. It can modify the transcriptional profile of specific cytokines, including Interleukin (IL)-6, IL-10, Tumour Necrosis Factor (TNF) α , TNF β , and TNF γ ²³⁻²⁶, and it can also influence the production of IL-4 by exerting inhibitory effects on interferon- γ expression²⁷. Furthermore, in contrast to other β -lactam antibiotics²⁸, this antibiotic seems to recognise a membrane receptor present on neutrophils capable of activating Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-oxidase. The resulting pro-oxidant effect likely strengthens the bactericidal action of this drug²⁹.

In cattle, amoxicillin was found to exert a relaxing effect on basal⁸ (Fig. 1) and oxytocin-induced myometrium contractility during both follicular and luteal phases³⁰. Therefore, treatment of

post-partum diseases using amoxicillin (both alone and in association with oxytocin) is contraindicated⁸: amoxicillin could affect uterine cleaning. While, during early pregnancy, amoxicillin could be useful for hindering embryonic resorption, for its relaxing effect and its mechanism of action³⁰. Csányi et al.³¹ found that pre-treatment with amoxicillin enhanced uterine contractions on the final day of pregnancy in rats. The authors believed that this effect may have been achieved through the decrease in aquaporin 5 levels, a water channel protein, predominant before parturition, and not by a direct action of amoxicillin on uterine smooth muscle cells³¹.

Cephalosporins

Cephalosporins are grouped into 5 generations based on their target organism. While the use of first- and second-generation cephalosporins is approved primarily for the treatment of mastitis, third- and fourth-generation cephalosporins are used to treat respiratory infection, foot rot disease, metritis, and mastitis³².

Ceftiofur, a third-generation cephalosporin indicated for the treatment of bovine puerperal metritis caused by susceptible organisms³³, also exhibits immunomodulatory activities. It impairs pro-inflammatory cytokine secretion through the inhibition of NF- κ B and mitogen-activated protein kinase activation.

Ceftiofur inhibits p65-NF- κ B translocation to the nucleus and down-regulates TNF α , IL-1 β , and IL-6³⁴. Ci et al.³⁵ demonstrated that pre-treatment with ceftiofur reduced the mortality rate in mice that received a lethal dose of lipopolysaccharides (LPS) by modulating the production of these cytokines. Moreover, ceftiofur significantly decreased inflammation in a murine model of LPS-mediated acute lung injury (ALI) by reducing the levels of TNF α , IL-6, and IL-8³⁶.

An in vitro study demonstrated that high doses of cephalosporins (500 μ M, cumulative 1000 μ M and 2000 μ M) influenced uterine contractility by reducing the frequency, whereas they did not affect the amplitude and increased the area under the curve (AUC), that is an essential parameter for describing the effect of drugs as it reflects the exposure of tissue to the drug over time³⁷. This finding could have implications for its clinical use during pregnancy and in the post-partum period in cattle³⁷.

Agents that inhibit protein synthesis

Drugs that inhibit bacterial protein synthesis can be divided into 50S inhibitors and 30S inhibitors^{38,39}.

Drugs that inhibit the 50S ribosomal subunit

Antibacterial agents that inhibit the 50S ribosomal subunit include macrolides, lincosamides, phenicols, and linezolid^{40,41}. As the use of chloramphenicol is banned in food production

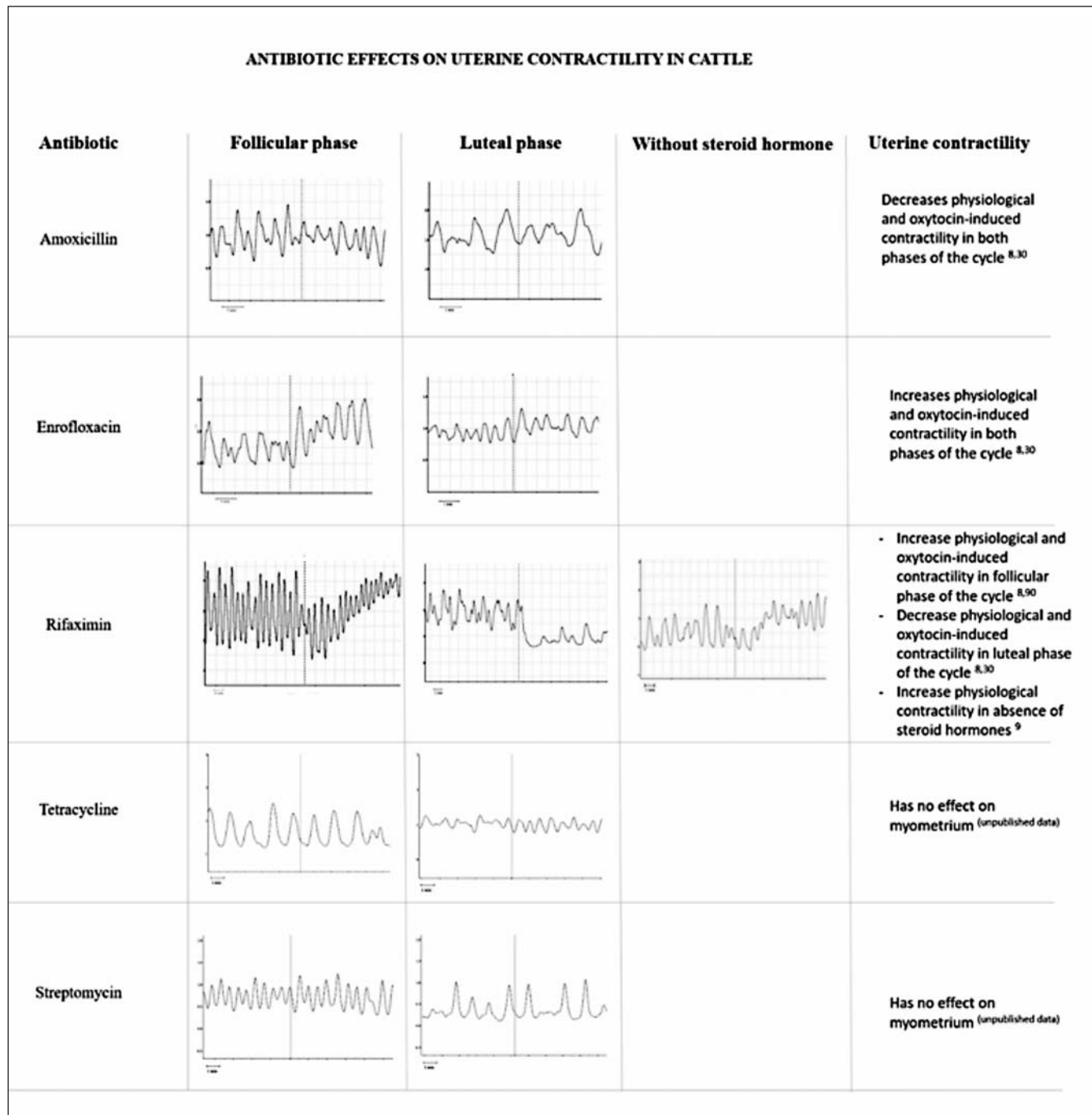


Figure 1 - Summary of the effects induced by antibiotics on in vitro contractility, in the bovine uterus under different hormonal conditions. Representative tracing of the drug has been performed by the authors. For each graph, the dotted line indicates the moment of the drug administration: the left side of the graphs represents the physiological contractility of the uterine strips; the right side shows the uterine strips behaviour after drug administration. Amoxicillin exerts a relaxing effect, whereas enrofloxacin promotes contractility in both phases of the cycle. Steroid hormones do not affect drug activity and for this reason their action has not been evaluated without steroid hormones. Rifaximin exerts a contractile effect only in the follicular phase⁸ or in the absence of predominant steroid hormones⁹. Tetracycline and streptomycin do not affect the contractility of the bovine uterus (unpublished data).

⁴², tiamphenicol and florphenicol are the only two phenicols approved for use in cattle. These molecules are mostly used for the treatment of respiratory infections and infectious keratoconjunctivitis^{43,44}. Florphenicol also exhibits immunomodulatory activity. Zhang et al.⁴⁵ demonstrated that florphenicol inhibited TNF and IL-6 release in mice infected with LPS by suppressing the NF- κ B pathway, which ultimately rescued them from LPS-induced death. Zhang et al.⁴⁶ also suggested the use of florphenicol as a potential treatment agent for ALI in LPS-infected mice based on a similar immunological effect on pro-inflammatory cytokines.

Macrolides and lincosamides are widely used to treat common infections in cattle, such as respiratory and genital infections and foot lesions⁴⁷. Macrolides are indicated in the treatment of metritis caused by susceptible organisms; however, therapeutic regimens often emphasise the evacuation of uterine contents as the primary treatment, which has been confirmed by in vitro studies: Granovsky-Grisaru et al.⁴⁸ demonstrated that erythromycin inhibited oxytocin- and carbachol-induced uterine contractions in pregnant rats. A study reported that erythromycin inhibited PGF 2α -induced uterus contractions in non-pregnant rats⁴⁹ and other study showed that clarithromycin inhibited contractions in the human uterus⁵⁰. Therefore, the use of antibiotics that exert a relaxing effect on myometrium could compromise the physiological self-cleaning phenomena and delay restitutio ad integrum in the treated subjects⁵¹⁻⁵⁴.

Macrolides act as anti-inflammatory agents by inhibiting NF- κ B, which further prevents the expression of other inflammatory mediators (IL-2, IL-6, IL-8, and TNF- α)⁵⁵. Macrolides also reduce neutrophil accumulation and encourage their apoptosis⁵⁶.

Agents that inhibit the 30S ribosomal subunit

Some of antibacterial agents that inhibit the 30S ribosomal subunit are tetracycline, streptomycin, and spectinomycin^{39,57}. In cows, tetracycline can be used to treat respiratory, urinary, enteric, soft tissue, and skin infections⁵⁸. Intra-uterine tetracyclines are extensively used in association with systemic penicillin to treat metritis and retained fetal membrane (RFM)^{22,59}. In cases of RFM, tetracyclines can interfere with the normal placental detachment mechanisms owing to their inhibitory effects on metalloproteinase activity^{60,61}. Moreover, these antibiotics suppress neutrophil chemotaxis⁶², act as scavengers of reactive oxygen species (ROS)⁶³, and are inhibitors of pro-inflammatory secretory phospholipase A₂⁶⁴.

Our in vitro study on uterine strips (unpublished data) indicates that tetracycline, at the concentrations of 10⁻⁶ M, 10⁻⁵ M, and 10⁻⁴ M, does not alter the contractility of the bovine uterus in pregnant animals (Figure 1). Csányi et al.³¹ showed that doxycycline pre-treatment did not alter the AUC of oxytocin-induced contractions in pregnant rats. Therefore, it can be confidentially excluded that the risk of placental retention described by Kaitu'u et al.⁶⁰ and Beagley et al.⁶¹ is related to the action of the antibiotic on uterine contractility.

Agents that react with the 30S ribosomal subunit and induce cell death

Aminoglycosides are primarily used to treat septicaemia and infections of the digestive tract, respiratory tract, and urinary tract in adult cattle and calves.

Aminoglycosides are believed to increase the phagocytic activity of leukocytes⁶⁵. Additionally, in an in vitro study, Frieling et al.⁶⁶ demonstrated the potential of gentamicin to induce the expression of higher levels of IL-1 β and IL-6 in whole blood samples upon stimulation with *Escherichia coli*, possibly due to the "remnants" of bacterial cell wall components broken down by gentamicin.

Among aminoglycosides, gentamicin is particularly indicated for the treatment of infection caused by *Pseudomonas aeruginosa* and *Mannheimia haemolytica* in calves, as well as for those caused by multiple gram-positive bacteria, *Mycoplasma* spp., and *Staphylococcus* spp.⁶⁷. Some studies have shown that gentamicin sulphate inhibits the induction of spontaneous contractions of the myometrium in non-pregnant⁵³ and pregnant cows⁵⁴. In the isolated bovine uterus, it was observed to inhibit oxytocin-, PGF 2α -, and KCl-induced contractions^{53,54,68}. Based on this, Yuksel et al.⁵⁴ claimed that gentamicin can be used as an antibacterial agent in cases of septic abortion, chorioamnionitis, pyelonephritis, and septic shock to prevent premature birth, miscarriage, and early contractions of the uterus. The relaxing effect was not exerted by streptomycin, as demonstrated in our in vitro study (unpublished data) (Figure 1). These results suggest that this drug may be used during pregnancy as well as in the treatment of post-partum pathologies, but more research is needed.

Nucleic acid synthesis inhibitors

Nucleic acid synthesis inhibitors include fluoroquinolones and rifamycins^{69,70}.

Fluoroquinolones

In buiatric practice, fluoroquinolones are mainly used for treating diarrhoea caused by *E. coli*⁷¹ in calves, and respiratory infections caused by *Pasteurella multocida*, *Pasteurella haemolytica*, *Haemophilus somnus*, and *Mycoplasma bovis* in adult cattle^{72,73}. In combination with other antibiotics (such as rifampicin and streptomycin), fluoroquinolones are used to treat infections caused by *Brucella* spp., as they can reach moderately high intracellular concentrations in macrophages and neutrophils⁷⁴.

Fluoroquinolones are also able to affect cytokine synthesis depending on the type of cell and cytokines⁷⁵⁻⁷⁷. Some fluoroquinolones modulate the leukocytes production and the synthesis of a wide spectrum of cytokines and chemokines with specific anti-inflammatory effects^{78,79}. Three mechanisms may explain the various immunomodulatory effects: (1) action on intracellular cyclic adenosine-3',5'-monophosphate and phosphodiesterases, (2) action on transcription factors, and (3) action on the eukaryotic equivalent of the bacterial SOS response (a global response to DNA damage), which is the bacterial response to DNA damage⁷⁸. On the other hand, enrofloxacin increases ROS production⁸⁰, which may be responsible for the typical side effects of the drug, such as cartilage damage and phototoxicity^{81,82}.

The effects exerted by fluoroquinolones on smooth muscles in different organs and animal species have been highlighted in some studies. These drugs act as gamma aminobutyric acid (GABA) receptor antagonists⁸³ by blocking ATP-dependent potassium channels⁸⁴⁻⁸⁷.

In the intestine, they induce the release of prostaglandin F 2α ⁸⁸ which is responsible for cholinergic transmission in the myenteric plexus⁸⁹. In vitro studies on rat myometrium have also

highlighted the effects of danofloxacin on oxytocin-induced uterine contractility. At lower concentrations (5-20 μmol), there is an increase in the frequency and amplitude of the peaks; however, at higher concentrations (40 and 80 μmol), this drug inhibits contractility as a consequence of hyperpolarisation⁸⁷. Enrofloxacin was shown to increase basal uterine contractions in strips collected from bovine uterus in a concentration-dependent manner; therefore, it was hypothesized to optimise uterine self-cleaning during post-partum complications, such as metritis and RFM⁸ (Figure 1). Moreover, its association with ecboic substances, such as oxytocin, could interfere with the formation of bacterial biofilms⁹⁰.

Rifamycins

In veterinary medicine rifamycins are mostly used to treat foal infections caused by *Rhodococcus equi* or diseases caused by intracellular bacteria (e.g. paratuberculosis)^{91,92}. In cattle, rifaximin can be administered by the endo-uterine or intra-mammary route for the prevention and treatment endometritis and mastitis^{93,94}. The endo-uterine administration of rifaximin, in foam formulation, in acute metritis, induce the expansion of the uterine lumen, which allows the uniform distribution of the active principle on the entire endometrial surface.

In vitro studies have shown that rifaximin exerts a contractile effect during the follicular phase under oestrogen control, and it has been speculated that it can be used alone or in association with oxytocin^{8,90}. Rifaximin can bind to the Pregnane X receptor (PXR), a phenomenon that can be affected by co-activation with steroids⁹⁵. Upon binding, there is a potentiation of the contracting/relaxing effect exerted by the steroid hormones predominantly expressed at that point⁸ (Figure 1). The administration of rifaximin could represent an optimal therapy for the treatment of retained placenta and acute metritis in cattle⁹.

Additionally, rifaximin exhibits anti-inflammatory and immunomodulatory properties that involve its binding to PXR⁹⁶. Activated PXR suppress the expression of NF- κ B and cAMP-response element (CREB) binding protein⁹⁷ and the release of pro-inflammatory cytokines⁹⁸, as indicated by the reduced levels of interferon- γ released by mononuclear cells (isolated from the gastrointestinal tract) after rifaximin treatment⁹⁹.

Agents that impair cellular metabolism

Sulfonamides and sulphonamide potentiators

Sulfonamides are commonly used owing to their efficacy in the treatment of several diseases of cattle and calf, including shipping fever complex, bacterial pneumonia, bacterial enteritis, peritonitis, metritis, mastitis, calf diphtheria, and foot rot disease¹⁰⁰. The association of sulfonamides with diaminopyrimidines, such as trimethoprim (TMP) (another competitive inhibitor that targets the bacterial folate production cascade), is commonly used with synergistic purpose¹⁰¹. The use of TMP and sulfonamides (TMP-SU) expands the spectrum of bacteria and protozoa affected, thus the combination of drugs is used more often than sulfonamides alone.

The intra-uterine administration of sulphonamides for the treatment of bovine endometritis is not recommended owing to the loss of efficacy in the presence of organic debris, pus, and necrotic tissues¹⁰². Unfortunately, data regarding contractility induced

by sulphonamides or TMP-SU are currently unavailable. A non-anti-infective effect of trimethoprim-sulfamethoxazole (TMP-SMZ) has been postulated. One study has suggested that the anti-inflammatory effects exerted by TMP-SMZ can be a consequence of the reduction in neutrophil production and interference with oxygen-derived free radicals owing to its action as a scavenger of reactive oxygen⁵.

CONCLUSION

While antibiotics are commonly classified based on their action, they are never classified based on the alternative effects exerted, such as immunomodulation and uterine contractility. This review outlines the non-antimicrobial effects of antibiotics, and suggest its knowledge is of great value when choosing the most suitable drug in different cases. Drugs that stimulate uterine contractility (cephalosporin, enrofloxacin, and rifaximin) or at least do not inhibit it (tetracyclines and streptomycin) are a preferred choice in the treatment of uterine diseases. Antibiotics that impair physiological uterine cleaning (amoxicillin, erythromycin, clarithromycin, gentamicin) are sub-optimal drugs. Substances that do not affect contractility can be used in pregnancy, and the relaxing properties of some agents (e.g., rifaximin) can be advantageously exploited to prevent premature birth, miscarriage, and early induction of uterine contraction together with more specific ones. Moreover, it could be interesting to select antibiotics based on their anti-inflammatory and/or immunomodulatory effect; e.g., ceftiofur and florphenicol may be particularly used to treat disease-associated endotoxemia, such as in the case of metritis, or to prevent septic abortion.

Therefore, a thorough understanding of the effects of antibiotics on the immune system and uterine smooth muscle cells may be useful in clinical practice, helping design efficient therapies, reduce the indiscriminate use of antibiotics, and prevent the antibiotic resistance. Moreover, this may help improve animal welfare and reduce the significant economic losses resulting from the impact of reproduction diseases on livestock.

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