Endocytosis in the placenta: An undervalued mediator of placental transfer

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Abstract

Endocytosis is an essential mechanism for cellular uptake in many human tissues. A range of endocytic mechanisms occur including clathrin-dependent and -independent mechanisms. However, the role of endocytosis in the placenta and the spatial localisation of individual mechanisms is not well understood. The two principal cell layers that comprise the placental barrier to maternal-fetal transfer are the syncytiotrophoblast and fetal capillary endothelium. Endocytic uptake into the syncytiotrophoblast has been demonstrated for physiological maternal molecules such as transferrin-bound iron and low density lipoprotein (LDL) and may play an important role in the uptake of several other micronutrients, serum proteins, and therapeutics at both major placental cell barriers. These mechanisms may also mediate placental uptake of some viruses and nanoparticles. This review introduces the mechanisms of cargo-specific endocytosis and what is known about their localisation in the placenta, focussing predominantly on the syncytiotrophoblast. A fuller understanding of placental endocytosis is necessary to explain both fetal nutrition and the properties of the placental barrier. Characterising placental endocytic mechanisms and their regulation may allow us to identify their role in pregnancy pathologies and provide new avenues for therapeutic intervention.

Keywords: syncytiotrophoblast, hFcRn, TfR1, caveolin, clathrin, albumin

Highlights:

- Endocytosis contributes to placental uptake of maternal micronutrients and serum proteins
- Endocytosis may have a role in placental uptake of viruses, drugs, and nanoparticles
- The role of endocytosis in the placenta is understudied and needs further research

1. Introduction

The placenta ensures fetal growth and health by mediating nutrient uptake from maternal blood and waste removal from fetal blood, and by providing a protective barrier to toxins and pathogens. Placental transfer by passive diffusion or transfer via membrane transporters have been researched extensively within the placenta, whilst contributions by alternative mechanisms are not well understood [1].

To cross the human placenta, cargoes must traverse multiple layers within the villi to reach the fetal blood. Villi are surrounded by a multinucleate epithelial layer in direct contact with maternal blood called the syncytiotrophoblast. The syncytiotrophoblast is the outermost layer of the placental villi and is the first barrier to maternal-fetal transfer. Cytotrophoblast cells form an incomplete layer underneath the syncytiotrophoblast. Three layers of connective tissue separate the trophoblast from the fetal capillaries: the trophoblast basal lamina, stroma, and vascular basal lamina. The final barrier to maternal-fetal transport is the fetal capillary endothelium. Endocytosis is likely to be most important for uptake in the syncytiotrophoblast and fetal capillary endothelium as these are the primary transport barriers.

Transfer across the placenta can be mediated by para- or transcellular diffusion, by membrane transporters or by endocytosis. Small non-polar molecules such as oxygen can readily diffuse across the placenta via transcellular routes. There is also a poorly defined size selective paracellular route across the placental syncytiotrophoblast which allows diffusion of hydrophilic compounds [2]. Transfer of nutrients and wastes is thought to be primarily mediated by membrane transporters. Membrane transport of a molecule across the placenta requires transport across both the apical and basal plasma membranes of the syncytiotrophoblast and potentially across the fetal capillary endothelium [3]. While endothelial transfer is less well understood it is likely that water soluble molecules like glucose and amino acids can diffuse through endothelial cell-cell junctions while lipophilic or large molecules may require transcellular transport. The final and least well characterised mechanism is endocytosis. Endocytosis mediates uptake of substances into the placenta which can then be transported to the fetus by basal membrane transporters or coupled to transcytosis to deliver cargo to the fetus.

Endocytosis defines a class of regulated active transport mechanisms with varying levels of specificity and capacity that all drive the internalisation of cargoes into vesicles within the cell. Despite the likelihood that these endocytic mechanisms play a critical role in the regulated transfer of diverse cargoes across the placenta, they are often overlooked as a significant placental/maternal-fetal transport mechanism.

Well-established endocytic and transcytotic cargoes in the placental syncytiotrophoblast include transferrin-bound iron and immunoglobulin G (IgG). There has been little research into endocytosis in the fetal capillary endothelium, which is another major cellular barrier to maternal-fetal transfer where endocytic mechanisms are also likely to regulate uptake. Specific modes of endocytosis such as clathrin-mediated endocytosis are known to occur in the syncytiotrophoblast and there is evidence for caveolae-mediated endocytosis in the fetal capillary endothelium [4,5]. Further work is required to

establish the full range of endocytic mechanisms in the human placenta and the entire complement of cargoes transported in this way.

This review will summarise how various cargo-specific endocytic mechanisms may contribute to placental function and simultaneously expose the current lack of understanding of placental endocytosis, highlighting the requirement for more research in this field. While specific aspects of endocytosis in the placenta have been reviewed previously [6,7], this review takes a broader approach and poses questions about the range of endocytic mechanisms in the placenta and their localisation (Figure 1). Although endocytosis is also likely to play an important role in transport across the endothelium, most research to date has focused on the syncytiotrophoblast which will be the primary focus here.

2. Endocytosis in the placenta

Endocytosis involves internalisation of a region of plasma membrane which allows uptake of extracellular cargo and/or membrane-bound cargo. Endocytosis can be non-specific through fluid phase uptake or specific via receptor-mediated endocytosis. Macropinocytosis is the large-scale non-specific fluid phase endocytosis of cargo. Receptor-mediated endocytosis is a selective endocytic mechanism that begins with the interaction between a plasma membrane receptor and its cognate ligand. Clathrin- and caveolae-mediated endocytosis are examples of receptor-mediated endocytosis, utilising the intracellular proteins clathrin and caveolin respectively to form coated vesicles. Phagocytosis also relies on plasma membrane receptor engagement with target molecules alongside remodelling of the actin cytoskeleton to facilitate plasma membrane extension. However, this mechanism functions on a much larger scale to take up foreign particles and apoptotic host-cells.

2.1 Clathrin-mediated endocytosis

Clathrin-mediated endocytosis mediates placental uptake of a range of cargoes including transferrin-bound iron, albumin and Zika virus (Table 1). Clathrin-mediated endocytosis regulates cargo uptake through specific membrane-receptor interactions, with receptor-internalisation also regulating membrane activity [8]. Clathrin-mediated endocytosis can be identified in electron micrographs by the bristle coat bordering pits and vesicles [9]. This coat is formed from clathrin triskelion's that construct cages around the vesicle [8]. Adaptor proteins like the adaptor protein complex 2 (AP2) interact with both clathrin and transmembrane receptors (e.g. the transferrin receptor TfR1 and multi-ligand receptor megalin), and together with accessory proteins support vesicle and coat formation [8]. The GTPase dynamin catalyses scission of the vesicle from the plasma membrane which is followed by vesicle uncoating and delivery to the early endosome, whose more acidic pH allows ligands to dissociate from receptors which can be recycled to the plasma membrane by the recycling endosome or sorted to the lysosome for degradation [10].

Clathrin-mediated endocytosis occurs in the placental syncytiotrophoblast, with coated pits observed at the syncytiotrophoblast microvillous membrane [11]. Proteomic analysis demonstrates clathrin heavy

chain 1 protein expression in the syncytiotrophoblast microvillous membrane, and immuno-localisation shows clathrin localised to the microvillous membrane [4,12]. Consistent with this observation, the contents of coated vesicles from term placenta include transferrin and IgG demonstrating a functional role for clathrin-mediated endocytosis in the syncytiotrophoblast [13].

Endocytosis mediated by the megalin receptor directs internalisation of a broad range of ligands into clathrin-coated vesicles [14]. Megalin is frequently co-expressed with the cubilin receptor where the two can function synergistically or independently of one another to endocytose ligands [14]. Megalin and cubilin bind serum proteins like albumin, and through these interactions mediate the uptake of serum protein-bound cargo [14]. Protein and gene expression for megalin (*LRP2*) and cubilin (*CUBN*) has been demonstrated in first, second, and third trimester placentas [15]. Megalin and cubilin have been localised to the syncytiotrophoblast and cytotrophoblast, with some evidence indicating megalin localised primarily to the syncytiotrophoblast microvillous membrane [4,15]. This suggests these receptors may have a role in uptake of megalin ligands such as albumin, vitamin D binding protein, lipoproteins and transcobalamin into the syncytiotrophoblast.

2.2 Caveolae-mediated endocytosis

Uptake via caveolae-mediated endocytosis is not well described in the placenta but has been implicated in the transfer of Zika virus and its role may be more prominent in the endothelium [16]. The structures generated, caveolae, are flask-shaped invaginations of the plasma membrane. These are characterised by high levels of membrane cholesterol, sphingolipids and integral membrane caveolin proteins which aid membrane curvature [17]. Following membrane invagination, caveolin-rich vesicles are excised from the membrane by dynamin [17].

Caveolin 1 protein and caveolae have been detected in isolated term cytotrophoblasts, but levels decrease after several days in culture as they syncytialise [18]. At term, caveolin 1 protein can be detected in the fetal capillary endothelium, pericytes, and stromal cells, but little or no caveolin 1 has been detected within the syncytiotrophoblast [5,18]. Similarly, flask-shaped structures characteristic of caveolae can be seen by electron microscopy in the fetal capillary endothelium but not syncytiotrophoblast [18]. Caveolae-mediated endocytosis may have a role in mediating cargo uptake in the fetal capillary endothelium, consistent with the caveolae-mediated transport of macromolecules like albumin and LDL in the capillary endothelium elsewhere [19].

2.3 Clathrin/caveolae-independent endocytosis

There are a number of clathrin- and caveolae-independent mechanisms, for example flotillin-dependent endocytosis which employs flotillin-1 and -2 proteins that in some regards resemble caveolins [20]. Flotillins are concentrated in plasma membrane lipid rafts where they drive membrane invaginations and are necessary for endocytosis of molecules including membrane-bound receptors such as CD59 [20]. Another example is the clathrin-independent carriers (CLIC) and GPI-anchored protein-enriched early endosomal compartment (GEEC) pathway. The CLIC/GEEC pathway endocytoses

glycosylphosphatidylinositol-anchored (GPI-AP) proteins in uncoated tubulovesicular membrane structures which contributes to fluid uptake [17].

Clathrin- and caveolae-independent mechanisms are poorly characterised within the placenta. Immuno-localisation of flotillin-1 and -2 at term was found in the cytotrophoblast and fetal capillary endothelium, but very little immunoreactivity was seen in the syncytiotrophoblast [20]. Direct evidence of the CLIC/GEEC pathway in the syncytiotrophoblast is yet to be demonstrated. The CLIC/GEEC pathway is the endocytic mechanism used by GPI-modified folate receptors which are highly expressed in the syncytiotrophoblast microvillous membrane [21,22]. It is therefore plausible that endocytosis via the CLIC/GEEC pathway plays a functional role in cargo uptake into the syncytiotrophoblast.

3. Cargo taken up by endocytosis in the placenta

The placental uptake of nutrients, hormones, serum proteins, and xenobiotics by endocytosis has been investigated: a summary of these can be found in Table 1. Studies have begun to characterise the endocytic mechanisms utilised by endogenous and exogenous cargo to enter and cross the human placenta.

3.1 Physiological maternal molecules transported by endocytosis

IgG

The neonatal Fc receptor (hFcRn) is expressed in the syncytiotrophoblast and fetal capillary endothelium [23]. hFcRn plays an essential role in the transport of IgG across the human placenta as demonstrated in the *ex vivo* perfused placenta [24]. IgG has high affinity for hFcRn in the acidic pH environment of the endosome, while being unable to bind at neutral pH at the cell surface [24]. The initial uptake of IgG at the apical syncytiotrophoblast membrane may occur through fluid-phase endocytosis or selectively via an unidentified receptor interaction. The mechanism of IgG transport across the fetal capillary endothelium is yet to be fully understood but may involve the hFcRn receptor and/or the Fc gamma receptor IIb (*FCGR2B*) which are both expressed at the fetal capillary endothelium [23,25].

Albumin

Albumin is a serum protein that binds a variety of cargo in the blood. Albumin is a known ligand of megalin and cubilin which are expressed in the syncytiotrophoblast [15,26]. Current evidence from *ex vivo* placental explant culture suggests that albumin uptake into the syncytiotrophoblast involves a clathrin-mediated route, with partial contribution by megalin [4]. Uptake is significantly reduced at 4°C indicative of an energy-dependent mechanism such as endocytosis [4]. Albumin transports essential placental cargo such as vitamin D and fatty acids but also potentially toxic substances like pharmaceutical drugs [27]. Once taken up by the placenta, albumin-bound cargo is released to intracellular compartments while albumin can be recycled to the maternal blood or degraded within the syncytiotrophoblast for harvesting essential amino acids [4].

Lipoproteins and cholesteryl esters

Cholesterol forms an important component of cell membranes and is a precursor for synthesis of steroid hormones. Uptake of lipoproteins may provide cholesteryl esters to the placenta and fetus, and may also provide a mechanism for the delivery of fatty acids to the fetus [28]. The fetus synthesises cholesterol *de novo* from 20-weeks gestation but maternal cholesterol is required to meet fetal demand [29]. Placental transport of maternal cholesterol has been demonstrated in first trimester and term placenta [29,30]. The placenta expresses receptors including the LDL receptor (LDLR) and scavenger receptor class B type I (SR-BI), and may use both mechanisms to internalise maternal lipoproteins [28,30]. LDLR receptor-mediated LDL endocytosis has been demonstrated in cultured primary trophoblasts [31]. Delivery of cholesterol to the fetal blood then requires transport out of the syncytiotrophoblast across the basal membrane which may involve transporter-mediated mechanisms [32].

Thyroid hormones

Maternal thyroid hormones T3 and T4 are important for fetal neurodevelopment. Thyroid hormones are transported in the blood bound to serum proteins including thyroxine-binding globulin, albumin, and transthyretin (TTR). Clathrin-mediated endocytosis of albumin is likely to admit bound-cargo including T3 and T4 into the syncytiotrophoblast. Fluorescently-labelled TTR is internalised into vesicular structures in JEG-3 choriocarcinoma cells and *ex vivo* cultured term villous tissue implicating endocytosis as an uptake route [33]. The presence of T4, thought to enhance TTR tetramerization, significantly increased TTR uptake into JEG-3 cells suggesting T4-driven tetramerization may be a prerequisite for receptor-binding and uptake [33]. To facilitate fetal neurodevelopment, thyroid hormones must be transported out of the syncytiotrophoblast toward the fetal blood. Efflux may be mediated by the known thyroid hormone transporter T-type amino acid transporter 1 (TAT1), expressed at the syncytiotrophoblast basal membrane [3].

Serum TTR also associates with retinol-bound retinol binding protein (RBP), but this does not enhance TTR uptake [33]. Retinol-RBP uptake is reportedly mediated by a non-endocytic mechanism via the STRA6 receptor expressed in the human placenta, which is thought to bind RBP thereby releasing retinol to diffuse across the membrane through a hydrophobic cleft in the receptor [34].

Iron

Iron (Fe) has important roles in oxygen transport in complex with haemoglobin and is a cofactor for numerous enzymes. Transferrin-bound iron is thought to utilise clathrin-dependent receptor-mediated endocytosis courtesy of the transferrin receptor TfR1 which is expressed at the syncytiotrophoblast microvillous membrane [35]. Following delivery to the early endosome, Fe^{3+} is likely to be released from transferrin and reduced to Fe^{2+}, released into the cytoplasm, and subsequently transported across the basal membrane by ferroportin 1 [35]. However, despite this explanation of transferrin endocytosis at the syncytiotrophoblast being generally well-accepted, much of the understanding has come from choriocarcinoma cell culture therefore additional research is required in more physiological models of the human placenta [35]. It is not clear how Iron crosses the fetal capillary endothelium; although there

is some evidence of TfR1 in the fetal capillary endothelium, supporting a possible contribution by endocytosis in this process [35].

Folate (vitamin B9)

During pregnancy, folate (vitamin B9) requirements increase in order to meet the needs of the developing fetus. Placental folate uptake may be mediated by endocytosis and transporter mediated routes. The folate receptors, a family of GPI-AP receptors, are believed to endocytose folate through a clathrin-, caveolae- and dynamin-independent mechanism [21]. Folate receptor α, a member of this family, is highly expressed at the syncytiotrophoblast microvillous membrane [22]. In the BeWo cell line folate uptake was suggested to occur by an endocytic mechanism as a result of inhibition by the endocytic inhibitor monensin (Table 2) [36]. Folate is subsequently transported across the basal membrane to the fetus. This may be mediated by the multidrug resistance protein 1 (MRP1) which is localised to the syncytiotrophoblast basal membrane [22].

Riboflavin (vitamin B2)

Riboflavin participates in essential cellular redox reactions. Its uptake into the placental syncytiotrophoblast may occur by clathrin-mediated endocytosis. In BeWo cells co-localisation of riboflavin with clathrin- and Rab5-positive endosomes was demonstrated [37]. In addition there was a requirement for activity of the GTPase dynamin in the uptake process, together indicating an endocytic uptake mechanism [37]. However, these observations need to be confirmed in primary trophoblast or villous tissue. To exit the syncytiotrophoblast toward the fetal blood, riboflavin transport across the basal membrane may be mediated by the riboflavin transporter RFVT1 which is expressed in human placenta and is typically localised to the basal membrane of polarised epithelia [38,39].

Vitamin B12

During pregnancy, fetal delivery of vitamin B12 is essential for proper neurodevelopment. Vitamin B12 is transported in the blood bound to transcobalamin II, and uptake into cells occurs through receptor-mediated endocytosis of vitamin B12-bound transcobalamin via the transcobalamin II receptor *CD320* [40]. This receptor has been isolated and purified from human placental plasma membranes, although its role at each transport barrier is yet to be demonstrated [40]. Investigations in mouse models have demonstrated TCblR receptor-mediated endocytosis of transcobalamin-vitamin B12 in the placenta and have also suggested an additional route via the megalin receptor but this has not been investigated in the human placenta [41]. Subsequently, vitamin B12 delivery to the fetus may be facilitated by MRP1-mediated transport out of the syncytiotrophoblast across the basal membrane [42].

Vitamin D

Sufficient fetal supply of vitamin D is required for proper fetal bone development. Vitamin D is transported in the blood bound to vitamin D binding protein (DBP) or albumin. In the renal proximal tubule re-uptake of vitamin D has been demonstrated via megalin and cubilin receptors. As these receptors are expressed at the placental syncytiotrophoblast they may mediate vitamin D uptake in the

placenta [15]. The use of pharmacological inhibitors in *ex vivo* placental explants has provided some evidence that vitamin D uptake into the placenta is mediated by endocytosis [43]. Further research is required to establish the precise molecular mechanism(s) responsible. How vitamin D is transported across the basal membrane toward the fetus is not known but could involve transporter mechanisms or simple diffusion.

Summary of placental serum protein and micronutrient endocytosis

The mechanism of endocytic uptake into the syncytiotrophoblast for some physiological maternal cargo are well-established, including clathrin-dependent receptor-mediated transferrin-bound iron uptake. However, the mechanisms of endocytic uptake for other cargo, including many micronutrients, is not well understood. The fetus is dependent upon micronutrient transfer for growth and development, so impaired transfer may underpin fetal growth restriction and developmental disorders, for instance impaired folate delivery may result in neural tube defects. More thorough research of the endocytic machinery expressed in placenta is required to advance our understanding of these processes.

3.2 Exogenous particles

Exogenous particles can also exploit endocytic mechanisms to cross the placenta and reach the fetus. This includes environmental particulates, pathogens and drugs intended for maternal treatment which can have potentially harmful effects on the developing fetus [16]. However, this also offers a potential strategy for targeted therapeutic interventions [44].

Drugs

Placental transport of maternal drugs can result in fetal exposure with potentially harmful consequences. For example, the impact of fetal thalidomide exposure following maternal administration during pregnancy is well-established and highlighted an imperfect barrier of the placenta against maternal drugs. Placental drug transport may however be advantageous when fetal conditions require treatment. Whilst placental transport of aminoglycoside antibiotics like gentamicin is desirable for the treatment of intra-amniotic infections, they can accumulate in the fetal kidneys causing nephrotoxicity [45]. Using the BeWo cell model, uptake of gentamicin (a known megalin substrate) was significantly reduced at 4°C compared to 37°C indicating endocytosis may regulate gentamicin uptake since it is a temperature sensitive mechanism [45]. Furthermore, gentamicin uptake was significantly inhibited by the megalin inhibitors receptor-associated protein (RAP) and EDTA demonstrating that placental uptake may require megalin receptor binding (Table 2) [45]. Notably, the uptake of some drugs can be influenced by the extent of binding to serum proteins like albumin highlighting the importance of characterising drug-serum protein interactions at the maternal-placental interface [27].

Viruses

Transplacental transmission of viruses can have severe implications on the developing fetus. Zika virus can cross the human placenta barrier and cause fetal abnormalities [16]. The mechanism of Zika virus transport across the placenta was investigated using the choriocarcinoma cell line JEG-3 in conjunction

with fluorescence-labelled viral particles. The *in vitro* transcytosis of Zika virus was significantly reduced at 4°C and by inhibitors of macropinocytosis and caveolae- and clathrin-mediated endocytosis, indicating endocytosis is likely to have a role in placental transmission of the virus [16]. In addition to Zika virus, transmission of other viruses may be mediated by endocytic and transcytotic mechanisms. For example, the hFcRn receptor may have a role in the transplacental transport of human cytomegalovirus which can result in serious disabilities [46].

Targeted therapeutics

Endocytosis may also provide a mechanism for targeted placental or fetal therapies. Synthetic nanoparticles in the plasma become coated by serum proteins like albumin and IgG [44]. Serum proteins, and their nanoparticle cargo, may undergo receptor-mediated endocytosis into the syncytiotrophoblast through interactions with endocytic receptors (e.g. megalin) on the microvillous membrane [44]. Transfer of IgG across the placenta via transcytosis may provide an important avenue for targeted immune therapy [47]. Liposomes have been demonstrated to provide targeted therapy to the placenta where they are likely taken up by an endocytic mechanism although this needs to be confirmed [48].

4. Approaches to studying endocytosis in the placenta

Placental endocytosis has been explored using various models of the human placenta. Placental explant culture and *ex vivo* perfusion maintain the complexity of chorionic villi as the syncytiotrophoblast, stroma and the fetal endothelium are retained [4]. Explants are tissue fragments dissected from early and late gestation placentas which retain secretory activity [4]. *Ex vivo* perfusion of an isolated intact cotyledon allows transfer between circulations and has been useful for investigating placental transport of IgG [24].

In vitro cell models including primary trophoblast cells and placental cell lines have been used to study endocytosis in the trophoblast. Primary villous cytotrophoblasts are a good model for placental uptake but do not divide in culture, limiting their application. Human choriocarcinoma cell lines such as BeWo and JEG-3 exist, however these are derived from extravillous trophoblast and are not a good representation of villous syncytiotrophoblast [49].

New techniques are becoming available which may prove useful models. These include two-dimensional stem cell-derived trophoblast cell lines which are amenable to siRNA-mediated knockdown and can differentiate into syncytiotrophoblast-like cells [50,51]. Alternatively, three-dimensional trophoblast organoids recapitulate *in vivo* cell heterogeneity and trophoblast functions including trophoblast proliferation [52].

Pharmacological inhibition of endocytic pathways can help identify uptake mechanisms of defined cargo and has been utilised in models of the human placenta to investigate mechanisms discussed herein (Table 2). The *ex vivo* explant model was employed to investigate albumin uptake into the placenta,

and the application of a range of endocytic inhibitors, which together target several of the mechanisms described here, aimed to pinpoint the discrete machinery required for the uptake of this particular cargo [4]. A pharmacological approach has also been implemented in placental cell lines to study cargo uptake and trafficking. Pharmacological inhibitors of actin polymerisation and receptor-mediated endocytosis were used to elucidate the role of endocytosis in folate uptake in BeWo cells [36]. In addition, pharmacological inhibitors have been useful for characterising the endocytic mechanisms required for the transport xenobiotics and viruses across an *in vitro* trophoblast barrier [16,45]. However, pharmacological inhibition is not without limitations as inhibitors can exhibit cell-line dependency, produce variable results under different experimental conditions, and display poor specificity [53]. This highlights the need for careful experimental design and caution interpreting data. An alternative and more specific technique may be precise manipulation of gene expression for instance by siRNA-mediated knockdown. The use of choriocarcinoma cell lines is also problematic and confirming these findings in more physiologically relevant models such as tissue explants and primary cell culture is important to ensure these findings are applicable to human villous trophoblast.

5. Conclusions

Endocytosis is an important and under recognised mechanism for the transport of maternal nutrients, IgG, and exogenous molecules across the human placenta. While most research has focused on the syncytiotrophoblast, endocytosis is also likely essential in the fetal capillary endothelium. There are still significant gaps in our understanding of the endocytic mechanisms that occur at the two primary barriers to placental transport, the syncytiotrophoblast and capillary endothelium, and which essential cargoes are transported by these regulated mechanisms. A greater understanding of these endocytic mechanisms in the placenta is essential for the design of targeted therapies that can exploit these methods to allow precise delivery to the placenta and fetus.

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Declaration of competing interest

The authors declare no conflicts of interest.

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568 **Table 1. Substances transported into trophoblast the placental syncytiotrophoblast by**
569 **endocytosis**

Substance	Endocytic uptake Mechanism	Fate after endocytosis	Reference & model
Immunoglobulin G (IgG)	FPE or RME followed by receptor-mediated transcytosis	Transcytosed to fetus	[24] *Ex vivo* perfused placenta
Albumin	CME, MME	Metabolised or recycled to maternal blood	[4] *Ex vivo* explant culture
Lipoproteins	RME	Release from lipoproteins, transport to fetus	[31] Primary trophoblast culture
Thyroid hormones (T3 and T4)	RME	Transporter (possibly TAT1) mediated BM transfer to fetus	[33] *Ex vivo* explant culture and JEG-3 choriocarcinoma cells
Transferrin-bound Iron	RME	Transporter (possibly Ferroportin 1) mediated BM transfer to fetus	Reviewed in [35]
Folate (vitamin B9)	RME *perhaps CLIC/GEEC*	Transporter (possibly MRP1) mediated BM transfer to fetus	[21,22] Receptor expression in placental tissue
Riboflavin (vitamin B2)	RME	Transporter (possibly RFVT1) mediated BM transfer to fetus	[37] BeWo choriocarcinoma cells
Vitamin B12	RME	Transporter (possibly MRP1) mediated BM transfer to fetus	[41] Mouse models
Vitamin D	RME	Metabolised by placenta, likely transporter mediated BM transfer to fetus	[43] *Ex vivo* explant culture
Aminoglycosides	MME	Likely transporter mediated BM transfer to fetus	[45] BeWo choriocarcinoma cells
Zika Virus	FPE, CDE, CVME	Transcytosed to fetus	[16] JEG-3 choriocarcinoma cells

570 CME, clathrin-mediated endocytosis; MME, megalin-mediated endocytosis; RME, receptor-mediated
571 endocytosis; CLIC/GEEC, clathrin independent carriers and GPI-anchored protein-enriched early endosomal
572 compartment; FPE, fluid-phase endocytosis; CVME, caveolae-mediated endocytosis; BM, basal membrane;
573 TAT1, T-type amino acid transporter; MRP1, multidrug-resistance protein 1; DBP, vitamin D binding protein.

574 **Table 2. Pharmacological inhibitors of endocytosis and their use in placenta**

Inhibitor	Mechanism*	Endocytic Pathway	Example of use in placenta
Amiloride	Inhibits Na+/H+ exchange	Macropinocytosis	[4,16]
Chlorpromazine	Causes loss of clathrin and AP2 at the plasma membrane	Clathrin-mediated	[4,16]
Colchicine	Inhibits microtubule polymerisation	Fluid-phase endocytosis Intracellular trafficking	[4,16]
Cytochalasins	Caps actin filaments inducing their disassembly	Fluid-phase Clathrin- & caveolae-mediated	[4,36,45]
DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid)	Anion transport inhibitor	Megalin-mediated	[4,36]
EDTA	Inhibits binding to megalin and cubilin	Megalin & cubilin-mediated	[45]
Genistein	Tyrosine kinase inhibitor	Caveolae-mediated	[4]
Methyl-B-cyclodextran	Extracts cholesterol from plasma membrane	Caveolae- and clathrin-mediated Macropinocytosis	[4]
Monensin	Eliminates proton gradients across membranes	Receptor-mediated clathrin-dependent	[36]
NPPB (5-nitro-2-(3-phenylpropylamino)benzoic acid)	Chloride channel inhibitor	Megalin-mediated	[4]
Nystatin	Cholesterol sequestering agent	Caveolae-mediated	[16]
RAP (Receptor associated protein)	Inhibits binding to megalin and cubilin	Megalin & cubilin mediated	[45]
Sodium maleate	Induces megalin shedding from the plasma membrane	Megalin-mediated	[45]

575 *[4,16,45,53]

576 **Figure 1. Localisation of endocytic proteins in the human placenta.** Question marks indicate potential localisation which has not been experimentally validated. CD320, transcobalamin II receptor; *FCGR2B*, Fc gamma receptor IIb; FRα, folate receptor alpha; hFcRn, human neonatal Fc receptor; LDLR, low density lipoprotein receptor; TfR1, transferrin receptor 1.