The role of the placenta in fetus exposure to xenobiotics: importance of membrane transporters, human models for transfer studies

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Running title:
Placental membrane transporters and models for transfer studies

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A list of nonstandard abbreviations:
ATP Binding Cassette transporters: ABC transporters
Enzymes of Xenobiotic Metabolism: EXM
CYP: cytochrome P450
P-glycoprotein: P-gp, MDR1, ABCB1
Multi-drug Resistance-associated Proteins: MRPs, ABCCs
Breast Cancer Resistance Protein: BCRP, ABCG2
Solute Carrier Transporters: SLC transporters
Organic Anion-Transporting Polypeptides: OATPs
Organic Anion Transporters: OATs
Organic Cation Transporters: OCTs
Organic Cation/Carnitine Transporter: OCTN
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Serotonin Transporter: SERT
Norepinephrin Transporter: NET
Monocarboxylate Transporters: MCTs
Equilibrative Nucleoside Transporters: ENTs
Folate Receptor alpha: FRα
Reduced Folate Carrier 1: RFC-1
Proton-coupled folate transporter/heme carrier protein 1: PCFT/HCP1
Amino acid Transporter: ASCT
Single Nucleotide Polymorphisms: SNPs
The placenta is a key organ in fetal growth and development, as it controls maternal-to-fetal exchanges of nutrients and hormones. It also interferes with drug delivery to the fetus, by expressing active membrane transporters and xenobiotic metabolism enzymes. Developing strategies to understand the role of the placenta in drug delivery is a challenge in toxicology. Despite common physiological functions, the placentas of different species are heterogeneous in both their morphology and in their expression of membrane transporters and metabolizing proteins. This raises the difficulty of obtaining a good representative model of human placental transfer. Up to now, different in vitro, in vivo and ex vivo tools have been used to elucidate transport and metabolism processes in the human placenta. After having recapitulated the typical features of human placenta, this review presents the placental enzymes of xenobiotic metabolism, ATP Binding Cassette transporters, Solute Carrier Transporters, and their role in fetus exposure to xenobiotics. It also compares the characteristics of different models of human placenta, in terms of membrane localization of transporters, and the expression of xenobiotic metabolism enzymes. The use of these models for toxicological studies, in particular xenobiotic transfer, is described, and the advantages and limits of each model are summarized.
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References
1. Introduction

The placenta is a key organ for the growth and development of the embryo and fetus during pregnancy: this semi-permeable barrier separates the mother and the fetus and regulates the exchange of nutrients, gases, wastes, endogenous and foreign molecules between maternal and fetal circulations. It has traditionally been considered as a highly permeable organ for a large variety of substances with diverse molecular structures that are readily able to cross it from the maternal blood to reach the fetus. Indeed, fetal exposure to maternal intake was proved for the first time with the thalidomide disaster in 1957-1961. Consequently, it is recommended to limit the use of drugs during pregnancy as much as possible.

The human placenta barrier consists of a single limiting layer of multinuclear cells, known as syncytiotrophoblasts (Enders et al., 1999). A microvillous brush border membrane, in direct contact with maternal blood, constitutes the maternal-facing plasma membrane of the syncytium. On the opposite side, the basal membrane faces the fetal circulation and lacks microvillar projections. Both sides of the syncytium are not only structurally distinct, but also differ in the localization of transporters, enzymes, and hormone receptors. Existing knowledge underlines the existence of different systems, including plasma membrane carriers, biotransforming enzymes, and export pumps, that determine the selectivity and efficacy of the so-called placental barrier. Good understanding of the molecular bases of these processes and their regulation is crucial for predicting the risk of fetal exposure to active agents.

Beside drug exposure, which can be limited voluntarily during pregnancy, fetus exposure to food contaminants is characterized by chronic exposure to low doses. Very little information is available on fetal exposure to these pollutants and their long-term effects. Drug transporters are involved in the regulation of the chemical environment of the fetus by selectively transporting and removing toxic substrates. Thus placental epithelia expressing xenobiotic metabolism enzymes and protein transporters are very helpful models for the in vitro study of
quantitative and qualitative transfers of molecules to the fetus.

Different experimental trophoblast systems are available, including immortalized cell lines, which are derived from normal and malignant tissues. In this review, we present a synthesis on xenobiotic metabolism enzymes and transporters in the placenta, and update the different experimental models potentially available for the study of the barrier function.

2. Physiological function of the human placental barrier

As in other primate species, the human placenta is haemochorial and characterized by direct contact between maternal blood and trophoblast (figure 1a). The human placenta is divided into functional vascular units called cotyledons. Within the cotyledon, the villus tree contains a barrier separating maternal circulation from fetal circulation. The layer of tissue separating maternal and fetal circulation is composed of the endothelium of fetal capillaries and the trophoblast, containing the villus strom, the cytотrophoblast and the syncytiotrophoblast. The thickness of the syncytiotrophoblast layer decreases during gestation, the cytотrophoblast becomes discontinuous, and exchange-processes between mother and fetus are facilitated (van der Aa et al., 1998, figure 1b).

The major function of the placenta is the transfer of nutrients that support embryonic and fetal growth and development. During early gestation, the placenta mediates the implantation of the embryo in the uterus and produces hormones that prevent the end of the ovarian cycle. The luteinizing hormone and the human chorionic gonadotrophin have a stimulating action on estrogen and progesterone synthesis by the placenta (Pasqualini et al., 1985). After implantation, the placenta regulates the supply of nutrients and oxygen to the fetus. It also produces angiogenic factors, vasodilators, growth hormones, placental lactogens and different hormones, all of which stimulate maternal blood cell production, increase blood volume, regulate appetite, etc.). Other functions of the placenta are immune function (immunoglobulin
transfert), and removal of waste or toxic products from the fetus and metabolism. The presence of metabolism enzymes and transport systems in the placenta make these functions possible.

3. Enzymes of Xenobiotic Metabolism (EXM) content. The human placenta contains a wealth of enzymatic machinery responsible for both phase I and phase II reactions (table 1). However, data on the contribution of placental biotransformations in the conversion of xenobiotics into potential toxic metabolites are very scarce. Compared to the liver, placental metabolism seems to be minor and does not limit the extent of xenobiotic passage across the placenta (Pasanen, 1999). Several cytochromes P450 (CYP) including CYP1, CYP2 and CYP3 have been isolated from the placenta. These proteins are largely responsible for the detoxification of drugs and toxins. However, the members and the quantity of CYPs vary as a function of placental development, length of gestation, and maternal health status (Hakkola et al., 1996a,b). Expression and activities of human CYP enzymes decline during gestation from the first trimester of pregnancy to the second and third trimesters (Syme et al., 2004). In the placenta, a large number of enzymes are involved in progesterone and estrogen synthesis from fetal and maternal precursors (Pasqualini, 2005, Smuc et al., 2009). Enzymes involved in steroid hormone metabolism are sulfatase (Salido et al., 1990), 3β-hydroxysteroid dehydrogenase (Luu-The et al. 1990), aromatase and 17β-hydroxysteroid dehydrogenase (type 1 and type 2) (Luu-The et al. 1989). CYP19 and CYP11A are the major isoforms expressed (mRNA quantification, Nishimura et al., 2003). Human placenta contains a few forms of phase II enzymes: glutathione S-transferase alpha and pi (Pacifici et al., 1981; Pasanen et al., 1997), epoxide hydrolase (Pacifici et al., 1983), N-acetyltransferase (Derewlany et al., 1994) and sulfotransferases isoforms such as SULT1A1 and SULT1A3 (Mitra et al., 2009). UDP-glucuronyltransferase isoforms such as UGT1A and UGT2B are also expressed
Data on EXM contents in placenta cell lines are very scarce compared to those for the whole organ: only a few CYP, including CYP19, and hydroxysteroid deshydrogenase enzymes have been reported (table 1).

4. Membrane transport in placenta

4.1. Transplacental transfer of xenobiotics. The transfer of molecules between maternal and fetal circulation occurs across the endothelial-syncytial membrane of placenta. Drug permeation can be influenced by numerous factors: drug properties (degree of ionization, lipophilicity, protein binding, molecular weight), placental characteristics (blood flow, concentration gradient of the drug across the barrier, pH gradient, thinning and aging barrier with advancement of pregnancy, increasing surface area as pregnancy progresses, developing metabolism, protein gradients), maternal and fetal factors (fetal growth and development, fetal metabolism, fetal tissue binding, maternal metabolism, maternal health condition) (Poulsen et al., 2009). Most of the drugs crossing the human placenta diffuse passively. In this case, placental blood-flow, pH of maternal and fetal blood, physicochemical characteristics of the compounds and protein binding determine ability to cross placental membranes (Pacifici and Nottoli, 1995; Audus, 1999). Facilitated diffusion, phagocytosis and pinocytosis are less important routes of placental drug transfer (Syme et al., 2004).

In 1998, the report of carrier-mediated transport of xenobiotics in the placenta stressed the role of ATP Binding Cassette (ABC) transporters in this organ (Lankas et al.1998). Depending on the localization of these transporters in the apical (maternal facing brush border membrane) and basal (fetal facing basal membrane) membrane of the syncytiotrophoblast, the substrates are preferentially transported either in maternal circulation or in fetal circulation (Unadkat et al., 2004). Placental xenobiotic transporters and their polarized distribution are summarized in table 2.
4.2. ATP Binding cassette (ABC) transporters.

4.2.1. P-glycoprotein (P-gp, MDR1, ABCB1). The placental barrier had been commonly considered as a permeable barrier until the publication of the work by Lankas et al. in 1998. These authors stressed the role of P-gp in the mouse placenta by exposing pregnant dams, which contained a spontaneous mutation in the mdr1 gene, to the teratogenic isomer of avermectin (1.5mg/kg/day, oral administration, 6-15 days gestation) which is a substrate of P-gp. They demonstrated that the degree of chemical exposure of fetuses within each litter was inversely related to the expression of placental P-gp, linking the fetal genotype to pharmacokinetic changes and subsequently developmental toxicity.

Other studies confirmed the involvement of P-gp in the limited fetal penetration of various potentially harmful or therapeutic compounds such as digoxin, saquinavir, paclitaxel (study performed in P-gp deficient mice, Smit et al., 1999) and indinavir (study using an in vitro model of human placenta perfusion, Sudhakaran et al., 2007). Indeed, Smit et al. (1999) used mice with a targeted disruption of Mdr1a and Mdr1b genes. Mdr1a+/−/1b+/− females were mated with Mdr1a+/−/1b+/− males to obtain fetuses of 3 genotypes (Mdr1a+/+/1b+/+, Mdr1a+/−/1b+/−, and Mdr 1a+−/1b−−) in a single mother. Intravenous administration of a single dose of P-gp substrate digoxin, saquinavir and paclitaxel to pregnant dams (gestation day 15) revealed that 2.4-, 7-, and 16-times more drug, respectively, crossed the placenta and entered the mdr1a−/−/1b−− fetus, when compared to the wild-type (mdr1a+/+/1b+/+) fetus. This study also demonstrated that P-gp activity was completely inhibited by oral administration of the P-gp blockers PSC833 and GG918 to heterozygous mothers, indicating that P-gp function in the placenta can be cancelled by pharmacological means.

ABCB1 mRNA is strongly expressed in the human placenta, comparable with mRNA levels in the intestine and liver (Bremer et al., 1992). Protein expression, confirmed by immunohistochemical studies, is located on the maternal side of the trophoblast layer (St-
Pierre et al., 2000), and on the syncytiotrophoblast microvillous border of the first-trimester and term placentas (MacFarland et al., 1994; Nakamura et al. 1997).

The mRNA expression of MDR3 (MDR1 homolog, ABCB4) has also been reported in human term and pre-term placenta by Patel et al. (2003). These results were confirmed by Evseenko et al. (2006a) who identified MDR3 protein in the basolateral membrane of the syncytium of the term placenta and suggested that MDR3 mediated the vectorial transport of substrates toward the fetus. MDR3 functions as a lipid translocator and may also interact with pharmaceutical drugs (Kimura et al., 2007). Although an overexpression of MDR3 was reported in resistant tumor cells (Scheffer et al., 2000), the role of MDR3 in multidrug resistance is probably minor and remains to be confirmed.

4.2.2. Multidrug Resistance-associated Proteins (MRPs, ABCCs). The human term placenta expresses at least three members of the MRP family: MRP1, MRP2, and MRP3 (St-Pierre et al., 2000). The localization of MRP1 has been studied using human placental brush-border, basal membrane vesicles and immunohistochemical studies, but the results are controversial, as both apical (St-Pierre et al., 2000) and basal (Nagashige et al., 2003) localization have been reported. MRP2 was clearly detected by both immunofluorescence and western blotting in the apical membranes of syncytiotrophoblasts, while immunoblotting suggested an apical localization facing the maternal blood of MRP3 (St-Pierre et al., 2000). MRPs mRNA have been identified in placental cell lines: MRP1 and MRP2 transcripts were found in the human choriocarcinoma cell line BeWo (Pascolo et al., 2000; Prouillac et al., 2009). Serrano et al. (2007) also reported the comparative mRNA expression of members of the MRP family (MRP1, MRP2, MRP3, MRP4) in human trophoblast and choriocarcinoma cells (BeWo, Jeg-3, JAR). MRP1, MRP2 and MRP3 are known to mediate the transport of various glucuronides, including estradiol and bilirubin, and handle various xenobiotics and their metabolites as substrates (as a review see Deeley et al., 2006). However their role in the
placenta has not been clearly established. It is probably related to the efflux of polar conjugates of xenobiotics or metabolites of endogenous compounds as steroid conjugates.

Protein and mRNA expression, localization, and the function of MRP5 in human placenta have been studied by Pascolo et al. (2003) and Zu Schwabedissen et al. (2005). Mostly located in the basal membrane of syncytiotrophoblasts, and in and around fetal vessels, MRP5 has also been detected in the apical membrane. MRP5 acts as an export pump for cyclic nucleotides, especially cGMP, which play a major role in cytotrophoblast differentiation. Although the role of MRP5 in this process is still unknown, MRP5 has been shown to confer resistance to nucleobase and nucleoside analogs used in anticancer and antiviral therapy, by cellular export of nucleoside monophosphate (Wijnholds et al., 2000).

4.2.3. Breast Cancer Resistance Protein (BCRP, ABCG2). Evidence of a BCRP-mediated transport in the placenta was highlighted by Jonker et al. (2000). Using P-gp-deficient pregnant mice, the authors demonstrated that the co-administration (at gestation day 15) of Bcrp1 inhibitor GF120918 (50 mg/kg, oral administration) increased the fetal concentration of topotecan (a BCRP/Bcrp1 substrate) after drug administration (1 mg/kg, oral administration). Other in vivo studies using Bcrp1-knockout mice demonstrated the transport activity of placental bcrp1 (Enokizono et al., 2007; Zhang et al., 2007) and its role in limiting fetal exposure to phytoestrogen (30 µmol/kg genistein, coumestrol or daidzein, oral administration, 2 weeks gestation) and nitrofurantoin (5mg/kg, retro-orbital injection, 2 weeks of gestation). The feto-protective function of BCRP was highlighted by studies using a human placental perfusion model, demonstrating an increase in the fetal to maternal concentration ratio of glyburide (100 ng/ml perfusion, Pollex E. et al., 2008) and PhIP (2 µM perfusion, Myllinen P. et al., 2008) after pharmacological inhibition of BCRP. The limitation of fetal glyburide distribution by BCRP was also confirmed in mice by Zhou et al. (2008), and in vesicles of human brush border by Gedeon et al. (2008).
The expression of BCRP mRNA is known to be higher in human placenta than in other organs (Allikmets et al., 1998). The corresponding protein is expressed in the apical membrane of syncytiotrophoblasts, and in the fetal vessels of chorionic villi (Maliepaard et al., 2001; Evseenko et al., 2006a; Yeboah et al., 2006). BCRP structure, substrate selectivity and localization in the placenta suggest that, like P-gp, it may have a protective role, removing cytotoxic drugs from fetal tissues.

### 4.3. Solute Carrier (SLC) transporters

#### 4.3.1. Organic Anion-Transporting Polypeptides (OATPs)

Members of the organic anion-transporting polypeptides (OATP/SLCO) are expressed in the placenta (OATP1A2, OATP1B1, OATP-2B1, OATP-3A1, OATP-4A1). These proteins are involved in the Na+-independent uptake of bile acids in hepatocytes. As shown in vitro in transfected epithelial cells, OATP2B1 could be involved in the transepithelial transport of steroid sulfates in human placenta (Grube et al., 2007). The role of other OATPs in placental drug transport is not well known, although they could transport drugs such as methotrexate, antibiotics and NSAID (Mikkaichi et al., 2004, Ugele et al., 2003).

#### 4.3.2. Organic Anion Transporters (OATs)

OAT-4 is the most expressed OAT. It is located in cytotrophoblast membranes and the basal surfaces of syncytiotrophoblasts (Cha et al., 2000; Ugele et al., 2003). Involved in the transport of steroid sulfates that allow estrogen synthesis in the placenta, it is also a multi-specific xenobiotic transporter which could interact with environmental toxins and drugs (You et al., 2004 a,b), and contribute to fetal exposure to xenobiotics.

#### 4.3.3. Organic Cation Transporters (OCTs)

Different organic cation transporters have been identified in the human placenta. The organic cation/carnitine transporter 1 (OCTN1), a member of a subfamily of OCTs (SLC22A subfamily), is expressed on the apical side of human syncytiotrophoblasts (Tamai et al., 1997; Ganapathy et al., 2005). OCTN2 is also
present on the maternal side (microvillous membrane) of trophoblast cells, as shown by Lahjouji et al. (2004). OCTN2 transports L-carnitine in a Na+-dependent manner, from maternal circulation to fetal circulation. It could also mediate the transport of drugs such as antidepressants, amphetamines and β-lactam antibiotics (Grube et al., 2005). OCT3 is a Na+- and Cl- independent monoamine transporter mostly found in the placenta (Kekuda et al., 1998). OCT3 transcript and protein have been detected at the basal membrane of human trophoblast cells (Sata et al., 2005). As it shows a high affinity for monoamines (serotonin, dopamine, norepinephrine and histamine), human OCT3 is considered as an extraneuronal monoamine transporter. However, antidepressants (desipramine, imipramine, amphetamines) could also interact with OCT3.

4.3.4. Monoamine transporters

The serotonin transporter (SERT) and the norepinephrin transporter (NET) have been identified in the placenta at the maternal facing membrane (Prasad et al., 1996; Bzoskie et al., 1997). Both SERT and NET proteins are functionally active in the human placenta at term. Since these transporters critically regulate extracellular monoamine concentrations, they could play a role in maintaining homeostasis in amniotic fluid and fetal circulation (Prasad et al., 1994a): they clear the maternal blood of excessive amounts of catecholamines and serotonine, preventing pre-eclampsia (Bottalico et al., 2004). In addition, the monoamine oxidases A (MAO-A), which allow the intracellular degradation of monoamines after uptake from the extracellular space, are less expressed and less active in placentas from pre-eclamptic pregnancies (Kaaja R.J. et al., 1999; Carrasco G. et al., 2000; Sivasubramaniam S.D. et al., 2002).

Monoamine transporters have been involved in the adverse effects of cocaine and amphetamines, because they are cellular targets for these abusable drugs that block
catecholamine transport. (Ramamoorthy J.D. et al., 1995; Prasad et al., 1996; Bzoskie L., 1997).

4.3.5. Monocarboxylate Transporters (MCTs). There are several isoforms of monocarboxylate transporters (MCT1, MCT3, MCT4, MCT5, MCT7, MCT8) (Price et al. 1998, Settle et al., 2004) and dicarboxylate transporters (NaDC3) (Wang et al., 2000) in the placenta. These proton-coupled transporters are essential for the transport of lactate, ketone bodies and other monocarboxylates through the plasma membrane. They may contribute to the net transport of lactate through the placental barrier (Nagai et al., 2010). MCT8 could be involved in the transport of thyroid hormones and implicated in the development of trophoblast cells and the fetus (Chan et al., 2006). Other substrates are benzoic acid, acetic acid, acetylsalicylic acid and antibiotic cefdinir (Utoguchi et al., 1999).

4.3.6. Equilibrative Nucleoside Transporters (ENTs). Both Na+-independent equilibrative nucleoside transporters ENT1 and ENT2 are expressed in placenta (Barros et al., 1995). Physiological substrates of these transporters are purine and pyrimidine nucleosides but a number of anticancer nucleoside analogs are also substrates (Griffiths et al., 1997).

4.3.7. Folate Transporters. As folates are essential nutrients for cell division and growth, folates deficiencies by insufficient dietary intake could impair fetal development. Besides their involvement in folate transport, these transporters could interact with a variety of antifolates used in the treatment of cancer and immune disorders.

Folate receptor alpha (FRα), localized on the apical side of human placental villi, could be involved in folate uptake from maternal blood to the placenta (Prasad et al., 1994b; Yasuda et al., 2008). Reduced Folate Carrier (RFC-1, SLC19A1) is distributed to both the microvillous plasma membrane of the syncytiotrophoblast and basal syncytiotrophoblast in term-placenta (Solanky et al., 2010; Yasuda et al., 2008), and mediates bidirectional reduced folate...
transport. Recently, a heme carrier protein 1 (PCFT/HCP1, SLC46A1) implicated in folate transport has been characterized in the apical side of the trophoblast.

Most studies on the placental expression of transporters have been performed in various models representing different stages of gestation. Therefore it is very difficult to compare the levels of expression from one stage of gestation to another. Data on the expression of transporters throughout gestation are necessary to understand their importance in fetal development. Indeed, the expression of numerous transporters varies during pregnancy.

5. Variation of transporter expression during pregnancy

The continuous development of placenta defines different stages of pregnancy and results in modifications of permeability, potentially leading to modulation of fetus exposure.

Among ABC transporters, ABCG2, ABCB1, ABCB33 and ABCC1 have been shown to vary during pregnancy.

5.1. ABCG2. Different in vivo studies have shown a clear variation in ABCG2 expression as gestation progresses. Indeed BCRP protein expression and mRNA increased 2-fold in a group of pre-term placentas (28±1 week of pregnancy, 15 placentas), compared to term placentas (39±2 week of pregnancy, 29 placentas) (Zu Schwabedissen et al., 2006). On the contrary, Yeboah et al. (2006) examined BCRP in placenta-tissues at week 6-41 of pregnancy and reported an increase in protein expression at term, although mRNA levels did not change as gestation progressed. In the rat placenta, a decrease in the expression of Bcrp (mRNA and protein) from the mid-stage to the end of gestation was observed (Yasuda et al., 2005). Bcrp mRNA expression increased 3-fold on the 15th day of gestation compared to the 21st (Cygalova et al., 2008). In agreement with the latter study, two recent studies on mouse placenta reported peaked Bcrp1 levels on the 15th gestation day (Wang et al., 2006) and a
gestation-dependent decrease in the mRNA expression of Bcrp1 from the 9\textsuperscript{th} to the 18\textsuperscript{th} day of gestation (Kalabis et al., 2007).

5.2. ABCB1, ABCB3. The mean expression of P-gp measured by the Western blutting method in early (13–14 gestation week) compared to late (full-term) placentas was found to be 2-fold higher (Sun et al., 2005; Gil et al., 2005). In agreement with this finding, Mathias et al. (2005) demonstrated that the P-gp expression protein decreased dramatically as pregnancy proceeded and was lowest at term. On the other hand, Novotna et al. (2004) showed increasing expression of P-gp in rat syncyiotrophoblasts from the 11\textsuperscript{th} to the 18\textsuperscript{th} day of pregnancy. This was in agreement with a possible role in fetal protection soon after the establishment of chorioallantoic placenta and until the end of pregnancy.

Unlike human MDR1, human MDR3 was more abundantly expressed in the last stage of gestation (Patel et al., 2003).

Existing data strongly suggest that ABCG2 and ABCB1 play an important role in protecting the fetus against the potential toxicity of drugs, xenobiotics, and metabolites. The decrease in ABCG2 and ABCB1 at the end of gestation could be related to higher fetal susceptibility to xenobiotic toxicity in the last part of gestation, and higher fetal protection against xenobiotics early in pregnancy. Indeed, the fetus is most vulnerable to xenobiotic toxicity during organogenesis.

5.3. ABCC1. In a study using placental samples (cytotrophoblasts and endothelial cells), MRP1 expression was increased 4-fold in the third trimester, compared to the first trimester (Pascolo et al., 2003). MRP1 was also increased 20-fold in polarized BeWo cells, compared to non-polarized cells.

5.4. OATPs. Among SLC transporters, OATP1A2 and OATP3A1 were down-regulated between the first and third trimester of pregnancy (Patel et al., 2003).

The discrepancies observed in observations in rats, mice and humans suggested different
mechanisms in the regulation of transporter expression among these species. However the molecular mechanisms underlying the modulation of placental ABC and SLC transporters by pregnancy have not been elucidated yet.

5.5. Folate transporters

An increase in the expression levels of FRα, RFC, and PCFT with the progress of gestation in rat placenta has been shown and could play an important role in the response to increased need by placenta and fetus for folate during development (Yasuda et al., 2008).

Due to obvious ethical reasons, studies of fetal risk from maternal exposure to chemicals and pharmaceuticals are not performed in humans. Besides in vivo/ex vivo models, cell lines have been developped to provide important knowledge on the transplacental transfer in humans of new chemical substances and on environmental exposures to hazardous compounds.

The use of non differentiated models, such as choriocarcinoma cell lines, is also an alternative in the study of the expression of transporters during early stages of gestation.

6. The use of cell lines as placental barrier models

The properties and main uses of these cell lines are summarized in table 3.

6.1. Human choriocarcinoma cell lines

6.1.1. BeWo cell line. Developed from a malignant gestational choriocarcinoma of the fetal placenta, the BeWo cell line (Patillo et al., 1968) displays morphological and biochemical enzymes of trophoblasts, showing hormonal secretion properties (hCG, progesterone, placental lactogen) (Pattillo et al., 1979; Nickel et al., 1991) and cytokine expression (IL4, Sacks et al., 2001; IL6 and IL8, Fujisawa et al., 2000; IFNα, IFNβ and IL10, Bennett et al., 1996).
The BeWo cell line is widely used as a model system to study trophoblast differentiation (Hussa et al., 1997; Vogt et al., 1997; Rao et al., 2003; Xu et al., 1999; Rote, 2005), placental metabolism, and nutrient and drug distribution across the placental barrier (Furesz et al., 1993; Moe et al., 1994; Way et al., 1998; Shah et al., 1999; Vardhana et al., 2002; Schmidt et al., 2003; Takahashi et al., 2001; van der Ende et al., 1989; Prasad et al., 1996; Eaton et al., 1998; Ellinger et al., 1999, Ushigome et al., 2000). BeWo cells form a confluent polarized monolayer on a permeable support, as shown by the development of a transepithelial electric resistance and a high density of microvilli on the apical surface of the monolayer (Liu et al., 1997). The b24 and b30 BeWo clones (developed by Dr. Alan Schwartz) have shown better monolayer-forming ability than the original BeWo clone available from the American Type Culture Collection (ATCC) (Bode et al., 2006). This contradicts studies by Parry et al. (2006) and Mark and Waddell (2006) who used the BeWo clone from ATCC as a representative model of transepithelial barrier. The ability of cells to form a monolayer on a permeable support depends on the formation of tight junctions. Many factors such as the choice of media (essential nutrients and growth factors), seeding density, extent of cell confluency, exposure time, environmental conditions, and choice of permeable insert (diameter, pore size, supporting matrix) may influence tight junction development. Validity of the monolayer is assessed by the measuring trans-epithelial electrical resistance (TEER) and determining the transfer of paracellular markers (inulin, sucrose, fluorescein, FITC-dextran, Lucifer yellow) (Liu at al., 1997, Konsoula et al., 2005). BeWo monolayers have been used for transport studies but the latter all report different cell culture conditions (Saunders et al, 2009).

The BeWo cells consist of undifferentiated cytotrophoblasts with few syncytialized cells (Wice et al., 1990). Their differentiation into syncytiotrophoblasts does not occur spontaneously, but the syncytialization process can be induced by forskolin or 8-bromoadenosine 38,58-cyclic monophosphate treatment (Borges et al., 2003). For this reason,
BeWo cells are used for studies on the maturation of trophoblasts: they can model either cytotrophoblasts of early gestation or syncytiotrophoblasts of late gestation. Treatments with syncytium inducers result in an increase of monolayer permeability (Liu et al., 1997), which can be a disadvantage for transfer studies.

BeWo cells constitute a good model for studying the fusion process that occurs during syncytium formation. Envelope proteins such as Syncytin-1, derived from human endogenous retroviruses, seem to play an essential role in these cellular fusion events (see the review by Pötgens et al., 2004). Syncytin-1 expression is up-regulated after stimulating the fusion of primary cytotrophoblast into syncytia by forskolin, a cAMP analogue. Recently, Vargas et al. (2009) demonstrated a more important role for syncytin-2 in trophoblast fusion, showing that syncytin-1 expression changed slightly in BeWo on stimulation, compared to syncytin-2 fluctuation. Moreover, using two different targeted siRNAs for each transcript, the authors demonstrated that a reduction of syncytin-2 expression led to a significant decrease in the number of BeWo cell fusions. Two amino acid transporters ASCT1 and ASCT2 expressed in trophoblast are potential receptors of syncytin-1. The receptor of Syncytin-2 protein is also placenta-specific (Esnault et al., 2008).

ABC efflux transporters including P-gp, MRPs and BCRP are expressed in BeWo cells (table 2). The pattern of expression is similar to that of syncytiotrophoblasts isolated from human placenta. BCRP is highly expressed in BeWo cells, whereas P-gp transcript and protein are minimally expressed or even absent. Both transporters are localized predominantly in the apical plasma membrane and show efflux activity. mRNA of MRP1, MRP2, MRP3, MRP5 and MRP8 are variably expressed, depending on differentiation status (Pascolo et al., 2000, Pascolo et al., 2003, Serrano et al., 2007). Folate transporters (Yasuda et al., 2008) and OCTN2 (Rytting et al., 2005; Rytting et al., 2007) are present, while OATs and MCTs
expression has not been reported up to now. Expression of ABC transporters (BCRP, MRP1, MRP2) is modulated by cell differentiation (Evseenko et al., 2006b; Prouillac et al., 2009).

BeWo cells are stable, easily maintained by trypsinization, and form a confluent monolayer on a permeable support in a relatively short period of time (4–5 days). These advantages make this cell line an attractive in vitro model for studying trans-trophoblast transport and drug metabolism (Poulsen et al., 2009), as shown by numerous studies on the transport of digoxin, vinblastine, vincristine (Ushigome et al., 2000), opioids peptides (Ampasavate et al., 2002), iron (Heaton et al., 2008), and cholesterol (Schmidt et al., 2003) (table 4).

6.1.2. JAR and JEG-3 cell lines. Derived from choriocarcinoma cell lines, they are used in studies of placental function including differentiation, invasion, hypoxia/oxidative stress response, endocrinology, maternal fetal immunology and transplacental transfer. The JEG-3 cells possess many of the biological and biochemical characteristics of syncytiotrophoblasts, although they are mononucleated and proliferative (Matsuo et al., 1994). They produce placental hormones (progesterone, hCG, steroids) (Chou, 1982; Kato et al., 1991) and express various enzymes (Sun et al., 1998; Tremblay et al., 1999). Although they show biochemical differentiation, such as increased hCG secretion, JEG-3 cells do not morphologically differentiate with forskolin treatment (Al-Naisiry et al., 2006). However, JEG-3 cells have a lower rate of proliferation and a higher degree of differentiation than BeWo and JAR cells (Borges et al., 2003; Kitano et al., 2004; White et al., 1988; Aplin et al., 1992).

When compared to cytotrophoblast cells stemming from isolated from human placenta, JAR cells are more similar to non-differentiated cytotrophoblasts (Evseenko et al., 2006a). The JAR cell line is probably an unsuitable model for studies of transepithelial transport (Mitchell et al., 1995), exactly like JEG-3 cells, if one refers to the few studies reporting the use of JEG-3 as a model for vectorial transepithelial transport (Hardman et al., 2007). Indeed, the ability of both cell lines to form a polarized monolayer is controversial (Bode et al., 2006). The
transcriptional profile and protein expression of many transporters, in particular export pumps (P-gp, MRPs and BCRP) and organic anion transporters (OATPs), show quantitative variation in both cell lines (table 2, Serrano et al., 2007).

6.2. Human primary cells

Primary cell lines of undifferentiated cytotrophoblasts can be produced from human placenta. Unless primary cells are allowed to differentiate, they represent early stages of gestation. These nonproliferative, multinucleated cells are able to syncytialize spontaneously. They form aggregates with large intercellular spaces, making studies of polarized transport of xenobiotics impossible when they are grown on semi-permeable membranes (Liu et al., 1997, Yui et al., 1994). Hemmings et al. (2001) reported that primary cytotrophoblasts formed tight junctions after three successive cycles of seeding and differentiation. Although this method produced multiple overlapping layers of syncytialized cells, areas of microvillar projections were formed on the apical surface and functioned as a barrier to low and high-molecular-weight molecules. Besides the fact that obtaining a reproducible cell population may be tricky, primary cell lines also have high contamination levels and are not viable for many passages. They are mostly used in investigations involving parasites or virus (Abbasi et al., 2003).

7. Other models for placental transfer studies

Experimental models have to replicate the full features and functionality of in vivo trophoblast cells. For obvious ethical reasons, in vivo experimentation of placental drug transport in humans cannot be performed. Therefore the risk assessment of fetal injury (teratogenic and fetotoxic potential of xenobiotics) from maternal exposure to xenobiotics is based on results from animal studies. Animal models provide the advantages of a complete physiological system. However, interspecies differences in placental morphology and length of gestation
demand caution when assuming that the maternal-to-fetal transfer of substances observed in animal models will be observed in the same way in humans (Enders et al., 1999; Moe et al., 1995). For these reasons, mechanisms of placental transport, metabolism and placental toxicity are best investigated in models of human origin (Myllynen et al., 2002). Primates such as rhesus macaques and baboons have also been used to evaluate the transfer of compounds since their haemochorial placentation is similar to that of humans (Patterson et al., 2000). Recently, an original study has reported the use of positron emission tomography imaging to monitor the placental P-gp function during pregnancy in non-human primates (Chung et al., 2010).

7.1. Feto-maternal blood concentration ratio. Human maternal and umbilical cord blood are simultaneously sampled during labor. Further analysis for the presence of compounds constitutes a simple and ethical method that illustrates the drug concentration between both circulations. However, the lack of comparison with a proper reference compound, the difficulties in evaluating the influence of placental metabolism on drug transfer and the lack of information on drug distribution and accumulation in tissue are definite drawbacks (Waddell et al., 1981; Levy, 1981; Chamberlain, 1986; Simone et al., 1994).

7.2. Human coelocentesis. This alternative to fetal blood sampling was introduced in 1991 (Jauniaux et al., 1991). It consists of sampling from human exocoelomic and amniotic fluid by transvaginal puncture at week 6-10 and week 7-12 respectively (Jauniaux et al., 2000). This method does not allow building a kinetic model which is necessary to extrapolate quantitative drug transfer.

7.3. Perfused single human placental cotyledon model. This technique of ex vivo perfusion of human placenta was first described in 1967 by Panigel et al., then modified by Schneider et al. (1972). It is commonly used to investigate the mechanism and predict the rate of transfer of drugs through the placenta in vivo (Bourget et al., 1995). This model has been used to
elucidate the mechanisms of placental transfer of HIV protease inhibitors and anti-epileptic drugs (Forestier et al., 2001; Myllynen et al., 2003). Ethical problems are minimal because this technique is non invasive and placentas are discarded or incinerated after birth. The critical point of the model is that it does not represent the first trimester placenta, when the fetus’ susceptibility to toxic hazards is high. However, since placental thickness and the number of cell layers decrease at the end of pregnancy, it is possible that the term placenta might be more sensitive to environmental agents than first anticipated (Vähäkangas et al., 2006). Another disadvantage is possible underestimation of the contribution made by transporters and metabolism during pregnancy, since transporters and metabolism differ between first trimester and full term placenta. Inter-individual variation also raises the question of the number of placentas that have to be perfused to validate the method. However, the influence of placental metabolism, the preferential direction of transport and the presence of overall active transport can be evaluated. Results are generally compared with data on reference compounds such as inulin or antipyrine.

7.4. Human trophoblast tissue preparations. Tissue explants can be used to study the transport of substances from the maternal circulation into the syncytiotrophoblast, as well as the metabolism, endocrine function, enzyme function, cellular proliferation and differentiation in the placenta at early gestation or near term (Miller et al., 2005). This method requires taking into careful consideration the potential contribution of mesenchymal and endothelial cells to the metabolic process (Syme et al., 2004). Moreover, a recent publication has reported the limits of this model: the functional characterization of individual transporters is difficult, due to the lack of selective substrates and/or specific inhibitors. Furthermore, the authors suggested the presence of compensatory regulatory mechanisms, which could explain the difficulty of performing a good kinetic transfer study (Vaidya et al., 2009).
7.5. Membrane vesicles. Isolated membrane vesicles can be obtained from either the membrane of the brush border, or the basal surface of the trophoblast. Therefore the study of transport mechanisms is possible in both fetal and maternal plasma membranes (Bissonnette et al., 1982; Murer et al., 1980). This model enables characterizing the expression and functionality (susceptibility to inhibitors, specificity, saturability) of transporters, but does not reflect the in vivo situation, due to a lack of regulatory factors.

8. Conclusions and perspectives

The expression of both ATP and SLC transporters has been demonstrated in many polarized epithelial barriers (i.e. kidney, liver, blood-brain barrier, intestine, lung, etc.) and many different species. Cellular localization combined with knowledge of the protein function gives insight into the role of these proteins in the accumulation and removal of specific substrates from the cells at each barrier. However, despite the considerable efforts invested in studying these transporters, few works have been dedicated to the cellular localization of these transporters in the placenta. Moreover, too little information is available on the mechanisms of fetal exposure to pollutants and the role of the placenta in this exposure.

Firstly, placental models efficient for studying xenobiotic transport are far from being fully characterized. Better knowledge of cell properties (content in xenobiotic metabolism enzymes and transporters, number of passage, phenotype, differentiation) is required and numerous external factors must be considered and optimized (Audus et al., 1990):

- culture conditions (culture medium composition, cell seeding density, stage of differentiation);
- permeable support characteristics (diameter, pore size, supporting matrix like collagen);
- transport study conditions (transport medium composition, pH, temperature, etc.);
- diffusion apparatus (stirring or not).
In vitro placental transfer studies show high variability, occasionally leading to controversial results. Further investigation into transporter activity and membrane localization in the polarized placental epithelium will help to define the physiological and pharmacological functions of transporters in placental cells.

Secondly, the modulation of the expression of placental transporters may influence their role. Besides variation of expression due to the gestation stage, in vivo genetic variability may also exist in xenobiotic transport by placenta. This is due to the presence of single nucleotide polymorphisms (SNPs), as has been shown for ABC transporter expression. This genetic polymorphism has been described for placental P-gp (Tanabe et al., 2001) and BCRP (Kobayashi et al., 2005), and associated with altered P-glycoprotein expression (Hitzl et al., 2004). However, it remains to be determined whether these SNPs influence the pharmacokinetic and dynamic properties of clinically useful drugs. Rahi et al., (2008) reported that although altering the expression levels of P-gp in the human placenta, SNPs did not have any consequences on P-gp-mediated placental transfer of saquinavir. On the contrary, recent studies have shown that some homozygous P-gp variants (C1236T, C3245T) are associated with an increase in placental P-gp efflux of paclitaxel (Hemauer et al., 2010). Transfected cell lines expressing the polymorphic variant (Q141K) of ABCG2 exhibit altered placental pharmacokinetics of glyburide (Pollex et al., 2010). Further investigations are needed to define the significance of SNPs in the placental transport of xenobiotics.

Thirdly, in addition to the trophoblast cells, human fetal membranes such as the amnion, chorion and yolk sac, may constitute an additional site of drug transfer in pregnant humans and rodents. Data are very scarce on transporter expression and function in these fetal annexes. The amnion can be considered as a barrier separating maternal and fetal compartments and expresses functional transporter proteins such as MRPs and BCRP (Aye et al., 2007), as well as the chorion, which expresses BCRP (Yeboah et al., 2008). RFC protein
is expressed in the yolk sac (Maddox et al., 2003), and the existence of xenobiotic efflux transporters in extraembryonic fetal membranes has recently been demonstrated in mice (Aleksunes et al., 2008; Kalabis et al., 2007). It is now necessary to study the contribution of fetal membranes in the transfer of xenobiotics between the mother and the fetus, opening up a new field of research.

ABC and SLC proteins are able to transport nutrients as well as xenobiotics. These xenobiotics may compete with the physiological substrates of the placental transporters, and interfere with the delivery of nutrients to the fetus. Future studies should deal with the possibility of an interaction that alters exchanges between fetus and mother. Exchange modulation may have consequences on the growth and development of the fetus, a crucial stage where small alterations may lead to long-term modulation of metabolic regulation and programming.
References


Bièche I, Narjoz C, Asselah T, Vacher S, Marcellin P, Lidereau R, Beaune P, de Waziers I (2007) Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP)1,


FIGURE LEGEND

Figure 1: Schematic drawing of human placental structure. a) Transverse section through a full-term placenta. b) Localization and structure of human placental barrier
Table 1: Metabolism enzyme expression as a function of pregnancy stage and placental models (+: positive signal, ++: strong positive signal, -: negative result; nd: no data available)

<table>
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<tr>
<th>CYP isoform</th>
<th>mRNA level</th>
<th>Protein level</th>
<th>Enzyme Activity</th>
<th>Reference</th>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>++</td>
<td>-</td>
<td>nd</td>
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</tr>
<tr>
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<td>+</td>
<td>nd</td>
<td>+</td>
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</tr>
<tr>
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<td>-</td>
<td>nd</td>
<td>Pasanen et al. (1997)</td>
</tr>
<tr>
<td>CYP2B</td>
<td>-</td>
<td>-</td>
<td>nd</td>
<td>Bièche et al. (2007)</td>
</tr>
<tr>
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<td>nd</td>
<td>nd</td>
<td></td>
</tr>
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<td>+</td>
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<td><strong>Full term placenta</strong></td>
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<td></td>
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</tr>
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<td>CYP19</td>
<td>++</td>
<td>nd</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>JAR cells</strong></td>
<td>CYP19</td>
<td>+</td>
<td>+</td>
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<td>nd</td>
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<td></td>
<td>CYP2C9</td>
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<td>nd</td>
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<td>nd</td>
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Table 2: Comparison of the polarized distribution and relative expression at mRNA/protein level (immunohistochemistry, Western blot and functionality) of placental transporters relevant to drug disposition across the maternal-fetal interface. +: positive signal; ++: strong positive signal; -: negative result; nd: no data available; a: limit detection.

<table>
<thead>
<tr>
<th>Localization</th>
<th>Transporter</th>
<th>Placenta</th>
<th>BeWo</th>
<th>Jeg-3</th>
<th>JAR</th>
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<td>Sata et al. 2005</td>
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<tr>
<td></td>
<td>MCT-1/SLC16A1</td>
<td>+/-</td>
<td>nd/nd</td>
<td>nd/nd</td>
<td>nd/nd</td>
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<tr>
<td>Unknown or controversial localization</td>
<td>MRP1</td>
<td>++/+</td>
<td>++++</td>
<td>++/+</td>
<td>++/</td>
<td>St Pierre et al., 2000; Nagashige et al., 2003; Evseenko et al., 2006</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>MRP3</td>
<td>++++</td>
<td>+/nd</td>
<td>+/nd</td>
<td>+/nd</td>
<td>+/nd</td>
<td>St Pierre et al., 2000</td>
</tr>
<tr>
<td>MRP4</td>
<td>+/nd</td>
<td>+/nd</td>
<td>++/nd</td>
<td>+/nd</td>
<td>+/nd</td>
<td>Serrano et al., 2007</td>
</tr>
<tr>
<td>MCT-8</td>
<td>+/-</td>
<td>nd/+</td>
<td>nd/nd</td>
<td>nd/nd</td>
<td>nd/nd</td>
<td>Utoguchi et al., 1999; Chan et al. 2006</td>
</tr>
<tr>
<td>MCT3, MCT5, MCT7</td>
<td>+/nd</td>
<td>nd/nd</td>
<td>nd/nd</td>
<td>nd/nd</td>
<td>nd/nd</td>
<td>Price et al., 1998; Settle et al. 2004</td>
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<td>hENT2/SLC29A2</td>
<td>nd/+</td>
<td>nd/nd</td>
<td>nd/nd</td>
<td>nd/nd</td>
<td>nd/nd</td>
<td>Barros et al., 1995</td>
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### Table 3: Properties and use of placental cell lines

<table>
<thead>
<tr>
<th></th>
<th>BeWo</th>
<th>Jeg-3</th>
<th>JAR</th>
<th>Primary placental cells</th>
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<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>Choriocarcinoma cells undifferentiated</td>
<td>Choriocarcinoma cells derived from BeWo</td>
<td>Choriocarcinoma cells</td>
<td>Normal trophoblast Contamination by other</td>
</tr>
<tr>
<td></td>
<td>cytotrophoblasts Mononucleated and</td>
<td>Mononucleated and proliferative</td>
<td>Early placental trophoblast</td>
<td>cell types (endothelial cells, etc.)</td>
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<tr>
<td></td>
<td>proliferative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Differentiation</strong></td>
<td>Not Spontaneously (forskolin, c-AMP</td>
<td>Not spontaneously (forskolin, c-AMP</td>
<td>Morphological differentiation</td>
<td>Spontaneously (Morrish D.W. et al., 1997)</td>
</tr>
<tr>
<td></td>
<td>analog)</td>
<td>analog)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biochemical and morphological differentiation</td>
<td>Only biochemical differentiation</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(Al-Nasiry et al., 2006)</td>
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<tr>
<td><strong>Hormonal secretion</strong></td>
<td>Progesterone, β-hCG, Steroids, placental lactogen</td>
<td>Progesterone, β-hCG, Steroids (Chou, 1982)</td>
<td>Progesterone, β-hCG, Steroids (Kato et al., 1991)</td>
<td>Progesterone, β-hCG, steroids (Morrish D.W. et al., 1997)</td>
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<tr>
<td></td>
<td>(Pattillo et al., 1979; Nickel et al., 1991; Prouillac et al., 2009)</td>
<td></td>
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</tr>
<tr>
<td><strong>Ability to form confluent monolayer on permeable support</strong></td>
<td>Yes (Liu et al., 1997)</td>
<td>Controversial (Hardman et al., 2007; Bode et al. 2006)</td>
<td>Controversial (Mitchell et al., 1995; Bode et al. 2006)</td>
<td>Controversial (Hemming et al., 2001)</td>
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<tr>
<td><strong>Use</strong></td>
<td>Transplacental transport of drug (Saunders, 2009)</td>
<td>Gene expression (Rena et al., 2009)</td>
<td>Apoptosis (Saulsbury, 2008)</td>
<td>Gene expression Physiological stimuli, hormone secretion Apoptosis, parasites, virus (Abbasi et al., 2003; Kilani et al., 2007)</td>
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<td></td>
<td>Trophoblast differentiation (Nampoothiri L. et al., 2007)</td>
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Table 4: Studies of therapeutic agents, abused drugs and food contaminants transfer using in vivo and in vitro models of placenta

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<th>Perfused human placenta</th>
<th>In vivo transplacental transfer</th>
<th>BeWo</th>
<th>Jeg-3</th>
<th>JAR</th>
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<td>Intracellular accumulation</td>
<td>Vectorial transport</td>
<td>Intracellular accumulation</td>
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<td>Physiological molecules</td>
<td>ND</td>
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<td>Amino acid $^{(3)}$,</td>
<td>ND</td>
<td>Copper $^{(24)}$</td>
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<td></td>
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<td>Glucose $^{(6)}$,</td>
<td></td>
<td>Folic acid $^{(22)}$</td>
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<td>Glucocorticoids $^{(7)}$,</td>
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<td></td>
<td></td>
<td></td>
<td>Folic acid $^{(7)}$,</td>
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<td>Fatty acid $^{(8)}$,</td>
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<td>Transferrin $^{(9)}$,</td>
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<td>Serotonin $^{(10)}$,</td>
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<td>Bilirubin $^{(11)}$,</td>
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<td></td>
<td></td>
<td></td>
<td>Choline $^{(11)}$,</td>
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<td></td>
<td>IgG $^{(11)}$,</td>
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<td>Cholesterol $^{(12)}$,</td>
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<td>Leptin $^{(13)}$</td>
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<td>Abused agents</td>
<td>Cocaine, alcohol,</td>
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<td>nicotine, morphine</td>
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<td>Medicinal drugs</td>
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<td>Antidepressants,</td>
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<td>Antibiotics, Anti-acid,</td>
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<td>Antidiabetic Anaesthetics, Anti-epileptic, Heart condition</td>
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<td>Pregnancy related treatment $^{(1,2)}$</td>
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<td>Antiviral drugs,</td>
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<td>Antimitotic, colchicines, HIV inhibitors,</td>
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<td>Antidepressants,</td>
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<td>Antibiotics, Anti-acid,</td>
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<td>Antidiabetic Anaesthetics, Anti-epileptic,</td>
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<td>Heart condition $^{(1)}$</td>
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<td></td>
<td>Levofloxacin $^{(3)}$,</td>
<td>Riboflavin $^{(4)}$</td>
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<td>Valproic acid $^{(14)}$,</td>
<td>Opioid peptides $^{(15)}$,</td>
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<td>Vincristine, vinblastine, digoxin $^{(16)}$,</td>
<td>Monocarboxylic acid $^{(17)}$</td>
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<td>Nitrofurantoin $^{(23)}$</td>
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<td>Food contaminant</td>
<td>Caffeine, Glyphosate,</td>
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<td>Benzoic acid $^{(19)}$,</td>
<td>2-amino-1-methyl-6-phenylimidazo[4,5-</td>
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<td>Caffeine, Glyphosate,</td>
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<td>Benzoic acid $^{(18)}$</td>
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Figure 1a
Figure 1b

Chorionic villous
Intervillous space
Syncytiotrophoblast (SCT)
Cytotrophoblast (CT)
Umbilical artery
Umbilical vein
Chorion
Maternal tissue
Conjonctival tissue
SCT
CT
Intervillous space
Maternal erythrocyte
Fetal capillary

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