Placenta-derived angiogenic proteins and their contribution to the pathogenesis of pre-eclampsia.

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Abstract

Placental angiogenesis is critical to the success of human pregnancy. Angiogenesis is defined as the formation of new blood vessels from existing vasculature. Angiogenesis is necessary for the establishment of adequate placental perfusion, which is important for providing the optimum *in utero* environment to support fetal development. Defective placental angiogenesis is associated with several pregnancy complications, the most clinically important of which is pre-eclampsia; the multisystem disorder characterised by maternal hypertension, proteinuria and endothelial dysfunction. Here, we review our current understanding of several key angiogenic factors that are associated with placental angiogenesis. We also discuss their importance with respect to pre-eclampsia, where aberrant expression and release of these factors into the maternal circulation is thought to contribute to the pathogenesis and pathophysiology of pre-eclampsia.

Pre-eclampsia

Pre-eclampsia (PE) is the most clinically important complication of human pregnancy and is defined by new onset hypertension and proteinuria that becomes evident after 20 weeks' gestation (Duley, 2009). The condition has health implications for both mother and fetus and is estimated to complicate between 2-8% of pregnancies worldwide. PE is a leading cause of maternal and neonatal morbidity and mortality (Duley, 2009). Treatment for PE is supportive and symptomatic, with the only cure being delivery of the placenta. The placenta is key to the development of PE, with shallow placentation and maternal endothelial dysfunction being strongly associated with its pathogenesis (Brosens *et al.,* 1972; Roberts *et al.,* 1989). The search for preventative strategies and treatments for PE is of particular importance as it is associated with both short and long term health consequences for a pre-eclamptic mother and her child (Wilson *et al.,* 2003). Current strategy to reduce PE includes the use of anti-platelet therapies early in pregnancy such as low-dose aspirin (LDA). Clinical trials of LDA administration show benefit in some, but not all, women at risk of the condition (Bujold *et al.,* 2009).

Clinical features of PE

Typically, a woman with PE presents in the clinic with a blood pressure of greater than 140/90 mmHg and proteinuria of greater than 300mg/day (Sibai *et al.,* 2005). Additional maternal clinical manifestations include oedema, altered liver and kidney function and abnormal clotting (Sibai *et al.,* 2005).

Health consequences during and after a PE pregnancy

A woman diagnosed with PE may be at increased risk of liver and kidney malfunction , blood clotting abnormalities, HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome and respiratory distress syndrome during her pregnancy, all of which are associated with significant

maternal morbidity and mortality (Bellgamy *et al.,* 2007 and Smith *et al.*,2001). Rare but serious maternal complications of PE include eclampsia (seizures superimposed on the syndrome of PE) and stroke. Following a pre-eclamptic pregnancy, a woman is at increased risk of developing cardiovascular complications, with an estimated 20% of these women developing cardiovascular disease within 7 years of the pre-eclamptic pregnancy (Nisell *et al.,* 1995).

In a pre-eclamptic pregnancy the fetus is at increased risk of poor growth due to a poorly perfused placenta which cannot provide sufficient blood supply to meet metabolic needs. Poor fetal growth is commonly known as fetal growth restriction (FGR). FGR is not exclusively associated with pre-eclamptic pregnancies and may be linked to other factors such as maternal nutrition (Romo, 2009) and existing maternal cardiovascular issues (Irving *et al.,* 2000). The fetus of a pre-eclamptic pregnancy is also at increased risk of pre-term birth (Goldenberg *et al.,* 2008), which may occur spontaneously or may be medically induced in order to resolve the PE and for fetal indications (Goldenberg *et al.,* 2008). These complications all require skilled neonatal healthcare and prolonged hospitalisation and may have long-term health consequences for the infant such as increased future risk of chronic diseases such as high blood pressure and diabetes (Irving *et al.,* 2000).

Maternal risk factors

There is much heterogeneity in the characteristics of women who develop PE. Despite this, several risk factors have been identified that increase a woman's risk of developing this condition. A genetic influence is reflected in the 2-5 fold increased risk of PE in women whose mothers also experienced the complication during pregnancy (Zhang *et al.,* 1997). Women are at increased risk of PE during their first pregnancy (Sibai *et al.,*1997) or if they have existing medical conditions such as CVD (Roberts *et al.,* 1989) or insulin resistance (Said and Dekker 2003).

Treatment for PE

Central to the clinical management of a PE pregnancy is maintaining the health of both mother and fetus (Sibai, 2003). Following a diagnosis of PE, close and regular monitoring of maternal and fetal health is recommended for the remainder of the pregnancy; this enables the application of timely and appropriate medical intervention should the complication increase in severity (Sibai, 2003). If a woman is thought to be at risk of developing PE, or clinically presents with severe PE, she will often receive antihypertensive drugs to attenuate her increased blood pressure, and magnesium sulphate for seizure prophylaxis in severe cases (Aaserud *et al.,* 2005).

Placentation in healthy pregnancies

Placental development involves the coordinated and proper function of many different cell types (Gude *et al.,* 2004). Trophoblasts are the most important cell type in the placenta. They differentiate into two lineages with distinct properties; the villous and extravillous trophoblast (Figure 1). Villous trophoblast (VT) fuse with neighbouring cells to form a single large, multinucleated and non-migratory syncytium called the syncytiotrophoblast. The primary functions of the syncytiotrophoblast are in endocrine regulation, immune tolerance and mediation of nutrient and waste exchange (Linzer & Fisher 1999). Extravillous trophoblasts (EVT) are migratory and invasive and are responsible for anchoring the placenta to the maternal uterine wall and for remodelling the uterine spiral arterioles in the decidua and myometrium during placentation (Brosens *et al.,* 1967).

The remodelling of the uterine spiral arterioles culminates in the transformation of smooth muscle-rich, high-resistance narrow blood vessels into dilated, low-resistance compliant vessels (Zhou *et al.,* 1997). This process allows increased maternal blood flow into the intervillous spaces of the placenta and is essential to meet the rising metabolic demands of the rapidly growing fetus (Figure

2). Trophoblast migration and invasion occurs from eight weeks' gestation, with the majority of the remodelling occurring between the 16th and 20th weeks of gestation (Pjinenborg *et al.,* 1986). Placental pathologies such as PE are associated with a disruption to these otherwise highly coordinated processes.

Placental endothelial cells, which line the interior walls of blood vessels, play important roles in the formation of new blood vessels, and act as mediators in the transport of bioactive molecules to and from the maternal and fetal circulations (Roberts and Cooper 2001). As with trophoblast dysfunction, endothelial cell dysfunction is associated with PE as well as with other placental pathologies (Roberts and Cooper 2001). A key feature of the latter stages of PE is a systemic condition that damages the maternal endothelium, and thus the dysfunction of maternal endothelial cells is also of clinical importance in PE.

Placentation in PE

Placentation in a pre-eclamptic pregnancy is compromised and incomplete. Pre-eclamptic pregnancies are typically characterised by an abnormal vascular response to pregnancy, where the spiral arteriole remodelling in the uterine decidua and myometrium does not occur adequately as a consequence of shallow extravillous trophoblast invasion and migration (Brosens *et al.*,1972). Although many hypotheses have been proposed to explain shallow trophoblast invasion, the underlying mechanisms are yet to be definitively established.

Blood vessel formation is critical to successful placentation and involves both vasculogenesis (the formation of new blood vessels *de novo*) and angiogenesis (the formation of new blood vessels from existing blood vessels). These processes are required to achieve sufficient placental

vascularisation and perfusion (Cross et al., 1994) and without them, optimal growth and function of the placenta does not occur.

The human placenta is a rich source of angiogenic molecules, which play an important role in blood vessel formation at the maternal-fetal interface. Key findings support the idea that an imbalance in placental production and release of angiogenic molecules not only impairs local cellular function and contributes to shallow trophoblast invasion, but also can contribute to the systemic endothelial cell dysfunction that is a characteristic feature of the latter stages of PE (Maynard *et al.*, 2008).

Angiogenic factors in pre-eclamptic pregnancies

Pro-angiogenic and anti-angiogenic factors are important in the regulation of placental development (Venkatesha *et al.*,2006). Numerous studies have reported the measurement of pro- and anti-angiogenic factors in maternal sera and have identified altered concentrations of these factors in women with pre-eclamptic pregnancies compared with women with normotensive pregnancies. These include those involved in angiogenesis that contribute to the endothelial dysfunction and are believed to be the major pathogenic factor underlying the maternal clinical manifestations of PE. Angiogenic factors have a complex and interactive pattern of expression and can influence subsequent release of other angiogenic molecules. Therefore, dysregulated expression and release of these factors early in pregnancy may act as a point of initiation for further angiogenic dysregulation as seen later in PE.

Vascular endothelial growth factors and their receptors

Members of the vascular endothelial growth factor (VEGF) family (VEGF-A, VEGF-B, VEGF-C, VEGF-D, placental growth factor (PlGF)), and their receptors (VEGFR-1/fms-like tyrosine kinase-1 (Flt-1), VEGFR-2/kinase insert domain receptor (KDR), VEGFR-3/fms-like tyrosine kinase

receptor-4 (Flt-4)), are important mediators of angiogenesis. A co-receptor for KDR is neuropilin-1 (NRP-1), and has also been studied in PE.VEGF-A in the placenta induces vascular permeability, endothelial cell proliferation and is important in maintaining the integrity of newly formed capillaries (Hoeben *et al.,* 2004). VEGF-A also modulates a number of trophoblast functions such as proliferation, differentiation and invasion, predominantly through the Flt-1 and KDR receptors (Athanassiades *et al.,* 1998). The effect of VEGF-A induced angiogenesis is enhanced with increased expression of co-receptor NRP-1, which potentiates VEGF-A binding to KDR (Soker *et al.,* 1998). KDR is a more potent receptor than Flt-1 for VEGF.

As depicted in Figure 3, VEGF-A, VEGF-B and PlGF can bind to membrane bound Flt-1 to exert their angiogenic effects (Maynard *et al.,* 2008). Flt-1 is measured as a soluble and membrane bound form and also exists as two splice variants, a membrane bound form and a secreted form known as sFlt-1. sFlt-1 antagonises this pro-angiogenic effect and is produced in excessive amounts by the VT of women with PE (Levine *et al.,* 2006) where increased placental expression of sFlt-1 is associated with increased maternal circulating concentrations of sFlt-1 (Maynard *et al.,* 2003). Moreover, circulating maternal sFlt-1 concentrations have been found to be high 5-6 weeks prior to the clinical onset of PE with a concurrent low concentration of both VEGF and PlGF (Maynard *et al.,* 2008). This observation implicated sFlt-1 in the pathophysiology of PE, with maternal blood concentrations of sFLt-1correlating with PE severity (Maynard *et al.,* 2003). This clinical observation is supported by *in vitro* studies where sFlt-1 was shown to antagonise VEGF. The addition of exogenous sFlt-1 inhibited trophoblast invasiveness and induced endothelial dysfunction (Zhou *et al.,* 2002).

Endostatin is an active fragment of collagen XVII, which is a potent inhibitor of angiogenesis. Endostatin interferes with angiogenesis by disrupting cellular motility and by directly blocking

VEGF signalling through interaction with KDR (Kim *et al.*, 2002). Endostatin is produced early in pregnancy by decidual cells (Pollheimer *et al.,* 2004) and inhibits trophoblast migration *in vitro* (Pollheimer *et al.,* 2011). Moreover, endostatin is detected at increased circulating concentrations at 16-20 weeks' gestation in women destined to develop PE compared to those proceeding with uncomplicated pregnancies (Wathen *et al.,* 2009). Together, these data suggest that endostatin potentially has a diverse, pathological role in PE by disrupting trophoblast function early in pregnancy and contributing to the maternal endothelial dysfunction that is characteristic of the latter stages of PE.

Soluble endoglin and endothelial cell dysfunction in PE

Trophoblast and endothelial cells express endoglin (Eng), a co-receptor for TGFβ1 and TGFβ3, which contributes to the regulation of vascular tone (St-Jacques *et al.,* 1994). Soluble endoglin (sEng) is a truncated form of Eng and as with sFlt-1, sEng is detected at increased concentrations in placental tissue from pre-eclamptic pregnancies when compared to normotensive pregnancies (Levine *et al.,* 2006). *In vitro,* Eng is anti-angiogenic and impairs the ability of endothelial cells to form capillaries (Dijke *et al.,* 2008). Both sFlt-1 and Eng are detected at increased concentrations in the last two months of uncomplicated pregnancies, but in PE-affected pregnancies, this increase is augmented and can be detected as early as in the first trimester (Romero *et al.,* 2008). Eng synergises with sFlt-1 and potentially contributes to the pathogenesis of PE (Mutter and Karumanchi, 2008), and as with sFlt-1, concentrations of sEng in the maternal circulation correlate with the clinical severity of PE (Venkatesha *et al.,* 2006).

Angiopoietin (Angpt) and the tyrosine kinase (Tie) receptors in PE

Angiopoietin-1 (Angpt-1), angiopoietin-2 (Angpt-2) and their receptor, Tie-2, have all been detected at the mRNA and protein level in decidual and placental tissue (Masonpierre *et al.,* 1997).

Their expression is localised to trophoblast and endothelial cells (Seval *et al.*, 2008). Angpt-1 and Angpt-2 bind the Tie-2 receptor with equal affinity but have different downstream effects. Angpt-1 acts to maintain the structural integrity of blood vessels and is thought to be involved in the latter stages of vascular remodelling (Geva and Jaffe, 2000), whilst Angpt-2 acts as a functional antagonist and disrupts endothelial cell adhesion interactions (Masonpierre *et al.*, 1997). Tie-1 has no identified ligand, but appears to modulate the activity of Tie-2 (Milner *et al.*, 2009). In addition to the regulation of vascular development during placentation, the spatial expression patterns of the angiopoietin family members are consistent with a role in the regulation of trophoblast function. For example, Angpt-1 is a chemotactic factor for tropohoblast and promotes EVT invasion into the decidua (Dunk *et al.*, 2000).

Extracellular matrix (ECM) remodelling and angiogenesis

Tumour necrosis factor receptor (TNFR) is a receptor for tumour necrosis factor alpha (TNFα). During the first trimester of pregnancy, TNFR is present at increased concentrations in the serum of women destined to develop PE (Leal *et al.*, 2009), and is also increased in women with established PE (Laskowska *et al.*, 2007). TNFR mRNA expression is up-regulated in both first and third trimester placental and decidual tissue (Wang and Walsh, 1996). Activity of this receptor influences the expression of a number of molecules involved in ECM degradation including matrix metallopepdidase-9 (MMP-9). MMP-9 is considered to be a key enzyme in EVT invasion and is present at increased concentrations in placental tissue from pre-eclamptic pregnancies when compared to tissue from normotensive pregnancies (Lockwood *et al.*, 2008). *In vitro*, signalling through TNFR causes increased expression of MMP-9, which may contribute to PE by disrupting critical steps involved in ECM degradation during both angiogenesis and EVT invasion (Lockwood *et al.*, 2008). *In vivo*, increased TNFR activity has been linked to an increased expression of plasminogen activator-inhibtor-1 (PAI-1). This has also been observed in *in vitro* placental explant

models where increased PAI-1 inhibits EVT migration (Bauer *et al.,* 2004). Compared with women experiencing normotensive pregnancies, women with PE have increased circulating concentrations of PAI-1 (Reith *et al.,* 1993).

Intercellular adhesion molecule-1 (ICAM-1) is an adhesion molecule detected at increased concentrations in the serum of women with established PE compared to those with normotensive pregnancies (Austgulen *et al.,* 1997). Interestingly however, ICAM-1 expression in PE-affected and non-PE-affected placental tissue has not been consistently observed, with reports indicating no difference in ICAM-1 expression in PE and non-PE affected placentae (Tziotis *et al.,* 2002), while others found increased expression (Goksu *et al.,* 2012). In contrast, intracellular adhesion molecule-3 (ICAM-3) was detected at increased concentrations in the placental bed of women with PE (Madazli *et* al., 2006), yet serum concentrations of the molecule have yet to be reported in PE. Further study is needed to establish the expression pattern and serum concentrations of the ICAMs in normotensive and PE pregnancies.

Clinical relevance of placental angiogenic markers to the diagnosis and prediction of early-on set PE.

Altered placentation and subsequent placental ischemia plays a critical role in the pathogenesis of PE. Whether release of anti-angiogenic factors is a cause or a consequence of these placental processes remains unclear. Altered placentation associated with PE occurs early in the first trimester, however at this stage of pregnancy serum concentrations of anti-angiogenic factors are not significantly different from those of pregnant women who progress to uncomplicated pregnancies. In contrast, low PlGF in the first trimester in combination with raised uterine artery Doppler velocimetry may be particularly useful in identifying early onset PE (Poon *et al.,* 2011). Understanding the role of early placentation defects in the aetiology of PE may be the key to early

diagnosis and prediction of PE. Noori *et al.* (2010) carried out a prospective study, starting at early pregnancy, to determine the sequence of changes in maternal circulating concentrations of PlGF, sEng and sFlt-1 and their relationship to maternal blood pressure and endothelial function, as well as uterine artery blood flow. The aim was to provide supporting evidence of a role for placenta-derived anti-angiogenic biomarkers in the control of maternal vascular resistance of PE. The authors investigated correlation between maternal serum sFlt-1, sEng, and PlGF concentrations hemodynamic factors (mean arterial pressure, MAP) at 4 fixed time points during pregnancy (10 to 17, 18 to 25, 26 to 33, and 34 to 40 weeks) and reported a strong relationship between all three angiogenic biomarkers and MAP during pregnancy. These observations support a progressive role for the placenta on maternal vascular resistance.

The implication of altered placenta-derived proteins in the pathogenesis of PE also opens the possibility for the development of novel targeted therapies. This will require appropriate animal models of PE to unravel how abnormal placental development contributes to early-onset PE. Replicating the PE human condition would require an animal model where changes in placental angiogenic factors occur secondary to impaired first trimester trophoblast invasion. Furthermore, animal models of PE should also model the adverse, characteristic fetal outcome. Using placenta-specific transgenesis and transduction of blastocyst-stage embryos with lenti-viral vectors, Okada *et al*. (2007) developed the unique sFLT1 over-expressing PE model. Using this animal model of PE, Kumasawa *et al.* (2010) demonstrated that the 3-hydroxy-3methyl-glutaryl-CoA (HMG-CoA) reductase inhibitor, pravastatin, induced PlGF and ameliorated sFLT1-induced PE in the mouse model. Future prospective studies and clinical trials are warranted to elucidate how the changes in the levels of placental angiogenic proteins can guide clinical management of PE in human pregnancy.

Therapeutic modalities to target placental angiogenic proteins

Early risk identification and stratification of PE pathogenesis would enable risk-tailored management of pregnancy. Identification of patients at risk for PE in subsequent pregnancies could allow preventative measures such as limiting the exposure of at risk pregnant women to potentially toxic medications and therapies. A recent meta-analysis demonstrated that aspirin has a "moderate but consistent" effect of decreasing the likelihood of a PE complication (Roberge *et al.,* 2013). Because the impact of aspirin can be observed only when administration begins as early as possible (i.e. ideally before the 16 weeks of pregnancy), the detection of angiogenic factor levels in early pregnancy would help identify those women who might have the greatest potential benefit from aspirin.

Clinical therapeutic trials targeting sFlt-1 will also benefit from the measurement of angiogenic biomarker levels. These data may assist in the recruitment of study participants and in the determination of dosage. Other therapeutic strategies such as the use of statins or dextran sulfate apheresis in preterm PE (Thadhani *et al.*, 2011) may benefit from the measurement of angiogenic biomarker levels. Two recent studies demonstrated that the measurement of angiogenic factors is not only useful in the triage setting for diagnosing PE, but also for the identification of patients at risk for adverse outcomes and preterm delivery (Chaiworapongsa *et al.,* 2011; Verlohren *et al.,* 2012). In routine clinical practice, uterine impedance to blood flow is determined by Doppler sonography (Harrington *et al.,* 1991). The resistance of the uterine artery is increased in PE, reflecting the mal-implantation of the trophoblast and insufficient spiral artery remodelling (Papageorghiou *et al.,* 2002; Spencer *et al.,* 2006). Nonetheless, Doppler assessment has a low positive predictive value in PE detection (Chien *et al.,* 2000). Extensive work clearly identifies maternal serum markers along with Doppler sonography measurements are useful in predicting early onset PE (Para et al., 2005; Stepan et al., 2007; Schlembech et al., 2007; Stepan et al., 2008).

Implications of these findings for the long-term cardiovascular complications of PE.

PE is a disease that has its origins in the placenta and culminates with systemic damage to maternal blood vessel endothelia. Most of the pathological conditions associated with PE resolve following delivery of the placenta and this constitutes a cure for PE can be cured. Nevertheless, women affected by PE have a life-time elevated risk of chronic adult diseases. There is growing evidence that women with a history of PE are more likely to develop cardiovascular disease (CVD) later in life (Brown *et al.,* 2013). Large cohort studies show that a woman's obstetric history is an important part of her risk profile for future CVD (Smith *et al.,* 2001; Wilson *et al.,* 2003; Ray *et al.,* 2005; Lykke *et al.,* 2009). Indeed, PE is associated with an up to eight-fold increased risk compared with women with no history of PE (Powe *et al.,* 2011). The mechanisms that account for this increased risk of cardiovascular disease in women with a history of PE are not yet well understood. Endothelial dysfunction, which has been linked to atherosclerosis, persists in women for many years after a PE-affected pregnancy (Shin et al., 2010). Evidence suggests that the maternal disease is attributable, at least in part, to release of antiangiogenic factors sFlt-1 and sEng from an abnormal placenta (Venkatesha *et al.,* 2006; Levine *et al.,* 2006; Noori *et al.,* 2010). These anti-angiogenic factors antagonize the effects of pro-angiogenic factors VEGF, PlGF, and TGF-β, which are important in the maintenance of the vascular endothelium. Although these anti-angiogenic proteins are likely to contribute to the maternal disease, and may prove to be useful diagnostic tools, the primary cause of the placental abnormality remains an area of intense investigation. Studies of cardiovascular function and atherogenic potential in animal models of PE where there is chronic exposure to altered levels of placental angiogenic proteins may reveal whether pro-angiogenic and anti-angiogenic molecules are possible contributors to cardiovascular disease in women.

Conclusion and future directions

The angiogenic imbalance in reproductive tissues and the maternal circulation observed in women with PE has been the subject of considerable research. Currently, we can appreciate that there are differences in the maternal angiogenic profile of a number of key angiogenic molecules in women affected by PE and that these differences may contribute to the altered physiological state both before and after the onset of PE. Further investigation into the mechanisms controlling the aberrant expression of these molecules in PE may provide novel insight and information that may be diagnostically and therapeutically relevant.

Figure 1. Trophoblasts at the feto-maternal interface during placentation in human pregnancies

This diagram was adapted from Qin *et al.* (2003). It shows the process of trophoblast differentiation and invasion into the maternal myometrium during the early stages of human placental formation. The direction of cell invasion is indicated. Multinucleated syncytiotrophoblast can be seen on the fetal placental side of the diagram and invasive, extravillous trophoblasts can be seen invading and remodelling the uterine blood vessels.

Cells of the anchoring villous form trophoblast columns from which extravillous trophoblast cells emerge to invade maternal tissue

Cells of the floating villous differentiate into syncytiotrophoblast.

(BV: blood vessel)

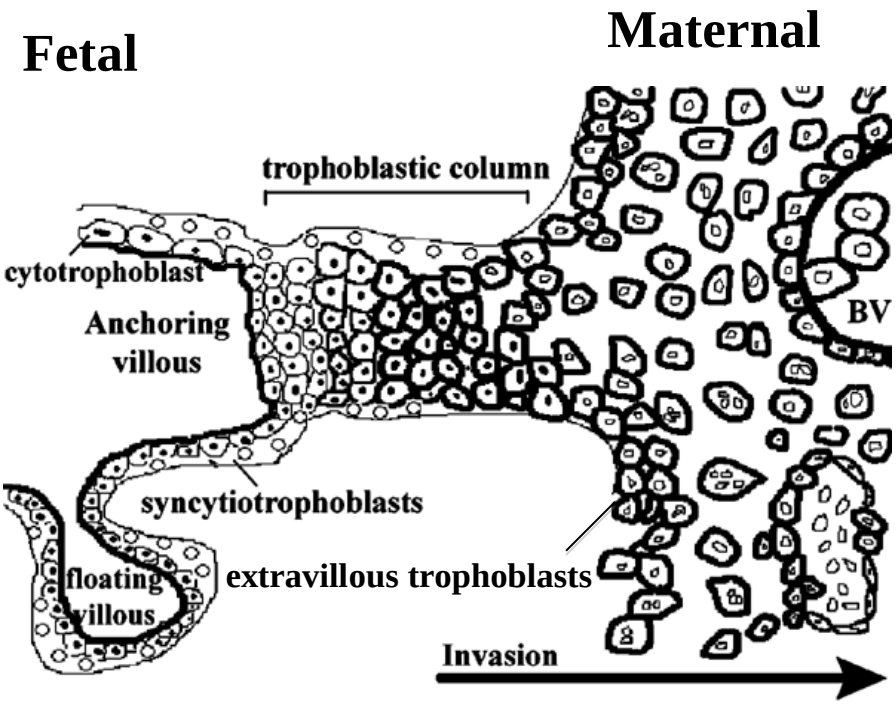


Figure 2. Trophoblast mediated remodelling of the uterine spiral arteries in non-PE and PE pregnancies

This figure adapted from VanWilk *et al.* (2000) shows uterine spiral artery re-modelling during pregnancies normal pregnancies compared to those complicated by pre-eclampsia. The diagram shows the retention of vascular smooth muscle lining the spiral artery and a shallow trophoblastic invasion into the decidua in a pre-eclamptic pregnancy.

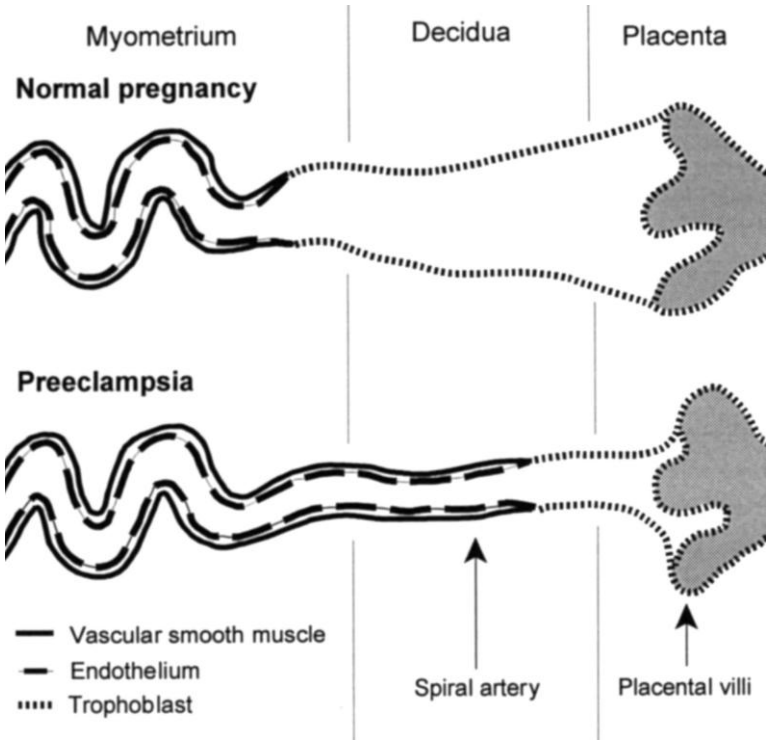
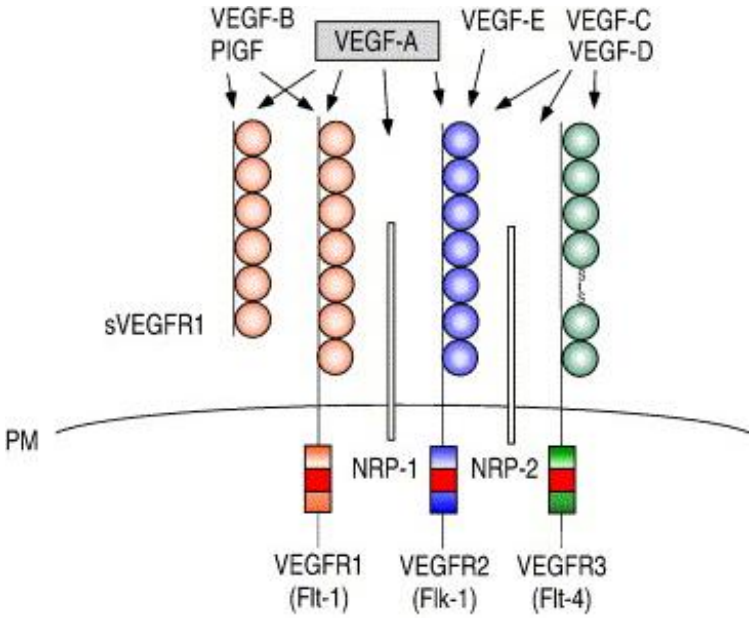


Figure 3. Vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor (VEGFR) interactions

This figure (adapted from Shibuya *et al.*, 2006) is a schematic representation of the VEGF family of angiogenic molecules (VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF) and their receptor (VEGFR-1, VEGFR-2 and VEGFR-3) interactions. Arrows have been used to indicate the binding of VEGFs to their receptors and co-receptors (NRP-1 and NRP-2). sVEGFR1 represents the soluble form of VEGFR-1 (denoted as sFlt-1 in the text).

PM: plasma membrane.



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