

## **Barusiban, a selective oxytocin receptor antagonist: placental transfer in rabbit, monkey, and human†**

Authors: Helmer, Hanns, Saleh, Leila, Petricevic, Ljubomir, Knöfler, Martin, and Reinheimer, Torsten M.

Source: *Biology of Reproduction*, 103(1) : 135-143

Published By: Society for the Study of Reproduction

URL: <https://doi.org/10.1093/biolre/ioaa048>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](http://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Research Article

# Barusiban, a selective oxytocin receptor antagonist: placental transfer in rabbit, monkey, and human<sup>†</sup>

Hanns Helmer<sup>1</sup>, Leila Saleh<sup>2</sup>, Ljubomir Petricevic<sup>1</sup>, Martin Knöfler<sup>2</sup> and Torsten M. Reinheimer<sup>3,\*</sup>

<sup>1</sup>Division of Obstetrics and Feto-Maternal Medicine, Department of Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria, <sup>2</sup>Reproductive Biology Unit, Department of Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria and <sup>3</sup>Ferring Pharmaceuticals A/S, Non-Clinical Development, Copenhagen, Denmark

\***Correspondence:** Ferring Pharmaceuticals A/S, Non-Clinical Development, Kay Fiskers Plads 11, 2300 Copenhagen, Denmark. E-mail: trr@ferring.com

<sup>†</sup>**Grant Support:** This study was funded by Ferring Pharmaceuticals A/S.

Received 13 December 2019; Revised 3 April 2020; Accepted 15 April 2020

## Abstract

The use of drugs in pregnancy always raises concerns regarding potential fetal exposure and possible adverse effects through their accumulation in fetal tissues and organs. Barusiban is an oxytocin antagonist under development for potential use as tocolytic in preterm-labor patients. It displays greater affinity for the oxytocin receptor compared to vasopressin V<sub>1A</sub> receptor and would thus not interfere with vasopressin-induced effects of the V<sub>1A</sub> receptor. Barusiban placental transfer was determined in the rabbit and cynomolgus monkey and in an ex vivo human cotyledon model. In the rabbit, there was an approximately 5% transfer of barusiban from the maternal to the fetal blood, without significant accumulation in any of the investigated fetal tissues. In the cynomolgus monkeys, the mean fetal plasma barusiban concentration was 9.1% of the maternal level. This was similar to the percentage of barusiban transfer in the human placental single cotyledon, which once equilibrated ranged between 9.3 and 11.0% over the observation period. The transfer of the small-molecule antipyrene as a comparator in this human model was approximately three times greater. The similarity in the degree of transfer in the cynomolgus monkey and human cotyledon, while being less in the rabbit, may reflect the species-specific placental barrier structure between the maternal and fetal compartments. In conclusion, limited placental transfer of barusiban occurred in all three models. The similarity of barusiban transfer in the cynomolgus and the human placental single cotyledon suggests the latter ex vivo model to be useful in assessing future drug candidates to be used in pregnant women.

## Summary Sentence

Limited barusiban placental transfer occurred in all three models, with the similarity of transfer in the cynomolgus and the human cotyledon, suggesting this ex vivo model to be useful in assessing human placental transfer of pharmaceuticals.

**Key words:** barusiban, human, labor, placental transport, primates, rabbit.

## Introduction

There are always potential concerns and regulatory expectations regarding maternal medications and potential fetal exposure during pregnancy. Placental drug transfer studies can help to identify possible hazard and thus the safety of such compounds, because resultant fetal circulatory levels may lead to exposure with or without accumulation in tissues and organs, possibly producing adverse effects.

Premature labor leads to early childbirth, associated with immediate and long-term health problems [1]. Therefore, tocolytic drugs are given for imminent preterm to prevent premature labor. This allows, for example, sufficient time for transfer to a neonatal intensive care unit and accelerated fetal lung maturation by antenatal hydrocortisone treatment, reducing the risk of related medical problems [2]. Molecular size plays a role in placental transfer [3]. Generally, tocolytic peptides display low placental transfer and tend to be less toxic than small molecules, some of which can readily cross the placenta and may have toxic metabolism issues, possibly affecting fetal health. Indeed, up to 100% transfer has been observed for MgSO<sub>4</sub> in humans [4, 5], greater than 60% for dihydropyridine nifedipine [6, 7], up to 90% for fenoterol [8], and 26% or 70% for ritodrine hydrochloride used in short- or long-term treatment, respectively [9, 10], whereas only 12% was reported for the peptide atosiban [11]. Several papers have described MgSO<sub>4</sub>-related reduced fetal heart rate (HR), HR variation (HRV), and bradycardia as well as fetal respiratory depression [12]. Nifedipine was shown to significantly reduce fetal HR while increasing HRV [13]. Ritodrine was reported to increase the fetal HR while lowering the long-term HRV, accompanied by increased left cardiac stroke volume, cardiac output, and pulsatility index of the middle cerebral artery [14, 15]. Although dihydropyridine nifedipine was reported to have a lower transfer of 17% [16], it was shown in rhesus monkey fetus to induce acidosis and hypoxemia [17]. Therefore, low transfer is important to reduce the risk of potential fetal adverse effects of tocolytics.

Barusiban (FE 200440) is a peptide oxytocin antagonist for potential future use as a tocolytic drug in preterm-labor patients, being slightly smaller than atosiban. It displays an approximately 300 times higher affinity for the cloned human oxytocin receptor (OTR) than the cloned human vasopressin V<sub>1A</sub> receptor (V<sub>1AR</sub>) when expressed in human embryonic kidney (HEK)-293 cell membranes [18]. Similarly, barusiban has a greater potency for the OTR when expressed in Chinese hamster ovary cells than the V<sub>1AR</sub> expressed in HEK-293 cells (K<sub>i</sub> 0.64 nM vs. 11 nM) (unpublished data). By contrast, atosiban displays a higher affinity for the cloned human V<sub>1AR</sub> than the OTR (K<sub>i</sub> 3.5/4.7 nM vs. 81/397 nM) [19, 20] and greater potency (K<sub>i</sub> 0.27 nM vs. 20 nM) (unpublished data). Barusiban binds to transmembrane regions 1 and 2 of the OTR rather than the agonist-binding extracellular N-terminus, while atosiban does not appear to bind to either of these sites [21]. Whereas the extracellular domain of the oxytocin and vasopressin receptors shares approximately 80% amino acid homology [22], this is only 30–50% over the entire receptor molecule [23]. The amino acid sequence difference between the receptors in the barusiban-binding region may thus contribute to barusiban's greater binding affinity for the OTR. Consequently, barusiban had practically no effect on vasopressin-induced contractions of isolated term-pregnant human myometrium [18], whereas it dose-dependently inhibited oxytocin-induced contractions of isolated human preterm and term myometrial strips with a greater tendency than atosiban [24]. In OTR-knockout mice, the dams exhibited normal parturition but displayed

an inability to lactate, such that the pups were unable to survive because of a lack of suckling [25]. Because barusiban is planned for use during pregnancy, this raises the question of the potential for placental transfer and possible fetal risks.

The rabbit is a standard model to test reproductive toxicology [26] and has been used previously in the toxicological evaluation of barusiban (unpublished data), is qualified as a relevant non-clinical species, and is accepted by the regulatory authorities for this study type. The rabbit placental barrier at term has two trophoblastic cell layers, syncytiotrophoblasts in direct contact with maternal blood and cytotrophoblasts overlying fetal blood vessels [27], compared to the single trophoblastic layer (syncytiotrophoblasts) of the human placenta at term [28]. The rabbit OTR shares 88% amino acid sequence identity with the human receptor, increasing to 92%, when also including highly similar amino acids (unpublished data). A further non-clinical model, in which barusiban is pharmacologically active, is the cynomolgus monkey, which has similarities to humans in reproductive physiology [29] and embryo/fetal development [30]. Like humans, the cynomolgus monkey is hemomonochorial, with a single syncytiotrophoblast layer separating maternal blood from fetal blood vessels [27, 28]. Moreover, its OTR amino acid sequence shares 97% identity with humans, increasing to 99%, when including highly similar amino acids (unpublished data). The investigation of barusiban's effect in the pregnant cynomolgus monkey found it to be more potent and its action longer lasting than atosiban in reducing oxytocin-induced uterine contractions, the effects being readily reversed by a high-dose oxytocin infusion [31]. This confirmed barusiban's apparent greater potency compared to atosiban observed previously using isolated human preterm and term myometrial strips [24]. Furthermore, barusiban was more effective than fenoterol in the cynomolgus monkey in reducing induced uterine contractions [32]. However, a Phase II clinical trial did not demonstrate a statistically significant effect of barusiban over the placebo group, although there was a tendency in all the barusiban treatment groups toward a higher proportion of women who did not deliver within 48 h with increasing predosing cervical length. [33]. Furthermore, with the high number (72%) in the placebo group who did not deliver within 48 h, this would have reduced the possibility of detecting a significant difference between the barusiban groups and the placebo group. A dually perfused model of a human placental single cotyledon has been used in transplacental studies since 1972 [34]. This model has been shown with numerous compounds to accurately predict *in vivo* transfer at steady state after modeling for differences in maternal and fetal/neonatal protein binding and blood pH [35].

Here, for pharmacokinetic safety reasons, we investigated barusiban placental transfer *in vivo* in the rabbit and cynomolgus monkey and in an *ex vivo* human perfusion model. The results will help to characterize barusiban's placental transfer kinetics in clinical use.

## Materials and methods

### Placental transfer in rabbits

This study was conducted in compliance with the good laboratory practice (GLP) regulations 1999 (SI 1999 No. 3106) for the United Kingdom and with the OECD GLP Principles (ENV/MC/CHEM (98) 17), which are accepted by the USFDA. Twelve healthy pregnant female New Zealand white rabbits aged 5–6 months and weighing 3.0–4.4 kg were obtained from Harlan UK (Bicester, UK). The rabbits were housed individually in stainless steel rabbit cages under a 12-h light/dark cycle and acclimatized for 4 days. The temperature and

relative humidity of the room housing the animals was 19–22 °C and 25.5–92%, respectively. Stanrab diet (Special Diets Services, Witham, UK) and mains tap water were provided ad libitum.

Barusiban supplied by Ferring AB (Limhamn, Sweden) (see [Methods Supplement](#) for dose preparation) was administered intravenously as a single bolus injection via an ear vein once daily for 7 days (days 24–30 of gestation). The mean daily dose was  $3.01 \pm 0.013$  mg/kg at 1 mL/kg in 0.9% saline, with a mean radioactive dose of  $522 \pm 1$  kBq/kg ( $14.1 \pm 0.021$   $\mu$ Ci/kg).

Blood was obtained via an ear artery immediately prior to sacrifice. Three maternal animals were sacrificed by barbiturate overdose at 10 min and 1, 3, and 10 h post-dose, and blood transferred to a heparinized tube. Necropsy was undertaken for each dam and one fetus of each sex. Fetal blood was obtained by cardiac puncture and various tissues were collected. Radioactivity in plasma and tissue samples was determined by liquid scintillation counting. (See [Methods Supplement](#) for preparation of blood, plasma, and tissue samples and determination of barusiban content.)

### Placental transfer in cynomolgus monkeys

This study was in compliance with the GLP regulations as outlined in the German Chemical Law, the OECD Principles, and the U.S. FDA for Nonclinical Laboratory Studies and was approved by the Münster District Council, planned experiment No. 14/87. Three female purpose-bred and sexually mature cynomolgus monkeys (*Macaca fascicularis*), were obtained from Best Engineering Company Ltd. and Vanny Chain Technology Ltd. (both Hong Kong). On gestation day 140, the animals weighed 3.9–4.2 kg and were at least 3 years old. On arrival, all animals received a clinical inspection and were subsequently regularly examined. The animals were acclimatized for a minimum of 6 weeks, being housed under a 12-h light/dark cycle in a climate-controlled room, providing a minimum of 10 air changes/h, with temperature and relative humidity routinely of 19–25 °C and 30–70%, respectively. The animals were individually housed in stainless steel cages (600 × 600 × 800 mm; E. Becker & Co GmbH, Castrop-Rauxel, Germany). Tools for environmental enrichment were provided in each cage. Each animal received twice daily 50 g of a commercial pellet diet for primates (Ssniff P10, Ssniff Spezialdiäten GmbH, Soest, Germany), fresh fruit normally twice weekly, and one slice of bread once weekly. Tap water was provided ad libitum.

Prior to caesarean section (C-section) on day  $150 \pm 1$  of gestation, barusiban (Ferring AB) at 10 mg/mL in isotonic 0.04 M acetate buffer pH 4.3 diluted using 0.9% saline was infused intravenously into the saphenous vein using an infusion pump for 2 h to a maximal dose of 300  $\mu$ g/kg (based on previous pharmacodynamic experiments [31]) in 5 mL/kg. Maternal blood samples were obtained from the brachial vein (approximately 1.5 mL) prior to initiation of dosing (as control) and 1 and 2 h thereafter.

For C-section, the animals were intramuscularly anesthetized using ketamine hydrochloride (Ketavet; Pharmacia & Upjohn GmbH, Erlangen, Germany) and xylazine hydrochloride (Rompun; Bayer Vital GmbH & Co. KG, Leverkusen-Bayerwerk, Germany). C-section was performed, and amniotic fluid (10 mL) and fetal blood (approximately 1.5 mL) from the umbilical vein were collected. Fetuses were subsequently sacrificed by an intravenous injection of ketamine hydrochloride via the umbilical vein. The fetuses were sexed and examined for external abnormalities. Following completion of the study, there were no signs of major illness in the

adults, and all healthy females were returned to the primate colony following CRO procedures.

Plasma samples were analyzed for barusiban by radioimmunoassay and amniotic fluid samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (see [Methods Supplement](#) for blood and amniotic fluid preparation and analyses).

The ratios of fetal plasma barusiban at C-section/maternal plasma after 2 h and amniotic fluid barusiban concentrations at C-section/maternal plasma after 2 h of infusion were calculated. Assuming that the maternal plasma barusiban concentrations after 2 h and the fetal plasma and amniotic barusiban concentrations at C-section were from normally distributed populations, parametric statistical analyses were performed, with a paired *t*-test for the barusiban concentrations using GraphPad Prism (Version 6, La Jolla, CA).

### Human cotyledon transfer

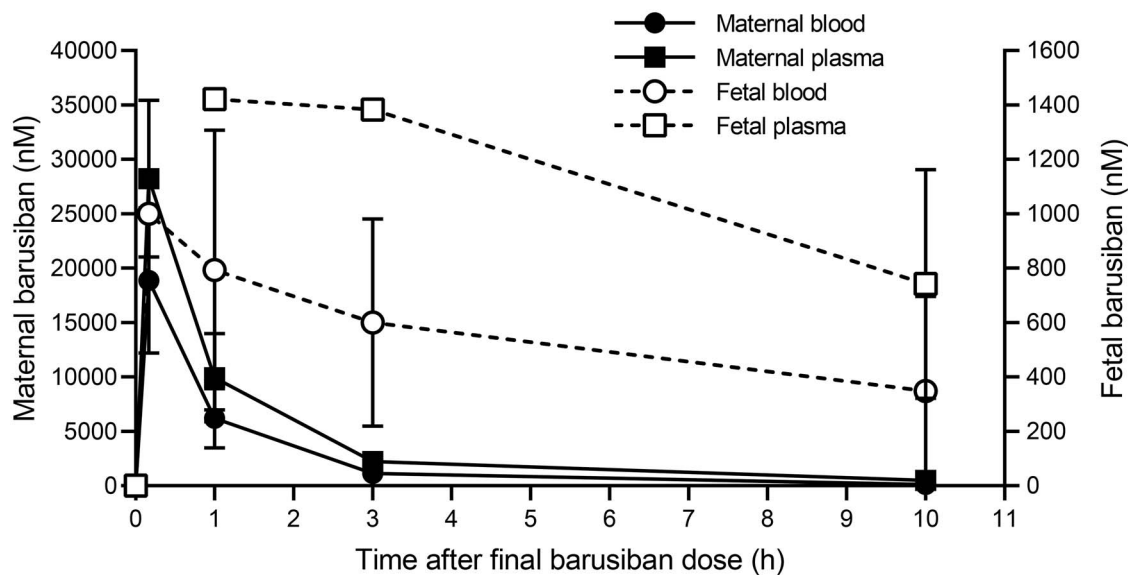
This study was approved by the Ethics Commission of the Medical Faculty of the University of Vienna and the General Hospital of Vienna AKH, Austria (approval no. EK Nr: 248/2003). Seventeen placentas with no signs of chorioamnionitis were obtained with written consent from women with uncomplicated obstetric histories after pregnancies of gestational weeks 39–41 and birth weights of 3–4 kg (see [Methods Supplement](#) for cotyledon preparation).

Ex vivo perfusions were performed according to published methods [34, 36], using a placental double-sided open cotyledon perfusion model (see [Methods Supplement](#) for preparation). Following a stabilization period, the fetal circulation flow rate was continued at 2 mL/min, and the maternal circulation was re-established at 10 mL/min, maintaining the perfusate at 36.5 °C using a water bath. Barusiban (Ferring Pharmaceuticals A/S, Copenhagen, Denmark; in isotonic 0.04 M acetate buffer pH 4.3) was applied at 1.2  $\mu$ M (1  $\mu$ g/mL) in the perfusate (corresponding to the maximum serum concentrations in clinical trials on intravenous administration of a 10-mg bolus [33]). Antipyrine (Sigma) as an inert reference compound was co-infused at 531  $\mu$ M (0.1 mg/mL) [37, 38]. Only experiments in which the fetal arterial inflow equaled the venous outflow were analyzed. Perfusate samples were obtained from the fetal venous cannula after 1, 5, 10, 30, 60, 90, and 120 min and stored at –20 °C. Following each experiment, membrane integrity was tested by adding 5 mL Evans blue dye (0.1 mg/mL NaCl; Sigma-Aldrich Co.), which cannot cross the placental barrier, to the fetal circuit. Barusiban was analyzed by automated protein precipitation followed by liquid chromatography and tandem mass spectrometry. Spectrophotometric quantitative determination of antipyrine was based on a published method [39]. (See [Methods Supplement](#) for details of the analytical methodology.)

The area under the curve (AUC) of barusiban and the reference small-molecule antipyrine of the transfer time course was calculated using the trapezoidal method. The AUC of the barusiban transfer time course was compared to that of antipyrine using a paired *t*-test and the Pearson correlation coefficient determined using GraphPad Prism. The mean of the ratios of barusiban to antipyrine transfer AUC time courses was calculated using GraphPad Prism. Normality of the distribution of the Pearson *r* and AUC ratio values was confirmed using the D'Agostino K2 test.

## Results

All data are presented as the mean  $\pm$  standard deviation.



**Figure 1.** Rabbit maternal and fetal blood and plasma mean barusiban levels. Note the much higher scale for the maternal (left) compared to fetal (right) barusiban levels. Only approximately 5% of the maternal barusiban was transferred to the fetus. Standard deviation is shown for all maternal time points and for fetal when  $n \leq 3$  ( $n = 3$  for maternal;  $n = 1-6$  for fetal when sample obtained).

### Rabbit placental transfer

At each time point, there was no maternal mortality ( $n = 3$ ), and one fetus of each sex was investigated from each dam (maximum  $n = 6$ ). The highest maternal blood and plasma barusiban levels were detected 10 min post-dose of  $18\,856 \pm 6\,679$  nM and  $28\,225 \pm 7\,189$  nM, respectively (both  $n = 3$ ) (Figure 1). For technical reasons, no fetal plasma samples were available for analysis and only one for blood containing 1000 nM barusiban (3.8% of the adult). Barusiban was detected in the fetal adrenal gland but only in two out of five fetuses, with minimal levels of 2456 and 5059 nM (9.3 and 19.1%, respectively, of the maternal blood), while in the other three fetuses, it was below the lower level of quantification (LLOQ) of 50 dpm ( $5.46 \times 10^{-3}$  nmoles total barusiban) above the background (Table 1). In other fetal tissues, the barusiban level was either extremely low (<1% of the maternal blood) or less than LLOQ.

The mean barusiban 1 h post-dose in maternal blood of  $6\,189 \pm 2\,728$  nM and plasma of  $9\,926 \pm 4\,063$  nM (both  $n = 3$ ) were 32.8 and 35.2%, respectively, of the maximum level (Figure 1). Fetal blood barusiban after 1 h was slightly lower at  $793 \pm 514$  nM ( $n = 3$ ), being 79.3% of that after 10 min and representing 4.2% of the peak maternal level. Fetal plasma barusiban after 1 h was  $1\,421 \pm 759$  nM ( $n = 2$ ) (representing 5.0% of the peak maternal level). Barusiban in most tissues samples was less than LLOQ or less than 1% of the maternal peak blood level (Table 2). In the single amniotic fluid sample available, a minimal amount of barusiban of 3070 nM (16.3% of the maximal parental plasma level) was found. In two fetal adrenals, the barusiban was 5.0 and 15.7% of the maximal parental plasma level, in the remaining four adrenals being less than LLOQ (Table 2).

At 3 h post-dose, the barusiban level had fallen further in maternal blood to  $1\,143 \pm 769$  nM and in plasma to  $2\,224 \pm 743$  nM (both  $n = 3$ ) (Figure 1), 6.1 and 7.9% of their respective maximum levels. The fetal blood barusiban was further slightly lower at  $600 \pm 381$  nM ( $n = 4$ ), while the plasma barusiban remained similar at  $1\,383 \pm 169$  nM ( $n = 2$ ) to that after 1 h. Barusiban was again

present at a minimal level of 3061 nM (16.2% of the maximum maternal plasma level) in the single available amniotic fluid sample (Table 3). In two fetal adrenals, barusiban was 26.6 and 16.4% of the maximum maternal plasma level, in the other three available adrenals being less than LLOQ (Table 3). The mean fetal carcass barusiban level was  $705 \pm 360$  nM ( $n = 5$ ), similar to that in fetal blood but less than in fetal plasma at this time.

By the final time-point of 10 h post-dose, the barusiban in maternal blood of  $111 \pm 37$  nM and plasma of  $488 \pm 55$  nM (both  $n = 3$ ) (Figure 1) were less than 1% and less than 2% of the first determination, respectively. Final barusiban in fetal blood was  $348 \pm 349$  nM and in plasma  $742 \pm 420$  nM (both  $n = 6$ ), less than 2% and less than 3% of initial maternal levels, respectively. In the two available amniotic fluid samples, again a minimal barusiban concentration could be detected of  $1\,077 \pm 243$  nM (5.7% compared to the maximal maternal blood level), being lower compared to 3 h (Table 4). Only one of the six adrenals in the fetuses was the barusiban above the LLOQ at a minimal level of 2258 nM (12.0% of the maximal maternal blood level). In all other fetal tissues, barusiban was present at less than 1% of peak maternal blood level or less than LLOQ.

The results indicate that following intravenous bolus barusiban administration, only a minimal amount of the barusiban crossed the placental barrier to reach the fetal blood. In most of the fetal tissues, the barusiban concentrations were generally much lower than in the blood/plasma, with many individual results below the limit of reliable quantification.

### Cynomolgus monkey placental transfer

There was no mortality or treatment-related clinical signs throughout the study. Live fetuses (one male, two females) displaying no abnormalities were delivered by C-section in the three adults.

The mean maternal plasma barusiban concentration was  $1\,952 \pm 628$  nM ( $n = 3$ ) 1 h after initiating dosing (Table 5). After 2 h, the mean maternal plasma barusiban was  $2\,666 \pm 1\,319$  nM, having increased in two animals by 40.4 and 54.6% while decreasing by



**Table 1.** Rabbit fetal tissue barusiban levels 10 min after the final maternal dose.

Tissue	Concentration (nmoles/kg tissue)						Mean ± SD
	1 M	2 M	3 M	1 F	2 F	3 F	
Adrenal	<LLOQ	<LLOQ	2456	<LLOQ	NS	5059	3119 ± 1527**
Amniotic	NS	1073	NS	929	NS	NS	831 ± 85**
Aorta	ND	<LLOQ	ND	ND	NS	ND	<LLOQ
Brain	ND	<LLOQ	<LLOQ	ND	NS	ND	<LLOQ
Heart	<LLOQ	<LLOQ	<LLOQ	24	NS	<LLOQ	24*
Kidney	49	72	147	72	NS	123	77 ± 34
Liver	61	80	95	54	NS	102	65 ± 17
Lung	25	59	28	36	NS	31	30.0 ± 11.0
Testes	<LLOQ	ND	<LLOQ	–	–	–	<LLOQ
Ovaries	–	–	–	<LLOQ	NS	ND	<LLOQ**
Carcass	322	383	376	288	NS	446	301 ± 50

M, male; F, female; SD, standard deviation; ND, not detected; NS, no sample; <LLOQ, below lower limit of quantification.

\*Based on single value.

\*\*Based on two values.

**Table 2.** Rabbit fetal tissue barusiban levels 1 h after the final maternal dose.

Tissue	Concentration (nmoles/kg tissue)						Mean ± SD
	4 M	5 M	6 M	4 F	5 F	6 F	
Adrenal	2959	<LLOQ	940	<LLOQ	<LLOQ	<LLOQ	1618 ± 1185**
Amniotic	NS	NS	3068	NS	NS	NS	3068*
Aorta	<LLOQ	<LLOQ	<LLOQ	<LLOQ	ND	<LLOQ	<LLOQ
Brain	ND	<LLOQ	<LLOQ	<LLOQ	ND	20	20*
Heart	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Kidney	60	20	75	63	36	40	41 ± 17
Liver	67	52	57	47	39	59	44 ± 8
Lung	89	35	75	98	80	63	61 ± 18
Testes	<LLOQ	ND	<LLOQ	–	–	–	<LLOQ
Ovaries	–	–	–	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Carcass	782	328	292	702	237	843	441 ± 227

M, male; F, female; SD, standard deviation; ND, not detected; NS, no sample; <LLOQ, below lower limit of quantification.

\*Based on single value.

\*\*Based on two values.

**Table 3.** Rabbit fetal tissue barusiban levels 3 h after the final maternal dose.

Tissue	Concentration (nmoles/kg tissue)						Mean ± SD
	7 M	8 M	9 M	7 F	8 F	9 F	
Adrenal	<LLOQ	5024	NS	<LLOQ	3100	<LLOQ	3372 ± 1129**
Amniotic	3061	NS	NS	NS	NS	NS	3061*
Aorta	<LLOQ	<LLOQ	NS	ND	<LLOQ	ND	<LLOQ A
Brain	<LLOQ	ND	NS	<LLOQ	<LLOQ	ND	<LLOQ
Heart	<LLOQ	<LLOQ	NS	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Kidney	92	89	NS	36	71	61	58 ± 19
Liver	192	100	NS	46	86	54	79 ± 48
Lung	122	72	NS	53	99	45	65 ± 27
Testes	ND	<LLOQ	NS	–	–	–	<LLOQ**
Ovaries	–	–	–	ND	<LLOQ	ND	<LLOQ
Carcass	1198	335	NS	364	811	816	585 ± 299

M, male; F, female; SD, standard deviation; ND, not detected; NS, no sample; <LLOQ, below lower limit of quantification.

\*Based on single value.

\*\*Based on two values.

**Table 4.** Rabbit fetal tissue barusiban levels 10 h after the final maternal dose.

Tissue	Concentration (nmoles/kg tissue)						Mean $\pm$ SD
	10 M	11 M	12 M	10 F	11 F	12 F	
Adrenal	<LLOQ	<LLOQ	<LLOQ	2258	<LLOQ	<LLOQ	2258*
Amniotic	NS	NS	NS	1249	905	NS	1077 $\pm$ 243**
Aorta	<LLOQ	ND	ND	<LLOQ	ND	<LLOQ	<LLOQ
Brain	ND	<LLOQ	<LLOQ	<LLOQ	ND	<LLOQ	<LLOQ
Heart	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Kidney	118	67	57	83	53	111	68 $\pm$ 23
Liver	59	71	57	76	51	77	54 $\pm$ 9
Lung	47	45	42	17	61	77	46 $\pm$ 16
Testes	ND	<LLOQ	ND	–	–	–	<LLOQ
Ovaries	–	–	–	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Carcass	265	840	649	330	1190	1098	605 $\pm$ 320

M, male; F, female; SD, standard deviation; ND, not detected; NS: no sample; <LLOQ, below lower limit of quantification.

\*Based on single value.

\*\*Based on two values.

**Table 5.** Cynomolgus monkey maternal and fetal barusiban levels.

Animal number	Maternal plasma after 1 h (nM)	Maternal plasma after 2 h (nM)	Fetal plasma at C-section $\pm$ SD (nM)	Plasma ratio: fetal at C-section/maternal after 2 h	Amniotic fluid at C-section (nM)	Ratio: amniotic fluid at C-section/maternal plasma after 2 h
1 (F)	2265	3181	257 $\pm$ 11	0.081	54	0.017
2 (M)	1229	1167	149 $\pm$ 7	0.128	27	0.023
3 (F)	2361	3650	234 $\pm$ 30	0.064	61	0.017
Mean $\pm$ SD	1952 $\pm$ 628	2666 $\pm$ 1319	213 $\pm$ 57*	0.091 $\pm$ 0.033	47 $\pm$ 18*	0.019 $\pm$ 0.004

Samples were obtained from three adult animals and their respective single fetus. Maternal plasma and the amniotic fluid represent a single value, while for fetal plasma, the values are the mean of triplicate determinations. C-section, caesarean section; SD, standard deviation; F, female fetus; M, male fetus.

\* $P < 0.05$  vs. mean maternal plasma level after 2 h.

5.0% in the third animal. Fetal plasma obtained during C-section contained a mean of 213  $\pm$  57 nM barusiban ( $n = 3$ ), being 9.1% of the mean maternal plasma level after 2 h ( $P = 0.0415$ ). The mean amniotic barusiban concentration at C-section of 47  $\pm$  18 nM ( $n = 3$ ) was 1.9% of the maternal plasma after 2 h ( $P = 0.0109$ ).

### Human placental transfer

In total, 17 placentas were perfused. Two were excluded before barusiban application, because the arterial inflow and venous outflow were unequal, while in the other Evans blue leakage occurred in the membrane-integrity test. In a further two placenta, the barusiban level was above the highest measurable limit for the inflow (maternal side), the data thus being excluded from calculations. Overall, a steady-state barusiban transfer from maternal to fetal side of approximately 11% was attained within 10 min in the 13 placentas included for analysis (Figure 2). For the reference small molecule antipyrine, the transfer in the same 13 placentas peaked after 10 min at 35% before falling back to a steady-state transfer of approximately 29% from 30 min. However, the time course pattern was quite variable from placenta to placenta for both barusiban and antipyrine, which may make the mean time course misleading. By contrast, for any given placenta, the time course patterns of barusiban and antipyrine were similar to each other. Therefore, the area under the curve (AUC) of the time course was a more representative quantification of the placental transfer, which was 1246  $\pm$  437%-min for barusiban vs. 3511  $\pm$  988%-min for antipyrine ( $P < 0.0001$ ,  $r^2 = 0.8922$ ), and

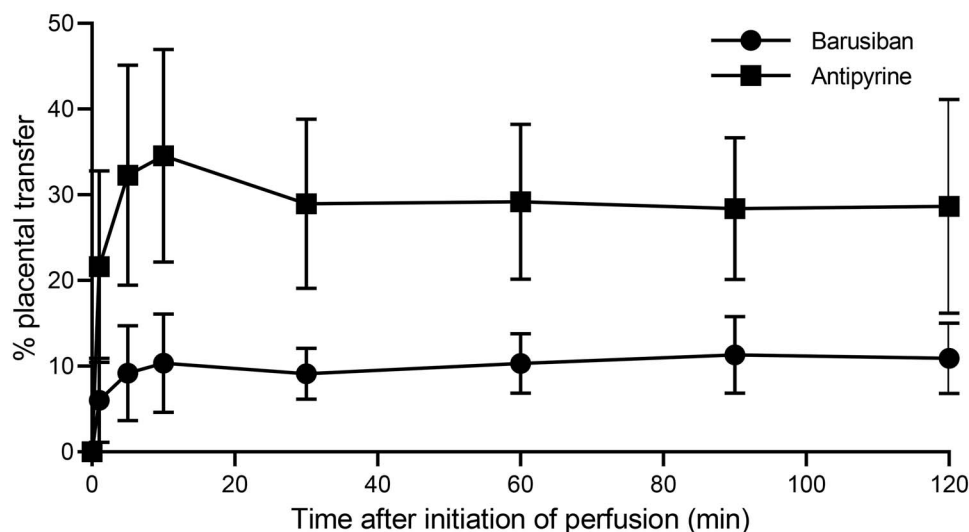
the AUC ratio of barusiban to antipyrine was 36  $\pm$  11% (95% confidence interval: 30–43%).

### Discussion

In the present study, three different model systems were investigated. The rabbit is a standard model used to test reproductive toxicology [26] and the cynomolgus monkey, a large non-clinical model with a similar placental barrier to the human. The ex vivo human cotyledon model provides an insight into the actual clinical transfer.

Following barusiban administration in rabbits, only a limited amount crossed the placental barrier, reaching a level in the fetal blood and plasma of approximately 5% of the corresponding maximum on the maternal side. In most of the fetal tissues investigated, the barusiban concentrations were generally much lower than in the blood/plasma, in many cases below the limit of reliable quantification. Similarly, on intravenous barusiban infusion for 2 h to pregnant cynomolgus monkeys, limited placental transfer occurred, reflected in the fetal plasma and amniotic fluid containing only 6.4–12.8 and 1.7–2.3% of the peak maternal plasma concentration, respectively. Again, in the perfused human placenta model, only approximately 11% barusiban diffusion or transport into the fetal circulation occurred, which was significantly lower compared to a peak of 35% for the small-molecule control antipyrine ( $P < 0.0001$ ).

The level of barusiban transfer was similar in the cynomolgus monkey and human cotyledon perfusion model compared with in vivo atosiban transfer, a non-peptide of slightly higher molecular



**Figure 2.** Percentage transfer of barusiban as well as antipyrine as a small-molecule control in isolated human cotyledons. Standard error bars are shown ( $n = 13$ ).

weight, reported in pregnant women at term of 12% [11]. The human cotyledon ex vivo barusiban transfer of approximately 11% was clearly below that reported in humans for the smaller tocolytics  $MgSO_4$ , nifedipine, fenoterol, and ritodrine hydrochloride, which ranged from 26% up to approximately 100% [4–10].

The tissue distribution pattern of barusiban in the rabbit fetuses was similar to that observed in a quantitative whole-body autoradiography (QWBA) study in the marmoset (unpublished data). Additionally, in both species, the radioactivity in fetal brain was normally below the limit of quantification (12 pmoles/g tissue for the marmoset).

The lower transfer of barusiban from maternal to fetal blood in the rabbit compared with the cynomolgus monkey and human cotyledon model may reflect species-specific placental barrier structure between the maternal and fetal compartments. Cynomolgus monkey and human placentas are hemomonochorial, with a single placental cell layer separating the maternal blood from fetal blood vessels [27, 28]. These displayed similar barusiban placental transfer in the present study, with approximately 9% of the maternal barusiban in the cynomolgus monkey and approximately 11% for the ex vivo human cotyledon reaching the fetal side. By contrast, this transfer was lower at approximately 5% in the rabbits, which have a hemodichorial placenta at term, with a double cell layer separating the maternal blood from fetal blood vessels [27]. While earlier in gestation the presence of the inverted yolk sack in the rabbit, which is absent in primates and humans [40], may contribute to drug transport or accumulation, this is no longer present at term in the current study. However, this structure would have to be taken into consideration when using this model to assess drug transfer earlier in gestation. Furthermore, in the rat, with a triple placental trophoblastic cell layer (hemotrichorial; one layer of cytotrophoblasts contacting the maternal blood and two layers of syncytiotrophoblasts) [27], barusiban in a QWBA study was below the limit of quantitation (40 pmoles/g) at all sampling times in the fetuses (unpublished data). A similar effect of placental barrier structure on transfer was observed for atosiban, which reached 12% in humans, but could not be detected in sheep, this animal with an epitheliochoral placenta of multiple cell layers between the maternal and fetal blood [11, 41].

Commonly used tocolytics are small-molecule drugs that were developed for clinical conditions other than preterm labor [42], for example,  $MgSO_4$ , to treat/prevent low blood magnesium, arrhythmias, and acute asthma; ritodrine in asthma; fenoterol in asthma and chronic obstructive pulmonary disease; and nifedipine as an antihypertensive. Atosiban was the first drug specifically developed to treat preterm labor, which is approved in the EU and numerous countries globally, although not in the USA. It is a mixed  $V_{1A}R$ /OTR antagonist and mainly acts in reproductive tissues, avoiding systemic adverse effects; however, it can still affect the vascular  $V_{1A}R$  [43]. The concentration of  $V_{1A}$  binding sites is high in human uterine tissues, particularly in the decidua, in all such tissues being approximately 50–60% of the level of oxytocin binding sites [44]. Barusiban, a new oxytocin antagonist, displayed considerably higher affinity in ligand-binding assays [18] and greater potency in functional, cell-based reporter gene assays for the human OTR than the human  $V_{1A}R$  (unpublished data). A selective action of this new analogue on oxytocin responses, without influencing the uterine  $V_{1A}R$ , was demonstrated in term-pregnant human myometrium [18]. Barusiban displayed concentration-dependent inhibition of oxytocin-induced contractions in both isolated human preterm and term myometrial strips, the effect at least as potent as atosiban [24]. Furthermore, in pregnant cynomolgus monkeys stimulated with oxytocin, barusiban displayed improved tocolytic potency and longer duration of action compared to atosiban [31]. Therefore, barusiban is a potential future alternative for atosiban, particularly with its greater affinity for the OTR than the  $V_{1A}R$  compared with atosiban.

Any drug that can cross the placental barrier may pose a potential risk in fetal development. Therefore, the present study was performed for pharmacokinetic safety reasons, with placental transfer of barusiban investigated in two animal models together with a human placental cotyledon model. Low transfer of barusiban in all three models and the limited accumulation in the rabbit fetal tissues were found. Furthermore, the amount of barusiban applied to the rabbits and cynomolgus monkeys was approximately 20 and two times greater, respectively, than the highest therapeutic dose [33] to ensure high but well-tolerated maternal exposure, to see any potential transfer, and to identify potential fetal target organs. Barusiban was applied in the perfusate to the human cotyledon at a



concentration corresponding to the maximum serum concentrations in clinical trials on intravenous administration of a 10-mg bolus, the maximum amount applied [33]. Therefore, considering the low barusiban transfer in all three models and the limited accumulation in the rabbit fetal tissues, at the therapeutic level, limited amounts of barusiban would be expected in the fetal tissues. Rasmussen et al. [45] reported that there were no barusiban-related effects in the mother or offspring on treating pregnant cynomolgus monkeys with up to 2.5 mg barusiban/kg body weight/day from day 85 of gestation until delivery. Detailed examination of the offspring over a 9-month postnatal period found no barusiban-related effects on developing organ systems or on behavior. Moreover, Thornton et al. [33] in a clinical study reported a very low level of fetal and neonatal adverse events with maternal barusiban, which did not differ from those observed on placebo treatment, and their frequency of occurrence did not alter with increasing maternal barusiban dosage. In the same study, barusiban did not result in any difference in physical or mental development in the offspring at a 1-year follow-up.

Maternal barusiban placental transfer needs to be determined in the clinical situation to confirm a limited transfer to human fetal blood. This would show how closely the transfer levels seen both in vivo in the cynomolgus, with a similar placental barrier structure, and ex vivo with the isolated human cotyledon reflect human maternal transfer. Consequently, this would indicate whether they are good models, as already shown, for example, for other drugs in the cotyledon model [35], to be used in further, more extensive studies of barusiban as well as in the future to test other potential peptide tocolytics. Future clinical trials of barusiban, including in comparison with other tocolytics, will elucidate any fetal side effects, which appear to be unlikely for barusiban based on current knowledge.

There were several limitations to the study. In the explorative rabbit study, the number of rabbits was limited, and not all fetuses were examined. Furthermore, only a limited number of time points were investigated, with available fetal blood/plasma samples restricted at the earlier time points and of amniotic fluid throughout. The cynomolgus study was restricted to three animals for ethical reasons, and in the fetuses, only plasma barusiban levels at a single time point were investigated and not the tissue distribution.

## Conclusions

The limited placental transfer of barusiban in both animal studies and in isolated human placenta suggests that in maternal therapy, there would be restricted fetal exposure. This contrasts with the greater transfer determined here of the small-molecule antipyrene and of small-molecule tocolytic treatments in the literature. Because the placental barrier structure may influence the level of tocolytic transfer, care must be taken in extrapolating from the transfer levels observed in animal studies to the clinical situation. The similarity of barusiban transfer in the cynomolgus, with its comparable fetal development to humans, with the human placental single cotyledon reflects the previously reported ability of the latter ex vivo model to accurately predict in vivo human drug transfer and thus be useful in assessing pharmaceutical drug candidates to be administered during pregnancy in women.

## Supplementary material

Supplementary material is available at *BIOLRE* online.

## Acknowledgments

We would like to thank the involved study directors and monitors, Covance Laboratories and R. Harrison, I. Osterburg, and M.B. Petersen. This manuscript was prepared by Robert Blakytyn PhD, a freelance medical writer, who was supported by Ferring Pharmaceuticals A/S.

## Conflict of Interest

The authors have declared that no conflict of interest exists.

## References

- McCormick MC, Litt JS, Smith VC, Zupancic JA. Prematurity: an overview and public health implications. *Annu Rev Public Health* 2011; 32:367–379.
- Roberts D, Brown J, Medley N, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev* 2017; 3:CD004454.
- Pommerenke WT. Placental interchange I. on the concentration of certain nitrogenous substances in the blood, before and after passing through the placenta. *J Clin Invest* 1936; 15:485–488.
- Cruikshank DP, Pitkin RM, Reynolds WA, Williams GA, Hargis GK. Effects of magnesium sulfate treatment on perinatal calcium metabolism. I. Maternal and fetal responses. *Am J Obstet Gynecol* 1979; 134:243–249.
- Green KW, Key TC, Coen R, Resnik R. The effects of maternally administered magnesium sulfate on the neonate. *Am J Obstet Gynecol* 1983; 146:29–33.
- Ferguson JE 2nd, Schutz T, Pershe R, Stevenson DK, Blaschke T. Nifedipine pharmacokinetics during preterm labor tocolysis. *Am J Obstet Gynecol* 1989; 161:1485–1490.
- Silberschmidt AL, Kühn-Velten WN, Juon AM, Zimmermann R, von Mandach U. Nifedipine concentration in maternal and umbilical cord blood after nifedipine gastrointestinal therapeutic system for tocolysis. *BJOG* 2008; 115:480–485.
- von Mandach U, Huch A, Huch R. Pharmacokinetic studies on fenoterol in maternal and cord blood. *Am J Perinatol* 1989; 6:209–213.
- Fujimoto S, Akahane M, Sakai A. Concentrations of ritodrine hydrochloride in maternal and fetal serum and amniotic fluid following intravenous administration in late pregnancy. *Eur J Obstet Gynecol Reprod Biol* 1986; 23:145–152.
- Fujimoto S, Tanaka T, Akahane M. Levels of ritodrine hydrochloride in fetal blood and amniotic fluid following long-term continuous administration in late pregnancy. *Eur J Obstet Gynecol Reprod Biol* 1990; 38:15–18.
- Valenzuela GJ, Craig J, Bernhardt MD, Holland ML. Placental passage of the oxytocin antagonist atosiban. *Am J Obstet Gynecol* 1995; 172:1304–1306.
- Verdurmen KMJ, Hulsenboom ADJ, van Laar JOEH, Oei SG. Effect of tocolytic drugs on fetal heart rate variability: a systematic review. *J Matern Fetal Neonatal Med* 2017; 30:2387–2394.
- de Heus R, Mulder EJ, Derks JB, Visser GH. The effects of the tocolytics atosiban and nifedipine on fetal movements, heart rate and blood flow. *J Matern Fetal Neonatal Med* 2009; 22:485–490.
- Gokay Z, Ozcan T, Copel JA. Changes in fetal hemodynamics with ritodrine tocolysis. *Ultrasound Obstet Gynecol* 2001; 18:44–46.
- Neri I, Monari F, Valensise H, Vasapollo B, Facchinetti F, Volpe A. Computerized evaluation of fetal heart rate during tocolytic treatment: comparison between atosiban and ritodrine. *Am J Perinatol* 2009; 26:259–263.
- Bartels PA, Hanff LM, Mathot RA, Steegers EA, Vulto AG, Visser W. Nicardipine in pre-eclamptic patients: placental transfer and disposition in breast milk. *BJOG* 2007; 114:230–233.
- Ducsay CA, Thompson JS, Wu AT, Novy MJ. Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes. *Am J Obstet Gynecol* 1987; 157:1482–1486.

18. Nilsson L, Reinheimer T, Steinwall M, Akerlund M. FE 200440: a selective oxytocin antagonist on the term-pregnant human uterus. *BJOG* 2003; **110**:1025–1028.
19. Akerlund M, Bossmar T, Brouard R, Kostrzewski A, Laudanski T, Lemancewicz A, Serradeil-Le Gal C, Steinwall M. Receptor binding of oxytocin and vasopressin antagonists and inhibitory effects on isolated myometrium from preterm and term pregnant women. *BJOG* 1999; **106**:1047–1053.
20. Cirillo R, Gillo Tos E, Schwarz MK, Quattropiani A, Scheer A, Missotten M, Dorbais J, Nichols A, Borrelli F, Giachetti C, Golzio L, Marinelli P et al. Pharmacology of (2S,4Z)-N-[(2S)-2-hydroxy-2-phenylethyl]-4-(methoxyimino)-1-[(2'-methyl[1,1'-biphenyl]-4-yl)carbonyl]-2-pyrrolidinedicarboxamide, a new potent and selective nonpeptide antagonist of the oxytocin receptor. *J Pharmacol Exp Ther* 2003; **306**:253–261.
21. Gimpl G, Postina R, Fahrenholz F, Reinheimer T. Binding domains of the oxytocin receptor for the selective oxytocin receptor antagonist barusiban in comparison to the agonists oxytocin and carbetocin. *Eur J Pharmacol* 2005; **510**:9–16.
22. Koehbach J, Stockner T, Bergmayr C, Muttenthaler M, Gruber CW. Insights into the molecular evolution of oxytocin receptor ligand binding. *Biochem Soc Trans* 2013; **41**:197–204.
23. Hoyle CH. Neuropeptide families and their receptors: evolutionary perspectives. *Brain Res* 1999; **848**:1–25.
24. 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* 2004; **11**:384–387.
25. Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, Onaka T, Yanagisawa T, Kimura T, Matzuk MM, Young LJ, Nishimori K. Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proc Natl Acad Sci USA* 2005; **102**:16096–16101.
26. Foote RH, Carney EW. The rabbit as a model for reproductive and developmental toxicity studies. *Reprod Toxicol* 2000; **14**:477–493.
27. Furukawa S, Kuroda Y, Sugiyama A. A comparison of the histological structure of the placenta in experimental animals. *J Toxicol Pathol* 2014; **27**:11–18.
28. Griffiths SK, Campbell JP. Placental structure, function and drug transfer. *Contin Educ Anaesth Crit Care Pain* 2015; **15**:84–89.
29. Shimizu K. Reproductive hormones and the ovarian cycle in macaques. *J Mamm Ova Res* 2008; **25**:122–126.
30. Martin PL, Weinbauer GF. Developmental toxicity testing of biopharmaceuticals in nonhuman primates: previous experience and future directions. *Int J Toxicol* 2010; **29**:552–568.
31. Reinheimer T, Bee WH, Resendez JC, Meyer JK, Haluska GJ, Chellman GJ. Barusiban, a highly potent and long acting oxytocin antagonist: pharmacokinetic and pharmacodynamic comparison to atosiban in a cynomolgus monkey model of preterm labor. *J Clin Endocrinol Metab* 2005; **90**:2275–2281.
32. Reinheimer TM, Chellman GJ, Resendez JC, Meyer JK, Bee WH. Barusiban, an effective long-term treatment of oxytocin-induced preterm labor in nonhuman primates. *Biol Reprod* 2006; **75**:809–814.
33. Thornton S, Goodwin TM, Greisen G, Hedegaard M, Arce JC. The effect of barusiban, a selective oxytocin antagonist, in threatened preterm labor at late gestational age: a randomized, double-blind, placebo-controlled trial. *Am J Obstet Gynecol* 2009; **200**:627.e1–627.e10.
34. Schneider H, Panigel M, Dancis J. Transfer across the perfused human placenta of antipyrine, sodium and leucine. *Am J Obstet Gynecol* 1972; **114**:822–828.
35. Hutson JR, Garcia-Bournissen F, Davis A, Koren G. The human placental perfusion model: a systematic review and development of a model to predict in vivo transfer of therapeutic drugs. *Clin Pharmacol Ther* 2011; **90**:67–76.
36. Brandes JM, Tavoloni N, Potter BJ, Sarkozi L, Shepard MD, Berk PD. A new recycling technique for human placental cotyledon perfusion: application to studies of the fetomaternal transfer of glucose, inulin, and antipyrine. *Am J Obstet Gynecol* 1983; **146**:800–806.
37. Reisenberger K, Egarter C, Vogl S, Sternberger B, Kiss H, Husslein P. The transfer of interleukin-8 across the human placenta perfused in vitro. *Obstet Gynecol* 1996; **87**:613–616.
38. Zheng Q, Zhou Q, Li J, Tian Y, Huang H, Yao Q, Wang J, Zhang J. Placental transfer of bromocriptine in an ex vivo human placental perfusion model. *J Matern Fetal Neonatal Med* 2019; **32**:1155–1159.
39. Pastrakuljic A, Schwartz R, Simone C, Derewlany LO, Knie B, Koren G. Transplacental transfer and biotransformation studies of nicotine in the human placental cotyledon perfused in vitro. *Life Sci* 1998; **63**:2333–2342.
40. Carney EW, Scialli AR, Watson RE, DeSesso JM. Mechanisms regulating toxicant disposition to the embryo during early pregnancy: an interspecies comparison. *Birth Defects Res C Embryo Today* 2004; **72**:345–360.
41. Greig PC, Massmann GA, Demarest KT, Weglein RC, Holland ML, Figueroa JP. Maternal and fetal cardiovascular effects and placental transfer of the oxytocin antagonist atosiban in late-gestation pregnant sheep. *Am J Obstet Gynecol* 1993; **169**:897–902.
42. Helmer H. Frequently asked questions on tocolytics. *BJOG* 2005; **112**(Suppl. 1):94–96.
43. Vrachnis N, Malamas FM, Sifakis S, Deligeorgiou E, Iliodromiti Z. The oxytocin-oxytocin receptor system and its antagonists as tocolytic agents. *Int J Endocrinol* 2011; **2011**:350546.
44. Ivanisevic M, Behrens O, Helmer H, Demarest K, Fuchs AR. Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists. *Am J Obstet Gynecol* 1989; **161**:1637–1643.
45. Rasmussen AD, Nelson JK, Chellman GJ, Golub M, McAnulty PA. Use of barusiban in a novel study design for evaluation of tocolytic agents in pregnant and neonatal monkeys, including behavioural and immunological endpoints. *Reprod Toxicol* 2007; **23**:471–479.