REPRODUCTION
REVIEW

Remodelling at the maternal–fetal interface: relevance to human pregnancy disorders

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Abstract

In human pregnancy, successful placentation and remodelling of the uterine vasculature require the integration of a number of stages, which are crucial for a healthy pregnancy. As the demands of the developing fetus for nutrients and oxygen increase, the capacity of the maternal blood vessels to supply this must be altered radically, with deficiencies in this process implicated in a number of dangerous pregnancy complications. The complex signalling networks that regulate these tightly co-ordinated events are becoming clearer as more studies of early pregnancy are performed. It is the aim of this review to draw together our knowledge of events that occur to facilitate a successful pregnancy ranging from the preparation for implantation, through the invasion and differentiation of the trophoblast and the regulation of these processes by other cells within the decidual environment, to the active role that the trophoblast and maternal immune cells play in facilitating the remodelling of the uterine spiral arteries. The events involved in a healthy pregnancy will then be compared to aberrant placentation and remodelling, which are characteristics of many pregnancy disorders, and recent advances in detection of abnormal placental development will also be discussed.

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Factors involved in setting up a successful pregnancy

Endometrial receptivity and implantation

The implantation of the blastocyst into a receptive endometrium in human pregnancy is a highly orchestrated reciprocal signalling process. However, the remodelling events required for a successful pregnancy begin before implantation with the decidualisation of the endometrium, which can occur even in the absence of a fertilised conceptus. Decidualisation commences in the mid-secretory phase of the menstrual cycle, when endometrial epithelial and stromal cells are under the control of rising progesterone levels and cease to proliferate and begin to differentiate (Dockery *et al.* 1988). Glandular epithelial cells begin to produce a number of secretory products and cytokines in preparation for implantation (Tabibzadeh *et al.* 1995, Cullinan *et al.* 1996). Stromal cells undergo the decidual reaction and differentiate, accompanied by an accumulation of maternal leukocytes mostly comprised of natural killer cells. Increased permeability of the uterine vasculature and maturation of the sub-epithelial capillary plexus prepare the vascular network for implantation (Demir *et al.* 2010). This period of ~5 days when the decidual reaction begins has been termed the 'window of implantation', and if pregnancy occurs and progesterone levels remain high, the decidua is maintained (Dimitriadis *et al.* 2010*b*). Decidualisation is a vital process for human pregnancy, functioning to provide maternal immune tolerance, protection of the fetus and to regulate placentation (Salker *et al.* 2010).

Many cytokines and growth factors secreted by the endometrium, which play a role in endometrial remodelling, have been identified, of which leukaemia inhibitory factor (LIF; a member of the interleukin 6 (IL6) family of proteins) is among the best characterised. Using mouse models, failure of blastocyst implantation in LIF-null mothers has been observed (Stewart *et al.* 1992) demonstrating that maternal LIF expression is vital for pregnancy, whereas LIF-null embryos implant normally (Dimitriadis *et al.* 2010*b*). LIF expression is dysregulated in many infertile women, indicating a function in setting up pregnancy, while *in vitro* studies have demonstrated that LIF (along with IL11) mediates expression of adhesion molecules on primary human endometrial epithelial cells (Marwood *et al.* 2009) implicating them in the process of blastocyst adhesion or trophoblast invasion. Endometrial expression of prokineticin 1 (PROK1) in the mid-secretory phase of the menstrual cycle has been implicated in the regulation of not only LIF but also several other genes involved in implantation such as cyclooxygenase-2 and heparin-binding epidermal growth factor (EGF; Evans *et al.* 2009). In addition to LIF, various chemokines are elevated at this stage of the menstrual cycle including chemokine ligand 7 (CCL7; also known as macrophage

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chemoattractant protein-3) and chemokine ligand 4 (CCL4; also known as macrophage inflammatory protein 1β), and have been suggested to play a role in leukocyte or blastocyst chemoattraction to the endometrium (Power 2003, Jones *et al*. 2004). However, as no chemokine or chemokine receptor knock out mouse has yet displayed an impaired reproductive phenotype, there may be a high degree of redundancy.

Implantation is a dialogue between the mother and the fetus. Signals from the blastocyst also direct the remodelling processes of the uterus allowing adhesion and invasion. Several signalling processes in the endometrium are only activated by an implanted blastocyst, such as the Wnt/β-catenin signalling pathway (Mohamed *et al*. 2005), and there is evidence that human chorionic gonadotrophin (hCG) produced by the blastocyst can stimulate LIF expression by the endometrium (Evans *et al*. 2009). Furthermore, in contrast to many animal pregnancies, the anti-adhesive mucin 1 (MUC1) is up-regulated during the implantation period in humans, but the implanting blastocyst is able to down-regulate MUC1 in the maternal epithelia, as demonstrated by its disappearance from endometrial cells located beneath and around the blastocyst *in vitro* (Meseguer *et al*. 2001).

Trophoblast invasion and differentiation

During the first trimester of human pregnancy, the placenta develops into a branching villous structure with differentiation of specialist trophoblast types that differ in function. Cytotrophoblast progenitors found in the villi follow two differentiation pathways; some fuse to form the multinucleated syncytiotrophoblast layer, which encases the floating villi of the placenta and provides the barrier to maternal blood, regulating oxygen and protein transport. Other cytotrophoblasts follow an invasive pathway and differentiate into extravillous trophoblast (EVT). These cells migrate from villous tips in columns anchoring the placenta to the maternal decidua, and EVT forms the cytotrophoblast shell over the decidua as well as migrating and invading into the decidua. Invasive EVT plays an active role in the remodelling events that occur in the uterine spiral arteries. This leads to both loss of vascular cells and alterations in extracellular matrix (ECM) proteins resulting in arteries that are dilated, non-vasoactive vessels by the mid-second trimester, allowing greater transport of maternal blood to the intervillous space (Brosens *et al*. 1967, reviewed by Whitley & Cartwright (2010)). Invasive EVT can be classified as either interstitial, which migrates into the decidua and later differentiates into giant cells in the myometrium, or endovascular, which is present within the lumen of uterine vessels. The importance of these different populations in the remodelling process will be discussed later.

As EVT detaches from the cell column and invades the decidua, it undergoes a differentiation process resulting in altered expression of surface antigens and function. The point of commitment to an invasive lineage is unclear, as there is evidence for both bipotential cytotrophoblasts and the existence of two separate progenitor cell types (James *et al*. 2007). In the decidua, EVT interacts with decidual stromal, epithelial and immune cells, all of which are able to regulate their differentiation and invasive potential. As EVT invades, it exits the cell cycle thereby preventing proliferation. The expression of a number of transcription factors that are implicated in cell cycle arrest have been identified in EVT (Loregger *et al*. 2003) including the up-regulation of the activator protein (AP-1) family of transcription factors, which control expression of a number of genes involved in growth and differentiation (Bamberger *et al*. 2004). Other factors thought to play a role in EVT differentiation include STAT3 and peroxisome proliferator-activated receptor-γ (PPARG; Knofler 2010).

Invasion into the decidua requires the up-regulation of proteases to degrade the ECM. Urokinase plasminogen activator (PLAU) promotes migration of EVT through matrix degradation as well as non-degradatory pathways (Liu *et al*. 2003). PLAU is also able to activate several matrix metalloproteinases (MMPs) produced by EVTs, and *in vitro* studies have shown that migrating trophoblasts up-regulate MMP2 (Staun-Ram *et al*. 2009), MMP3, MMP9 (LaMarca *et al*. 2005) and cathepsins (Varanou *et al*. 2006).

EVT comes into contact with a number of different maternal cell types, and invasion is both stimulated and inhibited by contact with the decidua. Several decidual factors have been identified which stimulate EVT migration, and these include hepatocyte growth factor (Cartwright *et al*. 2002) and EGF (Staun-Ram *et al*. 2009). Other factors that limit trophoblast invasion have been described, including interferon-γ (IFNG) and transforming growth factor-β (TGFB), highlighting the importance of controlling the extent of EVT invasion (Knofler 2010). Furthermore, factors associated with decidualisation and implantation are also implicated in the regulation of EVT invasion; for example, IL11 inhibits EVT invasion, while LIF alters the protease and adhesion molecule expression profile of EVTs, as well as promoting invasion (Dimitriadis *et al*. 2010*a*). The microenvironment into which EVT invades will influence expression of factors, and oxygen concentration is known to alter EVT behaviour. The physiologically hypoxic conditions (~2–3% oxygen) in the first trimester are thought to promote an invasive EVT phenotype (James *et al*. 2006).

The role of decidual natural killer (dNK) cells in regulating cellular interactions in successful placentation is considered to be particularly important, with their potential roles summarised in Fig. 1. Decidualisation is accompanied by substantial recruitment of NK cells, which constitute ~70% of maternal immune cells in the decidua basalis (Moffett-King 2002). dNK and peripheral blood NK (PB-NK) cells have phenotypically and

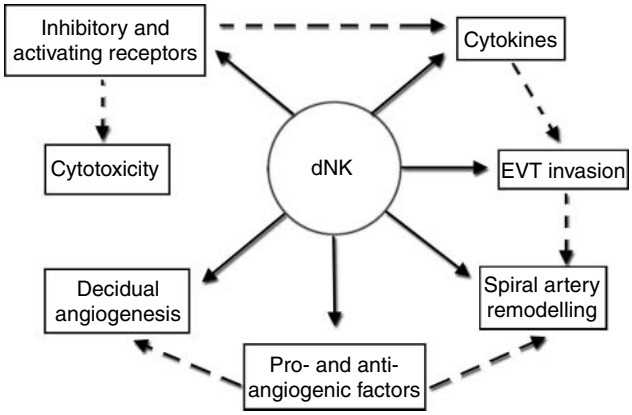

Figure 1 Possible roles of dNK cells in placentation and spiral artery remodelling. Direct effects are indicated by solid arrows, and indirect effects are indicated by dashed arrows.

functionally distinct populations. PB-NK cells, mainly (~90%) $CD56^{dim}CD16^{+}$, represent a granular cytotoxic population, with a small number exhibiting a $CD56^{bright}CD16^{-}$ non-cytotoxic phenotype. In contrast, the majority of dNK cells are $CD56^{bright}CD16^{-}$ and are considered a cytokine-producing rather than cytotoxic population (Tabiasco *et al*. 2006). Other immune cells present in the decidua include macrophages (~20–30%) and T-cells and B-cells (~2%). The effector functions of dNK cells are regulated by their expression of inhibitory and activating receptors, which interact with major histocompatibility complex (MHC) antigens. These receptors are classified structurally into the following families: the killer immunoglobulin-like receptors (KIR), the C-type lectin heterodimer family (CD94/NKGs), the natural killer cytotoxicity receptors (NCR) and the immunoglobulin-like transcripts (ILT; Moffett-King 2002, Tabiasco *et al*. 2006). The majority of the ligands for these receptors are HLA class I molecules expressed on EVTs: HLA-G, HLA-C and HLA-E. The expression profile of different inhibitory and activating receptors will influence cell behaviour and cytokine production, which will, in turn, regulate the extent of trophoblast invasion.

dNK cells express many cytokines and chemokines such as IL10, IL8, granulocyte macrophage colony stimulating factor (CSF2), CSF1 and tumour necrosis factor α (TNF), which may signal to trophoblast to regulate its invasion (Hanna *et al*. 2006). In addition, dNK cells express pro- and anti-angiogenic factors, including angiopoietin-1 (ANGPT1), ANGPT2, TGFB, vascular endothelial growth factor (VEGF) and placental growth factor (PGF), which not only act on trophoblasts but are able to influence the uterine vessel vascular cells, discussed below (Hanna *et al*. 2006, Lash *et al*. 2006). A balanced production of these regulatory proteins coupled with the pattern of expression of activating and inhibitory receptors would be crucial for controlling their potentially cytotoxic functions while promoting their roles in successful placentation and remodelling. Decidual macrophages are at a high concentration around invading trophoblast and may have roles in phagocytosis, tissue remodelling, interacting with trophoblasts, as well as in antigen presentation and innate defence. Differentiation is required to develop the decidual macrophage phenotype, with the state of activation as well as their secretion of cytokines influencing EVT invasion (Renaud *et al*. 2007).

Factors involved in spiral artery remodelling

Spiral artery remodelling begins in the first few weeks of pregnancy and modifies the arteries from low-flow, high-resistance to high-flow, low-resistance vessels capable of meeting the demands of the developing fetus, in what was first described as the 'physiological changes of pregnancy' (Brosens *et al*. 1967). The transformation of the spiral arteries following EVT invasion increases blood flow to the placenta with the alterations in vessel properties occurring as a result of remodelling of the vessel wall, and the extent of these processes is unique to human pregnancy. The remodelled vessel results from the culmination of a number of biological processes taking place in an ordered fashion over many weeks with crosstalk between immune cells, EVT and vascular cells key to these events (summarised in Fig. 2). The remodelling of maternal spiral arteries can be considered to occur in stages. The first stage involves trophoblast-independent mechanisms; the second stage involves the removal of vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) by invasive EVT and other decidual cell types. Finally, ECs are replaced with endovascular EVT, and extracellular fibrinoid is deposited. Endovascular EVT is detected in decidual vessels from 8 weeks, after the earlier plugging of the vessels, with deep invasion of the myometrial sections seen after 15 weeks (Pijnenborg *et al*. 1980). Endovascular EVT invasion continues until mid-second trimester reaching the inner third of the myometrium (Pijnenborg *et al*. 1981). Several interdependent mechanisms are involved in these changes including ECM restructuring, vascular cell de-differentiation, migration, changes in cellular adhesion and sensitivity to death-inducing stimuli. The importance of these events occurring in a regulated manner is illustrated by pregnancy complications associated with insufficient spiral artery remodelling, such as pre-eclampsia and intra-uterine growth restriction (IUGR; Pijnenborg *et al*. 1981), which are discussed further later. The mechanisms by which spiral artery remodelling occurs have not been fully elucidated, although we and others have now implicated a number of maternal and fetal processes in facilitating these crucial events (reviewed by Pijnenborg *et al*. (2006) and Whitley & Cartwright (2009)).

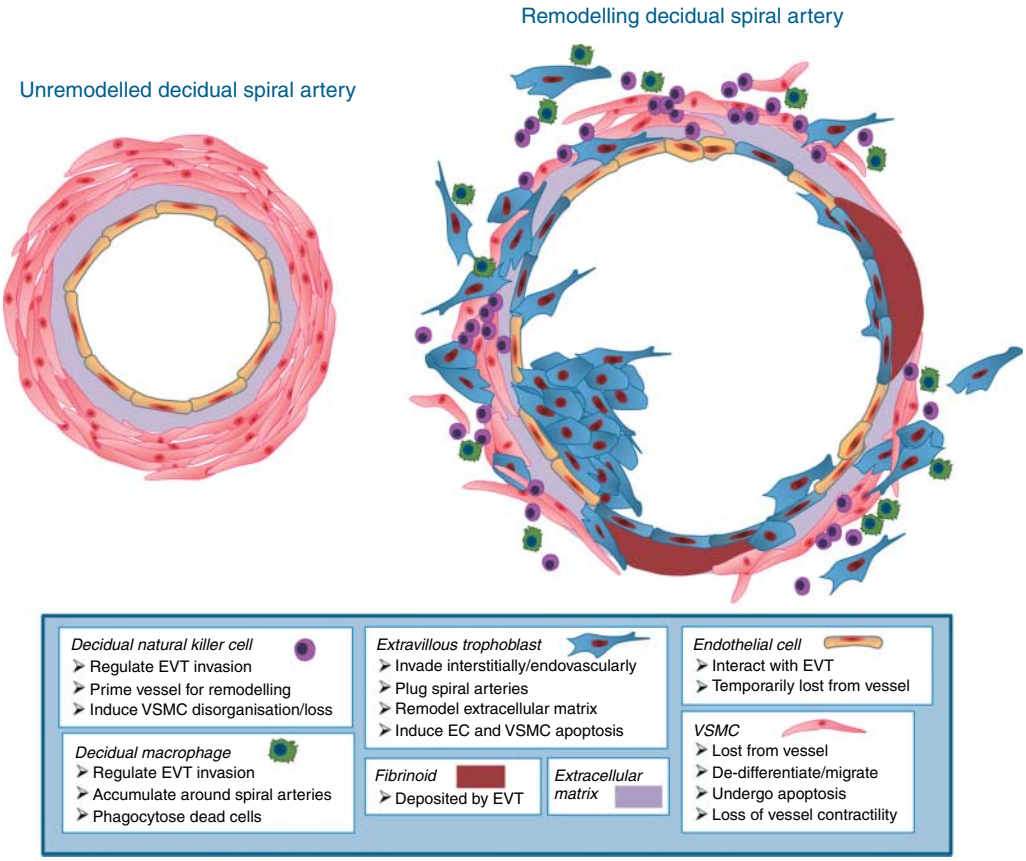

Figure 2 Diagram of the cellular interactions involved in decidual spiral artery remodelling. Prior to remodelling, low-flow, high-resistance spiral arteries have intact endothelium with ECM proteins and a layer of VSMCs. During spiral artery remodelling, the vessel structure changes with loss of vascular cells, and this increases the size of the arteries and creates a high-flow, low-resistance vessel. These changes are brought about partially by maternal immune cells (dNK cells and macrophages) with the changes completed by invading interstitial and endovascular EVT. The remodelled vessel consists of trophoblasts embedded in a fibrinoid material as a replacement for the VSMCs, with subsequent re-endothelialisation occurring later in pregnancy.

Several changes in EC and VSMC characteristics of vascular remodelling, such as vacuolisation, dilation, muscular hypertrophy, disorganisation and the start of fibrinoid change, can be detected in spiral arteries prior to trophoblast interaction (Craven *et al.* 1998). These initial stages may be regulated by immune cells (dNK cells and macrophages), which are localised near the vessel walls of first trimester decidual arteries ahead of trophoblast invasion at a time at which vascular changes can be detected *in vivo* (King *et al.* 1998, Smith *et al.* 2009). Studies in mice have implicated dNK cells in the modification of uterine blood vessels via an IFNG pathway (Ashkar *et al.* 2000). IFNG regulates the expression of a large number of genes involved with cell adhesion, smooth muscle cell proliferation and apoptosis, which could be important in the initial remodelling stages. Macrophages and dNK cells can also produce many MMPs, such as MMP7 and -9 (Smith *et al.* 2009), which along with pro- and anti-angiogenic factors such as VEGF, PGF and angiopoietins could influence vessel stability. Recent studies using placental–decidual co-culture model have implicated MMP2 and -9 in the remodelling events initiated by leukocytes (Hazan *et al.* 2010). Studies have indicated that dNK cells begin to disperse after spiral artery remodelling is complete, and are found in much reduced numbers at term (Williams *et al.* 2009*b*). However, the role that dNK cells play in remodelling is likely to be limited to the decidual segments of vessels since dNK cells are not as abundant in the inner myometrium (Pijnenborg *et al.* 2006).

Invasive trophoblast, located both interstitially and endovascularly, plays an active role in inducing loss of VSMCs and ECs and further remodelling of the ECM. Endovascular EVT migrates in a retrograde manner through the lumen of the vessels and forms trophoblast plugs. It is likely that its interaction with ECs will be most important in the remodelling process, whereas interstitial EVT will be positioned to firstly interact with VSMCs. It is unclear whether endovascular EVT arises from the migration of trophoblasts down the spiral artery or from invasion of the decidual interstitial EVT, and it may be a combination of these two mechanisms (Kaufmann *et al.* 2003, Pijnenborg *et al.* 2006).

The differentiation of EVT as it invades towards the uterine vessels may be influenced by other cell types such as dNK cells (Hu *et al*. 2006), and is thought to result in trophoblasts which may partially mimic ECs (Zhou *et al*. 1997*b*), although the relevance of this has also been debated (Pijnenborg *et al*. 2006). Invasive trophoblasts up-regulate expression of VE-cadherin, platelet endothelial adhesion molecule (PECAM)-1, vascular cell adhesion molecule (VCAM)-1, the α4 integrins and $\alpha_v\beta_3$ integrin, which are typically expressed by the endothelium that they replace (Zhou *et al*. 1997*b*). *In vitro* studies have shown the importance of these molecules in invasion and interactions with ECs (Bulla *et al*. 2005). The $\alpha_v\beta_3$ integrins increase adhesion of trophoblasts to ECs, and α4 integrins bind to VCAM expressed by ECs. VE-cadherin and $\alpha_v\beta_3$ have been shown to enhance trophoblast invasion, while E-cadherin expression decreases during trophoblast differentiation and may inhibit trophoblast invasion. Endovascular EVT in trophoblast plugs also expresses the adhesion molecule NCAM/CD56, which may aid its binding to the EC lumen. The reason why invasive trophoblast interacts with arteries rather than veins is unclear, and the up-regulation of specific ephrins and their ligands (Red-Horse *et al*. 2005) as well as the vascular cell production of specific chemokines such as IL8 and chemokine (C-X-C) motif ligand 1 (CXCL1; formerly Gro-α) (Aldo *et al*. 2007) may be important in regulating this.

The mechanisms by which EVT (and immune cells) brings about loss of the vascular cells are likely to involve a number of different processes co-ordinated in a temporal and spatial manner. Studies by our group and those of others have implicated trophoblast-induced apoptotic signalling events and remodelling of the ECM proteins in these events (reviewed by Whitley & Cartwright (2009, 2010)). EVT has been shown to produce cytokines such as TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FASLG) which on binding to their receptors (members of the TNF receptor family) initiate a signalling cascade resulting in caspase activation and apoptosis. A number of studies (*in vitro* co-cultures, an *ex vivo* dissected spiral artery model and an *in vivo* model) have shown that EVT can induce caspase-dependent apoptosis in both ECs and VSMCs (Ashton *et al*. 2005, Harris *et al*. 2006, 2007, Red-Horse *et al*. 2006). EVT production of FASLG is important in both the EC and VSMC events (Ashton *et al*. 2005, Harris *et al*. 2006), while an additional effect of TRAIL has been demonstrated in EVT-dependent VSMC apoptosis (Keogh *et al*. 2007). Apoptotic markers have been detected immunohistochemically in first trimester spiral arteries at a stage at which remodelling is taking place, although before much trophoblast was present (Smith *et al*. 2009, Hazan *et al*. 2010). Trophoblast involvement in the clearance of apoptotic vascular cells is likely since it has been demonstrated that they can induce EC death and then rapidly phagocytose the apoptotic cells (Chen *et al*. 2005), a role that has also been suggested for the surrounding macrophage cells (Hazan *et al*. 2010).

In addition to the induction of vascular cell apoptosis, other events occurring in the actively remodelling spiral artery may include detachment of the vascular cells from the surrounding ECM (which can also induce apoptosis), migration away from the vessel and de-differentiation of VSMCs (Whitley & Cartwright 2010). Many of the cell types involved in remodelling synthesise and secrete proteases that degrade ECM proteins and remodel the matrix; for example, invasive EVT expresses MMP1, -2, -9 and -12 (Harris & Aplin 2007), and dNK cells have an active PLAU system (Naruse *et al*. 2009). Proteases may also amplify pro-apoptotic effects since they are involved in the production of TRAIL and FASLG.

These physiological changes give rise to a vessel that has completely lost both vascular cell layers and vasomotor function with replacement by a layer of trophoblast embedded in a fibrinoid matrix. At this point, leukocytes are absent from around the vessels (Smith *et al*. 2009). Studies of third trimester placental bed biopsies have shown that there is later re-endothelialisation of the majority of remodelled spiral arteries (Khong *et al*. 1992), although the mechanisms by which this occurs have not been investigated. There is also thickening of the intima, with cells present which may function as VSMC precursors and be of importance in the repair of the vessels *post partum*.

Consequences of aberrant placentation and spiral artery remodelling

As our understanding of these complex cellular and molecular interactions increases, it is clear that there are multiple stages of the placentation and remodelling process which could be compromised. In the final section, we will discuss the most prevalent pregnancy disorders with impaired placentation/remodelling and draw on our knowledge of normal pregnancy described above to suggest possible areas of aberrant function. Finally, we will discuss whether assessments that can be made non-invasively in the first trimester of ongoing pregnancies can accurately represent the biology of the maternal–fetal interface.

Recurrent miscarriage

Recurrent miscarriage (three or more consecutive pregnancy losses) affects 1–3% of couples, and ~50% of these cases are of unknown aetiology. A range of potential mechanisms have been suggested in these cases including the involvement of NK cells, oxidative stress, decidual angiogenesis and antiphospholipid antibodies (aPL). An increase in the density of uterine NK cells has been reported in mid-luteal phase

endometrium of women suffering from idiopathic recurrent miscarriage (Clifford *et al*. 1999), and this may act to increase angiogenesis and peri-implantation blood flow (Quenby *et al*. 2009). It has recently been suggested that recurrent miscarriage may be attributed to impaired decidualisation, and hence an altered receptivity window. In endometrial cells taken from women with recurrent miscarriage, the marker of decidualisation PROK1 showed increased and lengthened expression, indicating a longer period of receptivity. It has been postulated that this may perturb the normal process of embryo selection (Salker *et al*. 2010).

Premature loosening of the trophoblast plugs and consequent premature and disorganised flow of maternal blood into the intervillous space have been associated with recurrent miscarriage, with such placentae demonstrating increased levels of placental oxidative stress and trophoblast degeneration (Hempstock *et al*. 2003). It has been proposed that NK-mediated increases in peri-implantation blood flow could facilitate this process (Quenby *et al*. 2009). However, further work is required to understand the relationship between uterine NK cells and recurrent miscarriage and to attribute a causative role.

aPL are a heterogeneous group of autoantibodies reactive with negatively charged phospholipids and their associated plasma protein co-factors. Women with idiopathic recurrent miscarriage have an increased frequency of aPL in comparison to normal fertile women (Buckingham & Chamley 2009). The reason for this remains unclear, but aPL have been linked to a wide range of factors involved in successful pregnancy. Several pro-thrombotic mechanisms have been proposed including alterations in eicosanoid balance, cross reactivity with glycosaminoglycans and interference with the function of natural inhibitors of coagulation such as annexin V present on the surface of the syncytiotrophoblasts (Cervera & Balasch 2009). aPL have also been shown to have direct placental effects and are capable of inhibiting both trophoblast differentiation and invasion, and may also affect endometrial decidualisation to an extent that may be sufficient to cause the loss of a pregnancy that is compromised by other factors (Buckingham & Chamley 2009).

Pre-eclampsia

Pre-eclampsia affects ∼3–5% of pregnancies, and can result in serious health consequences for both the mother and baby. Pre-eclampsia is characterised by gestational hypertension, proteinuria and systemic EC activation, and is classified clinically by two separate blood pressure recordings of >140/90 mmHg at least 4 h apart in previously normotensive women, and either ≥300 mg of proteinuria in 24 h, or ≥2+ proteinuria by dipstick testing in the absence of renal disease or infection (Brown *et al*. 2001). It is evident that the placenta is both required and sufficient to cause the disorder, and removing the placenta by delivering the baby remains the only absolute cure. The symptoms of pre-eclampsia do not present until ∼20 weeks of gestation onwards; however, it is in the first trimester that the pathogenesis is established where invasion of trophoblast into the decidua and remodelling of the spiral arteries are occurring. Pre-eclampsia and IUGR have been associated with inadequate spiral artery remodelling (Brosens *et al*. 1972, Pijnenborg *et al*. 1991). In a third trimester normal pregnancy, the mean external myometrial spiral artery diameter is 500 μm, while in pre-eclampsia, the diameter is ∼200 μm (Brosens *et al*. 1972) and defective remodelling can be detected in both decidual and myometrial portions of the vessels. Impairment of the events involved in normal spiral artery remodelling has the potential to affect the blood supply to the placenta by altering the flow rate of blood into the intervillous space and by altering the consistency of the blood flow, which can lead to fluctuations in the supply of oxygen to the placenta.

As pregnancy continues, inadequate placental perfusion can, in turn, result in hypoxia–reperfusion-type injuries to the placenta which can lead to modifications of lipids and proteins, mitochondrial and endoplasmic reticulum stress and apoptosis/necrosis (Burton *et al*. 2009). For example, oxidative stress can induce hypoxia-inducible factor-1 (HIF-1)-mediated degradation of glial cell missing-1 (GCM1), a transcription factor involved in regulating syncytiotrophoblast formation and turnover. Expression of GCM1 is known to be down-regulated in pre-eclamptic placentae (Chiang *et al*. 2009). The increased or turbulent velocities resulting from inadequate spiral artery remodelling have been shown to increase the amount of syncytial debris shed, as well as altering its properties (Hutchinson *et al*. 2009). A combination of these effects is believed to lead to an increase in the volume of debris shed from the placenta in pre-eclampsia, and may favour necrotic rather than apoptotic death (Redman & Sargent 2009). In normal pregnancy, syncytiotrophoblast particles/material may be shed from the placenta by apoptotic mechanisms and since phagocytosis of apoptotic material is not generally pro-inflammatory, and indeed may even be immunosuppressive, this debris may play an important role in the success of pregnancy (Abumaree *et al*. 2006). In contrast, necrotic trophoblast debris is able to activate ECs and stimulate the release of the pro-inflammatory cytokines. Sub-microscopic pieces of syncytiotrophoblast debris (microparticles), released from pre-eclamptic placentae, or as a result of induced oxidative stress, have also been shown to be more inflammatory in nature (Redman & Sargent 2007). In addition to the placental factors involved in pre-eclampsia, the underlying cardiovascular profile of the mother and her handling of factors produced by an oxidatively stressed placenta appears to

be of relevance in the development, timing of onset and severity of the disease.

The reasons for impaired spiral artery remodelling in pre-eclampsia remain relatively unclear despite considerable investigation. All of the stages setting up successful placentation and remodelling discussed above may potentially have defects (summarised in Fig. 3). For example, the influx of NK cells during decidualisation and their subsequent role in interacting with invasive EVTs and spiral artery cells could be impaired, supported by immunohistochemical studies showing reduced decidual leukocytes in pre-eclampsia (Williams *et al.* 2009*a*). Other factors of importance in regulating trophoblast invasion such as EVT expression of a particular adhesion molecule repertoire (Zhou *et al.* 1997*a*), levels of MHC class I molecules such as HLA-G (Le Bouteiller *et al.* 2003), increased apoptosis (Genbacev *et al.* 1999) or production of MMPs (Reister *et al.* 2006) have also been suggested. The signalling events that occur firstly between leukocytes and vascular cells and then subsequently between trophoblasts and vascular cells may also be impaired; however, this remains to be comprehensively investigated.

Intra-uterine growth restriction

In ∼30% of cases, pre-eclampsia is associated with IUGR – the failure of the fetus to reach its optimal growth potential. However, IUGR is not necessarily secondary to pre-eclampsia and is also seen in 8–14% of normotensive pregnancies. IUGR is believed to arise as a result of inadequate blood supply to the placenta and/or inadequate transport of nutrients across the placenta to the fetus. This can result from a range of mechanisms including reduced uteroplacental blood flow, compromised feto-placental angiogenesis and subsequent

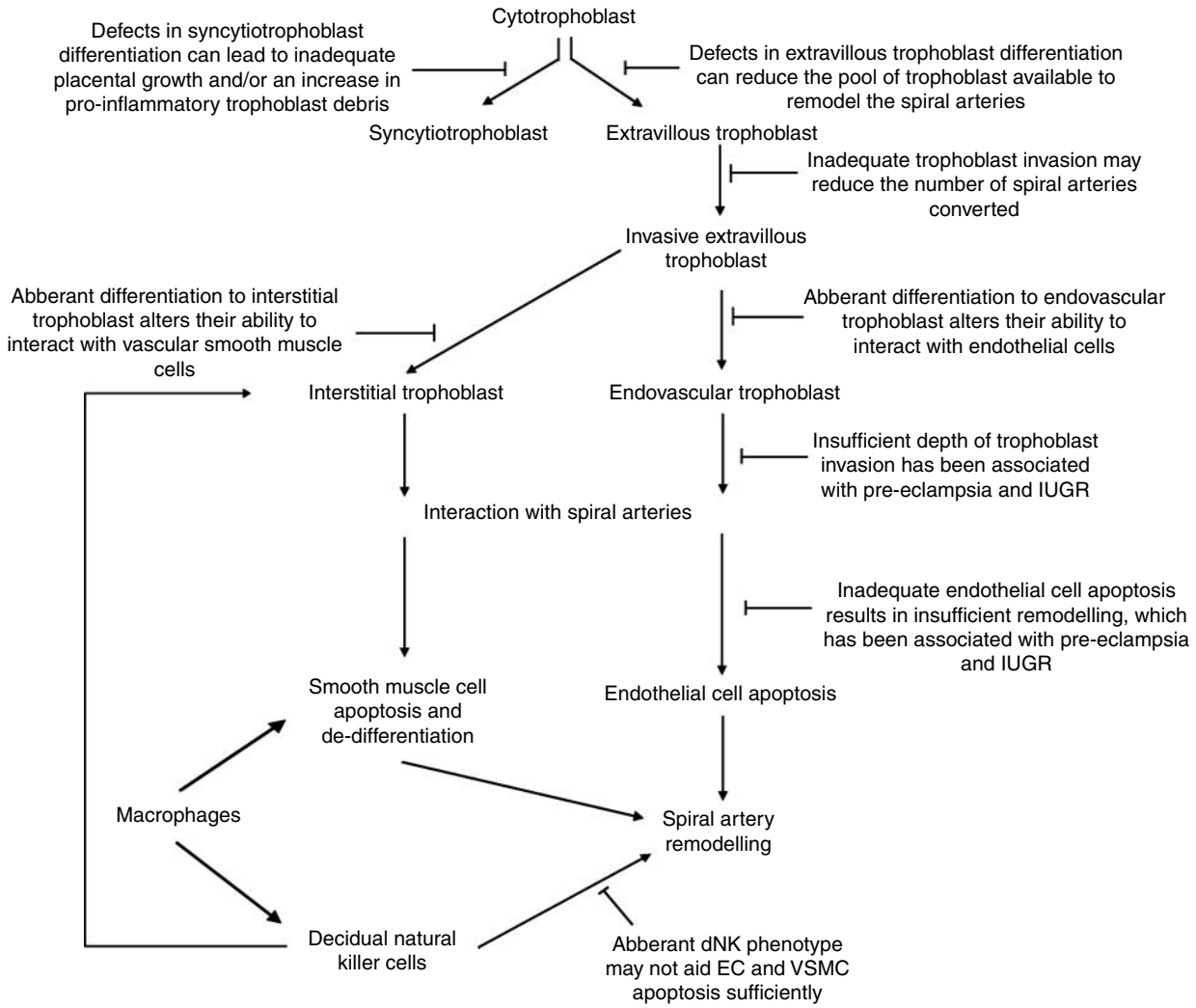

Figure 3 Schematic diagram representing the stages of trophoblast differentiation and invasion, with indication of where this may be aberrant in pathological pregnancies.

villous development, and/or a reduced expression of placental transporter activity. Immune cells resident in the decidua may play a role in both pre-eclampsia and IUGR. Placental bed biopsies from cases of IUGR show a reduction in dNK cells; this was also seen in biopsies from pre-eclamptic pregnancies along with a reduction in T lymphocytes and macrophages (Williams *et al.* 2009*a*). This suggests that differences in cytokines and growth factors produced by immune cells may be important in the pathogenesis of both pre-eclampsia and IUGR, potentially through effects on spiral artery remodelling and uteroplacental blood flow which are previously described. However, IUGR alone does not appear to exhibit the inflammatory and cardiovascular responses that are characteristic of pre-eclampsia; for example, the anti-angiogenic factors such as soluble endoglin, soluble fms-like tyrosine kinase 1 and endostatin have all been shown to be increased in cases of pre-eclampsia, but not in cases of normotensive IUGR (Shibata *et al.* 2005).

Detection of aberrant placentation/remodelling

Clinical diagnosis and reproductive research aimed at understanding both normal placentation and the pathological events that lead to pregnancy complications have been severely hampered by an inability to predict whether first trimester pregnancies are developing normally/abnormally. The search to date for preventative treatments has been disappointing, and it is likely that, to be effective, any future interventions will need to begin early in the disease pathology. Classification of risk early on will also allow appropriate planning of antenatal care.

Normal remodelling of the spiral arteries results in an increased delivery of maternal blood through a low pressure placental bed and, where this remodelling is incomplete, there will be an increased resistance to maternal blood flow. Assessment of impedance to uterine artery blood flow by Doppler ultrasound can provide a proxy measure of the degree to which successful remodelling has occurred (Campbell *et al.* 1983). In normal pregnancy, impedance to flow decreases with advancing gestation, while in pregnancies affected by early onset pre-eclampsia and IUGR, impedance is increased (Papageorghiou 2008). Histological examination of placentae from these pregnancies has shown deficient spiral artery remodelling, and there is also evidence to show that abnormal uterine artery Doppler in the first trimester is associated with deficient trophoblast invasion (Prefumo *et al.* 2004).

The use of uterine artery Doppler as a screening tool for pre-eclampsia and other pregnancy complications such as IUGR remains controversial. Criticism has tended to focus on the low positive predictive values for term disease reported in clinical trials in low-risk populations (Conde-Agudelo *et al.* 2004). It is, however, important that attention focuses on severe pre-eclampsia requiring delivery before 34 weeks. This is the severe end of the spectrum of disease and is less common than term disease (occurring in about 0.5% of pregnancies), but is the commonest cause of iatrogenic prematurity and is associated with increased perinatal mortality and morbidity. In the prediction of pre-eclampsia requiring delivery before 34 weeks, Doppler performs well with several large trials showing a detection rate of about 80% in the second trimester (Papageorghiou *et al.* 2001, Onwudiwe *et al.* 2008). Doppler also performs better at all gestations in predicting pre-eclampsia associated with IUGR (sensitivity 70%) than pre-eclampsia alone (25%) for a 5% false positive rate (Papageorghiou *et al.* 2001). The prediction of term pre-eclampsia is poorer with Doppler, and this may reflect fundamental differences in the underlying pathophysiology. It has been suggested that the term pre-eclampsia does not involve aberrant first trimester placentation and spiral artery remodelling as seen in the early onset pre-eclampsia and may be caused by atherosis of an initially normal placental bed (Papageorghiou 2008). Pre-eclampsia represents a heterogeneous spectrum of disease resulting from both placental and maternal factors, and it is therefore not surprising that no other screening modality will adequately predict all presentations.

Over the last 5–10 years, the focus has moved from second trimester screening to the first trimester. Although it is still not possible to categorically predict pre-eclampsia in the first trimester, it is possible to classify which pregnancies have a high- and low-risk of developing pre-eclampsia using Doppler scanning (Melchiorre *et al.* 2008). The largest study to date of uterine artery Doppler in 6015 women at 11 to 13+6 weeks of gestation had a sensitivity of 82% for pre-eclampsia requiring delivery before 34 weeks with a 10% false positive rate (Plasencia *et al.* 2007). Traditionally, a view has been held that a high-resistance pattern in the first trimester represents normal physiology and that it is only when women have a persisting high-resistance pattern beyond the second trimester that it becomes significant or pathological. This tied in with initial theories of two stages of trophoblast invasion and remodelling (Pijnenborg *et al.* 1983), with an early shallow wave in the first trimester followed by a second wave of deep trophoblast invasion in the second trimester that is completed by 18–20 weeks and leads to the conversion of the uteroplacental circulation into a low-resistance bed. This view has been questioned (Lyall 2002), with suggestion that remodelling progresses as a dynamic and continuous process, which will vary between individuals due to interacting fetal and maternal factors. It is therefore not surprising that some women who are in the highest centile of impedance to flow will have normal patterns in the second trimester and vice versa. However, at any point in gestation from

the late first trimester onwards, the group of women with the highest impedance to flow will contain those women who are at the highest risk of developing disease. Of course, the intriguing unanswered question remains; what factors regulate the defective placentation and vessel remodelling behind this?

This question may begin to be answered since the opportunity to obtain first trimester tissue from women screened by Doppler prior to termination of pregnancy represents an important advance in our ability to unravel the complex signalling events at the maternal–fetal interface in both a normal and pathological pregnancy. Indeed, the use of this resource is beginning to define inherent biological differences early on in tissue and isolated primary cells between pregnancies classified into risk groups for developing pre-eclampsia (Prefumo *et al*. 2004, Whitley *et al*. 2007).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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