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## Oxytocin Receptor Antagonists, Atosiban and Nolasiban, Inhibit Prostaglandin $F_{2\alpha}$ -induced Contractions and Inflammatory Responses in Human Myometrium

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Oxytocin receptor antagonists (OTR-A) have been developed as tocolytics for the management of preterm labour due to the significant role of oxytocin (OT) in the onset of both term and preterm labour. Similar to OT, prostaglandins (PGs) play key roles in myometrial contractility and cervical ripening. Inhibition of PG synthesis/activity is used to delay preterm birth. Thus, targeting the PG pathway in combination with an OTR-A may be an effective strategy for delaying preterm delivery. In this study, we examined the effects of atosiban and nolasiban on  $PGF_{2\alpha}$ -induced contractions and pro-inflammatory responses in human pregnant myometrium. Both OTR-As, atosiban and nolasiban, inhibited  $PGF_{2\alpha}$ -induced contractions in a dose-dependent manner ( $p < 0.001$  and  $p < 0.01$ , respectively). These inhibitory effects involved the suppression of  $PGF_{2\alpha}$ -mediated increase in intracellular calcium levels. In addition, the OTR-As significantly suppressed  $PGF_{2\alpha}$ -induced activation of pro-inflammatory pathways such as NF- $\kappa$ B and mitogen activated protein kinases (MAPKs), and the subsequent expression of contraction-associated-protein, COX-2. We have demonstrated that atosiban and nolasiban not only inhibit contractions elicited by OT, but also inhibit contractions and inflammation induced by  $PGF_{2\alpha}$ . This suggests a possible crosstalk between OTR and PG receptor signalling and highlights the importance of understanding G protein-coupled receptor interactions/crosstalk in the development of future tocolytics.

Preterm birth, defined as delivery before 37 weeks of gestation, is the major cause of perinatal morbidity and mortality worldwide<sup>1</sup>. Despite the growing focus in research, there is still limited understanding of the physiology of normal labour and preterm labour. Although a number of pharmacological agents also known as tocolytics have been introduced for the management of uterine contractions to delay preterm labour, there are no satisfactory tocolytics developed to date<sup>2</sup> as there is no clear evidence that they improve neonatal outcome. However, tocolysis is considered for a few days to allow completion of a course of corticosteroids for fetal lung maturation and to allow *in utero* transfer<sup>3</sup>. Most tocolytics currently available for use are aimed to suppress uterine contractions, however, are not utero-specific and therefore can exert multi-organ fetal, neonatal and maternal side effects<sup>4</sup>.

Primarily, myometrial contractility is believed to be regulated by the changes in the levels of intracellular calcium<sup>5-7</sup>. An increase results in the activation of calmodulin and myosin light chain kinase (MLCK), which in turn triggers actin filaments and the generation of a contraction force. The reduction in the intracellular calcium levels is thought to decrease calmodulin and MLCK interaction leading to relaxation, however, the mechanism through which such transient increase in intracellular calcium levels and phosphorylation of MLC maintain the tonic force is yet to be understood<sup>8</sup>.

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Oxytocin (OT) is a potent stimulator of myometrial contractions and plays an important role in the initiation of both term and preterm labour<sup>9</sup>. OT stimulates myometrial contractions through multiple signalling pathways<sup>10</sup>. Binding of OT to its receptor has been known to lead to G-protein coupling and subsequently, increase in intracellular calcium levels to mediate the generation of force<sup>10</sup>. Therefore, oxytocin receptor (OTR) is commonly used as a target for the development of tocolytics, and the only drugs developed specifically for the management of preterm labour are the OTR antagonists (OTR-As). Due to their increased specificity to the uterus, OTR-As such as atosiban can act as a suppressant of contractions with improved safety profiles<sup>11</sup>. Atosiban is primarily an arginine vasopressin (AVP) V1a receptor antagonist with lower affinity for the OTR. Its mechanism of action is via dose-dependent inhibition of OT-mediated increase in intracellular calcium levels which involves closing of voltage gated channels to prevent influx of calcium<sup>12</sup>. Atosiban has been approved in Europe for treatment of preterm labour but is administered through a bolus injection followed by an infusion and is not indicated for dosing beyond 48 hours<sup>11</sup>. Despite the previous reports of atosiban efficacy, it has been suggested to have a biased agonist effect in human amnion where it appears to act as a  $G_{\alpha q}$  antagonist whilst activating  $G_{\alpha i}$  signalling, leading to a pro-inflammatory response<sup>13</sup>. Nolasiban is a potent, selective, orally administered, non-peptide OTR-A with low affinity towards the AVP V1a and V2 receptors. Unlike atosiban, it was found to inhibit both  $G_{\alpha q}$  and  $G_{\alpha i}$  signalling induced by OT<sup>14</sup>.

Prostaglandins (PG) also play a central role in the onset as well as the maintenance of labour<sup>15</sup>.  $PGF_{2\alpha}$  is a naturally occurring prostaglandin that acts to induce uterine contractions and labour in pregnant women. Currently available PG inhibitors such as the NSAID, indomethacin, act by non-selective inhibition of PG-forming enzymes, thus blocking the generation and signalling of many PG sub-types, including  $PGF_{2\alpha}$ . Because they potentially adversely affect fetal physiology, NSAIDs are no longer recommended for pregnant women<sup>16</sup>. Through  $PGF_{2\alpha}$  receptors (FP receptors),  $PGF_{2\alpha}$  can elevate intracellular calcium in myometrium<sup>17</sup> and in turn, drive uterotonic effects that resemble an inflammatory response, whilst promoting the biophysical changes leading to cervical ripening<sup>18</sup>. Cervical ripening represents an integral part of the induction of labour process and prostaglandins such as dinoprostone are routinely used in clinical practice to promote cervical remodelling reducing the duration of labour and risk of caesarean section. In addition to promoting contractions and cervical ripening, it has been established that  $PGF_{2\alpha}$  is able to trigger upregulation of uterine activation proteins such as COX-2, OTR and connexin-43 (CX-43)<sup>19</sup>. Antagonism of the FP receptor decreases uterine contractions, and prevents membrane ruptures and cervical changes, which are the key features of preterm labour resulting in preterm birth. FP antagonists, such as the peptide THG113.31, have been developed for the management of preterm labour<sup>20</sup>, however, their clinical efficacy is yet to be demonstrated.

Initially, studies in animal models have demonstrated that OTR antagonism can affect  $PGF_{2\alpha}$ -mediated signalling and vice versa<sup>21,22</sup>. The aim of this study was to investigate and compare the inhibitory effects of nolasiban and atosiban on spontaneous, OT-, and  $PGF_{2\alpha}$ -stimulated contractions in an *ex vivo* model using strips of pregnant human myometrium. Furthermore, we aimed to identify the secondary intracellular effects, such as changes in intracellular calcium levels and to assess whether nolasiban and/or atosiban can inhibit  $PGF_{2\alpha}$ -induced downstream pro-inflammatory responses in human myometrial smooth muscle cells.

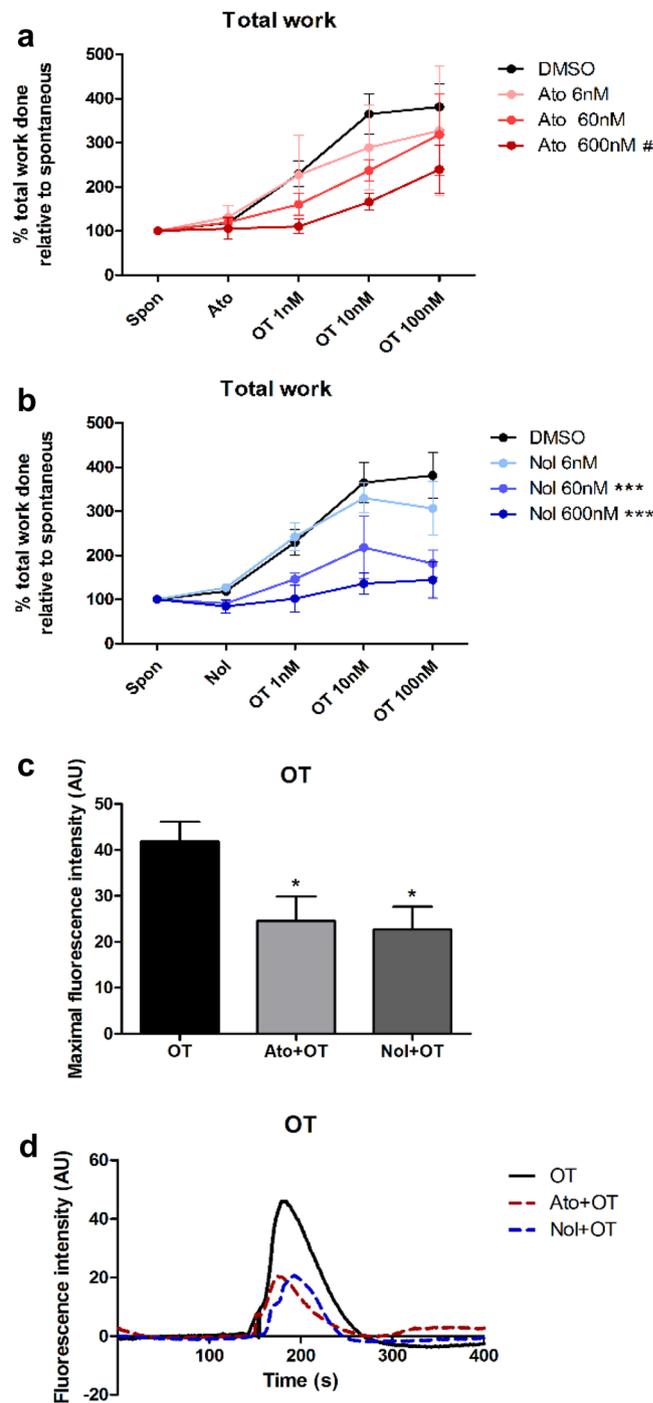
## Results

**Inhibition of OT-induced myometrial contractions.** We observed a robust progressive increase in myometrial contractility with increasing doses of OT in all experiments (Fig. 1). Incubation with atosiban or nolasiban had no significant effect on spontaneous contractility when compared to its vehicle control, however, the stimulatory effect of OT was suppressed by atosiban (Fig. 1a) and nolasiban (Fig. 1b). Both produced a concentration-dependent inhibition of OT-induced myometrial contractility, significantly reducing the rate of contraction, and the average area under the curve (Supplementary Fig. S1). Nolasiban at 60 nM was sufficient to significantly decrease OT-induced myometrial contractility ( $p < 0.001$  vs DMSO), whereas atosiban exerted its effects at 600 nM ( $p < 0.05$  vs DMSO). When comparing equimolar concentrations, nolasiban appeared more potent than atosiban at 60 nM and 600 nM but this did not reach statistical significance.

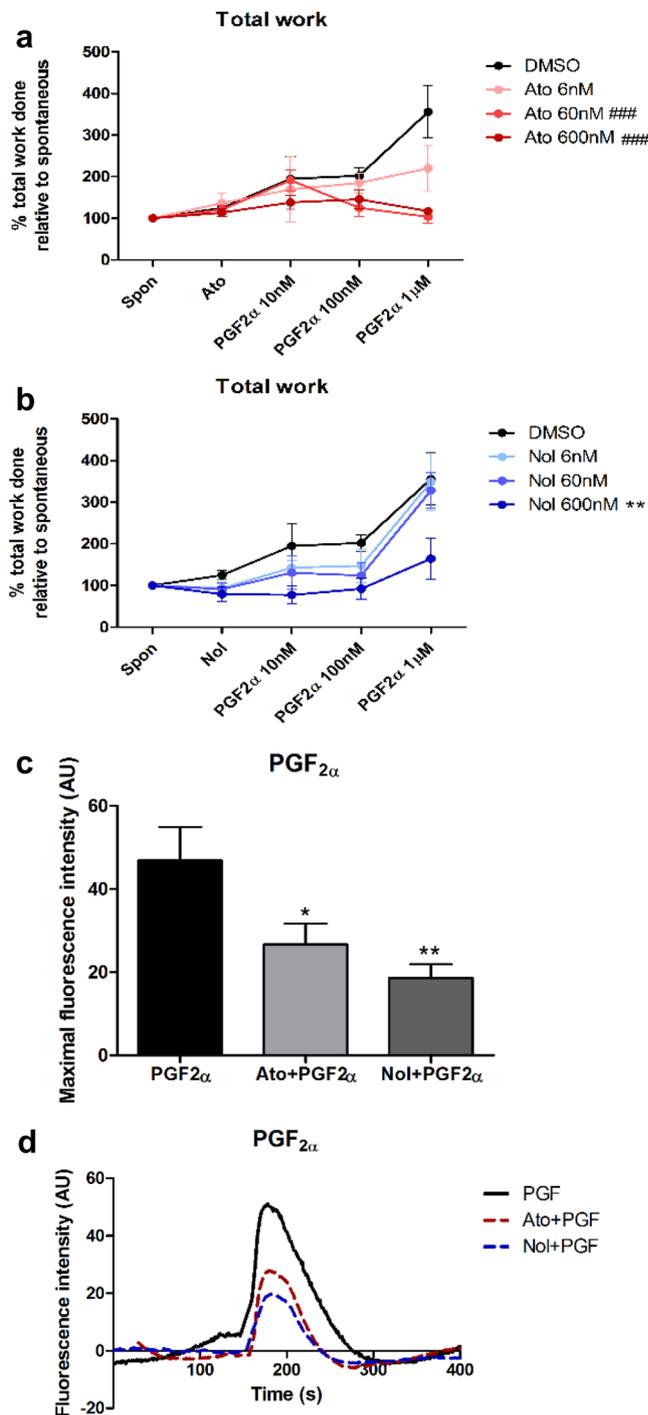
The effects of OTR-As on OT-mediated calcium responses were examined by loading the myometrial cells with Fluo-4-AM calcium dye. We have confirmed that the calcium response driven by OT stimulation is consistent in myometrial cells throughout passages 0 to 10 (data not shown). Human myometrial smooth muscle cells at passages 4–7 were used to observe the changes in their agonist-mediated calcium response in presence or absence of the OTR-As. The results showed a consistent response to OT stimulation with a substantial increase in Fluo-4-AM fluorescence and hence, intracellular calcium concentration. The maximal fluorescence intensity induced by OT was suppressed in presence of both atosiban and nolasiban by 41.5% ( $p < 0.05$ ) and 45.8% ( $p < 0.05$ ), respectively (Fig. 1c). Figure 1d shows the representative fluorescence intensity profile of an individual cell in response to OT with/without pre-treatment with OTR-As.

**Inhibition of  $PGF_{2\alpha}$ -induced myometrial contractions.** There was a consistent dose-dependent enhancement of myometrial contractile performance with  $PGF_{2\alpha}$  stimulation albeit to a lesser extent than OT (Fig. 2). There was a steady, dose-dependent increase in the overall contractile output ( $p < 0.001$  at 1  $\mu$ M vs spontaneous contractions), affecting the rate of contractions ( $p < 0.01$  at 1  $\mu$ M vs spontaneous contractions), average area under the curve ( $p < 0.05$  at 1  $\mu$ M vs spontaneous contractions), and peak amplitude ( $p < 0.01$  at 1  $\mu$ M vs spontaneous contractions) (Supplementary Fig. S2).

For both atosiban and nolasiban, we observed a significant reduction in  $PGF_{2\alpha}$ -induced rate of uterine contractions at 600 nM ( $p < 0.01$  and  $p < 0.05$ , respectively). Nolasiban decreased the contraction duration, however, this did not reach significance. Atosiban (60 nM) reduced contraction peak amplitude by 43.3% ( $p < 0.01$  vs DMSO) (Supplementary Fig. S2). The effect of  $PGF_{2\alpha}$  on the total work done was inhibited by 60 nM atosiban and 600 nM nolasiban by 67.1% and 53.8%, respectively, compared to vehicle (DMSO) control (Fig. 2a,b).



**Figure 1.** The effect of atosiban and nolasiban on spontaneous and OT-induced myometrial contractions. Pre-labour lower segment myometrial biopsies were subjected to stretch force of 4 g to attain spontaneous contractions. After 20 min of basal reading, vehicle control (DMSO), atosiban (Ato) or nolasiban (Nol) (6, 60, or 600 nM) was added and its effect on spontaneous contractions was measured for 10 min. The effect of the atosiban (a) or nolasiban (b) upon OT was then measured by adding increasing concentrations of the agonist (1, 10, and 100 nM) at 10 minute intervals. Total work (area under all contractions) was measured for each experimental time point and re-expressed as a ratio to the baseline period measurements ( $n = 6$ ,  $***p < 0.001$  Nol vs DMSO;  $^{\#}p < 0.05$  Ato vs DMSO; ANOVA). Primary myometrial cells were loaded with calcium sensitive dye, Fluo-4-AM, prior to stimulation with OT (100 nM) in presence or absence of atosiban (600 nM) or nolasiban (600 nM) pretreatment. Maximal fluorescence intensities are expressed as mean  $\pm$  SEM in arbitrary units (AU) ( $n = 6$ ,  $*p < 0.05$  vs OT; ANOVA) (c) and the fluorescence intensity profiles of a representative cell is shown in (d).



**Figure 2.** The effect of atosiban and nolasiban on PGF<sub>2α</sub>-induced myometrial contractions. Pre-labour lower segment myometrial biopsies were subjected to stretch force of 4 g to attain spontaneous contractions. After 20 min of basal reading, vehicle control (DMSO), atosiban (Ato) or nolasiban (Nol) (6, 60, or 600 nM) was added and its effect on spontaneous contractions was measured for 10 min. The effect of the atosiban (a) or nolasiban (b) upon PGF<sub>2α</sub> was then measured by adding increasing concentrations of agonist (10, 100, and 1000 nM) at 10 minute intervals. Total work (area under all contractions) was measured for each experimental time point and re-expressed as a ratio to the baseline period measurements (n = 6, \*\*p < 0.01 Nol vs DMSO; ###p < 0.001 Ato vs DMSO; ANOVA). Primary myometrial cells were loaded with calcium sensitive dye, Fluo-4-AM, prior to stimulation with PGF<sub>2α</sub> (1 μM) in presence or absence of atosiban (600 nM) or nolasiban (600 nM) pretreatment. Maximal fluorescence intensities are expressed as mean ± SEM in arbitrary units (AU) (n = 6, \*p < 0.05, \*\*p < 0.01 vs PGF<sub>2α</sub>) (c) and the fluorescence intensity profiles of a representative cell is shown in (d).

In human myometrial cells,  $\text{PGF}_{2\alpha}$ -driven calcium response was reproducibly obtained. This agonist-induced changes in intracellular calcium levels were significantly suppressed by nolasiban (60.2% decrease,  $p < 0.01$ ), and to a lesser degree, by atosiban (43.2% decrease,  $p < 0.05$ ) (Fig. 2c). Figure 2d shows the representative fluorescence intensity profile of an individual cell in response to  $\text{PGF}_{2\alpha}$  with/without pre-treatment with OTR-As.

**Inhibition of  $\text{PGF}_{2\alpha}$ -induced pro-inflammatory responses in human myometrial cells.** Human myometrial smooth muscle cells were treated with  $\text{PGF}_{2\alpha}$  in presence or absence of OTR-A, atosiban or nolasiban. As shown in previous studies, there was an increase in activation of p65 NF- $\kappa$ B subunit and MAPKs, p38 and ERK1/2, leading to upregulation of COX-2 with  $\text{PGF}_{2\alpha}$  stimulation (Fig. 3a,b). Atosiban treatment alone was able to drive the activation of NF- $\kappa$ B and MAPKs, however, it resulted in significant inhibition of  $\text{PGF}_{2\alpha}$ -induced p-p65 ( $p < 0.05$ ) and p-p38 MAPK ( $p < 0.01$ ) expression (Fig. 3a). This inhibition translated downstream to suppress  $\text{PGF}_{2\alpha}$ -mediated COX-2 upregulation ( $p < 0.05$ ) (Fig. 3b).

Similar to atosiban, nolasiban also led to significant inhibition of  $\text{PGF}_{2\alpha}$ -induced p-p65 ( $p < 0.001$ ), p-p38 MAPK ( $p < 0.001$ ), and p-ERK1/2 ( $p < 0.01$ ) expression (Fig. 4a), as well as the downstream COX-2 expression ( $p < 0.05$ ) (Fig. 4b). However, unlike atosiban, there was no increase in p-p65, p-p38 or p-ERK1/2 when treated alone.

## Discussion

We have shown that atosiban and nolasiban reduced not only OT-induced myometrial contractions in a dose-dependent manner but also  $\text{PGF}_{2\alpha}$ -induced myometrial contractions in an *ex vivo* myometrial contractility model. The two OTR-As have comparable potency in suppressing OT- and PG-augmented contractions. These effects appear to involve the suppression of agonist-driven increase in the intracellular calcium levels. The inhibitory effects of these OTR-As extended to affect  $\text{PGF}_{2\alpha}$ -induced activation of pro-inflammatory transcription factors, NF- $\kappa$ B and MAPKs, as well as the expression of contraction-associated-protein, COX-2.

Labour is defined clinically by the initiation of rhythmic uterine contractions. OT as well as PGs are potent stimulators of contractions and are commonly used to induce labour<sup>23,24</sup>. However, both OT and PGs are more than just a stimulator of myometrial contractions. They also play an important signalling role during the onset of labour, contributing to the transformation of the uterus by establishing a pro-inflammatory environment<sup>25,26</sup>. Therefore, the receptors of OT and PGs are attractive targets for development of drugs aimed at managing pre-term labour.

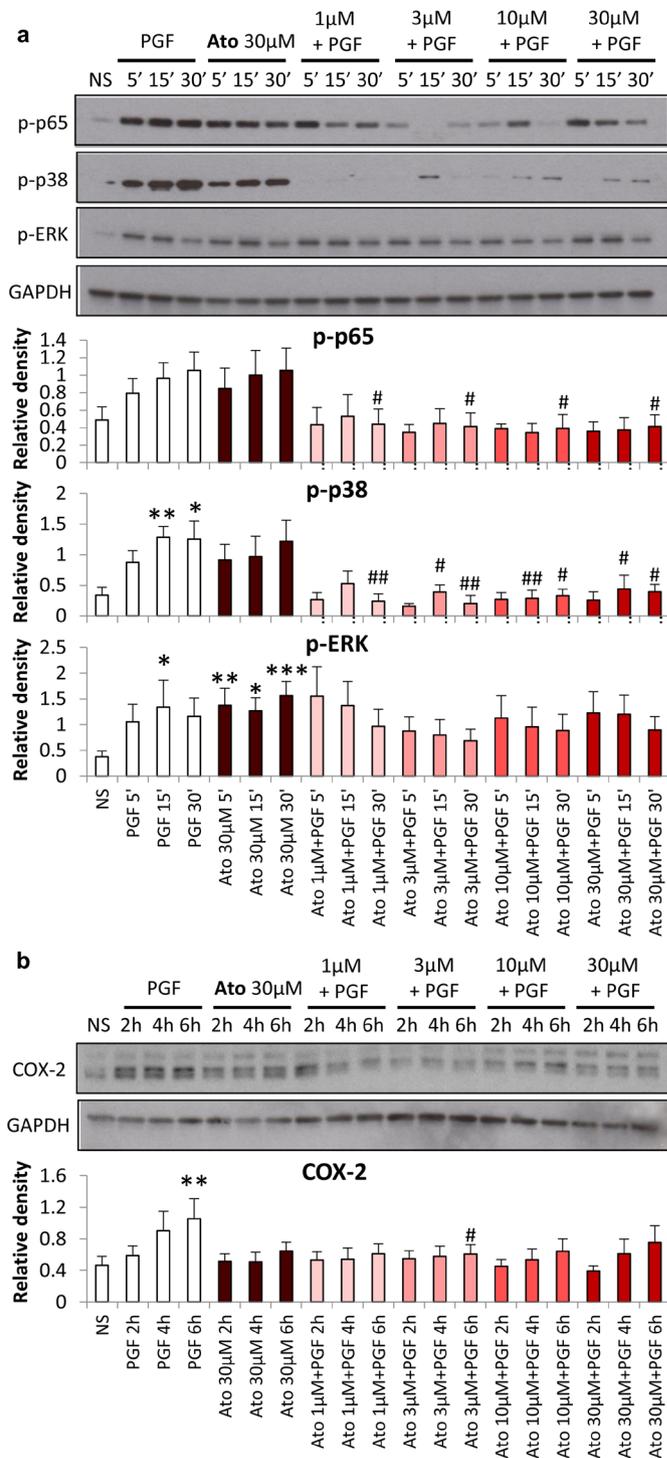
Atosiban is a well-established OTR-A which is approved in Europe. Although the efficacy of atosiban in clinical trials is disputed<sup>27</sup>, it has been repeatedly shown to successfully inhibit OT-induced myometrial contractions *in vitro*<sup>28–30</sup> by inhibiting inositol triphosphate ( $\text{IP}_3$ ) production with decrease in intracellular calcium mobilisation in the myometrial cell<sup>12</sup> as we have shown in this study. Nolasiban is a non-peptide OTR-A, which has a higher selectivity for OTR than V1a receptors when compared to atosiban<sup>14</sup>. Here, we have demonstrated that the inhibitory effects of nolasiban on OT-augmented myometrial contractions and calcium response are similar to that of atosiban. However, unlike atosiban, nolasiban appears to have no biased agonistic effect on the pro-inflammatory signalling pathways<sup>14</sup>, thus highlighting the promising potential of nolasiban as a new tocolytic.

Our results demonstrate a robust concentration-dependent increase in myometrial contractions in presence of  $\text{PGF}_{2\alpha}$  at 1  $\mu\text{M}$ , albeit to a lesser extent than OT. This finding complements previous reports demonstrating significant increases in myometrial contractility in presence of  $\text{PGF}_{2\alpha}$ <sup>30</sup>. The levels of PGs have been found to increase in both amniotic fluid and the maternal blood during the onset labour<sup>31</sup> and it is commonly believed that PGs directly stimulate myometrial contractions<sup>32</sup>. Thus  $\text{PGF}_{2\alpha}$  agonists (eg. carboprost) are clinically used for the induction of labour as well as management of postpartum haemorrhage<sup>33</sup>. Based on the existing evidence, there is little doubt that PGs have an important role in the process of labour, however, some studies have reported PGs as ineffective contractile agents and implied an indirect role for PGs in inducing contractions<sup>34,35</sup>. It was suggested that PGs may exert their effects on contractility via a complex interplay of processes with various targets, possibly by inducing calcium influx, driving the production of other uterotonins and/or contraction-associated proteins, or by modulating the uterotonin receptivity of the myometrium<sup>34,35</sup>.

In binding assays, nolasiban at 10  $\mu\text{M}$  showed no inhibition of FP receptors expressed in recombinant cell lines (data not shown, on file) indicating that nolasiban's inhibitory effects on  $\text{PGF}_{2\alpha}$ -induced contractions and inflammatory responses are not mediated by direct inhibition of cognate FP receptors. This suggests that our results may be due to a possible crosstalk between OTR and FP receptor signalling as a result of receptor-receptor interaction and negative cooperativity, or overlap of downstream signalling pathways. The first evidence of interplay between OT and PG receptors was reported in 1980 when Baxi *et al.* found that low  $\text{PGF}_{2\alpha}$  concentrations can enhance OT-induced uterine contractions<sup>36</sup>. A subsequent study suggested that this may be due to the increase in OTR binding affinity in term myometrium when stimulated with  $\text{PGF}_{2\alpha}$ <sup>37</sup>. More recently, further evidence of possible crosstalk between OT and  $\text{PGF}_{2\alpha}$  at receptor level was provided. THG113.31, a non-competitive FP receptor antagonist peptide, led to a concentration-dependent suppression of OT-augmented contractions in term, non-labouring human myometrium<sup>30</sup>.

As tocolytics are designed to delay preterm labour, the main limitation of our study is the use of myometrial samples obtained from term pregnancies before labour and not from preterm labouring pregnancies. It is possible that the findings from this study may not translate directly to clinical settings, however, by limiting our sample collection to breech- or previous caesarean-indicated sections, we were able to study the *in vitro* effects of these OTR-As on myometrial contractions in a homogenous study population.

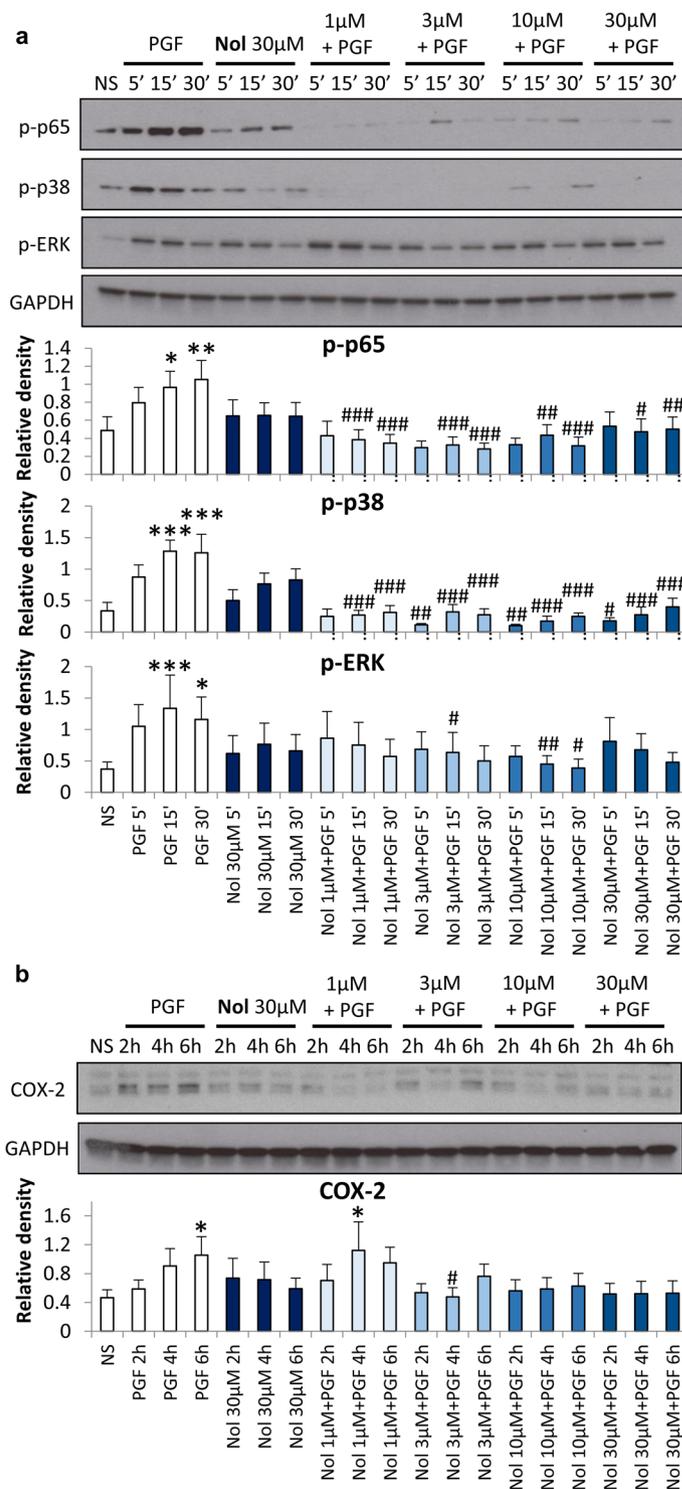
In conclusion, both OTR-As, atosiban and nolasiban, suppress OT- and  $\text{PGF}_{2\alpha}$ -induced calcium response to dose-dependently inhibit pregnant myometrial contractions. Moreover, the OTR-As suppressed the effect of  $\text{PGF}_{2\alpha}$  on downstream signalling and gene expression indicating that there may be a shared mechanism between OTR and FP receptors. Further insight into this potential crosstalk/interaction between OTR and FP receptors would lead to identification of a novel approach to improve current tocolytic efficacy via combination tocolysis.



**Figure 3.** The effect of atosiban on PGF<sub>2 $\alpha$</sub> -induced pro-inflammatory response in myometrial cells. Pre-labour myometrial smooth muscle cells were treated with atosiban (Ato; 1, 3, 10, or 30  $\mu$ M) and PGF<sub>2 $\alpha$</sub>  (1  $\mu$ M) for 5 min, 15 min, 30 min, 2 h, 4 h and 6 h. Whole cell proteins were subjected to Western blot analyses with antibodies against phosphorylated NF- $\kappa$ B p65 subunit, ERK1/2 and p38 MAPK (**a**), as well as COX-2 (**b**), and matching densitometry analyses have been added below the representative blots. Membranes were probed with GAPDH to confirm equal loading (n = 6; \**p* < 0.05, \*\**p* < 0.01 compared with non-stimulated (NS); #*p* < 0.05, ##*p* < 0.01 compared to PGF<sub>2 $\alpha$</sub> -treated, ANOVA).

## Materials and Methods

**Human myometrial tissue collection.** Myometrial tissues were collected from pregnant women undergoing scheduled elective caesarean section at term (38<sup>+0</sup> - 40 weeks of pregnancy), prior to the onset of labour. All participating women were informed about the nature of the study in advance and informed written consents were



**Figure 4.** The effect of nolasiban on  $\text{PGF}_{2\alpha}$ -induced pro-inflammatory response in myometrial cells. Pre-labour myometrial smooth muscle cells were treated with nolasiban (Nol; 1, 3, 10, or 30  $\mu\text{M}$ ) and  $\text{PGF}_{2\alpha}$  (1  $\mu\text{M}$ ) for 5 min, 15 min, 30 min, 2 h, 4 h and 6 h. Whole cell proteins were subjected to Western blot analyses with antibodies against phosphorylated NF- $\kappa$ B p65 subunit, ERK1/2 and p38 MAPK (**a**), as well as COX-2 (**b**), and matching densitometry analyses have been added below the representative blots. Membranes were probed with GAPDH to confirm equal loading ( $n = 6$ ; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with NS; # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  compared to  $\text{PGF}_{2\alpha}$ -treated, ANOVA).

provided with the approval from the Riverside Research Ethics Committee (RREC 3357). All experiments were performed in accordance with the committee's guidelines and regulations. Women with multiple pregnancies or medical conditions such as diabetes, pre-eclampsia, obstetric cholestasis were not included in this study.

**Sample processing.** The myometrial biopsies were obtained from the upper margin of the incision made at the lower segment of the uterus, and were stored in phosphate-buffered saline (PBS) at 4 °C for dissection. All samples were used for contractility experiments within 24 hours of collection. The biopsies were dissected into 8 longitudinal myometrial strips of 7 mm × 2 mm × 1 mm and mounted to thermostatically-controlled isolated organ baths (DMT Myograph 800MS) containing 7 ml of oxygenated (95% O<sub>2</sub> and 5% CO<sub>2</sub>) Krebs solution (D-Glucose 2.0 g/L, Magnesium sulphate (anhydrous) 0.141 g/L, Potassium phosphate monobasic 0.16 g/L, Potassium chloride 0.35 g/L, Sodium chloride 6.9 g/L, Calcium chloride dihydrate 0.373 g/L, Sodium bicarbonate 2.1 g/L) at 37 °C, pH 7.4.

**Drugs and reagents.** Dimethyl sulfoxide (DMSO) and atosiban were purchased from Sigma-Aldrich (Dorset, UK). PGE<sub>2</sub> was from Tocris Bioscience (Bristol, UK), and PGF<sub>2α</sub> from Cayman chemicals (Ann Arbor, MI, USA). OT (Syntocinon®) was from Alliance Pharmaceuticals (Chippenhams, UK). Nolasiban was provided by ObsEva SA (Geneva, Switzerland).

Vehicle for OT was Krebs's solution, atosiban was dissolved in double deionized water, and nolasiban in DMSO. The DMSO concentration was adjusted to 0.1% v/v in all dose formulations, and control wells for nolasiban experiments were treated to contain 0.1% v/v DMSO.

**Data acquisition.** The longitudinal myometrial strips were subjected to 4 g (19.62mN) of tension to attain spontaneous contractions and the experiment was abandoned if more than 2 strips failed to initiate stable spontaneous contractions. After recording 20 min of stable basal contractions, OTR-A (atosiban or nolasiban; 6, 60 and 600 nM) or vehicle (equivalent volume) was added. The effect of OTR-A or vehicle was recorded for 10 min prior to cumulative dose responses for agonists (OT and PGF<sub>2α</sub>) that were added every 10 mins. OT concentrations ranged from 1 to 100 nM and PGF<sub>2α</sub> concentrations ranged from 0.01 to 1 μM. Myometrial contractility was recorded by a force transducer with Powerlab and was analysed using LabChart5 with peak parameters extension (version 5.5.6; ADI instruments, Oxford, UK).

Acquired data were transferred from the datapad of the LabChart5 software for further analysis. The changes in the contractility in response to different treatments were measured by normalising to the basal spontaneous contractions of each strip and then to the equivalent time-point for the vehicle control strip.

**Measurement of intracellular calcium levels.** Primary myometrial smooth muscle cells were isolated from non-labouring myometrial biopsies as previously described<sup>14</sup>. Once the cells reach confluence, they were passaged using 0.25% trypsin with 0.02% EDTA in PBS and seeded into 35 mm glass bottomed cell culture dishes (MatTek Corporation). Prior to treatment, cells were serum starved in 1% FCS DMEM overnight. For calcium mobilisation study, the cells were loaded with calcium sensitive Fluo-4 Direct buffer with 5 mM probenecid (Invitrogen) for 30 min at 37 °C in a 5% CO<sub>2</sub> incubator and subsequently at room temperature for further 30 min. The dishes were then subjected for live cell imaging on the TCS-SP5 confocal microscope (Leica) with a x20 dry objective. Cells were imaged for 3 min and for 15 min after treatment with agonist/antagonist. The images were captured every 1.385 sec and the time-lapse movies were generated with the LAS-AF software (Leica). The changes in the fluorescence intensities were measured using open-source software, ImageJ/Fiji (US National Institutes of Health).

**Western blots.** Non-labouring primary myometrial smooth muscle cells were treated with PGF<sub>2α</sub> for 5 min, 15 min, 30 min, 2 h, 4 h or 6 h in presence/absence of atosiban or nolasiban (1, 3, 10 or 30 μM). Whole cell lysates were extracted using the modified radioimmunoprecipitation assay (RIPA) buffer<sup>25</sup>. Proteins were separated on a pre-cast SDS-polyacrylamide gel (Bio-Rad) and transferred to a PVDF membrane using the Trans-Blot® Turbo™ Blotting System following manufacturer's protocol. All primary antibodies were optimized to minimize background and/or non-specific binding using full length blots (Supplementary Fig. S3). Once optimized, membranes were cut at 55 kDa and top halves were incubated in primary antibody, p-p65 (Cell Signalling) or COX-2 (Santa-Cruz Biotechnology), and bottom halves in p-p38 and p-ERK (Cell Signalling) overnight at 4 °C and in the appropriate HRP-tagged secondary antibody (Cell Signalling) for 1 h at room temperature the next day. Signal detection was done using ECL reagent (Bio-Rad) and ImageQuant LAS4000 (GE Healthcare). Equal loading was confirmed by blotting bottom halves of the membranes for GAPDH (Proteintech).

**Statistical analysis.** For contractility and Western blot studies, all results were expressed as mean ± SEM with n = 6 experiments performed on myometrial samples from different patients. For measurements of intracellular calcium levels, all results were shown as maximum fluorescence ± SEM with n = 4. Two-way analysis of variance (ANOVA) and one-way ANOVA were conducted with *Bonferroni* and *Dunnett's post-hoc* test, respectively, using Graphpad Prism (version 5.02; GraphPad Software, San Diego, CA, USA). Values were considered to be statistically significant at *p* < 0.05.

## Data Availability

All data generated or analysed during the currently study are available from the corresponding author on reasonable request.

## References

- Slattery, M. M. & Morrison, J. J. Preterm delivery. *Lancet* **360**, 1489–1497, [https://doi.org/10.1016/S0140-6736\(02\)11476-0](https://doi.org/10.1016/S0140-6736(02)11476-0) (2002).
- Lamont, C. D., Jorgensen, J. S. & Lamont, R. F. The safety of tocolytics used for the inhibition of preterm labour. *Expert opinion on drug safety* **15**, 1163–1173, <https://doi.org/10.1080/14740338.2016.1187128> (2016).
- Peebles, D. M. Intrauterine infection and perinatal brain injury (2007).

4. Kam, K. Y. & Lamont, R. F. Developments in the pharmacotherapeutic management of spontaneous preterm labor. *Expert opinion on pharmacotherapy* **9**, 1153–1168, <https://doi.org/10.1517/14656566.9.7.1153> (2008).
5. Sanborn, B. M., Ku, C. Y., Shlykov, S. & Babich, L. Molecular signaling through G-protein-coupled receptors and the control of intracellular calcium in myometrium. *J Soc Gynecol Investig* **12**, 479–487, <https://doi.org/10.1016/j.jsjg.2005.07.002> (2005).
6. Lopez Bernal, A. Mechanisms of labour—biochemical aspects. *BJOG: an international journal of obstetrics and gynaecology* **110**(Suppl 20), 39–45 (2003).
7. MacIntyre, D. A. *et al.* Contraction in human myometrium is associated with changes in small heat shock proteins. *Endocrinology* **149**, 245–252, <https://doi.org/10.1210/en.2007-0662> (2008).
8. Woodrum, D. A. & Brophy, C. M. The paradox of smooth muscle physiology. *Molecular and cellular endocrinology* **177**, 135–143 (2001).
9. Fuchs, A. R., Fuchs, F., Husslein, P., Soloff, M. S. & Fernstrom, M. J. Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. *Science* **215**, 1396–1398 (1982).
10. Kim, S. H., Bennett, P. R. & Terzidou, V. Advances in the role of oxytocin receptors in human parturition. *Molecular and cellular endocrinology* **449**, 56–63, <https://doi.org/10.1016/j.mce.2017.01.034> (2017).
11. Tsatsaris, V., Carbonne, B. & Cabrol, D. Atosiban for preterm labour. *Drugs* **64**, 375–382 (2004).
12. Lamont, R. F. The development and introduction of anti-oxytocic tocolytics. *BJOG: an international journal of obstetrics and gynaecology* **110**(Suppl 20), 108–112 (2003).
13. Kim, S. H. *et al.* The oxytocin receptor antagonist, Atosiban, activates pro-inflammatory pathways in human amnion via G(alpha) signalling. *Molecular and cellular endocrinology* **420**, 11–23, <https://doi.org/10.1016/j.mce.2015.11.012> (2016).
14. Kim, S. H. *et al.* Differential Effects of Oxytocin Receptor Antagonists, Atosiban and Nolasiban, on Oxytocin Receptor-Mediated Signaling in Human Amnion and Myometrium. *Mol Pharmacol* **91**, 403–415, <https://doi.org/10.1124/mol.116.106013> (2017).
15. Keelan, J. A. *et al.* Cytokines, prostaglandins and parturition—a review. *Placenta* **24** Suppl A, S33–46, doi:S0143400402909483 [pii] (2003).
16. Hammers, A. L., Sanchez-Ramos, L. & Kaunitz, A. M. Antenatal exposure to indomethacin increases the risk of severe intraventricular hemorrhage, necrotizing enterocolitis, and periventricular leukomalacia: a systematic review with metaanalysis. *Am J Obstet Gynecol* **212**(505), e501–513, <https://doi.org/10.1016/j.ajog.2014.10.1091> (2015).
17. Molnar, M. & Hertelendy, F. Regulation of intracellular free calcium in human myometrial cells by prostaglandin F2 alpha: comparison with oxytocin. *J Clin Endocrinol Metab* **71**, 1243–1250, <https://doi.org/10.1210/jcem-71-5-1243> (1990).
18. Narumiya, S., Sugimoto, Y. & Ushikubi, F. Prostanoid receptors: structures, properties, and functions. *Physiol Rev* **79**, 1193–1226 (1999).
19. Xu, C. *et al.* Effects of PGF2alpha on the expression of uterine activation proteins in pregnant human myometrial cells from upper and lower segment. *J Clin Endocrinol Metab* **98**, 2975–2983, <https://doi.org/10.1210/jc.2012-2829> (2013).
20. Olson, D. M. & Ammann, C. Role of the prostaglandins in labour and prostaglandin receptor inhibitors in the prevention of preterm labour. *Frontiers in bioscience: a journal and virtual library* **12**, 1329–1343, doi:2151 [pii] (2007).
21. Miranda, M. A. *et al.* A proposed role for oxytocin in regulation of endometrial prostaglandin F2 alpha secretion during luteolysis in swine. *Adv Exp Med Biol* **395**, 421–433 (1995).
22. Engstrom, T., Bratholm, P., Christensen, N. J. & Vilhardt, H. Effect of oxytocin receptor blockade on rat myometrial responsiveness to prostaglandin f(2)(alpha). *Biol Reprod* **63**, 1443–1449 (2000).
23. Hamilton, B. E., Martin, J. A. & Ventura, S. J. Births: preliminary data for 2012. *National vital statistics reports: from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System* **62**, 1–20 (2013).
24. Obstetrics, A. C. O. P. B. ACOG Practice Bulletin No. 107: Induction of labor. *Obstet Gynecol* **114**, 386–397, <https://doi.org/10.1097/AOG.0b013e3181b48ef5> (2009).
25. Kim, S. H. *et al.* Oxytocin activates NF-kappaB-mediated inflammatory pathways in human gestational tissues. *Molecular and cellular endocrinology* **403**, 64–77, <https://doi.org/10.1016/j.mce.2014.11.008> (2015).
26. Xu, C. *et al.* PGF2alpha modulates the output of chemokines and pro-inflammatory cytokines in myometrial cells from term pregnant women through divergent signaling pathways. *Mol Hum Reprod* **21**, 603–614, <https://doi.org/10.1093/molehr/gav018> (2015).
27. Navathe, R. & Berghella, V. Tocolysis for Acute Preterm Labor: Where Have We Been, Where Are We Now, and Where are We Going? *Am J Perinatol* **33**, 229–235, <https://doi.org/10.1055/s-0035-1571147> (2016).
28. Buscher, U. *et al.* Effects of oxytocin receptor antagonist atosiban on pregnant myometrium *in vitro*. *Obstet Gynecol* **98**, 117–121 (2001).
29. Pierzynski, P., Lemancewicz, A., Reinheimer, T., Akerlund, M. & Laudanski, T. Inhibitory effect of barusiban and atosiban on oxytocin-induced contractions of myometrium from preterm and term pregnant women. *J Soc Gynecol Investig* **11**, 384–387, <https://doi.org/10.1016/j.jsjg.2004.02.008> (2004).
30. Friel, A. M., O'Reilly, M. W., Sexton, D. J. & Morrison, J. J. Specific PGF(2alpha) receptor (FP) antagonism and human uterine contractility *in vitro*. *BJOG: an international journal of obstetrics and gynaecology* **112**, 1034–1042, <https://doi.org/10.1111/j.1471-0528.2005.00658.x> (2005).
31. Dray, F. & Frydman, R. Primary prostaglandins in amniotic fluid in pregnancy and spontaneous labor. *Am J Obstet Gynecol* **126**, 13–19 (1976).
32. Wqvist, N., Bryman, I., Lindblom, B., Norstrom, A. & Wikland, M. The role of prostaglandins for the coordination of myometrial forces during labour. *Acta Physiol Hung* **65**, 313–322 (1985).
33. Bai, J., Sun, Q. & Zhai, H. A comparison of oxytocin and carboprost tromethamine in the prevention of postpartum hemorrhage in high-risk patients undergoing cesarean delivery. *Experimental and therapeutic medicine* **7**, 46–50, <https://doi.org/10.3892/etm.2013.1379> (2014).
34. Word, R. A., Kamm, K. E. & Casey, M. L. Contractile effects of prostaglandins, oxytocin, and endothelin-1 in human myometrium *in vitro*: refractoriness of myometrial tissue of pregnant women to prostaglandins E2 and F2 alpha. *J Clin Endocrinol Metab* **75**, 1027–1032, <https://doi.org/10.1210/jcem.75.4.1400867> (1992).
35. Chiossi, G. *et al.* The effects of prostaglandin E1 and prostaglandin E2 on *in vitro* myometrial contractility and uterine structure. *Am J Perinatol* **29**, 615–622, <https://doi.org/10.1055/s-0032-1311986> (2012).
36. Baxi, L. V., Petrie, R. H. & Caritis, S. N. Induction of labor with low-dose prostaglandin F2 alpha and oxytocin. *Am J Obstet Gynecol* **136**, 28–31 (1980).
37. Fukai, H. *et al.* Study of oxytocin receptor: II. oxytocin and prostaglandin F2 alpha receptors in human myometria and amnion-decidua complex during pregnancy and labor. *Endocrinologia japonica* **31**, 565–570 (1984).

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### Author Contributions

S.H.K., O.P., A.C., J.P.G., A.H., P.R.B. and V.T. designed the experiments. S.H.K., L.R. and H.A. performed the experiments and analysed the data. S.H.K., O.P., A.C., J.P.G., A.H., P.R.B. and V.T. contributed to the writing of the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship and all those who qualify for authorship are listed.

### Additional Information

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**Competing Interests:** V.T. has worked as a consultant to GlaxoSmithKline. P.B. has worked as a consultant to Boehringer Ingelheim, GlaxoSmithKline, Merck Serono, ObsEva SA and Tokyo Tanabe Pharmaceuticals, drug companies with an interest in the pharmacological effects of oxytocin and oxytocin antagonists. P.B. holds shares of ObsEva SA. J.P.G. and O.P. are salaried employees of ObsEva SA. A.C. is a consultant to ObsEva SA.

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