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REVIEW ARTICLE

Uterine contractions in rodent models and humans

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Abstract

Aberrant uterine contractions can lead to preterm birth and other labour complications and are a significant cause of maternal morbidity and mortality. To investigate the mechanisms underlying dysfunctional uterine contractions, researchers have used experimentally tractable small animal models. However, biological differences between humans and rodents change how researchers select their animal model and interpret their results. Here, we provide a general review of studies of uterine excitation and contractions in mice, rats, guinea pigs, and humans, in an effort to introduce new researchers to the field and help in the design and interpretation of experiments in rodent models.

KEYWORDS

animal models, contraction, myometrium, parturition, pregnancy, species differences, uterus

1 | INTRODUCTION

Problems in pregnancy, labour and delivery affect millions of people per year and contribute to significant mortality and morbidity worldwide.[1–3] Dysfunctional uterine contractions in labour can cause complications such as preterm birth, dystocia, uterine atony and post-partum haemorrhage. Therefore, research in this field is of paramount importance to public health. Current understanding of uterine contractions relies on evidence from both human subjects and animal models. Because of practical and ethical concerns with research on pregnant women, uterine biologists have turned animal models, primarily using mice, rats, and guinea pigs. However, because of physiological differences between species, experiments on rodent models must be carefully designed and interpreted.

The basic architecture of pregnancy differs considerably between humans and rodents (Figure 1). Typically, humans have a 40-week gestation and deliver one to two offspring, whereas rodent gestations are shorter in length with larger litter sizes. The shape and structure of the uterus is different between humans and rodents: humans have pyriform (lightbulb-shaped) uteri, whereas mice, rats and guinea pigs have bicornuate (two-horned) uteri with multiple implantation sites in each uterine horn.[4] In all species, the uterus consists of an inner mucosal layer called the endometrium or decidua, an intermediate muscular layer called the myometrium, and an outer serosal layer called the perimetrium. The myometrium consists of both longitudinal and circular muscle fibres. In rodents, the myometrium has a clearly defined outer longitudinal layer and inner circular layer. However, in humans, the longitudinal and circular fibres are not easily distinguishable.[5]

The precipitating factors that trigger parturition also differ between humans, mice, rats and guinea pig. In mice

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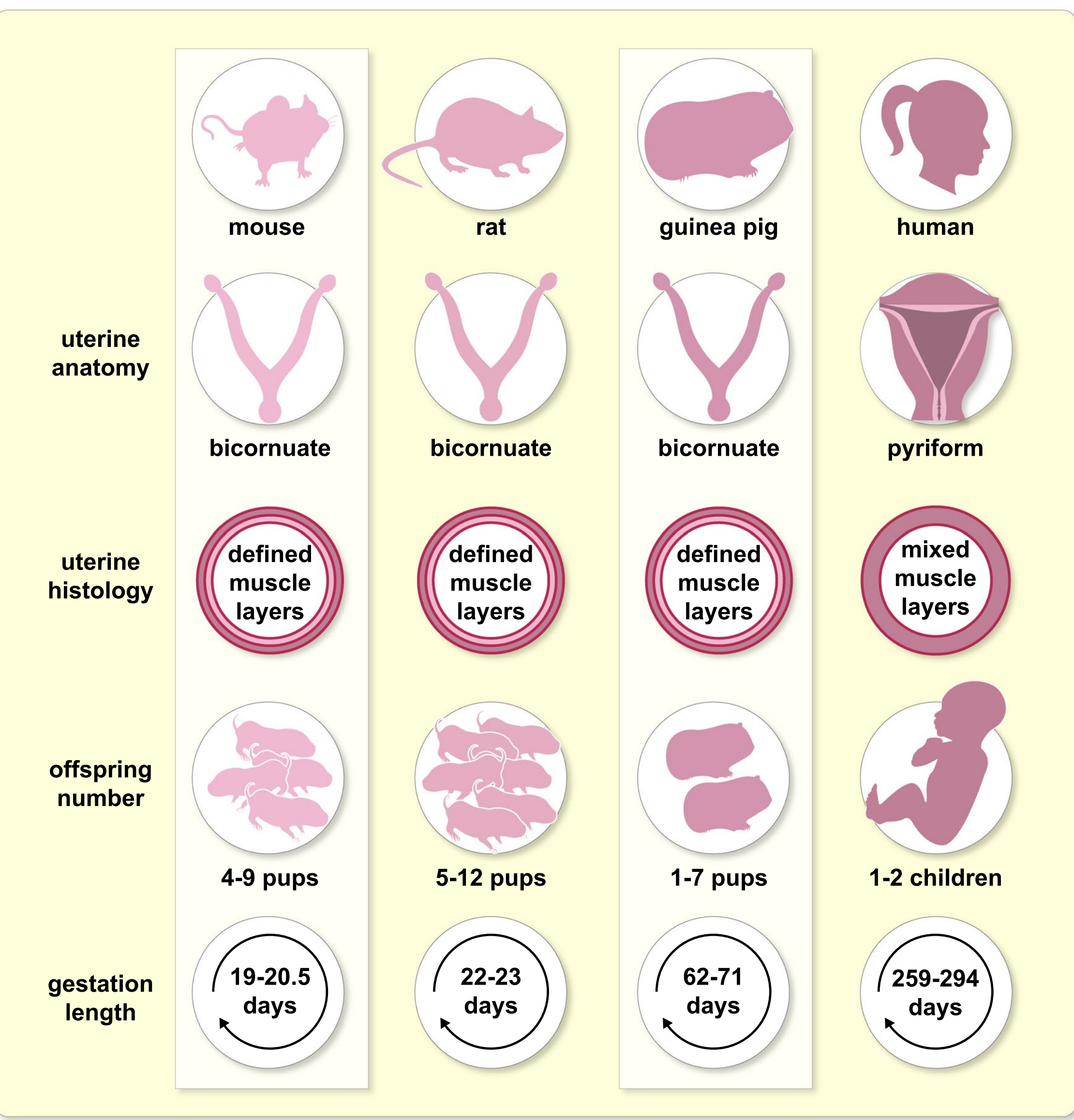

FIGURE 1 Uterine anatomy, histology, and pregnancy compared across species

and rats, labour is likely triggered by progesterone withdrawal.[6] During gestation, progesterone is produced by the corpus luteum.[6] At term pregnancy, prostaglandin production causes destruction of the corpus luteum (luteolysis) and consequently a decline in serum progesterone levels.[6] Early progesterone withdrawal, caused by treatment with a progesterone blocker such as RU486, causes premature labour, whereas progesterone supplementation causes prolongation of pregnancy.[7,8] These conditions differ in the human and the guinea pig. In both these species, the corpus luteum involutes early in pregnancy and progesterone is instead produced by the placenta. Progesterone levels remain high into late pregnancy in the human and guinea pig; further supplementation with progesterone does not prolong gestation in the guinea pig and has varying efficacy at preventing preterm birth in humans.[9,10] Thus, progesterone withdrawal alone is not the trigger for labour in guinea pigs or humans, although potential mechanisms for "functional" progesterone withdrawal are an area of active investigation.[11] Mitchell and Taggart[6] have hypothesized that labour in humans and guinea pigs is instead caused by multiple processes that occur in parallel to alter the balance between pro-quiescent and pro-contractile stimuli, as well as pro- and anti-inflammatory stimuli. Still, further research is necessary to determine whether these processes are similar between humans and guinea pigs.

When choosing an experimental model, researchers should consider the practical and ethical constraints that inform the types of experiments that can be performed with each species. In the United States, federal regulations provide special protections for pregnant women in research because of the risks that experimental interventions may pose to foetuses. For example, a pregnant woman can only be included

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in a trial if the potential benefit to the mother or foetus significantly outweighs any risks, or if the risks are negligible. Thus, pregnancy-related experimental studies in humans are rare, constituting only 0.27% of the drug trials registered on Clinicaltrials.gov.[12] To supplement these in vivo studies, researchers use tissue collected from pregnant participants. Intact uterine specimens can be used for ex vivo contractility experiments, or human myometrial smooth muscle cells can be isolated and studied in culture. Many of the experiments described in this review were performed on uterine biopsies taken at the time of Caesarean section (C-section). C-sections are routinely performed on labouring and non-labouring patients at term and preterm. However, these biopsies are necessarily samples of convenience: samples from pre-pregnancy, mid-pregnancy, or the postpartum period are rare, or nearly impossible to obtain ethically. Moreover, tissue strips are usually resected from the lower uterine segment, so results may or may not be applicable to the uterine fundus, the contractile portion of the uterus.[6] Finally, environmental and genetic factors vary widely between the patients who provide biopsies, resulting in heterogeneous samples and sometimes inconsistent results.

In contrast, when working with rodent models, researchers can obtain tissue at any time point prior to and during pregnancy and from any location within the uterus. Heterogeneity is minimal because environmental influences are similar in animals housed together, and genetic differences are negligible in inbred mouse strains commonly used for research. However, this homogeneity can limit the relevance of animal studies to human pathophysiology. For example, mice and rats rarely experience spontaneous preterm labour, so researchers must inoculate animals with infectious agents or treat them with lipopolysaccharide or progesterone blockers to induce early delivery.[13] Additionally, studies in rats and mice cannot recapitulate the myriad causes of idiopathic preterm labour in humans. In contrast, guinea pigs experience preterm labour and uterine dystocia at similar rates to humans, so researchers interested in dysfunctional labour might prefer the guinea pig model.[6] For guinea pigs, as for humans, the causes of dysfunctional labour are unknown.

Other practical considerations may inform a researcher's choice of experimental animal model (Figure 1). One consideration may be gestation length: compared to guinea pigs, mice and rats have shorter gestations. Mice are the least expensive mammalian model to maintain, and inbred mouse strains are ideal targets for genetic manipulation. As reviewed by Ratajczak and Muglia,[14] phenotypes in knockout mouse models have provided gold-standard evidence to support the importance of certain molecules, such as progesterone and prostaglandins, in parturition. However, with the advent of CRISPR-Cas9 gene editing, genetic manipulation in rats and guinea pigs may soon be equally practical. Ultimately, selection of the most appropriate animal model must be based on the scientific question at hand. In the next section, we review the current literature on the physiology of uterine contractions, focusing on key similarities and differences in physiology between species, highlighted in Table 1.

2 | PHYSIOLOGY OF UTERINE CONTRACTIONS

2.1 | Mechanism of uterine contractions

In all species, smooth muscle contractility is driven by a transient increase in intracellular calcium (Ca^{2+}) (reviewed in 15). Ca^{2+} binds to calmodulin, and the Ca^{2+}-calmodulin complex activates myosin light chain kinase (MLCK) (Figure 2). MLCK phosphorylates the regulatory light chain of myosin, which then allows myosin to hydrolyse ATP and move along the actin filament. A decrease in intracellular Ca^{2+} concentration deactivates MLCK, allowing myosin to be dephosphorylated by myosin light chain phosphatase (MLCP), leading to muscle relaxation.

Although these basic players are conserved across species and smooth muscle types, their activities can be differentially regulated. For example, the pro-labour hormones oxytocin and prostaglandin F2 increase contractility towards the end of pregnancy by increasing the ratio of active MLCK to active MLCP.[16,17] These hormones first bind to their respective GPCRs, causing activation of the small G protein RhoA.[18–21] RhoA in turn activates Rho kinase (ROCK), which phosphorylates MLCP to inactivate it (Figure 2).[18–21]

In contrast, GPCRs that signal through Gs and cyclic AMP (cAMP), such as the β2 adrenergic receptor (β2AR), decrease myometrial contractility (Figure 2). In this process, increases in cAMP and cGMP work through protein kinase A and protein kinase G to decrease the ratio of active MLCK to active MLCP and promote myosin dephosphorylation.[21] While the mechanism for this has yet to be defined in myometrial smooth muscle, experiments in other smooth muscle types suggest that protein kinase A phosphorylates MLCK to decrease its affinity for the Ca^{2+}-calmodulin complex.[21] Protein kinase A and protein kinase G can also phosphorylate the protein telokin, which activates MLCP.[21] Given that telokin expression in the uterus increases during pregnancy, this mechanism may contribute to the maintenance of uterine quiescence.[22–24]

In myometrial cells, the increase in intracellular Ca^{2+} that drives contractions can occur in several ways. Like most smooth muscle cells, myometrial cells in all species have an extensive sarcoplasmic reticulum (SR) network that maintains a high luminal Ca^{2+} concentration. The SR takes up Ca^{2+} from the cytosol through the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA, Figure 2) and stores Ca^{2+} via Ca^{2+}-binding proteins. Thus, the SR acts as a Ca^{2+}

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TABLE 1 Key similarities and differences in myometrial physiology between species

		Mouse	Rat	Guinea pig	Human
Mechanism of uterine contractions	IP3 receptor induces calcium release from endoplasmic reticulum.	Yes	Yes	Yes	Yes
	RyR modulates contractions.	No	No	No	No
	SOCE components are present/active in myometrium.	Yes	Yes	No Data	Yes
Action potentials and ion channels	Both simple and complex action potentials are present.	Yes	Yes	Yes	Yes
	Kv4 channel function is downregulated by oestrogen.	No Data	Yes	No Data	Yes
	SK channels promote myometrial quiescence.	Yes	Yes	No Data	Yes
	K_{ATP} openers inhibit contractions at parturition.	No Data	No	No Data	Yes
	BK_{Ca} is pro-contractile in pro-inflammatory conditions.	No Data	No Data	No Data	Yes
	BK_{Ca} alternative splicing decreases channel calcium sensitivity at parturition.	Yes	Yes	No Data	Yes
	LTCC expression (nifedipine binding sites) increases towards parturition.	No Data	Yes	Yes	No Data
	TTCC inhibition decreases contraction frequency.	No Data	Yes	No Data	Yes
Timing and propagation of action potentials	Multiple pacemakers are active in the uterus.	No Data	Yes	Yes	Yes
	Uterine contractions are peristaltic.	No Data	Yes	No	No
	ICC-like cells have been identified in uterus.	Yes	Yes	No Data	Yes
	Gap junctions coordinate contractions.	Yes	Yes	Yes	Yes
Uterotonics	Oxytocin promotes contractions.	Yes	Yes	Yes	Yes
	Oxytocin binding sites increase towards parturition.	Yes	Yes	No	Yes
	Relaxant prostaglandin receptors are downregulated at parturition and pro-contractile prostaglandin receptors are upregulated at parturition.	No Data	Yes	No	Yes
	Prostaglandins promote contractions at parturition.	Yes	Yes	Yes	Yes

buffer, providing Ca^{2+} to activate actin/myosin contractility and then quickly removing Ca^{2+} from the cytosol to allow relaxation following a contraction.

Signalling from plasma membrane-associated Gq-coupled receptors, such as the oxytocin receptor (OXTR) and prostaglandin F2 receptor (FP), leads to production of inositol triphosphate (IP3), which activates the IP3 receptor on the sarcolemma (Figure 2).[25] The IP3 receptor is also a Ca^{2+} channel that allows Ca^{2+} to flow down its concentration gradient from the SR lumen into the cytosol.[26] In non-myometrial cells, including cardiac, skeletal, and some smooth muscle cells, the SR can also release Ca^{2+} upon entry of Ca^{2+} through plasma membrane channels, a process called Ca^{2+}-induced Ca^{2+} release (CICR).[27] CICR occurs when entry of Ca^{2+} through L-type Ca^{2+} channels (LTCCs) activates ryanodine receptors (RyRs) on the SR, causing the RyR channel domain to open transiently. This transient opening leads to calcium "sparks" that activate calcium-gated potassium channels, contributing to repolarization. Researchers initially hypothesized that this could occur in myometrial cells, but studies in both pregnant and nonpregnant animals have shown that these calcium sparks do not occur in mouse or rat myometrium.[28–30] Furthermore, pregnant and nonpregnant mouse myometrium express a dominant negative splice variant of RyR3 that inhibits full length RyR3.[28,31] Accordingly, knocking out RyR3 in mice had no effect on pregnancy and labor.[28] While RyR3 is the only RyR isoform found in mice, RyR1 and RyR2 have been found in human pregnant myometrium.[32,33] Still, treatment with the RyR inhibitor ryanodine had little effect on mouse, rat or human myometrium.[28,34,35] Thus, RyRs are likely not important contributors to SR calcium release in mouse, rat, guinea pig or human.[36]

Both intracellular and extracellular Ca^{2+} sources help regulate myometrial contractility via the process of store-operated

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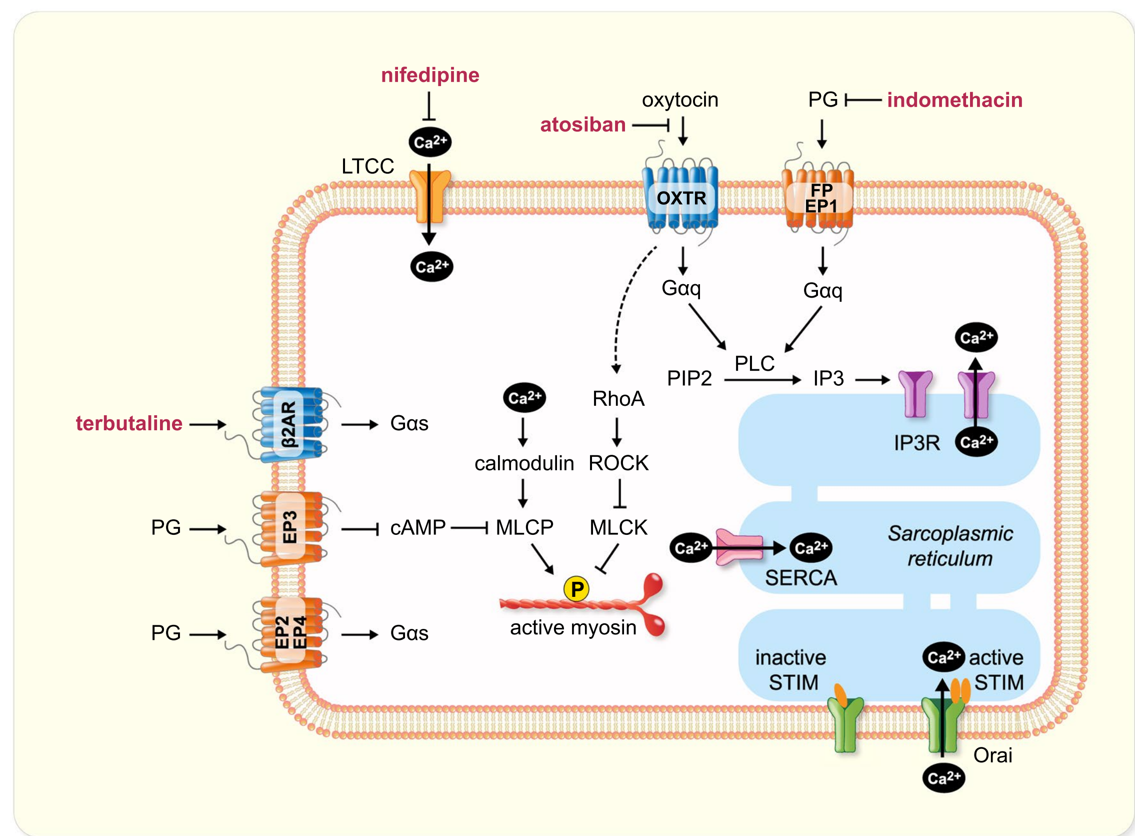


FIGURE 2 Mechanisms of uterine contractility. Ca^{2+} entry into the cell can occur through voltage-gated Ca^{2+} channels (LTCC) and STIM/Orai, members of the store-operated calcium entry complex. Ca^{2+} also effluxes from the sarcoplasmic reticulum through the IP3R to increase intracellular Ca^{2+} concentration. Intracellular Ca^{2+} promotes myosin phosphorylation, which in turn enables uterine contraction. Hormones and tocolytic agents modulate myosin phosphorylation through G protein-coupled receptor-mediated pathways. cAMP, cyclic AMP; EP, prostaglandin E receptors 1-4; FP, prostaglandin F receptor; IP3, inositol triphosphate; IP3R, IP3 receptor; LTCC, L-type calcium channel; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; OXTR, oxytocin receptor; PG, prostaglandins; PIP2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; ROCK, Rho kinase; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; STIM, stromal interaction molecule; β2AR, β2 adrenergic receptor

calcium entry (SOCE). When the SR releases Ca^{2+} through the IP_3 receptor, Ca^{2+} concentration in the SR lumen drops, leading to a conformational change in the stromal interaction molecule (STIM) protein located on the SR membrane (Figure 2).[37] Activated STIM then binds to and activates Ca^{2+} release-activated Ca^{2+} channel protein (CRAC, or Orai) and transient receptor potential cation (TRPC) Ca^{2+} channels located in the plasma membrane, allowing extracellular Ca^{2+} to enter the cell.[37] SOCE was originally described in non-excitable cells and striated muscle, but several lines of evidence suggest that this process also occurs in myometrial smooth muscle cells.[38] First, STIM and Orai are expressed in both human and mouse myometrium.[38–40] Second, STIM1 knockout mice have thin uterine walls, and explanted uterine strips from these mice do not contract normally.[38] Finally, Noble *et al* showed in rat myometrium that depleting Ca^{2+} from the SR caused an increase in intracellular Ca^{2+} and phasic contractions that were inhibited by the SOCE blocker Lanthanum.[41]

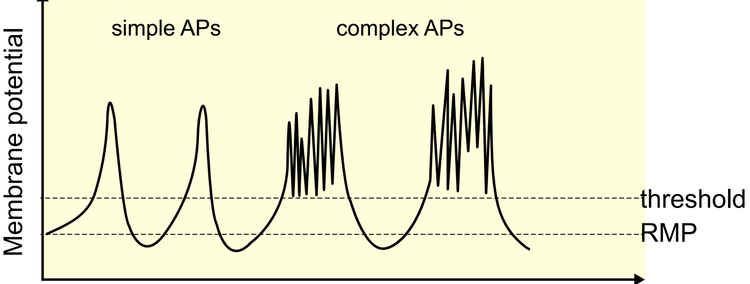
Recent studies have shown that mitochondria might also serve as a myometrial intracellular Ca^{2+} source. Much like the SR, mitochondria can "buffer" intracellular Ca^{2+}, taking up and releasing Ca^{2+} to drive the rhythmic contraction and relaxation seen in smooth muscle tissues such as the gastrointestinal tract. In the "pacemaker" cells of the gastrointestinal tract and prostate gland, inhibition of mitochondrial Ca^{2+} transport dampens pacemaker activity.[42,43] Additionally, Gravina *et al* found that drugs that modulate mitochondrial activity altered the force and frequency of contractions in uterine tissue isolated from nonpregnant mice.[44] However, more work is necessary to describe the exact mechanism of action of these drugs on uterine contractility and to clarify the role of mitochondrial Ca^{2+} in the myometrium.

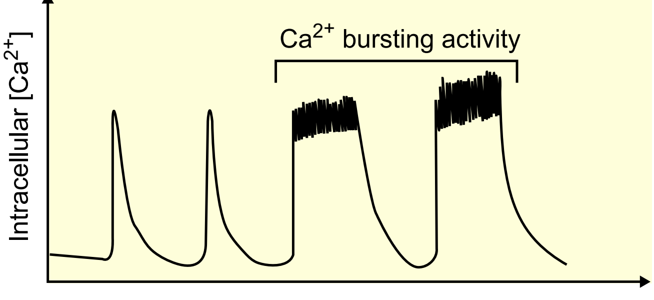
2.2 | Action potentials and ion channels

2.2.1 | Action potentials

Myometrial cells maintain a negative resting membrane potential, meaning that the inside of the plasma membrane is negative relative to the outside of the cell. In the basal state, voltage-gated Ca^{2+} channels are closed and intracellular Ca^{2+} concentrations are low, promoting a non-contractile state. Studies in isolated human myometrium have shown that as the third trimester progresses, the resting membrane potential becomes less negative, rising from −70 mV at week 29 to −55 mV at week 40.[45] Similarly, the membrane potential in the rat myometrium is the most negative during mid-pregnancy (−60 mV to −68 mV during days 11 through 15) and becomes less negative (−54 mV) closer to term (rats generally deliver around day 21).[46,47] In contrast, the resting membrane potential in guinea pig uterine tissue remains at −58 mV throughout the second half of pregnancy.[48]

Slow depolarization of the myometrial cell membrane to around −50 mV allows Ca^{2+} to enter the cell through nifedipine-sensitive, voltage-gated Ca^{2+} channels, otherwise known as LTCCs.[49,50] Ca^{2+} entry through LTCCs depolarizes the myometrial membrane and causes the upstroke of the uterine action potential. Voltage-, Ca^{2+}-, and time-dependent potassium (K^+) channels are responsible for repolarizing the membrane potential.[51,52] Action potentials in the myometrium can take the form of a simple spike or a complex plateau (Figure 3).[53] Complex "plateau"-type action potentials are formed by long depolarizations that often have several superimposed simple spikes, known as calcium bursts. In pregnant rats, simple spikes have been reported to be more common in the longitudinal myometrium, and complex action potentials more common in the circular muscle layer.[53–55] The opposite is true in guinea pigs, as complex action potentials predominate in the longitudinal myometrium, and simple spikes predominate in the circular myometrium.[53] Both types of action potentials have been described in human myometrium, which does not have clearly defined circular and longitudinal layers.[56–59] Hormones and drugs can modulate the shape of these action potentials in both humans and rats.[54,57] For example,





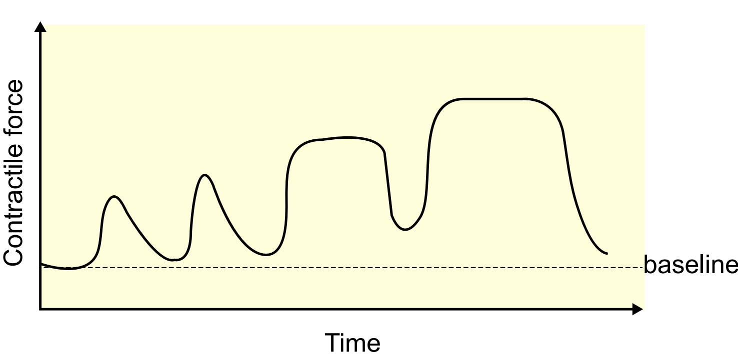


FIGURE 3 Examples of simple and complex action potentials (APs), Ca^{2+} bursts, and the resultant contractile force. Bursting activity can increase the duration of a contraction. RMP: resting membrane potential

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oxytocin can extend the depolarization phase so that simple spikes become complex plateaus with calcium bursting activity,[57] which can increase both the strength and duration of a contraction (Figure 3).[30]

2.2.2 | Sodium channels

As reviewed recently by our group, studies have provided conflicting evidence as to the role of voltage-gated sodium (Na^+) channels in the myometrium in any species.[60] Voltage-insensitive Na^+ channels may slowly depolarize the myometrial cell membrane to the threshold required to activate LTCCs.[60] Reinl *et al* demonstrated that, in both humans and in mice, Na^+ leaks into the myometrial cell through the voltage-insensitive channel sodium leak current, nonselective (NALCN).[61,62] Smooth-muscle specific deletion of NALCN in mice shortens myometrial action potential bursts and leads to delayed and dysfunctional labour.[62] Thus, NALCN was initially thought to increase myometrial excitability, at least in mice. However, a recent study showed that NALCN is upregulated by the pro-quiescent hormone progesterone and downregulated by the pro-contractile hormone oestrogen in human myometrial cells, consistent with a role for NALCN in maintaining myometrial quiescence.[63] This raises the possibility that NALCN functions differently in the mouse and human myometrium, although further studies are necessary to test this hypothesis.

2.2.3 | Calcium channels

Ca^{2+} currents in human myometrial cells can be separated into two components: long-lasting L-type currents and transient T-type currents (TTCCs).[50] Early studies showed that nifedipine-sensitive LTCCs cause the rapid depolarization phase of the myometrial action potential. Expression of mRNAs encoding the α1c pore-forming subunit and β2 regulatory subunit of LTCCs increase before parturition in both guinea pigs and rats.[64–67] These mRNA changes seem to functionally increase both LTCC drug binding sites and LTCC function in both species, consistent with a conserved role for LTCCs in initiating myometrial contractions.[65,66]

Researchers have used the LTCC inhibitor nifedipine and TTCC inhibitors nickel and mibefradil to dissect out the role of TTCCs in uterine contraction. Inhibiting TTCCs decreases the frequency of spontaneous contractions in both human and rat explanted uterine tissue, leading researchers to hypothesize that TTCCs play a role in initiating and pacing contractions.[68–70] This hypothesis is particularly attractive given findings that TTCCs contribute to pacemaker currents in sinoatrial nodal cells in the heart,[71] interstitial cells of Cajal in the intestine,[72] and lymphatic smooth muscle cells.[73]

The nonselective cation channel TRPV4 may also allow Ca^{2+} to enter myometrial cells. Whereas LTCCs and TTCCs are activated by the less negative membrane potential at the end of pregnancy, TRPV4 is activated by stretch or heat.[74] TRPV4 expression and activity increases throughout gestation in rats, and TRPV4 expression is higher in myometrium from pregnant women than in myometrium from non-pregnant women.[75,76] Silencing TRPV4 expression in human myometrial cells prevents them from contracting in a collagen gel matrix.[75] Similarly, a TRPV4 antagonist attenuates contractions in both rat and mouse myometrium.[75–77] Furthermore, treatment with TRPV4 antagonists delays parturition in two experimental models of preterm labour in mice.[75] These lines of evidence suggest that TRPV4 enables myometrial contractions in pregnant humans, mice and rats.

2.2.4 | Potassium channels

Potassium channels underlie the repolarizing current to bring the myometrial cell back to basal states. The number of potassium channels that contribute to myometrial excitability is vast, as reviewed previously.[78,79] Here we will highlight only a few K^+ channels know to contribute to uterine activity.

The repolarization phase of the myometrial action potential is driven by the action of voltage- and Ca^{2+}-gated potassium (K^+) channels. The voltage-gated, rapidly-inactivating K^+ currents initially identified by Mirroneau[51,52] are predominantly carried by the Kv4 family of K^+ channels, which are abundant in both human and rat myometrium.[79] In ovariectomized rats and isolated human cells, oestrogen decreases Kv4 expression and function; consistent with this, Kv4 expression decreases throughout gestation in rats.[80,81]

The Ca^{2+}-activated large conductance K^+ channel (BK_{Ca}) also contributes to this repolarizing current and has been extensively studied in the myometrium. In both rats and humans, activation of BK_{Ca} causes myometrial relaxation, whereas inhibition of BK_{Ca} causes myometrial contractions.[78] In guinea pigs and humans, chorionic factors (i.e. human chorionic gonadotropin) that promote myometrial relaxation do so by activating $BK_{Ca.}$[82,83] However, BK_{Ca} knockout mice do not deliver preterm, perhaps indicating that other K^+ channels compensate for loss of BK_{Ca}.[84] Nonetheless, recent evidence suggests that BK_{Ca} increases myometrial contractions under certain inflammatory conditions. Specifically, Wakle-Prabagaran *et al* found that the pro-inflammatory cytokine alpha-2-macroglobulin interacted with BK_{Ca} to potentiate Ca^{2+} oscillations in oxytocin-treated human myometrial cells.[85]

BK_{Ca} activity can be regulated by alternative splicing, interaction with auxiliary subunits, post-translational modifications, hormonal modulation and several other mechanisms.[78] Here, we will focus on alternative splicing, a mechanism that appears to be species-specific. In all species in which BK_{Ca}

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isoforms have been studied (rats, mice and humans), splice forms expressed at term are less sensitive to Ca^{2+} than those expressed earlier in pregnancy. The splice forms expressed at term would allow intracellular Ca^{2+} levels to rise without increasing K^+ efflux and repolarizing the membrane. For example, in human myometrium, alternative splicing produces the mK44 isoform, which has 44 extra amino acids and is less sensitive to Ca^{2+} than the canonical form.[86] The 44 amino acid insertion also causes BK_{Ca} to undergo proteolytic cleavage so that only the N-terminus reaches the cell membrane.[87] Production of mK44 increases at labour, while overall BK_{Ca} production decreases,[88] thus reducing the overall repolarizing current. In rats and mice, alternative splicing produces a different BK_{Ca} isoform containing the stress axis regulated exon (STREX). The 59 amino acids in STREX make this isoform more sensitive to Ca^{2+} than canonical BK_{Ca}, thus promoting myometrial repolarization.[89] However, STREX regulation is slightly different in mice and rats. In mouse myometrium, STREX is expressed throughout pregnancy, whereas BK_{Ca} without the STREX insertion is upregulated at term.[90] In rat myometrium, STREX expression is reduced by oestrogen and increased by progesterone; accordingly, its expression diminishes at the end of gestation.[91]

Another family of K^+ channels, SK channels, may be important for hyperpolarization after an action potential and therefore also promote myometrial relaxation.[92] Pierce *et al* showed that mice overexpressing SK3 channels had weaker uterine contractions.[93] Conversely, inhibiting SK channels with the selective inhibitor apamin increased spontaneous contractions in myometrial tissue samples from pregnant rats[94] and prevented relaxation in myometrial tissue samples from pregnant and non-pregnant women.[95,96] Moreover SK3 expression is downregulated at term in both human and mouse myometrium,[93,97] consistent with a conserved role for SK3 in promoting myometrial quiescence.

Functional studies suggest that ATP-sensitive inward rectifying K^+ channels (K_{ATP}) also play a role in promoting myometrial quiescence. In general, activation and overexpression of K_{ATP} channels leads to myometrial relaxation in both rat and human tissue.[79] Consistent with this, in humans, K_{ATP} channel expression decreases at parturition, and K_{ATP} function further decreases during active labour.[98,99] However, in rats, K_{ATP} channel activity is similar between mid-pregnancy and parturition.[100] Additionally, K_{ATP} channel openers could inhibit agonist-induced contractions in human myometrial tissue,[99] but not in rat myometrial tissue.[101] Thus, K_{ATP} channels may play different roles in the rat and human myometrium, although further studies directly comparing the two species are necessary to confirm this conclusion.

Finally, the inward rectifying K^+ channel Kir7.1 may play a role in regulating the resting membrane potential in the myometrium. Activation of Kir7.1 hyperpolarizes the cell membrane, thereby decreasing myometrial excitability.[102] Consistent with this role, Kir7.1 expression is highest during mid-pregnancy in mice and declines towards term.[102] In humans, Kir7.1 expression decreases as the uterus transitions from the non-labouring to the labouring state.[102] Pharmacological inhibition of Kir7.1 induces contractions in both mouse and human myometrial tissue strips.[102] Taken together, these results suggest that Kir7.1 promotes myometrial quiescence in both mice and humans.

The examples highlighted above show that while ion channels often have similar roles in several species, more extensive study almost always reveals species-specific differences in channel regulation. Researchers must keep this possibility in mind when designing ion channel studies in rodent models.

2.3 | Timing and propagation of action potentials

2.3.1 | The uterine pacemaker

When Alvarez and Caldeyro measured intrauterine pressure and uterine electrical activity for the first time in the 1950s,[103] they proposed that labour contractions originated from a single "pacemaker" region.[104] However, with more advanced electromyography techniques, later investigators observed that multiple possible pacemaker regions could be active during a contraction.[105] Further work with newer technologies have confirmed this idea of multiple pacemakers.[106] In particular, Eswaran *et al* and Ramon *et al* used a 151-channel magnetomyographic array and showed that electrical waves appeared "suddenly in random locations that were not contiguous or anatomically related to each other".[107,108] More recently, Wu *et al* developed electromyometrial imaging, a non-invasive technology that combines electrical data with magnetic resonance imaging to map electrical activity onto the entire three-dimensional uterus.[109] Data collected by using this technique support the existence of multiple pacemaker sites in sheep, although this technology has not yet been reported in humans.

These studies in humans and large animals at parturition have been complemented by ex vivo multi-electrode array (MEA) studies on rodent myometrium.[110] Several studies from Lammers and collaborators, who pioneered this technique in the myometrium, suggested that the coordinated uterine contractions at parturition result from action potentials propagating from many possible initiating pacemaker regions.[110–112] In guinea pigs, contractions can originate at the cervical or the ovarian end of the uterine horn and propagate in either direction.[112,113] In contrast, in rats, most electrical activities start at the ovarian end of the uterine horn and proceed toward the cervical end in a "peristaltic" contraction pattern.[112] These differences may be due to the fact that rats can carry up to 10 pups, whereas guinea pigs carry

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fewer offspring. Investigators recently proposed that multiple pacemakers in rat uterus originate from structures located in each implantation site called "myometrial-placental pacemaker zones".[114] These zones have not (yet) been identified in guinea pigs or in mice, and it is unclear whether these findings translate to humans.

Most MEA studies show that, in all animals tested (humans, sheep, guinea pigs and rats), action potentials do not propagate in one direction; instead, they tend to travel in tortuous paths before terminating. Thus, at least in large animals such as humans, an action potential originating at one point could not travel throughout the whole uterus fast enough to coordinate a contraction. Given these MEA studies and magnetomyography data showing that multiple pacemaker regions contribute to each contraction in humans, Young proposed a dual model for contractions.[115] In this model, a burst of action potentials in one pacemaker region propagates to produce a "local" contraction, which causes an increase in wall stress that activates another mechanically sensitive pacemaker region. This process theoretically continues until enough pacemaker regions are recruited to produce a coordinated contraction. However, more research is necessary to support this theory.

Although researchers may never identify a definitive uterine pacemaker region, the search is still on for a pacemaker cell type. In the gut, slow electrical waves initiate in pacemaker cells called interstitial cells of Cajal (ICCs) and then propagate to the rest of the gut myocytes. Several groups have sought to find an "ICC-like cell" in the uterus, searching for interstitial cells that produce similar slow waves or express similar membrane markers. So-called ICC-like cells have been found in the uterus of humans, rats, mice, and monkeys,[116–120] but thus far, no group has definitively shown that these cells are capable of producing slow waves in isolation. Furthermore, myometrial cells may not exhibit the slow waves seen in the gut.[113] Thus, further research is needed to determine whether these ICC-like cells act as pacemakers in any species.

2.3.2 | Gap junctions

Regardless of where action potentials originate, their coordination and propagation depend on uterine gap junctions. Gap junctions consist of connexin 43 proteins that form connexons, or pores, between neighbouring myometrial cells.[121] These low-resistance pathways allow electrical signals to travel through the uterus.[122] Early studies by Garfield's group in rat, guinea pig and human myometrium showed that gap junctions are scarce or absent in non-pregnant and mid-pregnant myometrium but become abundant at term.[123–125] Induction of preterm labour with RU486 or prostaglandin E2 induces gap junction formation in rats and guinea pigs, respectively.[122,125] In participants undergoing C-section at various points during labour, gap junction abundance correlated with cervical dilation and the frequency of uterine contractions.[124] Finally, conditional knockout of connexin 43 delays parturition in mice.[126] Together, these lines of evidence indicate that gap junctions play a conserved role in synchronizing uterine contractions at parturition.

2.4 | Pro-contractile agents

2.4.1 | Oxytocin and the oxytocin receptor

With many uses in both clinical practice and basic science research, oxytocin is perhaps the best-known modulator of uterine contractility. Oxytocin is a nonapeptide hormone produced and secreted in a pulsatile fashion by the hypothalamus, as well as by the placenta, decidua and myometrium during labour.[127] In the United States, clinicians prescribe exogenous oxytocin to about half of patients to induce or augment labour and recommend that all patients receive oxytocin to prevent postpartum haemorrhage.[128,129] Researchers use oxytocin to induce contractions and simulate a pro-labour environment in ex vivo experiments on uterine tissue and cells isolated from humans and animal models. In humans, rats, and mice, oxytocin binding sites in the uterus increase by 6- to 12-fold throughout pregnancy and reaches a peak at or just before labour.[130–133] However, in guinea pigs, oxytocin binding capacity does not change significantly between mid-pregnancy and late gestation (40 and 69 days of gestation, respectively).[134]

As mentioned above and reviewed in detail elsewhere,[127] OXTR works classically by coupling to Gq to induce the release of intracellular Ca^{2+} stores and allow for muscle contraction (Figure 2). OXTR may also promote an increase in intracellular Ca^{2+} concentration by increasing SOCE and preventing Ca^{2+} efflux through the Ca^{2+} ATPase.[127] Additionally, OXTR inhibits the K^+ efflux channel Slo2.1, thereby depolarizing the cell membrane toward the threshold required to induce an action potential.[135] In the longer term, oxytocin can work in the myometrium and other tissues to increase NFκB-dependent transcription and increase production of pro-inflammatory cytokines, cyclooxygenase 2, and prostaglandins.[136–138] In fact, some researchers have hypothesized that the most important function of oxytocin is to increase production of prostaglandins, which are also potent inducers of uterine contractions.[139]

Pharmacological inhibition of oxytocin impairs parturition to varying degrees in mice,[140] rats,[141] and guinea pigs,[142] but clinical trials have shown that the oxytocin antagonists atosiban and barusiban may not be effective tocolytics in humans.[143] Mice that lack oxytocin or OXTR deliver normally,[144–146] showing definitively that mice can compensate

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for lack of oxytocin signalling when initiating labour. The *OXTR* gene has not been deleted in guinea pigs or rats, so it is unclear whether this is true in other rodents. In humans, however, loss-of-function *OXTR* mutations have been linked to preterm labour rather than to delayed or absent contractions.[147] These lines of evidence have led researchers to conclude that signalling via OXTR is sufficient, but not necessary, to initiate uterine contractions in all species studied.

2.4.2 | Prostaglandins and prostaglandin receptors

Like oxytocin, synthetic prostaglandins are used clinically to induce and augment labour and to prevent postpartum haemorrhage. In vivo, prostaglandins are produced from arachidonic acid by the enzyme cyclooxygenase 2 (COX2). COX2 is upregulated in the uterus as pregnancy progresses towards labour, in part due to increased oxytocin signalling, causing an increase in local prostaglandin concentration.[136,148,149] The synthetic prostaglandins misoprostol (prostaglandin E1), dinoprostone (prostaglandin E2) and carboprost (prostaglandin F2) act on several GPCRs: the prostaglandin F2 receptor (FP) and four prostaglandin E receptors (EP1-4). The pro-contractile prostaglandin receptors—FP, EP1 and EP3 – work similarly to OXTR. FP and EP1 couple to Gq, whereas EP3 couples to Gi (Figure 2).[150] The relaxant receptors EP2 and EP4 couple to Gs to produce cyclic AMP and activate protein kinase A (Figure 2).[150] During parturition in human and rat myometrium, expression of the relaxant receptors EP2 and E4 is downregulated, while expression of the pro-contractile prostaglandin receptors FP, EP1 and EP3 is upregulated.[151,152] Therefore, increased expression of prostaglandin receptors and induction of prostaglandin production by COX2 at parturition appear to stimulate uterine contraction in the rat and human uterus. However, in the guinea pig, EP2, EP3 and EP4 mRNA expression does not change at parturition.[153]

Evidence from knockout mice highlights the necessity of prostaglandins for labour. Sugimoto *et al* found that mice that lacked the prostaglandin F receptor had normal fertility, but never delivered their pups.[154] However, when these knockout mice were ovariectomized at 19 days of pregnancy, the mice delivered normally. These results suggest that in mice, prostaglandin F is necessary for luteolysis and progesterone withdrawal but not for causing uterine contractions.

Clinicians and researchers hypothesized that COX2 inhibitors that prevent prostaglandin production would be effective tocolytics (see "Tocolytics" section below). Treatment with COX2 inhibitors delayed parturition in rats[155,156] and prevented lipopolysaccharide-induced preterm labour in mice.[157] Of note, both rats and mice require luteolysis and progesterone withdrawal for parturition, unlike humans and guinea pigs.[6] The COX2 inhibitor indomethacin has been shown to inhibit uterine contractions in explanted tissue strips from mice, rats, guinea pigs, and humans. 164,172-174 . Indomethacin is used clinically as a tocolytic, although results from clinical trials have been mixed.[158] Thus, although prostaglandins are clearly essential for parturition in rats and mice, prostaglandin signalling may be dispensable for labour induction in humans. However, more systematic study is necessary to support this conclusion.

2.5 | Tocolytics

The agents most commonly used by obstetricians to inhibit contractions in preterm labour take advantage of the molecular mechanisms of uterine contractility described above. Their broad classes include Ca^{2+} channel blockers, which target LTCCs; COX2 inhibitors, which target prostaglandins; β2 adrenergic receptor agonists, which increase cAMP to cause Ca^{2+} desensitization; and the oxytocin receptor antagonists atosiban and barusiban. As shown in Table 2, all of these tocolytics were tested for efficacy in mice, rats and/or guinea pigs before being approved for human use. However, tocolytics that effectively prevent parturition in rodent models have varying success in actually preventing preterm labour in humans.[159] Although these failures could reflect physiological differences between animal models and humans, it is important to note that no experimental model can capture the range of environmental and genetic factors that contribute to human preterm birth. Thus, it is impossible to compare the results of a randomized controlled trial to an animal study.

Nevertheless, experiments comparing the effects of tocolytic drugs on explanted uterine tissue may shed some light on how tocolytics affect uterine contractions in different species. In Table 3, we compiled IC_{50} values of several tocolytics in ex vivo uterine tension recording experiments. These results are easiest to interpret when researchers use the same experimental conditions to assay contractions in multiple species, as in one study by Kantas.[165] Comparing results from separate studies is more difficult, especially when considering systematic differences between experimental setups. For example, researchers may use nonpregnant or pregnant animals and may measure spontaneous or induced contractions, as indicated in Table 3. Other methodological differences—such as different tension recording setups, dissection methods, or salt concentrations in buffer solutions—are even more difficult to account for.

3 | CONCLUSIONS AND OPPORTUNITIES FOR FURTHER RESEARCH

As mentioned in the introduction of this review, each animal model has experimental benefits and limitations that make it difficult to determine whether certain features of pregnancy

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TABLE 2 Common tocolytics

Class	Calcium channel blockers	COX inhibitors	β2AR agonists	OXTR antagonists
Preferred agent	Nifedipine	Indomethacin	Terbutaline	Atosiban
Other drugs in class	Nicardipine Diltiazem (not used)	Ibuprofen Sulindac Nimesulide COX-2 specific inhibitors (-coxibs)	Salbutamol Hexoprenaline Ritodrine	Barusiban
Mechanism of action	Block calcium entry from voltage-gated calcium channels	Block prostaglandin synthesis	Activate Gs signalling to inhibit MLCK	Inhibits oxytocin signalling
Clinical use[160,161]	Nifedipine is first-line therapy (32-34 weeks); second-line therapy (24-32 weeks)	Indomethacin is first-line therapy (24-32 weeks)	Terbutaline is second-line therapy (32-34 weeks)	Not available in the United States
Species considerations	Efficacy originally demonstrated in rat.[162,163] Nifedipine also inhibits oxytocin-induced contractions in explanted uterus from mice.[92]	Indomethacin initially tested in rats,[156] successfully inhibits LPS-induced preterm birth in mice.[157] Indomethacin inhibits spontaneous contractions in explanted guinea pig uterus.[164]	Direct comparison of effect of ritodrine on isolated myometrial strips from rat and human myometrium showed identical results.[165]	Efficacy originally demonstrated in rat and guinea pig.[166,167] Efficacy in humans in is contested.[143]

and parturition are conserved between species. Knockout studies and randomized controlled trials are considered the gold standards of evidence in animal and human research, but there is no human equivalent of a knock-out mouse and no rodent equivalent of a randomized controlled trial. Thus, results that appear divergent may reflect unavoidable experimental differences rather than physiological differences.

Here, we attempted to draw conclusions about species-level differences by comparing results from different studies, often conducted by different groups, that use similar experimental approaches (Table 1). However, even this imperfect strategy reveals significant gaps in the literature. In particular, relatively few molecular studies have been performed in guinea pig myometrium to characterize ion channels and receptors that have been extensively studied in other animals. Because both humans and guinea pigs maintain high progesterone levels even in late pregnancy,[6] molecular studies may provide insight into hormonal regulation of contractile-associated proteins that may be more translatable to human physiology than similar studies in the mouse or rat.

Our review of the literature for this article revealed a lack of true comparative studies, in which researchers use identical techniques to analyse uterine tissue or cells from multiple species. One rare example was performed by Kantas et al, who used tension recording to compare ex vivo contractility between rat and human myometrial strips.[165] Other techniques such as patch clamping, MEA, and gene and protein expression analyses are equally suited for species comparison.

Furthermore, the advent of unbiased, high-information approaches such as RNA sequencing now allows researchers to conduct systematic studies of pregnancy and uterine contractions across species. These studies would identify species differences in the expression of important pro-contractile and pro-quiescent genes throughout pregnancy, which would help to confirm the fragmented conclusions drawn from disparate studies in Table 1. Similarly, proteomic and metabolomic studies may provide insight into how hormones and inflammatory markers vary between species. These systematic studies would help investigators determine the most appropriate animal model for their questions, potentially improving the translatability of their study. In the absence of such a systematic comparison, researchers must rely on the existing literature to determine the most appropriate animal model for their study that takes into account the study question and needs for experimental tractability and translatability.

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CONFLICT OF INTEREST

The original draft for this review was adapted from a white paper commissioned by Corteva Agriscience on species

TABLE 3 IC50 values for tocolytic drugs

	Mouse	Rat	Guinea pig	Human
Nifedipine (calcium channel blocker)	«1 × 10^{-6} mol/L[a] (nonpregnant, spontaneous contractions)[92]	~1 × 10^{-7} mol/L (gestation day 22, contractions induced by 25 mmol/L KCl)[168]	~1 × 10^{-6} mol/L (gestation day 62-66, PGF-induced contractions)[169]	5.5 × 10^{-8} mol/L (gestation weeks 38-40, spontaneous contractions)[170] ~1 × 10^{-7} mol/L (gestation weeks 36-40, spontaneous contractions)[171]
Indomethacin (COX inhibitor)	3 × 10^{-5} mol/L (gestation day 19, spontaneous contractions)[172]	1.8 × 10^{-5} mol/L (gestation day 18, spontaneous contractions)[173]	«1 × 10^{-5} mol/L (gestation day 7-20, spontaneous contractions)[164]	~1 × 10^{-4} mol/L (term pregnancy, spontaneous contractions)[174]
Ritodrine (β2AR agonist)	«1 × 10^{-10} mol/L[a] (nonpregnant, spontaneous contractions)[175]	~1 × 10^{-6} mol/L (gestation day 19-21, spontaneous contractions)[165] 3.1 × 10^{-7} mol/L (gestation day 18, spontaneous contractions)[173]	Not available	~1 × 10^{-6} mol/L (term pregnancy, spontaneous contractions)[165] ~1 × 10^{-6} mol/L (gestation weeks 38-40, oxytocin-induced contractions)[176]
Atosiban (OXTR antagonist)	3 × 10^{-7} mol/L (gestation day 19, spontaneous contractions)[172]	1 × 10^{-7} mol/L (gestation day 18, spontaneous contractions)[173]	~4 × 10^{-7} mol/L (gestation day 60-65, oxytocin-induced contractions)[177]	~6 × 10^{-8} mol/L (gestation weeks 38-40, oxytocin-induced contractions)[176]

[a]Indicates dose used to achieve maximal inhibition (no dose-response curve shown in study).

differences in uterine contractions. This draft was rewritten and expanded to be more useful to a general audience.

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