REVIEW

Molecular and cellular underpinnings of normal and abnormal human placental blood flows

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Abstract

Abnormal placental function is well-established as a major cause for poor pregnancy outcome. Placental blood flow within the maternal uteroplacental compartment, the fetoplacental circulation or both is a vital factor in mediating placental function. Impairment in flow in either or both vasculatures is a significant risk factor for adverse pregnancy outcome, potentially impacting maternal well-being, affecting immediate neonatal health and even influencing the long-term health of the infant. Much remains unknown regarding the mechanistic underpinnings of proper placental blood flow. This review highlights the currently recognized molecular and cellular mechanisms in the development of normal uteroplacental and fetoplacental blood flows. Utilizing the entities of preeclampsia and fetal growth restriction as clinical phenotypes that are often evident downstream of abnormal placental blood flow, mechanisms underlying impaired uteroplacental and fetoplacental blood flows are also discussed. Deficiencies in knowledge, which limit the efficacy of clinical care, are also highlighted, underscoring the need for continued research on normal and abnormal placental blood flows.

Introduction

Placental blood flow is a critical component of pregnancy outcome. Human placental blood flow comprises two separate compartments – the maternally derived uteroplacental circulation and the fetoplacental vasculature. This hemochorial villous placenta allows for maternal blood to directly bathe syncytiotrophoblast (STB), which becomes progressively apposed to the fetoplacental endothelium as gestation progresses, allowing for nutrient/waste and gas exchange to occur. While trophoblast, stromal and endothelial functions and interactions are also vital factors influencing pregnancy outcome, abnormal uteroplacental and fetoplacental blood flows, both individually and in combination, are strongly associated with adverse perinatal outcome, posing risks to maternal well-being and to immediate and long-term health of the infant.

Clinical implications of abnormal placental blood flow

Abnormal placental blood flow, either on the maternal side, fetal side or both, has been implicated in various complications of pregnancy such as preeclampsia (PE) and fetal growth restriction (FGR). For instance, one key component to the pathogenesis of PE and perhaps placently mediated FGR, as well, is thought to be defective trophoblast invasion into the maternal spiral arteries (Zhou et al. 1993, 1997a, Kaufmann et al. 2003).
This results in abnormally narrow and tortuous uterine vessels that result in placental hypoperfusion and uteroplacental ischemia.

Within the fetal compartment, placental vascular resistance should normally be low, allowing for forward flow through the umbilical arteries during both fetal cardiac systole and diastole (Giles et al. 1985, Trudinger et al. 1985c). In high-risk pregnancies that can be seen in PE and/or FGR, placental findings that lead to abnormally elevated placental vascular resistance include vasoconstricted stem villous vessels and impaired fetoplacental vascular angiogenesis can be seen in PE and/or FGR (Salafia et al. 1995, 1997, Kingdom et al. 1997, Su et al. 2015). This is seen clinically through abnormal umbilical artery Doppler velocimetric indices, where diastolic forward flow is initially impaired and can eventually become absent or reversed (Giles et al. 1985). When severe enough, the fetus is at a high risk for prolonged exposure to in utero hypoxemia/acidemia or stillbirth (Baschat & Weiner 2000, Alfirevic et al. 2017).

Clinically, the ‘treatment’ for both entities is delivery. With PE, it is removal of the placenta after delivery that cures the maternal manifestations of the disease. With isolated FGR, delivery is the only method to prevent prolonged exposure to an abnormal in utero environment and stillbirth (Group 2003, Lees et al. 2015). The problem, though, is that in severe cases, delivery oftentimes needs to occur at very preterm gestational ages with the attendant consequences of prematurity such as blindness, deafness, mental retardation, cerebral palsy and chronic medical problems (Group 2003, Thornton et al. 2004). Furthermore, even if these infants are fortunate enough to evade the potentially severe complications of the perinatal period, they are at an increased risk for developing obesity, cardiovascular disease and metabolic syndrome later in life (Barker & Thornburg 2013). Thus, a better understanding of the mechanisms underlying normal and abnormal placental blood flows in both maternal and fetoplacental compartments is vital if clinical outcomes are truly to be improved.

**Development of uteroplacental blood flow**

Uteroplacental blood flow develops soon after implantation. By post-fertilization day 6, the blastocyst has attached to the endometrial surface, with partial embedding and contact with the endometrial stroma by day 8 (Schlaufke & Enders 1975, Sadler 1995). Lacunae, spaces within the early syncytiotrophoblastic layer of the chorion, begin to appear within the syncytiot and these fuse to maternal sinusoids by day 12 after fertilization (Enders 1989). Once maternal blood flows through this compartment, the rudimentary uteroplacental circulation is established. The fusion of lacunae to maternal sinusoids eventually forms the intervillous space of the placenta, and ultimately, the spiral arteries of the uterus directly communicate with this intervillous space, resulting in uteroplacental blood flow that is clinically evident during pregnancy.

Approximately two to three weeks after fertilization, interstitial trophoblasts, one type of extravillous trophoblasts (EVTs), further migrate through the endometrial stroma, penetrate the decidua and adjacent myometrium and gather around spiral arteries. This is thought to prepare the spiral artery for endovascular trophoblast invasion (Pijnenborg et al. 1980). Endovascular trophoblasts, another type of EVT, then invade and migrate down the lumens of the spiral arteries (Fig. 1) (Zhou et al. 1997a, Red-Horse et al. 2005, Fisher 2015). They initially form cell plugs within the terminal portions of the spiral arteries, which results in destruction of maternal vascular endothelium via apoptotic mechanisms (Kaufmann et al. 2003, Harris et al. 2006). Vascular smooth muscle cells and elastic fibers of the vascular media are replaced with fibrinoid, aiding transformation into a low-resistance circuit (Brosens et al. 1967, Harris 2010). Simultaneously, these plugs are dissolved and the functional maternal circulatory component of the placenta is established.

From a molecular perspective, multiple coordinated events that have yet to be completely elucidated occur. For successful invasion to occur, trophoblastic phenotype must change from an epithelial to endothelial composition. For instance, during normal pregnancy, downregulation of epithelial-like adhesion molecules such as α6β4 integrin and concomitant upregulation of adhesion molecules more typical of endothelial cells, including α5β1 and α1β1, occur in cytotrophoblasts (Damsky et al. 1992, 1994, Zhou et al. 1997b). Invasive EVT also produces various proteases, such as matrix metalloproteinases to degrade the extracellular matrix, but excessive trophoblast invasion is also being limited by EVT production of protease inhibitors, including tissue inhibitors of metalloproteinases and plasminogen activator inhibitors (Librach et al. 1991, Damsky et al. 1992, Shimonovitz et al. 1994). Together, these underlying mechanisms allow endovascular EVTs to migrate through the endothelium, where maternal uterine artery endothelial and vascular smooth muscle cells then undergo apoptosis and are replaced by these trophoblasts, which have assumed an endothelial-like phenotype.
**Mechanisms underlying placental blood flow**

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(Pijnenborg et al. 1983, Kam et al. 1999, Harris et al. 2006). Ultimately, these normal processes allow for development of a low-resistance maternal vascular compartment with maternal blood flowing from maternal spiral arteries to the intervillous space by about 10–12 weeks of pregnancy (Hustin & Schaaps 1987, Rodesch et al. 1992, Jaffe et al. 1997).

**Expansion of the fetoplacental circulation**

With regard to the fetoplacental circulation, development of the placental villi starts around day 13 after conception. Mesodermal cells within the villus core initiate the process of vasculogenesis by differentiating into small blood vessels starting approximately 21 days post-fertilization (Castellucci & Kaufmann 1982, Demir et al. 1989, Sadler 1995). These capillaries within the villus fuse with capillaries developing within the mesoderm of the chorionic plate and connecting stalk, and ultimately they establish contact with the intraembryonic circulation, forming the fetoplacental circulation (Castellucci et al. 1990, Sadler 1995).

The fetoplacental circulation then continues to expand via a gradual transition from vasculogenesis to angiogenesis between 32 days post-conception to approximately 9 weeks after fertilization (Kaufmann et al. 1985, Castellucci et al. 1990, van Oppenraaij et al. 2009). Thereafter, angiogenesis continues throughout gestation but exponentially accelerates beginning at approximately 25 weeks’ gestation (23 weeks post-fertilization), resulting in a vascular bed that is approximately 550 km in length and 15 m² in surface area (Fig. 2) (Burton & Jauniaux 1995, Mayhew 2002). Physiologically, this corresponds to decreasing fetoplacental vascular resistance as demonstrated by increasing end-diastolic velocities within the umbilical artery as gestation progresses (Thompson & Trudinger 1990, Guiot et al. 1992, Todros et al. 1992). Much remains unknown regarding the mechanistic underpinnings of normal human fetoplacental vascular development, but various growth factors have been shown to be key players in both vasculogenesis and angiogenesis.

**Vascular endothelial growth factor (VEGF) family**

One key group of factors is the VEGF family, and most notably for placental angiogenesis, VEGFA. The critical importance of VEGF and its two main receptors – (1) VEGF receptor 1, also known as FMS-like tyrosine kinase 1 (FLT1) and (2) VEGF receptor 2 or kinase insert domain (KDR) – is demonstrated by the finding of embryonic lethality due to abnormal blood vessel formation during embryogenesis in: (1) Vgf knock-out mice, (2) Flt1

Figure 1

Formation of the uteroplacental circulation. EVTs migrate from the cytotrophoblastic shell into the endometrium. Interstitial EVTs prepare the spiral artery for endovascular EVTs. Endovascular EVTs then invade and migrate down the lumens of spiral arteries as they approach the decidua. The cell plugs that are initially formed destroy maternal vascular endothelium via apoptosis and replace vascular smooth muscle cells and elastic fibers from the media with fibrinoid. After dissolution of these plugs, the maternal circulatory component of the placenta is established.
knock-out mice and (3) Kdr knock-out mice (Fong et al. 1995, Shalaby et al. 1995, Carmeliet et al. 1996). Even mice lacking a single Vegf allele demonstrate abnormal blood vessel development and embryonic lethality (Carmeliet et al. 1996). Within humans, VEGFA is highly expressed in cytotrophoblast cells early in pregnancy, whereas FLK1 and KDR are primarily expressed within the hemangiogenic cords, with a predominance of KDR (Demir et al. 2004). This suggests that paracrine signaling may help to drive fetoplacental vasculogenesis. As gestation progresses, the VEGFA expression decreases within trophoblast while increasing within fetoplacental endothelial, mesenchymal and Hofbauer cells (Demir et al. 2004).

Based upon animal studies, Vegfa binding to Flt1 and/or Kdr within endothelial cells triggers various signal transduction cascades that result in endothelial proliferation and migration. For instance, in ovine fetoplacental artery endothelial cells, VEGFA induces activation of several signal transduction cascades including the PI3-kinase (PI3K)/AKT1 pathway (Zheng et al. 2008, Liao et al. 2010). PI3K inhibition results in suppressed nitric oxide (NO) production and completely blocks VEGFA-mediated cell proliferation and migration (Zheng et al. 2008). In Akt1 knock-out mice, there is also a marked reduction in endothelial nitric oxide synthase (NOS3) phosphorylation (Lee et al. 2014). In vivo findings also demonstrate significance of the PI3K/AKT1 pathway. For example, in endothelial-specific, postnatal deletion of Akt1, retinal angiogenesis is impaired with delayed radial outgrowth and reduced endothelial coverage (Lee et al. 2014). Furthermore, the placentas of Akt1-null mice show decreased placental vascularization, which is thought to contribute to both growth restriction and neonatal mortality in the pups (Chen et al. 2001, Yang et al. 2003).

Placental growth factor (PGF) is also a member of the VEGF family and is expressed in trophoblast. Unlike VEGFA isoforms, however, it is only able to bind to FLT1 and not KDR. Initially considered a simple competitive inhibitor of VEGFA effects through FLT1, it is also capable of stimulating the growth of endothelial cells in vitro, promoting proliferation, survival and migration (Maglione et al. 1991). This was thought to occur by increasing VEGFA availability to bind to KDR (Park et al. 1994). More contemporary data, especially within the cancer literature, suggest that PGF also has direct angiogenic effects, with PGF homodimers and PGF/VEGFA heterodimers being pro-angiogenic both in vitro and in vivo (Autiero et al. 2003a,b, Fischer et al. 2007, Yang et al. 2013). However, other studies suggest that PGF, especially when elevated, can decrease angiogenic activity within tumors (Eriksson et al. 2002, Xu et al. 2006).

**Fibroblast growth factors (FGFs)**

The family of FGFs are known to regulate several cellular processes, including proliferation, differentiation,
migration and survival (Beenken & Mohammadi 2009). Specific to the human placenta, FGF2 is currently considered the main FGF. Its expression has been localized to the cytotrophoblast in the first trimester and primarily to the fetoplacental endothelium at term, with low expression in STBs (Ferriani et al. 1994, Shams & Ahmed 1994, Arany & Hill 1998). FGF10 has also been described in the placenta, but its role appears to primarily mediate chorionic villous growth (Natanson-Yaron et al. 2007). FGF activity is mediated by a family of FGF receptors (FGFR1-4) that are members of the receptor tyrosine kinase family. While the human placenta has been shown to express all the FGFRs, FGFR1 appears to be the only FGFR expressed within the fetoplacental vasculature to date (Arany & Hill 1998, Anteby et al. 2005).

The majority of knowledge surrounding the role of FGFRs and placental angiogenesis is derived from animal studies. Ovine fetoplacental arterial endothelial cells, which also express FGF2 in vivo and in vitro, have been demonstrated to undergo activation of ERK1/2 and PI3K/AKT1 pathways via phosphorylation of FGFR1 (Feng et al. 2012). In contrast to VEGFA stimulation of these endothelial cells, inhibition of either signaling pathway only partially inhibits FGF2-stimulated proliferation (Zheng et al. 2008). Interestingly, FGFR1 is confined within plasma membrane caveolae, with the regulatory protein caveolin-1 interacting with FGFR1 (Feng et al. 2012). This allows FGF2/FGFR1-mediated activation of both MAPK and PI3K/AKT1 cascades, which, in turn, enhances various measures of angiogenesis including endothelial cell migration and tube formation (Feng et al. 2012).

Angiopoietins

Angiopoietins are a group of four angiogenic growth factors (ANGPT1, ANGPT2, ANGPT3 and ANGPT4) that regulate angiogenesis via binding to the TEK receptor tyrosine kinase (TEK, also known as TIE2). ANGPT1, ANGPT2 and TEK are expressed within the human placenta (Dunk et al. 2000, Goldman-Wohl et al. 2000, Zhang et al. 2001, Geva et al. 2002, Seval et al. 2008, De et al. 2016). There is some discrepancy between studies regarding the exact localization of expression of ANGPT1, ANGPT2 and TEK during the course of gestation. However, there is consensus that TEK is expressed within endothelium of placental blood vessels, and the majority of studies suggest that the ANGPT1:ANGPT2 ratio increases as gestation progresses (Dunk et al. 2000, Zhang et al. 2001). More recently, data suggest that there are intraplacental variations, with the ANGPT1 and TEK expression being higher in the periphery of the placenta and higher expression of ANGPT2 within the central regions of the placenta (De et al. 2016). Taken in the context where ANGPT2 activation of TEK results in destabilization of the vessels, allowing them to remain plastic and capable of responding to other angiogenic factors such as VEGFA, this suggests that in early pregnancy and within the more central portion of the villous vascular tree, high levels of ANGPT2 allow for progression of angiogenesis. In contrast, as gestation progresses or as vessels grow outward to the periphery of the placenta, increases in ANGPT1-mediated phosphorylation of TEK allows for these newly formed vessels to be stabilized via EC survival and EC–EC interactions (Dunk et al. 2000, Zhang et al. 2001, Geva et al. 2002).

Mechanisms underlying abnormal placental blood flow

Clinically, abnormal placental blood flow can be detected in the maternal uteroplacental compartment, the fetoplacental vasculature or both. Aberrant uterine artery and/or umbilical artery Doppler velocimetry are suggestive of placental insufficiency and are individually associated with an increased risk for adverse pregnancy outcome (Alfirevic et al. 2013, Garcia et al. 2016). Based upon Doppler studies, impairment of flow in either vascular compartment can occur in isolation or concurrently (Brosens et al. 1977, Trudinger et al. 1985a,b,c). Ovine studies, however, suggest more interdependence between maternal and fetoplacental circulations. With decreases in uterine perfusion, there is a corresponding reduction in fetoplacental blood flow (Stock et al. 1980). Similarly, the umbilical circulation has also been shown to locally regulate uterine blood flow (Rankin et al. 1975). Nevertheless, based upon clinical studies and for the purpose of clarity in this review, the two vascular compartments are discussed separately.

The maternal uteroplacental vasculature

Abnormalities of maternal uteroplacental blood flow often manifest clinically as PE with and without FGR. It is now well-established that defects in both EVT invasion and spiral artery remodeling that are characteristic of hypertensive disorders in pregnancy and FGR lead to abnormal placentation (Kaufmann et al. 2003, Pijnenborg et al. 2006). In turn, the placenta becomes ischemic, which is then thought to result in the release of soluble factors
that cause maternal systemic endothelial dysfunction, ultimately leading to the phenotype of PE (Kaufmann et al. 2003, Pijnenborg et al. 2006).

**Deficient EVT invasion and impaired spiral artery remodeling**

Although likely not the only culprit, much of the impairment in maternal uteroplacental blood flow is initially triggered by compromised EVT invasion of the uterine stroma and vasculature. This is evidenced by histologic sections of placental bed biopsies in patients with PE, where cytotrophoblasts were located farther from uterine vessels than in sections of control placentas (Zhou et al. 1997a). Furthermore, when spiral arteriole endovascular cytotrophoblasts were detected, invasion within the vessel did not exceed the depth of the superficial decidua (Zhou et al. 1997a). These findings have been shown to be mediated, at least in part, by the failure of transformation from epithelial- to endothelial-like phenotype with an abnormally persistent expression of epithelial adhesion molecules such as E-cadherin and α6β4 integrin (Brosens et al. 1972, Zhou et al. 1993, 1997a).

Although impaired trophoblast invasion is an important source for abnormal spiral artery remodeling, a defective decidualization process has also been proposed to affect uteroplacental blood flow. Some evidence indicates that changes in the endometrium before EVT invasion are distinct in preeclamptic women. For instance, decidual-dependent secretion of insulin-like growth factor bind protein-1 is reduced during the first trimester of women who later develop PE as compared to normal pregnancies (Hietala et al. 2000, Vatten et al. 2008). This suggests a maternal component for altered uteroplacental perfusion. Other studies have shown that maternal macrophages are concentrated around suboptimally remodeled spiral arteries from preeclamptic women and that those macrophages secrete tumor necrosis factor-alpha and indoleamine 2,3-dioxygenase to induce apoptosis in EVTs (Reister et al. 1999, 2001).

Invasive trophoblasts are further regulated by their interaction with other cells including decidual natural killer (NK) cells. NK cells secrete various chemokines, and for NK cells and trophoblast to interface, the trophoblast must express matching chemokine receptors (Hanna et al. 2006). Additionally, NK cell function is also mediated by NK receptors that bind to MHC class I molecules among other ligands. EVTs express HLA-C as their sole polymorphic classical MHC class I molecule, and given paternal contributions, HLA-C alleles can be different to that from the mother. Furthermore, NK cells express an incredibly diverse range of killer immunoglobulin receptors (KIRs) resulting from differences in gene number between individuals and allelic diversity at individual KIR loci (Parham & Moffett 2013). Specific maternal KIR haplotypes have been found to increase the risk of PE, demonstrating that immune recognition also plays a role in the regulation of uteroplacental blood flow (Hilby et al. 2010).

**Uteroplacental hypoperfusion resulting in maternal systemic EC dysfunction**

Several lines of evidence support that abnormal uteroplacental blood flow with subsequent poor placental perfusion strongly contributes to the phenotype of PE. For example, the incidence of PE is higher in women who live at high altitudes, where there is some degree of relative hypoxia (Palmer et al. 1999, Zamudio 2007). Additionally, medical comorbidities resulting in vascular insufficiency, such as preexisting hypertension, acquired thrombophilias and renal disease increase the risk for impaired placenta, PE and FGR (Dekker 1999, Bartsch et al. 2016). Perhaps most compellingly, animal models whose uteroplacental blood flow has been reduced demonstrate phenotypes strongly suggestive of PE (Khalil & Granger 2002, Bird et al. 2003, Ianosi-Irimie et al. 2005, Karumanchi & Stillman 2006).

Much of the maternal clinical phenotype of PE is thought to arise from maternal endothelial dysfunction. During normal pregnancy, uterine artery vasodilation has been found to be mediated, at least in part, by enhanced NO production. This occurs not only via NOS3 phosphorylation, but also through other mechanisms including enhanced gap junction communication which contributes to amplified and sustained intracellular calcium bursts (Tran et al. 2009, Yi et al. 2010, 2011). In PE, uterine artery ECs are unable to produce the normally augmented levels of NO that occur during normal pregnancy, and this is thought to be one major mechanism resulting in insufficient vasodilation (Sladek et al. 1997, Bird et al. 2003, Savvidou et al. 2003).

**The fetoplacental vasculature**

As noted earlier, fetoplacental vascular resistance should normally decrease as gestation progresses, allowing for increased forward flow through the umbilical arteries during fetal systole. For this to occur, there are at least two main requirements: (1) Proper placental vasomotor
tone and (2) appropriate expansion of the fetoplacental vasculature via angiogenesis. When either or both these core principles are disrupted, fetoplacental vascular resistance may become abnormally elevated.

**Constricted vasomotor tone**

In contrast to small arterioles of other vascular beds, vasomotor tone of placental chorionic plate and stem villous vessels, which are similar in size to these arterioles, are not under autonomic control as placental vessels lack innervation (Fox & Khong 1990, Poston et al. 1995, Sabry et al. 1995, Reilly & Russell 1977). Not only are these larger placental vessels controlled primarily by humoral influences, they also respond differently than other vessels outside of the placenta. For instance, the response of the placental vasculature to factors such as acetylcholine, angiotensin II and bradykinin is blunted (Mak et al. 1984, McCarthy et al. 1994). Issues such as vessel diameter have also been shown to affect sensitivity to factors such as thromboxane A2 (TXA2), with a selective decrease to TXA2 with decreasing vascular diameter in stem villous arteries (Broegger et al. 2016). Placental vessels also vasoconstrict when subjected to prostaglandin E2 (PGE2), whereas all other vascular beds, including maternal uterine vessels, vasodilate under the influence of PGE2 (Glance et al. 1986, Allen et al. 1989, Bours & Walters 1991, Sastry et al. 1997).

Much of the current knowledge surrounding regulation of human fetoplacental vasomotor tone is extrapolated from in vitro models and studies of humoral factors in cord blood. For example, results from a dual-perfused single cotyledon model showed that vessels within FGR placentas had reduced reactivity to PGE2 stimulation in comparison to control placentas (Luria et al. 2012). In a study where cordocentesis was performed in pregnancies complicated by FGR, all of whom had umbilical artery Dopplers suggestive of abnormally elevated placental vascular resistance, significantly higher fetal concentrations of endothelin-1 were found in contrast to gestational age-matched, appropriately grown controls (Rizzo et al. 1996). The same authors also found that the stable metabolite of the vasodilator prostacyclin, 6-keto prostaglandin F1-alpha, was lower in cord blood of FGR pregnancies.

The specific molecular mechanisms underlying the changes in prostanoid levels or of these unique placental vascular responses remain unidentified. There is a small body of literature suggesting that cyclooxygenase-2 (PTGS2) may play a role, although the data are conflicting as to whether it is upregulation or downregulation of PTGS2 that is resulting in abnormal vasoconstriction. In general, PTGS2 inhibition has been shown to alter the prostacyclin to thromboxane A2 ratio, leading to a prostanoid phenotype that favors vasoconstriction (Howard et al. 2003). A different study found that polymorphisms of the fetal PTGS2 gene that correlate with decreased PTGS2 expression are associated with abnormal placental blood flow and FGR (Polydorides et al. 2007). Chronic glucocorticoid treatment of chorionic plate arteries results in decreased PTGS2 expression and increases vascular resistance, although glucocorticoid treatment was shown to have effects on several other vasoactive mediators (Nugent et al. 2013).

In contrast, we have found an upregulation of PTGS2 expression and activity within the endothelium of stem villous vessels in pregnancies complicated by FGR with abnormally elevated fetoplacental vascular resistance in comparison to gestational age-matched controls (Su et al. 2011). However, our studies also suggest that this may be regulated by estrogen receptor-beta, which concomitantly regulates downstream prostanoid synthases resulting in a vasoconstrictive prostanoid profile (Su et al. 2009, 2011). These differences in mechanistic findings may be explained by disparities in types of samples collected (e.g. normal vs pathologic, cell vs tissue studies) or may represent compensatory, rather than pathologic, mechanisms. As an example, NO also regulates placental blood flow and is produced primarily via NOS3 in the placenta. One group of investigators found that the NOS3 expression was significantly higher in endothelium of stem villous vessels in pregnancies complicated by FGR in comparison to controls, suggesting that NOS3 does not contribute to the pathologic phenotype of abnormal placental blood flow but may be an adaptive response to increased fetoplacental vascular resistance (Myatt et al. 1997).

**Deficient formation of the fetoplacental vasculature**

Even when there is no disturbance in vasomotor tone, vascular resistance of the fetoplacental circulation is also mediated by the structure of the placental vasculature itself. When fetoplacental vascular resistance is abnormally elevated, as clinically manifested by decreased, absent or reversed umbilical artery end-diastolic velocities, several groups of investigators have previously shown that there are fewer, more slender, and substantially longer and unbranched villous capillary loops than in placentas of uncomplicated pregnancies (Jackson et al. 1995, Macara et al. 1995, 1996, Krebs et al. 1996, Todros et al. 1999,
Chen et al. 2002) (Fig. 2). This results in fewer vascular conduits, resulting in a structural cause for abnormally elevated placental vascular resistance. These findings have been attributed to excessive non-branching angiogenesis (Benirschke 2012, Mayhew 2002, Mayhew et al. 2004). In actuality, however, whether the cause is too much non-branching angiogenesis, impaired branching angiogenesis or a combination of both entities remains unknown.

There are both limited and conflicting data regarding the expression of angiogenic factors in placental pathologies associated with abnormal fetoplacental blood flow. For instance, within a hyperthermic ovine model of placental insufficiency-derived FGR where these fetuses manifest abnormal umbilical artery Doppler indices, the cotyledonary VEGFA mRNA expression was actually increased in placentas of FGR fetuses at the equivalent gestational age of the late first trimester (50 days post-conception) (Regnault et al. 2002, Barry et al. 2008). This difference, however, disappeared by what could be considered the late second trimester (90 days post-conception) (Regnault et al. 2002). This suggests that there may be gestational age-dependent roles of certain angiogenic mediators. In a study of human placentas, one group found that total placental RNA expression of VEGFA was actually increased in the FGR group as compared to the control group (Szentpeteri et al. 2013). This study, however, did not discuss several key maternal characteristics, including gestational age at delivery and number of subjects with abnormal umbilical artery Doppler velocimetry. Additionally, use of total placental RNA further limits interpretation of this finding. Cell-specific VEGFA has also been investigated, primarily through immunohistochemistry, with various studies showing no difference in VEGFA protein expression in pregnancies complicated by FGR, unlike the previously mentioned studies that investigated mRNA expression (Helske et al. 2001, Gurel et al. 2003). Umbilical cord blood levels have also been investigated, and in a cohort of FGR from Spain that, on average, required a late preterm delivery, there was also no difference in umbilical arterial or umbilical venous VEGFA and sFLT1 concentrations (Borras et al. 2014). However, in this study, umbilical artery Doppler pulsatility indices did not significantly differ between the two groups and at worst were only mildly elevated in the FGR group, suggesting that these were not the fetuses that suffered from abnormally high fetoplacental vascular resistance. Taken together, these data suggest that the mechanisms underlying the development of abnormal fetoplacental vascular resistance are much more complex than just VEGFA mediation.

With regard to PGF, data also differ. In the hyperthermic ewe model of FGR that also demonstrates abnormal umbilical artery Doppler velocimetry, there was no difference in PLGF at either 50 or 90 days post-conception (Regnault et al. 2002). Recently, one study found that the total placental PGF expression was diminished in cases of severe FGR requiring preterm delivery, and the authors concluded that this may indicate a role of PGF in placental angiogenesis (Joo et al. 2017). No other specific data regarding PGF and fetoplacental angiogenesis have been described to our knowledge, but it warrants comment, especially given its potential effects on angiogenesis and its interaction with VEGFA.

Knowledge surrounding FGF, the angiopoietins and placental blood flow is also limited. Based upon cordocentesis data, FGF2, which again is considered the primary placental FGF, appears to peak at approximately 18–20 weeks with a slow decrease all the way until term (Hill et al. 1995). There was also a trend toward lower FGF2 levels in cord serum of pregnancies that resulted in SGA fetuses, but this was not statistically significant (Hill et al. 1995). As for the angiopoietins, in a model of FGR derived from overnourished, adolescent, pregnant ewes, the vascularity of the placental cotyledon was decreased, with elevated ANGPT2 expression within these cotyledons in comparison to appropriately grown controls (Carr et al. 2016). In humans, data are more discrepant. For instance, studies have found diminished ANGPT2 expression within total placentas of pregnancies complicated by severe FGR or PE as compared to gestational age-matched controls (Dunk et al. 2000, Zhang et al. 2001). In contrast, a more recent study showed that ANGPT2 and TEK expression was lower, while ANGPT1 was higher in preeclamptic or preeclamptic/FGR pregnancies (Kappou et al. 2014). This study, however, utilized cases that were all mild enough to be delivered at term, suggesting that these fetuses and placentas were less likely to have experienced abnormal placental blood flow.

In addition to the gaps in knowledge surrounding levels of angiogenic mediators and deficient placental vascularization, the molecular mechanisms underlying this impairment also remain incompletely understood. Murine studies demonstrate that knock-out of aryl hydrocarbon receptor nuclear translocator (ARNT), a heterodimeric partner to hypoxia inducible factor 1-alpha (HIF1A) that induces transcription of angiogenic genes such as VEGFA, results in embryonic lethality secondary to deficient placental vascularization (Kozak et al. 1997, Maltepe et al. 1997). Within human fetoplacental endothelial cells isolated from severely growth-restricted...
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fetuses with absent or reversed umbilical artery end-diastolic velocities, the expression of ARNT is decreased in comparison to gestational age-matched, appropriately grown controls (Su et al. 2015). Deficient ARNT expression leads to decreased binding of the ARNT/HIF1A transcription factor to hypoxia response elements within the VEGFA proximal promoter, diminished VEGFA expression and impaired proxies of angiogenesis such as tube formation (Su et al. 2015).

Conclusion

Placental blood flow, both within the fetoplacental and the maternal uteroplacental vasculatures, is critical for pregnancy outcome. Knowledge surrounding the mechanisms underlying both normal and impaired human placental blood flows is limited for several reasons. First, much of the determinants of uteroplacental blood flow are established at or soon after implantation, oftentimes prior to the parturient even knowing she is pregnant. Second, clinical modalities such as Doppler velocimetry of the uterine or umbilical circulations may identify pregnancies at high risk for placental insufficiency, but their utility is limited by the fact that mechanistic investigation cannot occur until after delivery of the infant and placenta. Finally, utilizing the diagnosis of FGR or PE to better investigate mechanisms of abnormal placental function has its limitations. For example, there are likely various mechanisms leading to the same clinical phenotype such as PE or FGR, which is likely why prophylactic measures such as heparin or baby aspirin have limited efficacy, even in high-risk pregnancies. (Bujold et al. 2010, Dodd et al. 2013, Odibo et al. 2015). This is supported by the discrepancies in expression or mechanistic findings in FGR and/or PE. Continued future investigation is warranted in mechanisms underlying maternal and fetoplacental blood flows.

Declaration of interest

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