

## Tocolytic Effects of Meloxicam on Isolated Cattle Myometrium <sup>[1]</sup>

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### Summary

Meloxicam is a selective, cyclooxygenase-2 (COX-2), enzyme inhibiting, non-steroid, and anti-inflammatory drug (NSAID). In this study, its tocolytic effects on the cattle myometrium strips pre-stimulated with oxytocin (OT) were investigated with the isolated tissue bath system. The uterine samples were from newly slaughtered, non-pregnant cattle which were older than two years old and in anoestrus stage at the time of sampling, in a registered slaughterhouse. Seven cattle samples (each sample consisted of four strips) were used in this study. The samples were incubated in the isolated tissue baths until regular spontaneous contractions occurred. 0.5 nM of OT was then applied to all strips. After OT application, 10 different doses of meloxicam (sodium salt) ranging between  $1 \times 10^{-7}$  and  $7 \times 10^{-4}$  M were administered, cumulatively. After each application, uterine contractions were recorded for 20 min. Mean peak amplitude (peak maximum, PMAX), frequency (beats per minute, BPM) and area under the curve (AUC) values of the uterine contractions, which were used to evaluate the effects of the drug, were calculated from the curve. In addition, the effective concentration 50 ( $EC_{50}$ ) values for the tocolytic effects of meloxicam were calculated  $4.22 \times 10^{-4}$  M (2.63-6.76  $\times 10^{-4}$  M, 95% confidence limits for mean, CLM) for frequency (BPM),  $3.61 \times 10^{-4}$  M (2.47-5.27  $\times 10^{-4}$  M, 95% CLM) for AUC and  $2.31 \times 10^{-4}$  M (1.97-2.70  $\times 10^{-4}$  M, 95% CLM) for peak amplitude (PMAX), by using the GraphPad Prism (5.0) program. At the end of this study, it is suggested that the use of meloxicam as a tocolytic agent may prevent preterm labour in cattle and the loss of calves. However, *in vivo* studies should be performed to show the clinical therapeutic and possible maternal and foetal adverse effects of meloxicam on live cattle.

**Keywords:** Meloxicam, Oxytocin, Cattle, Contraction, Tocolysis

## Meloksikamın İzole Sığır Miyometriumu Üzerine Tokolitik Etkileri

### Özet

Meloksikam seçici siklooksijenaz-2 (COX-2) enzim inhibitörü, steroid yapıda olmayan ağrı kesici ve yangı giderici ilaçların (NSAID) bir üyesidir. Bu çalışmada izole doku banyosunda önceden oksitosin (OT) ile uyarılmış inek miyometrium striplerine meloksikamın tokolitik (gevşetici) etkisi araştırılmıştır. Uterus örnekleri mezbahada iki yaşın üzerinde, yeni kesilen, anöstrüs evresinde gebe olmayan ineklerden sağlanmıştır. Çalışmada yedi örnek kullanılmış ve her örnek dört miyometrium stripinden oluşturulmuştur. Örnekler spontan uterus kasılmaları şekilleninceye kadar doku banyolarında bekletilmiştir. Daha sonra her izole miyometrium üzerine 0.5 nM OT eklenmiştir. Bunu takiben  $1 \times 10^{-7}$  M ile  $7 \times 10^{-4}$  M arasında 10 farklı meloksikam (sodyum tuzu) dozu kümülatif olarak uygulanmıştır. Her uygulama sonrası uterus kasılmaları 20 dk süresince kaydedilmiştir. Uterus kasılmalarının grafiğinden ilaç etkisinin ölçüleri olan ortalama pik yükseklikleri (peak maximum, PMAX), frekans (dakikadaki pik atım sayıları, beats per minute-BPM) ve eğrinin altında kalan alan (EAA) değerleri hesaplanmıştır. Bunlara ek olarak meloksikamın tokolitik etkisinin etkili konsantrasyon 50 ( $EC_{50}$ ) değerleri GraphPad Prism (5.0) programı ile pik frekansları (BPM) için  $4.22 \times 10^{-4}$  M (2.63-6.76  $\times 10^{-4}$  M, % 95 güven aralığı, GA), EAA için  $3.61 \times 10^{-4}$  M (2.47-5.27  $\times 10^{-4}$  M, %95 GA) ve ortalama pik yükseklikleri (PMAX) için  $2.31 \times 10^{-4}$  M (1.97-2.70  $\times 10^{-4}$  M, %95 GA) olarak ölçülmüştür. Çalışma sonunda meloksikamın tokolitik olarak kullanımının, sığırlarda erken doğumu ve buna bağlı buzağı kayıplarını önleyebileceği kanısına varılmıştır. Bununla birlikte canlı sığırlar üzerinde meloksikamın klinik olarak tedavi edici etkilerinin yanı sıra; anne ve yavru üzerine olası istenmeyen etkilerini göstermek için *in vivo* çalışmaların yapılması gereklidir.

**Anahtar sözcükler:** Meloksikam, Oksitosin, Sığır, Kasılma, Tokoliz



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## INTRODUCTION

Prostaglandins (PGs) are important in the mediation of several key processes during pregnancy and parturition<sup>1</sup>. They stimulate uterine contractions and cervical maturation, and their inhibition of cyclooxygenase (COX) results in prolongation of gestation and parturition in humans and animals<sup>2</sup>. Non-steroid anti-inflammatory drugs (NSAIDs) inhibit the COXs which convert arachidonic acid to PGG<sub>2</sub> and PGH<sub>2</sub> and lead to the production of thromboxane A<sub>2</sub> (TxA<sub>2</sub>) and a variety of other PGs which contribute to pain<sup>3</sup>.

There are three COX isoforms; COX-1, COX-2, and COX-3, with molecular weights of 70 to 74 kDa, each encoded by a different gene. COX-1 is constitutively expressed in most tissues (although not within all cells of a tissue) and produces PGs involved in homeostasis. COX-2 is an inducible enzyme, capable of being rapidly up-regulated in selected tissues by cytokines, growth factors, tumour promoters, hormones and bacterial endotoxins. However, COX-2 is also constitutively expressed in the brain, testes, tracheal epithelia, macula densa of the kidney and the gravid uterus<sup>4</sup>. The most frequent and clinically significant side effects of NSAIDs are irritant, ulcerogenic, erosive effects on the gastrointestinal tract. The other their adverse effects are renotoxicity (including occasional acute renal failure), hepatotoxicity (cholestatic or parenchymal), inhibition of haemostatic mechanisms leading to haemorrhage, blood dyscrasias, delaying of parturition, soft tissue healing and fracture healing<sup>5</sup>. Because studies have indicated that most of these side effects are caused by the inhibition of the COX-1 isoform, the COX-2 selective drugs may be a safer treatment option for tocolysis<sup>6</sup>. Differing findings on selectivity from studies in various laboratories, meloxicam can be classified as a non-specific COX inhibitor or a preferential and moderately selective COX-2 inhibitor<sup>5</sup>. In various assays, meloxicam has been estimated to be between 3 and 77 fold selective for COX-2<sup>7</sup>. Meloxicam administration has been shown to decrease PG production and uterine activity in sheep. The discovery of a new effective and relatively safe tocolytic agent would greatly improve the management of preterm labour and thus decrease the incidence of preterm birth<sup>6</sup>.

Therefore, the aim of the present study is to detect the tocolytic effect of meloxicam on the cattle myometrium strips pre-stimulated with OT in the isolated tissue bath system.

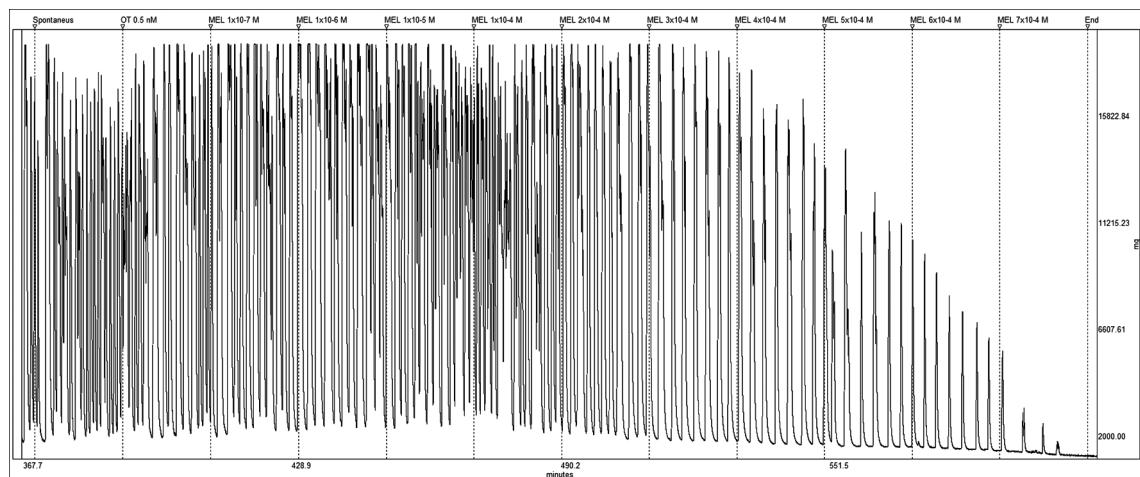
## MATERIAL and METHODS

The methods described by Çelik et al.<sup>7</sup> and Wrobel et al.<sup>8</sup> were employed in the current study for determination of the tocolytic effects of meloxicam on isolated cattle myometrium strips. The uterine samples were collected from seven (four Holstein and three Brown Swiss) newly slaughtered, non-pregnant cattle in a registered slaughterhouse. All the animals were older than two years old and in

anoestrus stage at the time of sampling. There were not extra vascularisation, thickening and caruncles in the uterine endometriums. Also no asymmetry was observed between two uterine horns, macroscopically. Each sample consisted of four longitudinal myometrium strips from the curvature major in a uterine horn (1 cm x 0.2 cm x 0.2 cm). Physiological salt solution (PSS) (for transportation of uterine parts to laboratory and incubation of myometrium strips in the tissue baths) was consisted of 116 mM NaCl, 4.6 mM KCl, 1.16 mM NaH<sub>2</sub>PO<sub>4</sub> x 2H<sub>2</sub>O, 1.16 mM MgSO<sub>4</sub> x 7H<sub>2</sub>O, 21.9 mM NaHCO<sub>3</sub>, 1.8 mM CaCl<sub>2</sub> x 2H<sub>2</sub>O and 11.6 mM dextrose (Merck, Germany). Its pH was modified 7.4 with 85% orthophosphoric acid (Merck, Germany). After collecting from slaughterhouse, uterine parts transported to the laboratory in the PSS at 4°C in the mornings. The experiment was performed at the same day which sample was collected. After preparation, four myometrium strips were hung vertically and attached to force transducers (Commat, Turkey) under 2 g of pre-tension in 10 ml tissue baths (Commat, Turkey) (95% O<sub>2</sub> + 5% CO<sub>2</sub>, pH 7.4, 38°C), simultaneously. The strips were left to stabilize for approximately 1 h. In the stabilisation period, the bath solutions were changed every 15 min. They were not changed from the recording of spontaneous contractions to the end of the experiment. Spontaneous contractions were then recorded with a data acquisition system (Biopac, USA) for a 20 min period. 0.5 nM OT (Sigma, USA) was applied on all strips. After that, meloxicam (sodium salt) (Sigma, USA) was applied cumulatively at 1x10<sup>-7</sup>, 1x10<sup>-6</sup>, 1x10<sup>-5</sup>, 1x10<sup>-4</sup>, 2x10<sup>-4</sup>, 3x10<sup>-4</sup>, 4x10<sup>-4</sup>, 5x10<sup>-4</sup>, 6x10<sup>-4</sup> and 7x10<sup>-4</sup> M to the strips. These doses were determined according to our preliminary experiments. After each drug application, myometrial contractions were recorded for a 20 min period. Mean and standard error of mean (SEM) values of peak amplitude (P<sub>MAX</sub>), frequency (BPM) and AUC were determined for myometrial samples from seven cattle. The SPSS program (15.0) was used for statistical data analysis. Statistical differences between drugs doses were determined with the Freidman Test, followed by the Wilcoxon-Signed Rank Test Differences were considered significant when P values were less than 0.05. Effective concentrations 50 (EC<sub>50</sub>) values for the tocolytic effects of meloxicam were calculated on myometrial strips which had been pre-contracted with 0.5 nM OT by using the GraphPad Prism (5.0) program.

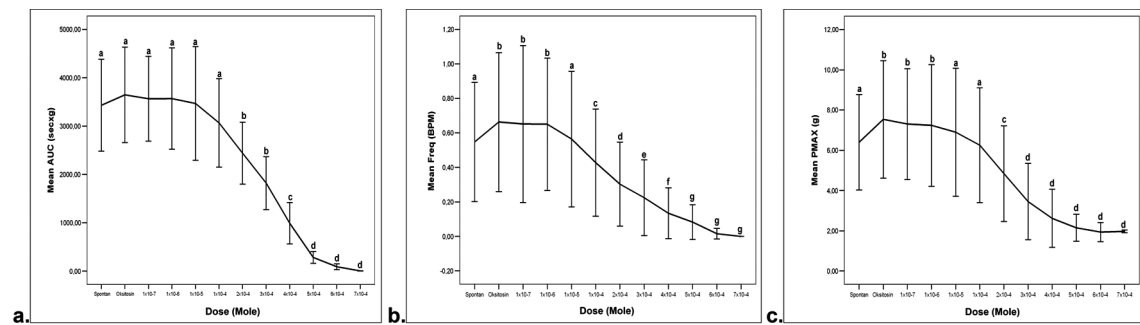
## RESULTS

The effects of OT and meloxicam on isometric myometrial contractions are shown in Fig. 1. The values used to infer drug effects, mean peak amplitude (peak maximum, P<sub>MAX</sub>), frequency of myometrial contractions (beats per minutes, BPM) and area under curve (AUC), were calculated from traces and are shown in Fig. 2. The EC<sub>50</sub> values of meloxicam were calculated 4.22x10<sup>-4</sup> M (2.63-6.76x10<sup>-4</sup> M, 95% CLM) for frequency (BPM), 3.61x10<sup>-4</sup> M (2.47-5.27x10<sup>-4</sup> M, 95% CLM) for AUC and 2.31x10<sup>-4</sup> M (1.97-2.70x10<sup>-4</sup> M, 95% CLM) for peak amplitude (P<sub>MAX</sub>).



**Fig 1.** The effects of OT and meloxicam on isometric myometrial contractions

**Şekil 1.** Oksitosin ve meloksikamın izometrik miyometriyum kasılmalarına etkisi



**Fig 2.** The effects of OT and meloxicam on (a) the mean ( $\pm$ SEM) AUC, (b) BPM and (c)  $P_{MAX}$  values of myometrial (n=7) contractions  
<sup>a-9</sup> Means within different letters are significantly different ( $P < 0.05$ )

**Şekil 2.** Oksitosin ve meloksikamın miyometriyum (n=7) kasılmalarının (a) ortalama ( $\pm$ SEM) EAA, (b) BPM ve (c)  $P_{MAX}$  değerlerine etkisi  
<sup>a-9</sup> Farklı harfler arasında istatistiksel olarak anlamlı fark vardır ( $P < 0.05$ )

## DISCUSSION

Most strategies for delaying preterm birth rely on the reduction of uterine contractions. It is well understood that uterine contractile activity at birth involves uterine activation and increased levels of contractile stimulators. Uterine activation proteins include the OT receptor (OTR),  $PGF_{2\alpha}$  receptor (FP),  $PGE_2$  receptors (EP1-4), and COX-2. The uterine stimulators include PGs and OT. The PGs have been a target for tocolysis because i) the myometrium contracts in response to exogenous PGs (*in vivo* and *in vitro*), ii) PG synthetic enzymes and PG levels in tissues and fluids increase before or at the time of labour, and iii) inhibitors of PG synthesis delay birth and prolong pregnancy<sup>9</sup>.

Tocolytic effects of meloxicam and indomethacin in uterine contractions in rats were investigated by Yousif and Thulesius<sup>1</sup>. Both compounds dose-dependently inhibited uterine contractions in non-pregnant rats and also during pregnancy. In general, meloxicam induced inhibition at lower concentrations than indomethacin in all gestation periods. The potency of meloxicam in inhibiting spontaneous uterine

contractions was slightly greater than that of indomethacin in the different gestation periods studied and particularly in early pregnancy.

The human uterine relaxant effect of COX-2 inhibitors was researched by Slattery et al.<sup>10</sup>. Nimesulide, meloxicam and celecoxib exerted a significant relaxant effect on contractility in non-pregnant, pregnant non-labour and pregnant labour myometrial strips. Celecoxib exhibited greater potency than nimesulide or meloxicam. The range of maximal relaxation values achieved in the three tissue types were 68-70%, 69-84% and 69-77% for nimesulide, meloxicam and celecoxib, respectively.

The effects of NSAIDs on human myometrial contractility were studied by Sawdy et al.<sup>11</sup>. SC 58236 (COX-2 selective inhibitor) and SC 58560 (COX-1 selective inhibitor) had modest inhibitory effects on contraction peaks at the highest concentration tested. Meloxicam had a similar effect on peak height but did not influence the rate of contractions. A COX-2 selective inhibitor 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl) phenyl-2(5H)-furanone (DFU) had no obvious effects. Nimesulid and indomethacin completely

inhibited myometrial contractility at the highest concentration tested. SC 58236 had a significant inhibitory effect on myometrial contractility only at the highest concentration tested (100  $\mu$ M). SC 58260 had no effect on contractility at concentrations up to 100  $\mu$ M. Myometrial contractility was unaffected by DFU at all concentrations tested, whereas meloxicam had a clear effect at 100-200  $\mu$ M. Contractions continued during the incubations with meloxicam but were diminished in amplitude and frequency. In contrast, nimesulid and indomethacin had potent inhibitory effects, with almost complete cessation of contractile activity at concentrations of 100 and 300  $\mu$ M, respectively.

An *in vitro* study of the tocolytic effect of rofecoxib was conducted by Doret et al.<sup>12</sup> in pregnant rats. The effects of rofecoxib were also studied in comparison and combination with other agents. Rofecoxib inhibited 95-100% of contractile activity at a mean concentration of  $1.6 \times 10^{-7}$  M. The mean  $EC_{50}$  values were  $1.5 \times 10^{-4}$ ,  $3.1 \times 10^{-5}$ ,  $1.9 \times 10^{-7}$  and  $7.5 \times 10^{-6}$  M for indomethacin, ritodrine, nicardipine and atosiban, respectively. Rofecoxib was effective at significantly lower concentrations were than those of indomethacin, ritodrine and atosiban. The rofecoxib-ritodrine combination involved a synergic effect, whereas. The rofecoxib-nicardipine and rofecoxib-atosiban combinations had additive effects.

The tocolytic effects of parecoxib on the spontaneous and PG-induced contractions of isolated rat myometrium were tested by Ayar<sup>13</sup>. The application of parecoxib in the range of 50-900 mM caused dose-dependent decreases in mean amplitude, mean frequency and mean AUC of both spontaneous and PG-induced contractions. Mean percentage inhibition of the AUC of spontaneous contractions was 29, 56, 74 and 84% in the presence of 50, 150, 300 and 600 mM parecoxib, respectively. In the case of  $PGF_{2\alpha}$ -induced contractions, 100, 300, 600 and 900 mM parecoxib resulted in 27, 43, 61 and 73% inhibition, respectively. Moreover, pre-treatment with 600 mM parecoxib reduced the responsiveness and maximum contractility of  $PGF_{2\alpha}$ -treated strips compared with non-treated strips.

The tocolytic effects of meloxicam in an induced preterm labour model for pregnant sheep were studied by Rac et al.<sup>6</sup>. Preterm labour was induced in chronically catheterized sheep with RU486. The animals were then randomized to receive maternal infusions of saline or meloxicam for 48 h or until delivery when the animals were killed and tissues and blood samples collected. Maternal infusion of meloxicam inhibited uterine contractions, increased contraction duration, and attenuated contraction frequency and amplitude. Saline-treated animals progressed to delivery. Administration of meloxicam was not associated with any change in foetal or maternal blood gas status, osmolality, arterial pressure, heart rate or foetal blood flows.

The effects of the specific COX-2 inhibitor rofecoxib on the female reproductive system were studied in rats by Borham and Abd-ElGalil<sup>14</sup>. Rofecoxib, given orally and daily for

15 days in female rats, produced a significant decrease in serum estrogen level between the control and treated groups. Acetylcholine (Ach) and  $PGF_{2\alpha}$  produced dose dependent contractions in isolated non-pregnant rat uterus of the control group. In the treated group, rofecoxib reduced the Ach and  $PGF_{2\alpha}$  induced contractions, with the shift of the dose response curves of both agonists to the right. Incubation with rofecoxib at different doses produced statistically significant reductions in the uterine contractions caused by Ach and  $PGF_{2\alpha}$ . In addition, at the same doses rofecoxib produced from 37.5 to 100% inhibition of the spontaneous uterine contractions of isolated pregnant rat uterus.

The synergistic tocolytic effect of the paracetamol and pyrilamine combination (PPC) in isolated human myometrium was investigated by Ortiz et al.<sup>15</sup>. The contraction of uterine strips was stimulated with KCl (60 mM) and treated with the vehicle or increasing concentrations of paracetamol (100-3200 mM), pyrilamine (3.2-100 mM) or the paracetamol-pyrlamine combination (PPC). The relaxing effects of the drugs alone and in combination were measured. Paracetamol, pyrilamine and the PPC produced a significant relaxing effect on non-pregnant human uterine strips pre-contracted with KCl (60 mM). The  $EC_{30}$  values for paracetamol and pyrilamine on uterine contraction were 2,391.3 mM and 14.7 mM, respectively. The derived experimental  $EC_{30}$  for PPC was 401.8 mM. This value was significantly lower than the theoretical  $EC_{30}$  expected for a purely additive interaction, which was 1,203.0 mM for PPC. The interaction index (g) of 0.33 for PPC was statistically different from unity.

The tocolytic effects and side-effects of the COX-2 inhibitor celecoxib, on dams and pups when using a lipopolysaccharide (LPS)-induced preterm delivery mouse model (preterm delivery rates, 95%) were investigated by Sakai et al.<sup>16</sup>. Celecoxib was administered at 50, 10, 1, 0.3 and mg/kg and the preterm labour percentages were decreased to 18, 30, 36 and 60%, respectively. Celecoxib reduced preterm labour; however it was stated that careful attention to constriction of the foetal ductus arteriosus should be given.

The tocolytic effects of DFU, indomethacin and nimesulide on myometrial strips isolated from rats in both LPS-induced preterm labour and term labour were investigated by Karadas et al.<sup>17</sup>. The constrictor effects of DFU and indo-methacin were also compared on the foetal ductus arteriosus. They significantly inhibited KCl, OT,  $PGE_2$  and  $PGF_{2\alpha}$  stimulated contractions of myometrial strips isolated from rats in preterm and term labour. The  $E_{MAX}$  value of indomethacin was significantly lower than those for DFU and nimesulide ( $P < 0.05$ ). Foetal ductus arteriosus was significantly constricted by DFU at 10 or 100 mg/kg in preterm and term rats, although the induced constriction ratios for DFU at 10 or 100 mg/kg were significantly lower than those for indomethacin ( $P < 0.05$ ). The authors suggested that DFU could be considered as a new therapeutic agent for preterm labour but continued that careful attention should be given to constriction of the foetal ductus arteriosus.



The comparative effects of DFU and nimesulide on the amplitude and frequency of the KCl, OT and PGF<sub>2α</sub>-stimulated contractions of isolated pregnant human myometrial strips were studied by Karadas et al.<sup>18</sup>. The inhibitory effect of DFU was more potent than that of nimesulide on KCl, OT and PGF<sub>2α</sub>-stimulated myometrial contractions; however, the inhibitory effects of nimesulide and DFU were much greater on KCl-stimulated contractions than on OT and PGF<sub>2α</sub>-stimulated contractions. In that study, DFU was a more potent inhibitor than nimesulide on KCl, OT and PGF<sub>2α</sub> stimulated contractions of pregnant human myometrium.

Kuc et al.<sup>19</sup> evaluated the inhibitory effects of dual combinations of atosiban, nifedipine and celecoxib on the contractility of human myometrial strips in an *in vitro* model of preterm labour. The atosiban/nifedipine combination showed an additive tocolytic effect on the contractility of myometrial strips in preterm and term patients. The other combinations (atosiban/celecoxib and nifedipine/celecoxib) caused only antagonistic effects in both studied groups.

The tocolytic effects of AS604872, a selective FP receptor antagonist, were studied in rodents by Chollet et al.<sup>20</sup>. They examined the effects of AS604872 in the inhibition of spontaneous uterine contractions in pregnant, near term rats. They also tested AS604872 for its ability to delay preterm birth in a mouse model in which the anti-progestin agent RU486 triggered parturition. AS604872, through both oral and intravenous dosing, markedly and dose-dependently reduced spontaneous uterine contractions in late-term pregnant rats during gestational days 19 to 21. In pregnant mice, AS604872 delayed the preterm birth induced with RU486. Its effects were dose dependent; it caused a significant increase in the mean delivery time from 16 to 33 h at oral doses of 30 mg/kg and 100 mg/kg, respectively, in labour triggered on day 14 of gestation. In both models, AS604872 appeared more effective than the β-agonist ritodrine.

NSAIDs were found effective on myometrial contractions in previous studies<sup>1,6,7,10-20</sup>. The tocolytic effects of meloxicam determined in the current study are similar to results of previous studies which were performed by Yousif and Thulesius<sup>1</sup> and Slattery et al.<sup>10</sup>. However, meloxicam affected the peak height of human myometrial contractions but did not influence the rate of contractions in the study performed by Sawdy et al.<sup>11</sup>. In this regard, that study is different from our study. In the study performed by Rac et al.<sup>6</sup>, meloxicam was administered to pregnant sheep which were preterm labour induced with RU486. In that study, maternal infusion of meloxicam inhibited uterine contractions, increased contraction duration, and attenuated contraction frequency and amplitude. Although it was an *in vivo* experiment, it could be considered similar to our study.

In the current study, meloxicam demonstrated tocolytic effects on isolated cattle myometrium preparations which were pre-constricted with oxytocin. Meloxicam, due to its

preferential and moderately selective COX-2 inhibition, may be a safer tocolytic agent than COX-1 inhibitors for cattle at risk of preterm labour. However, *in vivo* studies should be performed to show the clinical therapeutic and possible maternal and foetal adverse effects of meloxicam on live cattle.

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