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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.

Statistical analyses were carried out in the same way for the percentage indices of increase or decrease (basal/oxytocin, basal/association oxytocin-antibiotics, and oxytocin/association oxytocin-antibiotics). Values were considered statistically significant at $P < 0.05$.

3. Results

Spontaneous uterine contractility was observed in 24 strips of 28 uteri collected in the follicular phase and 24 strips of 32 uteri collected during the luteal phase. Twelve strips were discarded because they do not show any spontaneous contraction or comparable responses to carbachol (10^{-5} M). The representative tracings (Figs. 1–3) respectively show the effects induced by oxytocin (10^{-6} M) on basal contractility, and by associations oxytocin-antibiotics on oxytocin-induced plateau, in the follicular (Figs. 1A, 2A and 3A) and luteal phases (Figs. 1B, 2B and 3B), for amoxicillin (10^{-4} M), rifaximin (10^{-4} M), and enrofloxacin (10^{-4} M), respectively. The mean values ± SEM of amplitude, frequency, and AUC before (basal), after oxytocin administration, and subsequent doses of the associations oxytocin-antibiotics are reported in Tables 1–3. The relative percentage indices (baseline/oxytocin, basal/association oxytocin-antibiotics, oxytocin/association oxytocin-antibiotics) are reported in Tables 4–6, respectively, for amplitude, frequency, and AUC. At all stages of the experiment, the amplitude, frequency, and AUC of baseline contraction showed a statistically significant difference between follicular and the luteal phase (Tables 1–3) [23,31,32]. The dose of oxytocin resulted in a tonic effect, whose plateau was reached within 6 minutes and continued until washout was performed, in agreement with previous works [12,33]. The activity of the neuropeptide was higher in the follicular than that in the luteal phase. The contractile activity induced by the antibiotics on oxytocin plateau was different for intensity and duration of action (Fig. 1A, B; Fig. 2A, B; Fig. 3A, B). Amoxicillin reduced the contractility induced by oxytocin in both phases of the cycle; rifaximin increased the contractile tone in the follicular phase (Tables 4 and 6), whereas it reduced the contractile tone in the luteal phase. Enrofloxacin increased the contractile tone induced by oxytocin in both stages of the estrous cycle (Tables 4 and 6).

4. Discussion

The involvement of biofilms in infectious diseases is critical (about 80% of microbial infections) [34] and represents the leading cause of bacterial resistance, both in human and veterinary medicine [35,36]. Several groups of investigators have identified a potentially important suppressive action on bacterial resistance and on bacterials' biofilm formation induced by oxytocin-antibiotic associations [16–19]. In particular, Ahmad et al. [17] reported the benefits of the association of oxytocin and antibiotics in mares affected by endometritis, finding that in treated animals an increase in uterine cleaning was induced by the neuropeptide and, consequently, a better efficiency (potentiation) of the antimicrobial drug used. In this context, we tested, *in vitro*, the effects of three antibiotics (amoxicillin, enrofloxacin, and rifaximin), commonly used in bovine reproduction, on the uterine contractility induced by oxytocin, in the follicular and the luteal phases. The results showed that all three antibiotics have a modulatory effect on the tonic action induced by oxytocin, similar for intensity and duration of action to those determined by the same antibiotics on uterine strips basal contractility [23]. In particular, amoxicillin was responsible for a modest relaxing effect on the contraction induced by oxytocin. This modulatory activity happened in both phases of the cycle, although with a more important effect in the follicular phase. However, it was more limited than that determined by the amoxicillin on basal contractility [23], as demonstrated by the percentage differences for indices of contraction amplitude of amoxicillin alone (−7.95%) [23] versus oxytocin plus amoxicillin (−2.69%; Table 4). This minimal effect that amoxicillin had on the force of contraction may have been caused by the action of oxytocin on Ca^{2+}-ATPase pump. Indeed, the neuropeptide seems to exert its tonic effect through the inhibition of this pump responsible for the outflow of Ca^{2+} in the extracellular environment; a mechanism extremely important for the relaxation of smooth muscle cells [37–39]. Consequently, the use of amoxicillin is not indicated for the treatment of postpartum diseases even when it is administered with oxytocin. Indeed, the reduction of the force of contraction induced by amoxicillin (albeit modest) could promote adhesion of bacterial cells to the uterus and the subsequent formation of biofilms. Rifaximin exerted a modulatory action strictly dependent on the stage of the cycle: in the follicular phase, it strengthened the contractile effect of oxytocin, whereas in the luteal phase, it induced a relaxing action. The activity of this antibiotic on the tonic effect induced by the neuropeptide is more moderate compared to that obtained on basal contractility, possibly because rifaximin does not induce a direct effect on smooth muscle cells, but it acts only by amplifying the action of the predominant steroid hormone of each cycle phase [23].

In the light of these observations, the increase in contractile tone induced by the rifaximin under the influence of estrogen allows a consideration for possibility of using this molecule in association with oxytocin, in the treatment of subacute metritis to facilitate the uterine cleaning and to interfere with the establishment of the antibiotic resistance and/or with the formation of bacterial biofilm. Conversely, the use of rifaximin in the treatment of chronic metritis is not indicated. Indeed, this antibiotic could promote adhesion of microorganisms in planktonic form to the uterus and the subsequent formation of biofilms. Enrofloxacin is able to increase the contractions induced by oxytocin in both phases of the cycle, albeit with a more important effect in the luteal phase. We can speculate that the inhibition of the ATP-sensitive potassium channels induced by enrofloxacin [40–42], combined with the inhibitory action on Ca^{2+}-ATPase pump exerted by the neuropeptide [37–39], creates a synergism of potentiation on the uterine contraction, optimal to facilitate the self-cleaning, and hinder the formation the bacterial biofilm. In support of this conjecture, it is useful to compare the percentage indices relative to the magnitude of contraction induced by enrofloxacin in the luteal phase (6.20%) with those

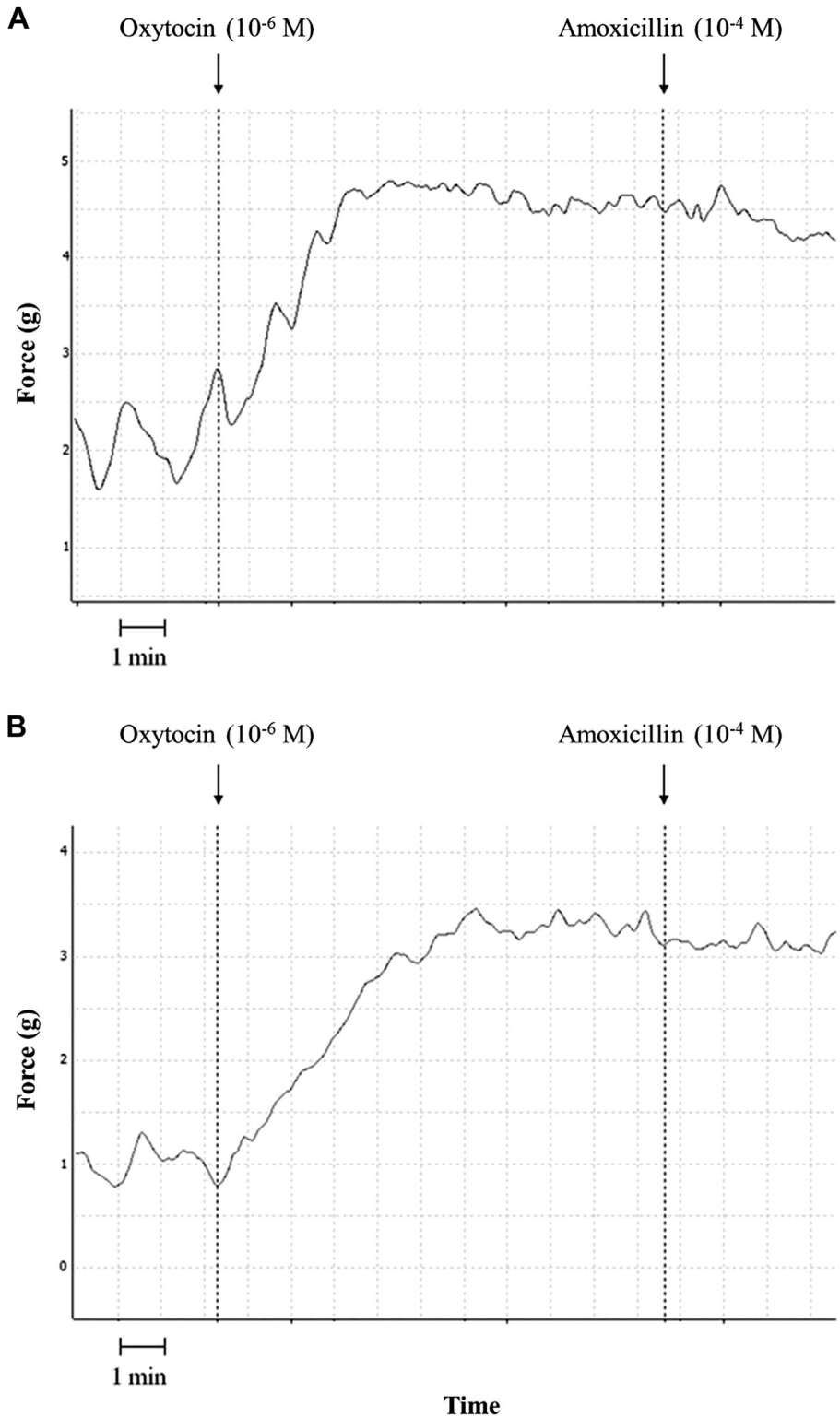

Fig. 1. Representative tracing of the effects induced by oxytocin (10^{-6} M) on uterine contractility and by amoxicillin (10^{-4} M) on tonic effect induced by neuropeptide, in follicular (A) and luteal (B). The amplitude (y axis) is expressed in g.

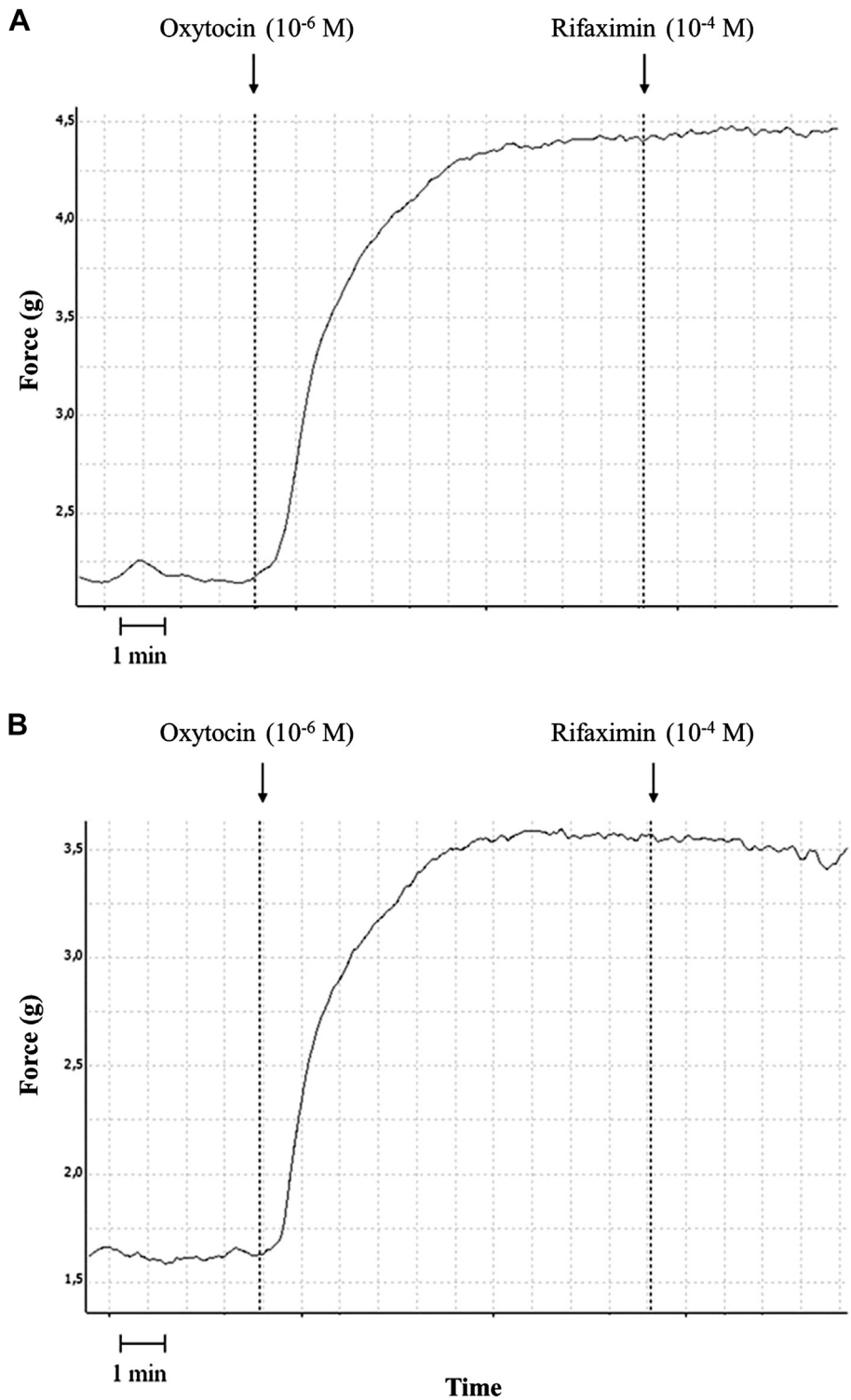

Fig. 2. Representative tracing of the effects induced by oxytocin (10^{-6} M) on uterine contractility and by rifaximin (10^{-4} M) on tonic effect induced by neuropeptide, in follicular (A) and luteal (B). The amplitude (y axis) is expressed in g.

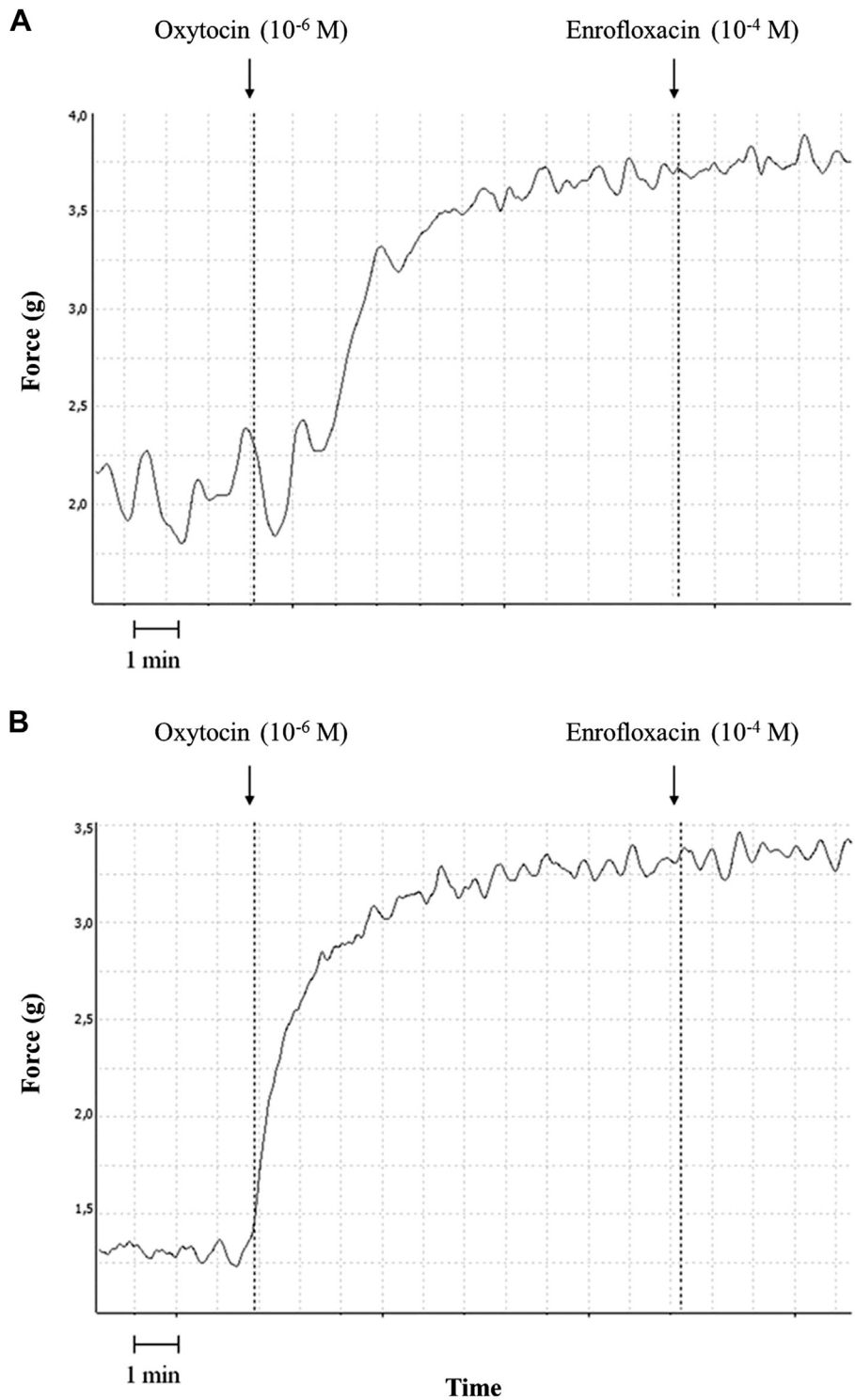

Fig. 3. Representative tracing of the effects induced by oxytocin (10^{-6} M) on uterine contractility and by enrofloxacin (10^{-4} M) on tonic effect induced by neuropeptide, in follicular (A) and luteal (B). The amplitude (y axis) is expressed in g.

obtained from Piccinno et al. [23] on the basal contractility in the same phase of the cycle (6.77%) and at the same concentration of antibiotic (10^{-4} M). These values are almost overlapping suggesting that there may be an effect of enhancement of the contractile activity induced by the oxytocin-enrofloxacin association. The enrofloxacin seems to produce an increase in uterine contractility by direct action on $GABA_A$ receptor and the release of $PGF_{2\alpha}$ [42], as

Table 1
Mean values ± standard error of the mean (SEM) of amplitude calculated before (basal), after oxytocin administration, and subsequently to the addition of oxytocin-antibiotics associations (amoxicillin, rifaximin, and enrofloxacin).

Amplitude (g)	Mean ± SEM	
	Follicular phase	Luteal phase
Amoxicillin		
Basal	2.31 ± 0.10^{eE}	1.47 ± 0.08^{fC}
Oxytocin	4.39 ± 0.19^{aF}	3.52 ± 0.29^{bD}
Association oxytocin-antibiotic	4.02 ± 0.25^{F}	3.47 ± 0.31^{D}
Rifaximin		
Basal	2.32 ± 0.11^{cE}	1.57 ± 0.13^{dE}
Oxytocin	4.32 ± 0.15^{cAF}	3.40 ± 0.25^{dAF}
Association oxytocin-antibiotic	4.55 ± 0.17^{eBF}	3.18 ± 0.19^{fBF}
Enrofloxacin		
Basal	2.46 ± 0.16^{eE}	1.40 ± 0.46^{fE}
Oxytocin	4.29 ± 0.16^{eF}	2.84 ± 0.20^{fCF}
Association oxytocin-antibiotic	4.37 ± 0.17^{eF}	2.90 ± 0.21^{fDF}

In line: $^{a,b}P < 0.05$; $^{c,d}P < 0.01$; and $^{e,f}P < 0.001$.
In intercompany column: $^{A,B}P < 0.05$; $^{C,D}P < 0.01$; and $^{E,F}P < 0.001$.

Table 2
Mean values ± standard error of the mean (SEM) of frequency calculated before (basal), after oxytocin administration, and subsequently to the addition of oxytocin antibiotics associations (amoxicillin, rifaximin, and enrofloxacin).

Frequency (no of contractions/min)	Mean ± SEM	
	Follicular phase	Luteal phase
Amoxicillin (2.5 min)		
Basal	1.10 ± 0.13^{eA}	0.46 ± 0.05^{fE}
Oxytocin	1.30 ± 0.07^{cB}	0.91 ± 0.06^{dF}
Association oxytocin-antibiotic	1.20 ± 0.01^{e}	0.86 ± 0.05^{fF}
Rifaximin (4 min)		
Basal	1.00 ± 0.01^{eAC}	0.57 ± 0.04^{fAC}
Oxytocin	1.19 ± 0.04^{D}	1.14 ± 0.18^{B}
Association oxytocin-antibiotic	$1.13 \pm 0.05^{cB*}$	0.89 ± 0.04^{dD}
Enrofloxacin (3 min)		
Basal	1.25 ± 0.16^{c}	0.48 ± 0.06^{dCE}
Oxytocin	1.50 ± 0.19^{a}	1.00 ± 0.09^{bF}
Association oxytocin-antibiotic	$1.25 \pm 0.05^{c*}$	0.90 ± 0.08^{dD}

In line: $^{a,b}P < 0.05$; $^{c,d}P < 0.01$; and $^{e,f}P < 0.001$.
In intercompany column: $^{A,B}P < 0.05$; $^{C,D}P < 0.01$; and $^{E,F}P < 0.001$.
In intergroup column: $^{*}P < 0.05$.

Table 3
Mean values ± standard error of the mean (SEM) of area under the curve (AUC) calculated before (basal), after oxytocin administration, and subsequently to the addition of oxytocin-antibiotics associations (amoxicillin, rifaximin, and enrofloxacin).

AUC (no of contractions/min)	Mean ± SEM	
	Follicular phase	Luteal phase
Amoxicillin		
Basal	345.98 ± 26.14^{aE}	235.35 ± 27.45^{bAC}
Oxytocin	605.39 ± 27.32^{EF}	565.81 ± 79.66^{D}
Association oxytocin-antibiotic	$573.67 \pm 25.03^{F*§}$	520.81 ± 72.68^{B}
Rifaximin		
Basal	417.72 ± 39.46^{aC}	293.98 ± 31.25^{bE}
Oxytocin	720.93 ± 92.33^{CD}	679.25 ± 44.93^{EF}
Association oxytocin-antibiotic	$750.33 \pm 88.76^{D*}$	634.00 ± 45.33^{F}
Enrofloxacin		
Basal	422.53 ± 36.15^{E}	317.48 ± 60.00^{E}
Oxytocin	706.92 ± 49.87^{aEF}	543.44 ± 55.90^{bF}
Association oxytocin-antibiotic	$818.39 \pm 38.55^{cF§}$	573.03 ± 62.20^{dF}

In line: $^{a,b}P < 0.05$; $^{c,d}P < 0.01$.
In intercompany column: $^{A,B}P < 0.05$; $^{C,D}P < 0.01$; and $^{E,F}P < 0.001$.
In intergroup column: $^{*}P < 0.05$; $^{§}P < 0.01$.

Table 4
Percentage indexes of average amplitude, calculated from baseline (basal/oxytocin and basal/association) and from contractility induced by neuropeptide (oxytocin/association) in bovine uterus during follicular and luteal.

Amplitude (g)	Percentage index (%)		
	Basal/oxytocin	Basal/association oxytocin-antibiotic	Oxytocin/association oxytocin-antibiotic
Amoxicillin			
Follicular phase	86.61^{E}	78.91^{aE}	-2.69^{F}
Luteal phase	200.98^{A}	146.54^{bC}	-1.76^{BD}
Rifaximin			
Follicular phase	86.07^{aE}	90.99^{EF}	5.38^{cF}
Luteal phase	119.12^{bAE}	106.38^{BE}	$-5.52^{dF§}$
Enrofloxacin			
Follicular phase	78.04^{E}	81.63^{E}	2.51^{F}
Luteal phase	118.79^{C}	123.89^{CD}	$6.20^{D§}$

In line: $^{A,B}P < 0.05$; $^{C,D}P < 0.01$; and $^{E,F}P < 0.001$.
In intercompany column: $^{a,b}P < 0.05$; $^{c,d}P < 0.01$.
In intergroup column: $^{§}P < 0.01$.

shown by studies carried out on the myenteric plexus [43,44]. This effect is added to that produced by the neuropeptide, which acts by blocking the most important mechanism deputed to the outflow of Ca^{2+} (Ca^{2+}-ATPase pump) and through the stimulation of the release of $PGF_{2\alpha}$ [45]. On the basis of the data obtained, the association oxytocin-enrofloxacin could represent an optimal therapy for the treatment of retained placenta and acute metritis, in cattle. This is possible because, in the first 7 days of postpartum, oxytocin receptors are still present [46]. The oxytocin-enrofloxacin association is not affected by hormonal changes; this would encourage the use of these substances for treatment of all forms of metritis. The synergism of contractile activity could interfere with the formation of biofilm and with the onset of antibiotic resistance, hindering the adhesion of bacteria in planktonic form and/or the transition from the reversible adherence to

Table 5
Percentage indexes of average frequency, calculated from baseline (basal/oxytocin and basal/association) and from contractility induced by neuropeptide (oxytocin/association) in bovine uterus during follicular and luteal.

Frequency (no of contractions/min)	Percentage index (%)		
	Basal/oxytocin	Basal/association oxytocin-antibiotic	Oxytocin/association oxytocin-antibiotic
Amoxicillin			
Follicular phase	25.00^{cC}	18.75^{eA}	-6.25^{BD}
Luteal phase	105.98^{dC}	110.87^{fE}	-4.85^{DF}
Rifaximin			
Follicular phase	18.75^{aC}	12.50^{cC}	-3.75^{D}
Luteal phase	102.08^{bA}	61.18^{dC}	-6.34^{BD}
Enrofloxacin			
Follicular phase	25.00^{c}	12.25^{c}	-4.18
Luteal phase	132.78^{dC}	105.17^{dC}	-8.60^{D}

In line: $^{A,B}P < 0.05$; $^{C,D}P < 0.01$; and $^{E,F}P < 0.001$.
In intercompany column: $^{a,b}P < 0.05$; $^{c,d}P < 0.01$; and $^{e,f}P < 0.001$.

Table 6
Percentage indexes of average area under the curve (AUC), calculated from baseline (basal/oxytocin and basal/association) and from contractility induced by neuropeptide (oxytocin/association) in bovine uterus during follicular and luteal.

AUC (no of contractions/min)	Percentage index (%)		
	Basal/oxytocin	Basal/association oxytocin-antibiotic	Oxytocin/association oxytocin-antibiotic
Amoxicillin			
Follicular phase	80.81^{E}	71.98^{CF}	$-5.06^{D\S *}$
Luteal phase	158.13^{AC}	141.16^{B}	$-7.26^{AD\square}$
Rifaximin			
Follicular phase	67.79^{AC}	80.39^{BC}	$5.19^{eD\S\square}$
Luteal phase	167.23^{A}	146.06^{BA}	$-6.90^{fB\square}$
Enrofloxacin			
Follicular phase	70.37^{C}	99.87^{DE}	$17.23^{aDF*\square}$
Luteal phase	100.10^{A}	112.61^{C}	$7.68^{bBD\square}$

In line: $^{A,B}P < 0.05$; $^{C,D}P < 0.01$; and $^{E,F}P < 0.001$.
In intercompany column: $^{a,b}P < 0.05$; and $^{e,f}P < 0.001$.
In intergroup column: $^{\S}P < 0.01$; * and $^{\square}P < 0.001$.

irreversible one. In fact, some workers have shown, *in vitro*, that the ciprofloxacin, as a metabolite derived from enrofloxacin, [47,48], are effective and the synergistic in association with oxytocin. This association has shown not only an increase of antimicrobial power but also the ability to suppress the formation of bacterial biofilms [18,19]. In addition, our results suggest a possible explanation, on the basis of contractile effect of the two molecules: oxytocin could remove the organic material, favoring the antimicrobial power of the antibiotic; in turn, enrofloxicin could strengthen oxytocin's contractility, through its $GABA_A$ antagonistic effects, further hampering the formation of bacterial biofilm.

4.1. Conclusions

This study shows, for the first time, the modulatory activity of three antibiotics (amoxicillin, enrofloxacin, and rifaximin) on the contractility induced by oxytocin, on bovine uterine tissue. The tested antibiotics showed different effects that made it possible to identify, only in some cases (rifaximin in follicular phase and enrofloxacin in both phases of the cycle), a synergistic enhancement of oxytocin-induced contraction. This outcome might enable, *in vivo*, a simultaneous increase of uterine cleaning and antimicrobial action against bacteria and biofilms. This study contributes to our understanding of uterine contractility and its pharmacologic control, furthering opportunities for veterinary applications in the care of postpartum females. Clinicians will want to go beyond a consideration of the canonical activity of antibiotics, to also consider their effects on uterine contractility in different phases of the estrus cycle.

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