Microbial vertical transmission during human pregnancy

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Abstract

Congenital infections with pathogens such as Zika virus, *Toxoplasma gondii, Listeria monocytogenes, Treponema pallidum*, parvovirus, HIV, varicella zoster virus, Rubella, Cytomegalovirus, and Herpesviruses are a major cause of morbidity and mortality worldwide. Despite the devastating impact of microbial infections on the developing fetus, relatively little is known about how pathogens associated with congenital disease breach the placental barrier to transit vertically during human pregnancy. In this review, we focus on transplacental transmission of pathogens during human gestation. We introduce the structure of the human placenta and describe the innate mechanisms by which the placenta restricts microbial access to the intrauterine compartment. Based on current knowledge, we also discuss the potential pathways employed by microorganisms to overcome the placental barrier and prospects for the future.

Introduction

During human pregnancy, the vertical transmission of infectious agents from an infected mother to her fetus can lead to devastating consequences in the developing fetus. Vertical transmission can be antenatal (before birth), perinatal (weeks immediately prior to or after birth) or postnatal (after birth). Antenatal fetal infections are major causes of global morbidity and mortality. In this review, we focus on proposed antenatal mechanisms of transplacental transmission of known "TORCH" pathogens, which includes **T**oxoplasma gondii, **o**ther (*Listeria monocytogenes, Treponema pallidium*, parvovirus, HIV, varicella

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zoster virus, amongst others), **R**ubella, Cytomegalovirus (CMV), and **H**erpesviruses (HSV) 1 and 2, and more recently Zika virus (ZIKV).

In the U.S., there are approximately 4 million deliveries each year, and in most of these cases, there is no evidence of *in utero* infection. However, infections with TORCH pathogens continue to occur. This issue came into focus last year when it became clear that ZIKV infection of pregnant women was associated with high rates of microcephaly and other congenital anomalies in their infants. Although ZIKV has received considerable attention since its recognition in the Western Hemisphere and is the newest mircroorganism classified as a TORCH pathogen (Schwartz, 2017), vertical transmission of other infectious agents is linked to adverse pregnancy outcomes worldwide, particularly in resource-limited countries where access to adequate prenatal care is scarce. Even in the United States, maternal infections with TORCH pathogens is associated with significant fetal disease. For example, between 400-4000 infants are affected with congenital toxoplasmosis annually. *T. gondii* infections *in utero* can be associated with pregnancy loss (miscarriage or stillbirth) or severe disease in the neonate, including blindness, developmental delay, or neurologic manifestations such as epilepsy. In many cases, neonates infected *in utero* display no obvious symptoms at birth but develop disabilities later in life, which are mostly ocular or neural in nature, including developmental delay and intellectual impairment (Faucher et al., 2012; Koppe et al., 1986; Lindsay and Dubey, 2011; Peyron et al., 2011). The magnitude of disability caused by congenital human CMV (HCMV) infection is immense. Approximately 40,000 children are born in the United States each year with congenital HCMV infection, and as many as 8000 of these require complex medical and surgical care (Cannon and Davis, 2005). These statistics highlight the need to better define the pathways by which infectious agents are vertically transmitted, which can be used to design therapeutic approaches to limit these events.

The placenta

To understand the mechanisms of vertical transmission, it is essential to define the structure of the human placenta, which forms the primary barrier between the maternal and fetal compartments throughout pregnancy. The human placenta is composed of fetal cells and is characterized by a close association between fetal-derived trophoblasts and the maternal tissues that they come into contact with. In humans and other eutherian organisms, the maternal-fetal interface consists of fetal-derived trophoblast progenitor cells which differentiate into cytotrophoblasts (CTBs) and have a proliferative capacity, and syncytiotrophoblasts, which are terminally differentiated, fused, multi-nucleated cells. In humans, the placenta begins to form within 5-6 days post fertilization, when a layer of syncytialized trophoblasts surrounds the blastocyst and begins to anchor into the maternal endometrium. By 10-12 weeks of gestation, the maternal circulation has been remodeled through the formation of spiral arteries and the human placenta becomes hemochorial, which indicates direct contact between maternal blood and the placenta. Together, trophoblasts form the placental villi and mediate the maternal–fetal exchange of gases, nutrients, and waste products (Figure 1A-C). (Cross et al., 1994; Hamilton and Boyd, 1960; Weisblum et al., 2011). The human placenta differentiates into two types of villi—floating and anchoring (Figure 1C). Floating villi are formed by an inner layer of CTBs that are covered by a layer

of syncytiotrophoblasts, which are bathed in maternal blood flowing into the intervillous space (IVS). The IVS can contain as much as 500mL of maternal blood, exposing the villous surfaces to microbes that might be present in maternal blood. Unlike floating villi, anchoring villi are attached to maternal decidual tissue (termed the decidua basalis, Figure 1A) by highly invasive CTBs called extravillous trophoblasts (EVTs) that form cell columns at the distal ends of the anchoring villi. EVTs that directly invade the decidua basalis and myometrium are referred to as interstitial EVTs whereas those that invade the maternal vasculature (spiral arteries) are referred to as endovascular EVTs. The decidua capsularis, in turn, forms the endometrial layer in direct contact with the chorioamniotic sac and diminishes through gestation and the decidua parietalis lines the entire uterus (Figure 1A). EVTs directly invade the decidua basalis, anchoring the placenta into the uterine implantation site where the EVTs are directly juxtaposed to maternal immune cells. On both floating and anchoring villi, syncytiotrophoblasts, which are formed by the fusion of the underlying CTBs, form the outermost cell layer and thus compose the key interface between maternal and fetal blood (Figure 1D). The core of the chorionic villous consists of fetal macrophages (termed Hofbauer cells), placental fibroblasts, and fetal endothelial cells lining villous capillaries and their associated basement membranes, which are thereby protected by the trophoblast cell layers from microbes in maternal blood.

Given its function as the sole interface between the maternal and fetal environments, the placenta protects the fetus from pathogens present in maternal blood. Yet, the defense mechanisms used by the placenta to limit microbial access to the fetus are largely undefined. Syncytiotrophoblasts provide potent protection against both viral and nonviral pathogens. In addition, CTBs also possess innate defense mechanisms against intracellular pathogens. Strategies used by pathogens to bypass these trophoblast-intrinsic defenses are varied, but common themes are emerging.

The maternal decidua is a specialized endometrium that makes up the implantation site. The decidua is a multicellular structure consisting of maternal cells and fetal interstitially invading extravillous CTBs. The decidua basalis consists of approximately 40% immune cells, 70% of which are unique decidual natural killer (NK) cells (dNK), 20 to 25% are macrophages, and 3 to 10% are T cells (Bulmer et al., 1988; King et al., 1997; Manaster and Mandelboim, 2010; Mor et al., 2006; Wicherek et al., 2009). The decidua basalis, apart from being a site of placental implantation, also functions in immunological tolerance of the semi-allogeneic fetal origin trophoblasts, which lie in direct contact with these maternal cells. The decidua is thought to maintain its immune privilege by virtue of its cell composition, which is characterized by limited lymphocyte access and control of cell trafficking by the tight regulation of local chemokine expression (Nancy et al., 2012; Red-Horse et al., 2004), which is maintained by adaptation of both fetal and maternal cells (Arck and Hecher, 2013; Erlebacher, 2013; Mor and Cardenas, 2010; Zenclussen, 2013). The expression of human leukocyte antigen (HLA)-G exclusively on extravillous trophoblasts (Chumbley et al., 1993; Proll et al., 1999; Yelavarthi et al., 1991) has been shown to play a role in maternal tolerance of the semiallogenic fetus. In addition, HLA-G expression on the stromal cells of the decidua may also play a direct role in maintaining fetal tolerance (Blanco et al., 2008).

Humans have a villous placenta with only one layer of syncytiotrophoblasts bathed in maternal blood. In contrast, mice have a labyrinthine placenta with two layers of syncytiotrophoblasts and one complete layer of mononucleated cytotrophoblasts, with the cytotrophoblast layer being in direct contact with maternal blood (Maltepe et al., 2010). Given the three layers of cells, the mouse placenta is referred to as a hemotrichorial placenta. These differences between mouse and human placental cellular structures may make it difficult to directly translate findings made using mouse models to studies of vertical transmission pathways in human pregnancy.

Syncytiotrophoblaste as a barrier to vertical transmission

The syncytiotrophoblast layer is highly resistant to infection by numerous pathogens, including *Listeria monocytogenes, Toxoplasma gondii*, and viruses such as HCMV, HSV1, and ZIKV (Bayer et al., 2015; Bayer et al., 2016; Delorme-Axford et al., 2013; Fisher et al., 2000; Koi et al., 2002; Maidji et al., 2006; Maidji et al., 2010; Robbins et al., 2010). Primary syncytiotrophoblasts isolated from full-term placentas potently resist infection by diverse viruses and confer broad antiviral resistance to non-trophoblast cells through effectors that operate in a paracrine manner (Bayer et al., 2015; Bayer et al., 2016; Delorme-Axford et al., 2013; Ouyang et al., 2016). Accordingly, the syncytiotrophoblast layer of first-trimester chorionic villi is largely resistant to HCMV infection, whereas CTBs and other cells of the villous core are susceptible (Fisher et al., 2000). Amniotic epithelium and CTBs of chorionic villi isolated from mid- and late-gestation placentas as well as explants from first-trimester chorionic villi are susceptible to ZIKV infection, whereas the syncytium is spared (Tabata et al., 2016). Interestingly, higher ZIKV titers are observed in amniotic epithelial cells from mid-gestation compared to late-gestation placentas, indicating a greater susceptibility to infection in the early placenta. Consistent with this, primitive trophoblasts, which represent the earliest phases of trophoblast development, are sensitive to ZIKV, but become increasingly resistant as the syncytium forms, suggesting that trophoblasts that form very early in gestation are sensitive to ZIKV (Sheridan et al., 2017).

Resistance of syncytiotrophoblasts to microbial infection is not specific to viruses. For example, *T. gondii* is reduced in partially syncitialized primary human trophoblasts relative to those with no syncytilization. (Abbasi et al., 2003). In addition, *T.gondii* infection across the syncytiotrophoblast layer appears to be rare *in vivo* (Buxton and Finlayson, 1986; Ferro et al., 2002; Shiono et al., 2007). Damage of the syncytium would allow parasite access to the villous core, which might increase parasite infection (Robbins et al., 2012). This process may occur in the context of a secondary infection and could be exacerbated by trauma. The effects may also depend on gestational time, as the layer of subsyncytial CTBs grows thinner and partially discontinuous after the first trimester (Jones et al., 2008; Mori et al., 2007). Remarkably, *T.gondii* is incapable of infecting syncytiotrophoblasts, but replicates well in underlying subsyncytial cytotrophoblasts (Robbins et al., 2012), suggesting that in the stages of pregnancy associated with an intact CTB layer, *T. gondii* targets these cells following its breach of the syncytium. The restriction of *T. gondii* replication in syncytiotrophoblasts is particularly striking given that the parasite is thought to replicate in all nucleated cells. Similarly, syncytiotrophoblasts also are highly resistant to *L. monocytogenes* infection in

human first trimester placental explants by either internalin-mediated invasion or cell-to-cell spread (Robbins et al., 2010).

HCMV can infect early and late gestation placentas (McDonagh et al., 2004; Mostoufi-zadeh et al., 1984; Muhlemann et al., 1992; Pereira et al., 2005; Revello and Gerna, 2004; Trincado et al., 2005). Importantly, most of the HCMV nucleic acids are observed in CTBs and only rarely in syncytiotrophoblasts. Concordantly, HCMV can infect cultured CTBs and syncytiotrophoblasts, albeit at low efficiency (Fisher et al., 2000; Halwachs-Baumann et al., 2006; Halwachs-Baumann et al., 1998; Hemmings and Guilbert, 2002; Hemmings et al., 1998; Maidji et al., 2002; Pereira et al., 2005; Revello and Gerna, 2004; Schleiss et al., 2007). However, these studies used lab-adapted as opposed to clinical HCMV strains and obtained CTBs from full term placentas, which may account for the modest observed replication efficiency. Thus, studies of diverse microorganisms point to a barrier formed by syncytiotrophoblasts that limits vertical transmission of pathogens into the fetal compartment at multiple stages of gestation.

Syncytiotrophoblast defenses

What are the mechanisms by which syncytiotrophoblasts restrict the growth of diverse pathogens? In most cases, the mechanistic basis for this restriction is unclear, largely given the difficulties in working with trophoblasts at various stages of gestation and modeling the human placenta using small animals. In experiments with primary syncytiotrophoblasts from full term placentas, we discovered that these cells resist viral infections and transfer this resistance to non-placental cells in a paracrine manner through pathways involving placental-specific miRNAs packaged in exosomes as well as type III interferons (IFNs) (Bayer et al., 2015; Bayer et al., 2016; Delorme-Axford et al., 2013; Ouyang et al., 2016). Others have shown that the physical properties of the syncytium function in the restriction of microbial infections. The syncytial surface has unique physical properties, such as the presence of dense, branched microvilli at the apical surface and a complex cortical actin network that might limit microbial invasion (Cantle et al., 1987; Fisher et al., 2000; Koi et al., 2002; Maidji et al., 2010; McDonagh et al., 2006; Robbins et al., 2010; Zeldovich et al., 2013; Zeldovich et al., 2011). Indeed, disruption of the actin cytoskeleton slightly enhances invasion of *L. monocytogenes* (Zeldovich et al., 2013), suggesting that the syncytium employs direct physical barriers to restrict pathogen infections.

The human placenta also expresses high levels of other antimicrobial defense components, such as the antimicrobial peptides defensins (King et al., 2007; Svinarich et al., 1997), toll-like receptors (TLRs) (Ma et al., 2007; Patni et al., 2009; Pudney et al., 2016; Tangeras et al., 2014), and nucleotide-binding oligomerization domain (NOD) proteins (Costello et al., 2007). However, whether these components play active roles in defending the human placenta from microbial infections *in vivo* remains unclear. Importantly, the expression of these components differs between primary human trophoblasts and trophoblast cell lines (Pudney et al., 2016), which may further limit the usefulness of these models for the study of microbial vertical transmission.

Bypassing the syncytiotrophoblast layer

It is unclear how pathogens might breach the syncytiotrophoblast layer to reach the underlying villous core. Placental villous inflammation associated with multiple pathogens including *T. gondii* induces syncytial expression of ICAM-1 and subsequent monocyte binding to the syncytial surface, which might lead to inflammatory syncytial damage (Juliano et al., 2006). However, increased ICAM-1 expression likely occurs in response to infection rather than providing the cause. Therefore, infection with one pathogen could predispose to infection with another via immune-mediated breakdown of the syncytium, as has been suggested (Mor and Cardenas, 2010).

CTBs can differentiate along two pathways. In floating villi, CTBs differentiate into syncytiotrophoblasts, aiding in the expansion of this continuous and ever expanding layer. In anchoring villi, CTBs differentiate into invasive EVTs, which are specialized cells that invade the uterine implantation site and contact maternal cells. EVTs represent a possible pathway into the placenta and are targeted by several microbes to facilitate vertical transmission. Since the maternal decidua has a multicellular phenotype composed of EVTs, uterine epithelium, stroma, endothelial cells and a variety of immune cells, several cell types that comprise the decidua may serve as replication sites for vertically transmitted pathogens.

Unlike syncytiotrophoblasts, EVTs are susceptible to infection by all three strains *T. gondii* (Robbins et al., 2012). Remarkably, EVTs occupy less than 5% of the surface area of the first trimester placental explants, but they contained 80% of the *T. gondii* parasitophorous vacuoles. *T. gondii* also may enter into the villous core by first infecting maternal immune cells that are enriched in the decidua, which might then facilitate parasite transfer to EVTs following this initial infection. This mechanism is consistent with data from *in vivo* studies (Buxton and Finlayson, 1986; Ferro et al., 2002; Shiono et al., 2007) demonstrating that maternal leukocytes in the uterine decidua are initially infected with *T. gondii*. This infection is followed temporally by infection of the trophoblast giant cells (Ferro et al., 2002). There is an 8-day delay between initial detection of *T. gondii* in the mouse placenta and the fetus, suggesting that infection begins in the blood, perhaps in maternal immune cells that subsequently transfer the parasite to EVTs or other cell types (Shiono et al., 2007). *T. gondii* cannot be detected in the mouse uterus, placenta, or fetus until 7 days following inoculation, also supporting a delay (Pezerico et al., 2009). In sheep experimentally infected with *T. gondii*, necrotic foci are initially detected in the uterus and then later observed in the placental villi (Buxton and Finlayson, 1986). Thus, the syncytiotrophoblast layer is not a significant site of *T. gondii* vertical transmission. Instead, the parasite likely first infects the decidua and then passes to the EVTs and subsequently to the villous core and fetal vasculature, such that infection of this interface occurs at approximately the same time as fetal infection.

Studies of placental explant infection by *L. monocytogenes* also are consistent with the preferential targeting of EVTs to allow entry into the intrauterine space. *L. monocytogenes* can infect non-phagocytic cells by two means—direct invasion or cell-to-cell spread. Syncytiotrophoblasts in first-trimester explants are resistant to infection, whereas EVTs, which express E-cadherin, are the preferred site for bacterial infection (Robbins et al., 2010).

EVTs eliminate 80% of the intracellular bacteria in 24 hours following inoculation via a mechanism that involves lysosomal-mediated degradation. Thus, although EVTs are a primary site of infection in the placenta, they also limit the spread of *L. monocytogenes* by inhibiting vacuolar escape (Zeldovich et al., 2011).

ZIKV appears to bypass the syncytium by virtue of its capacity to replicate in EVTs (Tabata et al., 2016), although other placental cell types also can support ZIKV replication. Therefore, in the case of ZIKV, multiple cell types might support viral replication, which could differ with gestational age, virus access to target cells, or both parameters. Given that maternal immune cells are likely to be targeted by ZIKV, the virus also might use these cells to reach EVTs to access the villous core. It is also important to note that ZIKV can be sexually transmitted, which would allow it to bypass the trophoblast layers entirely (D'Ortenzio et al., 2016; Hills et al., 2016).

For HCMV to reach CTBs, where replication occurs, the virus must first breach the syncytium. This breach could be facilitated by transcytosis of virions across the syncytium rather than by their direct infection. Syncytiotrophoblasts express the neonatal Fc receptor (FcRn), which transports IgG at high levels in the second half of pregnancy (Bright and Ockleford, 1995; Dancis et al., 1961; Garty et al., 1994; Gusdon, 1969; Malek et al., 1996). Immunohistochemical analysis of HCMV infection in human first-trimester floating and anchoring placental villi explants indicates that HCMV replicates in underlying villous CTBs, whereas the syncytiotrophoblasts are spared. Infection is detected in placentas with low-to-moderate CMV-specific neutralizing antibody titers, which is consistent with virion transcytosis across the syncytium via FcRn-mediated transcytosis (Fisher et al., 2000; Gabrielli et al., 2001; Maidji et al., 2007; Maidji et al., 2006; Pereira et al., 2005).

In addition to the antibody-mediated mechanisms described above, EVTs also might be targeted by HCMV to bypass the syncytial layer. Many cell types in *ex vivo* decidual organ cultures allow HCMV replication, including EVTs, microvasculature, and immune cells such as macrophages and dendritic cells (Weisblum et al., 2011). Therefore, in addition to EVTs, HCMV might target multiple cell types located in the decidua to reach the EVT layer. Concordantly, a wide range of cells in *ex vivo* human first-trimester decidual organ cultures are infected by both clinical and laboratory-adapted strains of HCMV (Weisblum et al., 2011). Notably, viral transmission in these cultures appears to be mediated by cell-to-cell spread. This pattern of tissue-associated spread, which is observed for low-passage clinical isolates as well as laboratory-adapted strains, closely mirrors the mode of HCMV spread *in vivo* and could confer a replication advantage within solid tissue, while potentially facilitating immune evasion (Weisblum et al., 2014).

Hofbauer cells are placenta-specific macrophages and are a potential target for ZIKV infection once it breaches the syncytium (Quicke et al., 2016). Once within the villous core where it replicates in Hofbauer cells, ZIKV may access the fetal compartment by spreading from the parietal decidua to the amniochorionic membrane (Tabata et al., 2016). Hofbauer cells also may facilitate HIV vertical transmission given that an HIV coreceptor, dendritic cell specific intercellular adhesion molecule-3-grabbing non integrin (DC-SIGN), is expressed on these cells, and DC-SIGN polymorphisms are associated with risk of mother-

to-child HIV transmission (Boily-Larouche et al., 2012). However, studies of animal and human fetuses indicate that there is almost no HIV transmission during the first and second trimesters of pregnancy (Brossard et al., 1995; Van Dyke et al., 1999), suggesting that this process has a limited biological role.

Final thoughts

Despite the growing number of pathogens associated with *in utero* fetal disease, the mechanistic basis of vertical transmission of infection across the placental barrier is unclear. Undoubtedly, the difficulty in establishing laboratory models that recapitulate the complexities of vertical transmission in humans, particularly at all stages of gestation, is a major roadblock to understanding mechanisms of vertical transmission. The placentas of many small animals display significant anatomic differences relative to the human placenta, which complicates an assessment of whether findings made using these systems mirror the biology in humans. In addition, *in vitro* trophoblast cell lines, such as BeWo and JEG-3 cells, do not spontaneously fuse to form syncytia and do not recapitulate the resistance of the placenta to microbial infections (Bayer et al., 2016; McConkey et al., 2016). While many groups, including ours, use primary trophoblast models, these culture systems may not represent cells at all gestational ages and do not recapitulate the structure of placental villi or contain signals from maternal blood that might act on these cells in a paracrine manner. Although explant models maintain villous structure, these systems are limited in the capacity to recapitulate the gestational ages of human pregnancy and also lack exposure to maternal factors. Furthermore, both primary cells and explant models are not genetically tractable, which limits mechanistic studies. Thus, it is essential that we recognize the limitations of models used in studies of microbial vertical transmission in humans and interpret our findings accordingly.

The placenta is arguably the most critical barrier in human life, yet the precise mechanisms by which microbes breach this barrier remain largely unknown. While it is possible that pathogens associated with congenital disease employ a common mechanism, it is more likely that these microbes have evolved distinct strategies, which might vary throughout gestation or with the level of maternal infection or corresponding immune responses. This is particularly true for ZIKV, which seems to be uniquely capable of penetrating the placental barrier at a variety of gestational ages (Brasil et al., 2016). Although the ZIKV outbreak appears to be waning, the critical role of the placenta in restricting vertical transmission of microbes must not be overlooked, and the mechanisms by which this organ does so should remain the focus of future investigation. Defining the means by which the placenta limits fetal infections is likely to provide important insights about previously unknown host-defense pathways and enhance knowledge about how these pathways could be targeted therapeutically.

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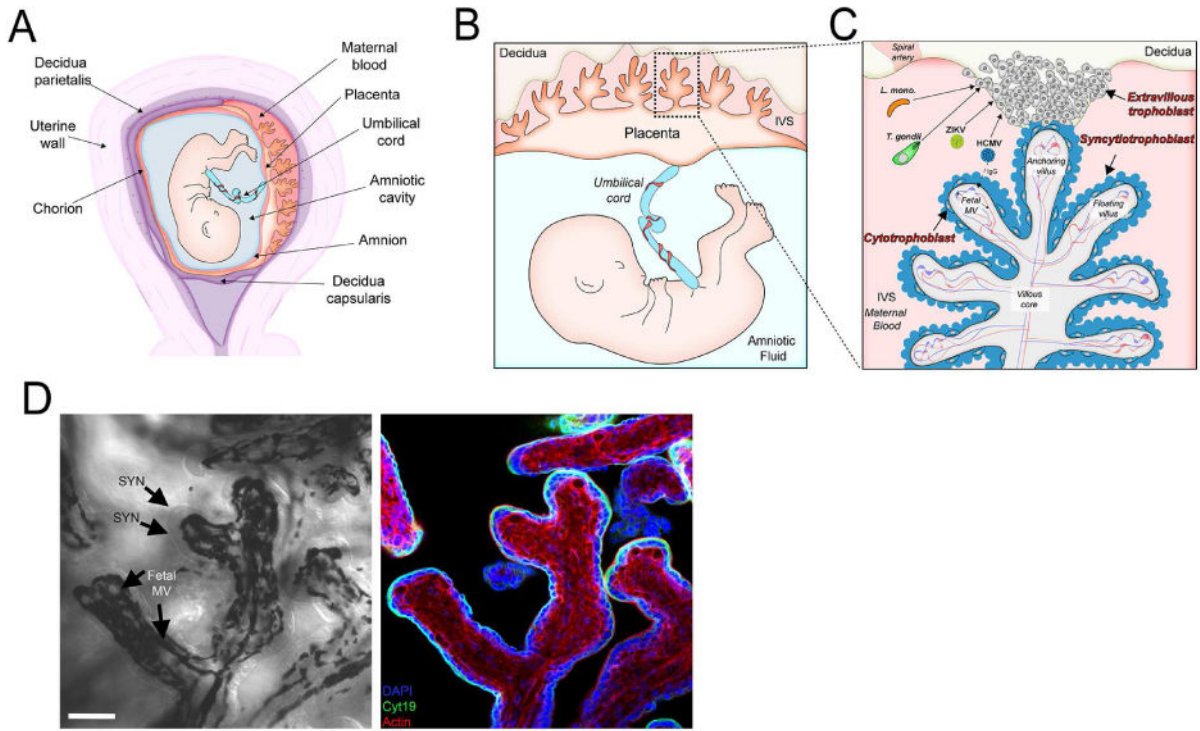**Figure 1. Structure and cellular composition of the human placenta**

(**A**) Schematic of the uterine cavity during pregnancy. The developing fetus is encased within the amniotic cavity, surrounded by the chorion and amnion, and anchored to the maternal decidua by the placenta (at the site of attachment, the decidua basalis). (**B**) Maternal blood fills the intervillous space (IVS) via spiral arteries that bathe the surfaces of the placenta in maternal blood (once the maternal microvasculature has been established). (**C**) The human hemochorial placenta is formed by villous trees composed of both floating villi and anchoring villi, which attach directly to the decidua basalis by the invasion of extravillous trophoblasts (EVTs). The human placenta villous trees are covered by syncytiotrophoblasts, with a layer of cytotrophoblasts (which become discontinuous throughout pregnancy) below this layer. Several pathogens, including *Listeria monocytogenes* (L. mono), *Toxoplasma gondii* (T. gondii), human CMV (HCMV), and Zika virus (ZIKV), are thought to access the villous core following replication in EVTs. (**D**) Left, differential interference contrast (DIC) image of a floating villous from an ~ 20-week gestation placenta highlighting the syncytiotrophoblast (SYN) layer and underlying fetal microvasculature (MV). Right, confocal micrograph of cytokeratin-19-positive SYNs and the underlying cytokeratin-19-negative core of the villous trees. Scale bar, 50 μm.