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# Oxytocin plus antibiotics: A synergism of potentiation to enhance bovine uterine contractility

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ABSTRACT

This *in vitro* study investigates the modulatory effect of three antibiotics (amoxicillin, enrofloxacin, and rifaximin) on contractility of the bovine uterine tissue, in follicular and luteal phases. The evaluation of the effects of these antibiotics ($10^{-4}$ M) was performed on oxytocin-induced contractility. The decision to test these antibiotics with the oxytocin ($10^{-6}$ M) comes from the reported ability of these combinations of hinder the antibiotic resistance and the formation of bacterial biofilms. The procedures were carried out in isolated organ bath, and the contractile functionality of the strip throughout the experiment was evaluated after a dose of carbachol ($10^{-5}$ M). The results demonstrate the different modulatory activity of these antibiotics, on the plateau of contraction induced by oxytocin, in both phases of the estrus cycle. The differing individual antibiotic effects of our testing made it possible to identify, only in some cases. Rifaximin in the follicular phase and enrofloxacin in both phases of the estrous cycle, induced a synergistic enhancement (potentiation) of uterine strip contraction induced by oxytocin. This result is thought important because these associations might enable, *in vivo*, a simultaneous increase of uterine cleaning and the antimicrobial action on bacteria in planktonic form and of those organized in biofilms.



## 1. Introduction

Treatment of metritis is aimed to eliminate, in a timely manner, any bacteria within the uterine lumen without interfering with the defence mechanisms therein [1–3]. Therefore, systemic administration of antibiotics is extremely useful but often not sufficient to achieve the local minimum inhibitory concentration [4,5], an essential parameter to avoid the occurrence of antibiotic resistance [6,7].

During the postpartum period, the activation of mechanisms involved in bacterial antibiotic resistance is facilitated by several organic substances inside the uterus (residual liquid delivery, cellular debris, and blood, pus) that may inactivate the action of antimicrobials [3,8], increasing the risk of antibiotic resistance [9]. Microorganisms may develop resistance not only against natural and semi-synthetic antibiotics but also against fully synthetic antibiotics, such as fluoroquinolones [10].

Therefore, the combination of antibiotics with ecbolic substances is important for treatment of postpartum disorders. Exogenous ecbolic substances (oxytocin, carbetocin, or prostaglandins) facilitate the action of antimicrobials [3,11] through stimulation of the self-cleaning activity and removal of organic material from the uterine lumen [12–14].

In particular, antibiotic treatment is often associated with the use of oxytocin [15]. Bukharin et al. [16] showed that combinations of antibiotics oxytocin have a significant effect on many infectious processes (mastitis, paraproctitis,

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endometritis, and so forth). In 2001, Ahmad et al. [17] reported the synergistic action of the association oxytocin-antibiotic in mares affected by endometritis. They found an increased uterine cleaning activity in animals treated with oxytocin and, consequently, a better efficiency of the antimicrobial drug [17].

Recently, *in vitro* studies demonstrated that ciprofloxacin-oxytocin association, in addition to the properties previously mentioned, has the ability to suppress the formation of bacterial biofilms [18,19]: sessile microorganisms are thousand times more resistant to antimicrobial agents than that found for the same microorganisms in planktonic form [20,21].

Therefore, current research includes a focus on the development of new means to decrease the spread of biofilm, not only through the discovery and development of antimicrobials but also with new strategies aimed to increase the sensitivity to the traditional drugs [22].

Several workers [18,19] demonstrated that oxytocin, when combined with some antibiotics, significantly reduces the formation of bacterial biofilm, but the mechanisms behind this synergistic action are not completely understood. Previous work demonstrates that some antibiotics have intrinsic contractile activity [23]. With this background, the present study was aimed to examine whether antibiotics previously identified as having intrinsic contractile activity could act synergistically with oxytocin in strengthening the contraction activity of bovine uterus. The effects of three antibiotics, commonly used in bovine reproduction (amoxicillin, enrofloxacin, and rifaximin), on oxytocin-induced uterine contraction were tested *in vitro*.

## 2. Materials and methods

### 2.1. Uterine strip preparation

A total of 60 uteri were obtained from cows slaughtered at a local abattoir. All uteri were found without diseases and so were considered in our study: 28 from cows in the follicular phase and 32 from cows in the luteal phase. The phase of the estrus cycle was identified as reported by Piccinno et al. [23]. After cows' slaughter, uteri were collected in about 20 ± 10 minutes.

From each uterus, a single circular portion of the middle part of the ipsilateral horn to the functional ovarian structure was excised and immediately placed in a flask containing prerefrigerated and oxygenated Krebs solution (NaCl 113 mM, KCl 4.8 mM, $CaCl_2 \cdot 2H_2O$ 2.2 mM, $MgSO_4$ 1.2 mM, $NaH_2PO_4$ 1.2 mM, $NaHCO_3$ 25 mM, glucose 5.5 mM, and sodium ascorbate 5.5 mM), which was prepared daily. The flask was then immediately transported (15 ± 5 minutes) to the laboratory in an insulated box. Uterine circular full-thickness portions were cut into strips (10 mm × 3 mm) parallel to the longitudinal muscle fibers [23].

### 2.2. Experimental design

The procedures were carried out in isolated organ bath, and the functionality of the strip throughout the experiment was evaluated by a dose of carbachol ($10^{-5}$ M), as described by Piccinno et al. [23]. After the stabilization period, strips were exposed to a single dose of oxytocin ($10^{-6}$ M; Sigma–Aldrich, Milano, Italy) ensuring maximal uterine stimulation *in vitro*, as reported in the literature [24,25], and were left in the bath for 10 minutes, to reach the plateau phase of the tonic effect. Afterward, without washing out the solution, amoxicillin (Sigma–Aldrich), rifaximin (Fatro, Ozzano Emilia, Italy), or enrofloxacin (Sigma–Aldrich) were added to the bath. In particularly, we tested 28 strips in follicular phase (respectively, 10 for amoxicillin, nine for rifaximin, and nine for enrofloxacin) and 32 strips in luteal phase (respectively, 11 for amoxicillin, 11 for rifaximin, and 10 for enrofloxacin). These associations (oxytocin-amoxicillin, oxytocin-rifaximin, and oxytocin-enrofloxacin) were left in the bath for 10 minutes and then washed.

All the antibiotics were tested at a concentration of $10^{-4}$ M which is, at the same time, the concentration that ensures the maximum uterine stimulation *in vitro* [23] and the minimum inhibitory concentration of amoxicillin, rifaximin, and enrofloxacin [26–28]. Rifaximin was dissolved in ethanol, whereas the solutions of oxytocin, amoxicillin, and enrofloxacin were made in distilled water. Previous studies from our group reported that ethanol has no effect on *in vitro* uterine contractility of the cow [23,29].

A solution of carbachol ($10^{-5}$ M) was used to test the functionality of the strips throughout the experiment. A variability ≤20% between tests was considered acceptable. Contractile activity of the strips was computed as average amplitude (grams), average frequency (number of contractions per minute), and area under the curve (AUC, g·s), before and after the administration of oxytocin and oxytocin-antibiotics. The time interval over which were made such determinations was identified after observing the behavior of oxytocin on basal contractility and that of the three antibiotics (amoxicillin, enrofloxacin, and rifaximin) tonic effect (plateau) induced by oxytocin.

For each administration, the percentage index of increase or decrease from baseline (basal vs. oxytocin and basal vs. association oxytocin-antibiotics) and from oxytocin plateau (oxytocin vs. association oxytocin-antibiotics) was evaluated using the following formula: $(T_{\text{Second value}} - T_{\text{first value}}/T_{\text{first value}}) \times 100$ [23,30].

### 2.3. Statistical analysis

Amplitude, frequency, and AUC were expressed as mean ± standard error of the mean (SEM) and were subjected to statistical analysis with SPSS Statistics 19 (IBM, NY). Area under the curve depended mainly on the amplitude of contraction and to a smaller degree on its time course and gives a more exact evaluation of the work performed by contracting motor units than the measure of tension alone. The comparison between the phases of the cycle was analyzed using Student's *t* test.

The action exerted by oxytocin on spontaneous contractility and that produced by the three antibiotics was analyzed, intragroup by general linear model for repeated measures and intergroups (amoxicillin, rifaximin, and enrofloxacin) with one-way ANOVA for independent parameters and posthoc least significance difference test.