

## Angiogenesis in implantation

Donald S. Torry · Jonathan Leavenworth ·  
Miao Chang · Vatsala Maheshwari · Kathleen Groesch ·  
Evan R. Ball · Ronald J. Torry

Published online: 7 July 2007  
© Springer Science + Business Media, LLC 2007

### Abstract

**Problem** Implantation failure and early pregnancy loss are common following natural conceptions and they are particularly important clinical hurdles to overcome following assisted reproduction attempts. The importance of adequate vascular development and maintenance during implantation has recently become a major focus of investigation.

**Materials and methods** Review of current published literature was undertaken to summarize the cells and cell products that regulate tissue vascularity during implantation. **Results** Vascular development at the maternal fetal interface can be regulated by a number of different cell types; two principal candidates are trophoblast and natural killer cells. A wide range of soluble factors, some with well established angiogenic functions as well as other more novel factors, can contribute to vascular development and maintenance at the maternal–fetal interface.

Vascular development during implantation is mediated by numerous cell types and cell products and aberrant vascularity likely contributes to implantation failure and early pregnancy loss.

D. S. Torry (✉) · J. Leavenworth · M. Chang ·  
V. Maheshwari · K. Groesch  
Department of Medical Microbiology,  
Immunology and Cell Biology,  
Southern Illinois University School of Medicine,  
P.O. Box 19626, Springfield, IL 62794-9626, USA  
e-mail: dtorry@siu.edu

D. S. Torry · J. Leavenworth · M. Chang ·  
V. Maheshwari · K. Groesch  
Department of Obstetrics and Gynecology,  
Southern Illinois University School of Medicine,  
Springfield, IL, USA

E. R. Ball · R. J. Torry  
College of Pharmacy and Health Sciences, Drake University,  
Des Moines, IA, USA

**Conclusions** Robust vascular development occurs during implantation and early placentation of normal pregnancies. Studies to define the extent and mechanisms by which defects in vascularity contribute to human implantation failure and early miscarriage need to be undertaken.

**Keywords** Angiogenesis · Growth factors · Implantation · Miscarriage · Pregnancy · Trophoblast · Vasculogenesis

### Introduction

Successful implantation, placentation and subsequent gestation require coordinated vascular development and adaptations on both sides of the maternal–fetal interface. Specifically, several temporally distinct vascular processes occur enabling successful pregnancy to ensue. First, adequate uterine vascularity is needed at the time of implantation to provide a richly vascularized endometrium for implantation. Shortly after implantation, development and expansion of the placental villous vasculature is needed to facilitate transport of nutrients and oxygen to the embryo. Conceptually, these first two vascular processes are not unlike those associated with the growth of solid tumors [1]. Subsequently, remodeling of the maternal endometrial/uterine vasculature is needed to accommodate the rapid growth demands of the embryo. Inhibition of angiogenesis with a single dose of an antiangiogenic compound (e.g. AGM-1470) either before, or shortly after implantation, interrupts placentation in mice and results in resorption of all embryos [2]. These results support the hypothesis that angiogenesis is a critical component of normal implantation/placentation and highlights the importance of the vasculature in early stages of pregnancy. Similarly, the importance of increasing uterine and/or

placental vascularity during later stages of pregnancy and the pathophysiological consequences of impaired vascularity are now well recognized in terms of suboptimal outcomes [3]. These findings have brought a great deal of recent interest in the factors and conditions which might regulate vascular growth and remodeling during pregnancy. A large amount of attention has focused on events in later pregnancy which effect fetal growth (IUGR) and/or maternal health (preeclampsia) [3] but it is clear that the molecular and cellular defects associated with these conditions are established early in gestation. Less attention has been given to aberrant vascular development that may lead to implantation failure or early miscarriage in humans.

Although analyses of vascularity in early human pregnancy is difficult to evaluate, there is evidence supporting a role of defective angiogenesis at the maternal–fetal interface contributing to miscarriage in humans. Initial reports indicated that the percentage of vascularized villi and vascular density within placental villi from elective termination samples was significantly higher than those in sporadic miscarriage samples [4]. Similarly, trophoblast expression of one potent angiogenic growth factor, vascular endothelial growth factor (VEGF) (see below), was lower in 8–9 week gestation samples from idiopathic recurrent spontaneous abortions (RSA) than from samples of gestational age matched elective terminations [5]. In addition, decidua endothelial cells of recurrent abortion samples expressed quantitatively fewer receptors for VEGF and angiopoetins [5]. As with most human studies, whether the decreases in vascularity and angiogenic growth factor gene expression were responsible for, or merely reflected the inevitable nature of, the miscarriages is difficult to determine with certainty.

The advent of sensitive Doppler ultrasound technology has lead to increased interest and ability to further evaluate vascularity before and soon after implantation in humans. The use of ultrasound in diagnosing and predicting early human pregnancy failures has recently been reviewed [6]. Adequate endometrial thickness concomitant with high vascularity are clear requirements for embryo implantation and can be useful indicators for successful outcomes in assisted reproduction procedures. However, ultrasound approaches to investigate vascularity and blood flow dynamics at the maternal–fetal interface early in human pregnancy have not proved reliable enough to currently predict subsequent miscarriages [6]. This may be due in part to technological limitations.

Although associations between vascularity and implantation success are known, mechanistic studies to explain these relationships will be difficult to perform in humans. In this regard, animal studies have been instrumental. Microvascular volume of the gravid uterine horn of ewes increases within 24 days of mating [7]. MRI analyses of

murine implantation sites showed significant increases in localized vessel permeability and vessel density early in gestation (Ed5.5) [8]. These effects were largely restricted to areas near the implantation site. Similarly, vessels near implantation sites also undergo selective dilation later in gestation in the mouse [9]. The spatial proximity of these vascular adaptations to nearby implant sites strongly suggest that embryo derived factors mediate the changes. Indeed, embryo-derived factors differentially alter angiogenic gene expression, among others, in mouse decidua [10].

There are a large number of proteases, metabolites, ions, growth factors, matrix proteins, cytokines and mechanical forces that can modulate, either positively or negatively, the processes of angiogenesis/vasculogenesis. However, space limitations prevent a complete review of all potential angiogenic molecules expressed during the course of pregnancy. Although also important to the overall success of pregnancy, this review will not address aspects related to angiogenesis in the nonpregnant cycling endometrium nor events related to the vascular remodeling and uterine/placental blood flow dynamics that occur later in pregnancy. There are recent excellent review articles that address these critical steps [11, 12]. We have focused this review on cells and soluble factors that may mediate angiogenic events in early human pregnancy. Since studies in humans are largely restricted to descriptive observations, we incorporate data from animal models of implantation angiogenesis to highlight potential molecular mechanisms. Clearly, there are differences in implantation and placentation events between species, but there are significant similarities as well. More importantly, the ability to manipulate events in the animal models enable valuable cause versus effect questions to be addressed [3].

## Factors mediating implantation angiogenesis

### Cells

#### *Trophoblast*

Trophoblast form the interface between fetal and maternal tissues and thus they play a pivotal role in promoting angiogenesis during implantation. Indeed, trophoblast are known to be rich sources of angiogenic growth factors [13].

In human placentae, chorionic villi develop and subsequently become vascularized via pluripotent mesenchymal precursor cells (vasculogenesis) at approximately day 21 [14]. Both cytotrophoblast and syncytiotrophoblast of the villi produce numerous angiogenic factors and their receptors [15]. In addition to trophoblast, mesenchymal cells and Hofbauer cells (see below) within the villi produce angiogenic growth factors [16, 17]. These factors

likely have autocrine and/or paracrine functions to promote vasculogenesis and angiogenesis in placenta villi. During implantation, cytotrophoblast migrate into maternal decidua where they differentiate into extravillous trophoblast (EVT). These migrating EVT also express various angiogenic growth factors and their receptors [18], suggesting they could influence angiogenic processes within the decidua.

Vascular formation in mouse placentae begins from extraembryonic mesoderm at ~day 8 [19]. Labyrinth trophoblast initiate formation of branching villi and also mediate fetoplacental vascular development [20]. Trophoblast giant cells (TGC), one of the major murine trophoblast cell types, acquire unique characteristics during differentiation [21] and produce several angiogenic factors, vasoactive factors, and hormones [22, 23]. These factors contribute to various steps of implantation, including vasculogenesis and angiogenesis in the placenta. Some invasive TGC localize to maternal spiral arteries [24]. These cells are a major source of IGF-II, which has angiogenic properties (see below) and influences growth of the mouse placenta [25].

Trophoblast proliferation, migration, differentiation and selective gene expression are tightly regulated processes. Spatial and/or temporal regulation of these functions are mediated at least in part by specific transcription factors. There are several genes expressed by trophoblast that affect vascularization of the mouse labyrinth [26]. The extent to which similar genes regulate vascular development either directly or indirectly in humans is not known. Similarly, whether dysregulation of transcription factors involved in human trophoblast differentiation and/or angiogenic growth factor expression contributes to implantation failures and/or miscarriage is not known.

#### *Uterine natural killer (uNK) cells*

During transition of the endometrium to the secretory phase, ungranulated uNK cell precursors are recruited to the endometrium [27] and will maintain their presence in the decidua [12, 27]. Decidua progesterone allows for maturation of pre-uNK into relatively large granulated uNK cells which comprise a majority of the leukocyte population at the implantation site [28]. Although they may share some common functional characteristics, a major function of uNK cells in the uterus seems to be the secretion of cytokines that are beneficial for successful implantation and placental development [28]. Principle among these may be cytokines which direct angiogenesis during early pregnancy and influence spiral arteriole modifications later in pregnancy [28].

Mouse and in vitro models suggest that decidua IL-15, up regulated by progesterone [29], serves as a key activator

of the uNK population [30]. In the context of this review, activated uNK cells proliferate and mature to produce several known angiogenic and angiomodulatory growth factors and cytokines like angiopoietin (Ang)-1 and -2, placenta growth factor (PlGF), VEGF-C, IL-18, and IFN- $\gamma$  [28]. Mature mouse uNK cells, abundant in the decidua basalis, are enhanced by IL-18 to modify spiral arteries through increased IFN- $\gamma$  secretion [28]. Implantation in mice devoid of all NK cells results in spiral arteries that were neither elongated nor dilated, relative to NK intact mice. Moreover, the arteries are restricted by the presence of a vascular smooth muscle coat, suggesting that absence of uNK impairs vascular remodeling [31].

Recent evidence suggests that human dNK cells also uniquely function to promote vascular development during early pregnancy [32]. In vitro and in vivo studies show that endogenous human decidual NK (dNK) cells, like mouse dNK cells, respond to IL-15 by expressing high levels of the key angiogenic growth factors VEGF and PlGF. Isolated human dNK cells are able to significantly promote vascularization and growth of ectopic choriocarcinoma tumors in an VEGF/PlGF dependent manner. Endogenous functional activity of these unique dNK cells may be due to ligation of activating type receptors by molecules on trophoblast and decidual stromal cells which stimulate secretion of PlGF and VEGF. Importantly, the properties of dNK are unique and not evident in peripheral NK cells. These results strongly suggest that dNK populations support reproductive tissue development principally by regulating the vascular biology at the maternal–fetal interface. NK cells are historically a fairly well studied cell type in reproduction. In light of these new findings however, the clinical importance of endometrial and decidual NK cells in influencing the efficacy of early placentation needs to be re-examined.

#### *B and T lymphocytes*

All lymphocytes, although B cells to a lesser extent, are present within the human decidua and likely contribute to the immunological acceptance of the semiallogeneic embryo [33]. There is limited information available concerning the roles of T and/or B cells in influencing implantation angiogenesis. B lymphocytes express c-Myc proto-oncogene which can stimulate angiogenesis via production of VEGF [34]. However, there does not seem to be a significant difference between the number of endometrial B cells [35] or peripheral B cells [36] in women with normal obstetrical histories compared to those with a history of recurrent miscarriage undergoing IVF treatments.

The contribution of T lymphocytes to successful implantation and pregnancy have been widely examined from an immune perspective, however studies regarding the

contribution of these cells to pregnancy-associated angiogenesis are just beginning [28]. Functionally, T lymphocytes are categorized as cytotoxic T cells (Tc) or helper T cells (Th). Helper T cells are further divided into two groups (Th1 and Th2) depending upon their general pattern of cytokine production. Involvement of Th cells in angiogenesis is mediated by the cytokines they produce, many of which can control endothelial cell proliferation, apoptosis, migration and activation [37]. Since pro-inflammatory/Th1 cytokines may act as negative or positive regulators of angiogenesis [37], their involvement in successful vascularization during implantation cannot be easily elucidated.

After implantation, lymphocytes (and macrophages) surround the embryo and are activated to secrete cytokines [38] such as TNF- $\alpha$ , a Th1 pro-inflammatory cytokine that influences angiogenesis in a context dependent manner [37]. TNF- $\alpha$  stimulates production of angiogenic factors from decidua fibroblasts yet suppresses their proliferation [38]. TNF- $\alpha$  has been associated with inhibiting embryonic and fetal development [39]; however, TNF- $\alpha$  also up-regulates VEGF production in first trimester trophoblast and therefore may indirectly modulate placental vascular permeability and angiogenesis [40]. These apparently conflicting findings suggest that secretion of TNF- $\alpha$  by T lymphocytes at the appropriate concentrations may be needed to maintain a normal/healthy pregnancy [38]. Continued investigations are needed to define the role of TNF- $\alpha$  in regulating angiogenic potentials at the maternal–fetal interface.

IFN- $\gamma$  and IL-12, also pro-inflammatory Th1 cytokines, act as negative regulators of angiogenesis *in vitro*. IFN- $\gamma$  inhibits growth of endothelial cells and capillary formation in a dose dependent manner [41], and lymphocytes are reported to be the major source of IFN- $\gamma$  within the endometrium of pigs [42]. The anti-angiogenic effects of IL-12 are currently being investigated in various tumor cell lines due to the *in vitro* evidence that IL-12 inhibits VEGF production by mammary adenocarcinoma cells [37]. Paradoxically, IL-12 and IFN- $\gamma$  are important mediators of uNK cell activation and function which are required for modulation of the maternal spiral arteries during pregnancy [43].

Secretion of Th2 cytokines (i.e., IL-4, -5, -6, -10 and -13) by T lymphocytes is involved in the release of human placental lactogen (hPL) and human chorionic gonadotropin (hCG) from trophoblast [44]. The role of hCG in mediating angiogenesis is discussed further in this review. Similar to Th1 cytokines, Th2 cytokines may also have dual roles as regulators of angiogenesis. IL-6 is generally considered a pro-angiogenic cytokine, while IL-4 may have a positive or negative effect on endothelial cell function [45]. Although, successful pregnancy likely requires a balance of immune cytokine production at the maternal–fetal interface during implantation [46], the decidua

cytokine profiles, T lymphocyte subsets producing these cytokines, and the involvement of these cells and cytokines in regulating angiogenesis at the maternal–fetal interface early in human pregnancy are yet to be elucidated.

### Macrophages

Macrophages are normal components of the nonpregnant endometrium and cell numbers increase in response to insemination [47]. Macrophages constitute 20–30% of the cells in the decidua and their numbers remain fairly consistent throughout pregnancy [48]. A reduction in number or maturity of macrophages has been associated with less successful pregnancy rates in mice. The absence of colony stimulating factor-1 (CSF-1), one major factor in recruitment of macrophages to the uterus, in osteopetrotic mice results in a significant decrease in pregnancy rates [49]. In addition to maternal decidua macrophages, the placenta harbors a distinct variety of macrophages known as Hofbauer cells. These cells express angiogenic growth factors such as VEGF [17, 50] and IL-17 [51], among others. Hofbauer cells also demonstrate immunoreactivity for the VEGF receptor VEGFR-1 (flt-1) suggesting they influence angiogenesis at the fetoplacental border [17, 50].

Decidual macrophages secrete various factors such as epidermal growth factor, transforming growth factor (TGF)- $\beta$ , platelet-derived growth factor, insulin-like growth factors (IGFs), fibroblast growth factor (FGF), among others [52]; all of which are thought to have proangiogenic effects. Conversely, macrophages can also inhibit angiogenesis through secretion of anti-angiogenic mediators, such as a soluble variant of the VEGF receptor-1 (sVEGFR-1, or sflt-1), that may play an important role in pregnancy loss [53] (see VEGF section below). In addition, macrophages influence trophoblast expression of various molecules *in vitro* [16] and thus can act indirectly through trophoblast to promote or inhibit angiogenesis.

Macrophages have been widely studied for their role in angiogenesis especially in osteolytic diseases [54], cardiovascular pathologies [55], endometriosis [56] and tumors [57]. Collectively, macrophages exert proangiogenic effects through expression of various pro-angiogenic and pro-inflammatory factors. Although similar mechanisms are likely in pregnancy, it remains to be determined how the unique placental microenvironment modulates macrophage involvement in angiogenesis at the materno–fetal interface.

### Soluble products

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

VEGF is a potent vasculogenic/angiogenic factor involved in physiological and pathological vascular growth [58, 59].

Multiple VEGF protein isoforms have been identified in humans and mice [60]. VEGF receptors include VEGFR-1 (or flt-1) and VEGFR-2 (KDR) [58] and VEGF isoforms which contain heparin-binding domains also bind neuropilin receptors 1 and 2 (NRP-1 and NRP-2) [61].

VEGF null mice die before mid-gestation with impaired angiogenesis and vasculogenesis [62, 63]. VEGFR-1 and VEGFR-2 are also required for early embryonic vasculogenesis and angiogenesis [64, 65]. Mice with both NRP-1 and NRP-2 receptors disrupted die at E8.5, and mice with either knockout (NRP-1(+/-)NRP-2(-/-) or NRP-1(-/-)NRP-2(+/-) die at Ed10 or Ed10.5 due to lack of angiogenesis [66]. Collectively, disruption of VEGF production and/or signaling by its receptors produce similar phenotypes of abnormal embryo and yolk sac vascularization and highlight the critical need for VEGF and its receptors in embryo vascularization.

In pregnant mice, VEGF expression is observed as early as day 1 by luminal epithelium and is also expressed by stromal cells by day 3 and TGC by day 8 [60, 67]. The relative early expression of VEGF accompanies placenta formation and embryo angiogenesis. Expression of VEGF receptors, VEGFR-1, VEGFR-2 and NRP-1, occurs in endometrium at peri-implantation stages and are involved in mediating vascular hyperpermeability necessary for blastocyst implantation [60, 68]. The earliest time of VEGF expression in human placenta was documented on ~Ed22 where it is expressed in villous cytotrophoblast and Hofbauer cells in the villous core [17]. Similarly, VEGFR-1 and VEGFR-2 expression is evident by day 22 in cytotrophoblast and haemangiogenic cell cords [17]. The expression of VEGF in villi coincides temporally with vasculogenesis in the placenta. VEGF is produced by early stage human embryos (2–8 cell) and can promote human endometrial microvascular endothelial cell tube formation *in vitro* [69].

The importance of VEGF in mediating angiogenesis/vasculogenesis has prompted studies to address mechanisms that regulate its expression and biological function at the maternal–fetal interface. It is thought that early placentation takes place in a relatively hypoxic environment [70] which is a strong inducer of VEGF expression in trophoblast [71–73]. However, the biological function of VEGF (and PIGF) is tightly regulated by antagonist sVEGFR-1 [74], which is also produced by hypoxic trophoblast [73]. Other sources of sVEGFR-1 production include macrophages suggesting that immune reactions may contribute to VEGF/PIGF bioavailability at the maternal–fetal interface. Indeed, a strong link between the immune and vascular systems has been demonstrated in a mouse model of spontaneous abortion and intrauterine growth restriction [53]. In this model, complement activation results in a significant decrease in peripheral concentrations of free

VEGF, a concomitant increase in sVEGFR-1 and high resorption rates in the mice. VEGF expression can also be regulated by hormones. VEGF expression in early pregnancy correlates with estradiol and hCG levels [75] and hCG can induce expression of VEGF in endometrial tissue [76]. Genetic polymorphisms within the human VEGF gene regulate its expression and a single-nucleotide polymorphism at -1,154 has a statistically higher association with a previous history of recurrent spontaneous abortion [77].

Collectively, these data suggest VEGF expression and function are tightly regulated in the maternal decidua and the placenta to ensure adequate vasculogenesis and angiogenesis during implantation and early placentation. Disruption of this balance could conceivably contribute to implantation failure and pregnancy loss. Additional studies are needed to further understand regulation of VEGF expression at the maternal–fetal interface, its function in early pregnancy and reproductive consequences of aberrant expression in humans.

### *Placenta growth factor*

Placenta growth factor (PIGF) is a constituent of the VEGF family of proangiogenic growth factors [78, 79]. PIGF is predominantly produced by trophoblast, exists as four different isoforms produced from a single PIGF primary transcript [80], and functions in both autocrine and paracrine manners [79, 81]. Receptors for PIGF include NRP-1 and NRP-2 and VEGFR-1 [82].

PIGF and VEGFR-1 are minimally expressed in quiescent vasculature, however both are significantly up-regulated under most pathological and hypoxic conditions [83]. In contrast, low oxygen tensions reduce PIGF expression in trophoblast [71] and induce sVEGFR-1 expression [72].

Gene knockout studies show that loss of PIGF impairs pathological angiogenesis in adult mice [78, 84] and PIGF is angiogenic *in vivo* and *in vitro* [85]. PIGF has been correlated with increased placental perfusion at the maternal/fetal interface [86] and it induces relaxation of placental vessels *ex vivo* [42]. Although PIGF null mice are viable [83], PIGF *-/-* pups exhibit lower fetal and placental weights when compared to wild types [84]. PIGF may stimulate angiogenesis either directly or indirectly by synergizing with VEGF [83], and is proposed to be a growth factor predominantly involved in mediating the molecular and morphological steps of vasculogenesis and angiogenesis within placental villi [87].

Little is known about PIGF expression during the first stages of human implantation. However, rhesus monkey trophoblast express VEGF initially and its expression decreases with a concurrent increase in PIGF expression as placentation advances [88]. This induction of PIGF may be critical for the establishment of placental development

and for normal angiogenesis to occur during the early stages of implantation [88]. In later stages of gestation, low PIGF expression coupled with high sVEGFR-1 in maternal sera correlate with on-set of preeclampsia [89, 90]. Low PIGF results in deficiencies in placental angiogenesis [91], although the mechanisms behind this correlation are unclear. Aberrations in PIGF expression, as of yet, have not been investigated as a biological marker regarding failed implantation and recurrent miscarriage.

### *Fibroblast growth factors (FGF)*

FGFs are a highly conserved family of polypeptide growth factors with high affinity for heparin sulfate proteoglycans (HSPG) [92]. There are at least 23 characterized FGF's that bind one or more of four known receptors (FGFR-1–4) in an HSPG-dependent mechanism [92]. While FGF/FGFR signaling pathways are critical for normal trophoblast and inner cell mass interactions in mice [93], their role in mediating angiogenesis during early implantation in humans is less characterized. The main FGF isoforms studied during implantation are FGF2, FGF4, FGF7, FGF9, and of these, FGF2 is the best characterized.

### *FGF2 (bFGF)*

Basic fibroblast growth factor (bFGF), first isolated from human placental tissue [94], is a known endothelial cell mitogen and is angiogenic *in vivo*. *In vitro* studies have confirmed that trophoblast produce and release biologically active FGF2 into culture media [95].

Prior to implantation, FGF2 is expressed in the uterus of a variety of species including humans. FGF2 has been immunolocalized in the basal lamina of glandular and surface epithelial cells, blood vessels in the myometrium as well as in stromal cells, extracellular matrix and myometrial cells in the nonpregnant uterus [96]. As described below, this pre-existing growth factor expression could play an important role in the angiogenic response at the time of implantation.

Strong FGF2 immunoreactivity is noted in endothelial cells and decidualized stromal cells during early pregnancy in rhesus monkeys [97]. Although FGF2 mRNA and protein are expressed in vascular endothelial cells, smooth muscle cells and in the cytotrophoblast/syncytiotrophoblast bilayer, its receptor is mainly localized to cytotrophoblast [98]. Thus, in addition to angiogenesis, FGF2 may also influence cytotrophoblast proliferation and migration during early placentation.

Decidualization in response to blastocyst implantation in rats is accompanied by robust angiogenesis in the mesometrial decidua. A concomitant lack of vascular growth in the anti-mesometrial deciduas suggests local expression and/or bioavailability of angiogenic growth factors drives

this spatially select angiogenic response. Interestingly, pseudo pregnancy selectively induces FGF2 mRNA in mesometrial tissue in rats and there is greater FGF2 immunolocalized in isolated mesometrial decidua cells than isolated anti-mesometrial cells [99]. FGF2 protein distribution also demonstrates temporal and spatial changes during the peri-implantation period in the rat [100].

A role for FGF2 in vascular development of the human placenta is also likely. FGF2 mRNA expression is associated with syncytiotrophoblast and cytotrophoblast of first trimester human placenta and FGF2 gene expression is greater in first trimester than term placenta, suggesting a developmental control of its expression [101]. FGF2 has been shown to be released by human embryos [95] as well as in gilts [102]. Collectively, the increased expression and subsequent sequestration of FGF2 in the mesometrial decidua suggest this serves as a reservoir of potent angiogenic signals as the trophoblast invade the tissue. Furthermore this mechanism, as well as FGF2 release from the embryo, provides a convenient temporal and spatial relationship between implantation and local angiogenesis.

There are few studies that directly assess the functional role of FGF2 in implantation and early pregnancy. However, pregnancy is inhibited in rodents immunized against FGF2 [103]. FGFR-1 protein expression is markedly reduced in women with menorrhagia compared to normal cycling women [104]. Further studies are needed to determine if FGF2 is required for adequate vascular development in early human implantation/placentation.

Although FGF2 is the most widely studied, other members of the FGF family are also expressed in reproductive tissues. The role of many of these members in implantation angiogenesis is largely unknown. The temporal and spatial expression of FGF4 in animal models suggests it could modulate placental angiogenesis. FGF4 is expressed in the villi stroma adjacent to fetal blood vessels [105]. FGF4 in mice is expressed during pre-implantation period, but becomes restricted to the inner cell mass at the blastocyst stage. FGF4 mutation in mice is lethal due to lack of trophoblast proliferation [93].

FGF7, also known as keratinocyte growth factor (KGF), is produced by stromal cells. In gilts, FGF7 protein is detected in endometrial epithelial cells, vascular smooth muscle cells and blood vessels [106]. FGF7 stimulates PLAC1, a recently described trophoblast-specific gene important in placental development [107].

FGF9 is an endometrial stromal cell growth factor that facilitates cyclic proliferation of uterine endometrial stroma [108]. Its role in implantation in humans is poorly understood; however, it is expressed in high levels in uterine endometrium and is induced by 17 $\beta$ -estradiol [109].

There is evidence that some FGF's are regulated by sex hormones and the specific hormonal signals may be critical

for FGF/FGFR expression. Several studies have shown that estrogen can induce FGF2 expression in human or rodent uterine tissue [110–112]. Indeed, FGF-2 and FGF-R1 mRNA is significantly higher in proliferative than in secretory endometrium in humans, suggesting estrogen mediation [104]. There is relatively little information available on the hormonal modulation of other key FGF species in reproductive tissues. In humans, the secretory endometrium expresses significantly higher FGF7(KGF) mRNA than that of proliferative endometrium suggesting progesterone regulation [113]. However, the opposite is noted in porcine [106]. In humans, exogenous 17-β Estradiol induces FGF9 expression in human endometrial stromal cells [109] and FGF9 is expressed at high levels especially during the late proliferative phase, which is associated with a rise in estradiol [108]. Hormonal modulation of growth factor/receptor expression *in vivo* is difficult to study in humans due to the variability in menstrual cycle inherent among women. Indeed, different human studies have produced results that conflict with one another, and animal models do not always correlate to humans [96]. Clearly more information on the hormonal regulation of FGF/FGFR expression during implantation is needed.

Collectively, the FGF family of growth factors are pluripotent and thus may exert many actions in reproductive tissues. The temporal and spatial expression of FGF's and their receptors suggest they may directly or indirectly influence angiogenesis during implantation and early placentation. The extent of the involvement, and their contributions to aberrant human pregnancy outcome warrants further investigation.

#### *Angiopoietins (Ang) and Tie signaling*

The angiopoietin receptors, Tunica interna endothelial cell kinase-1 and 2 (Tie-1 and Tie-2) are almost exclusively expressed on endothelial cells in humans and other primate species [114]. The Tie-2 receptor binds the angiopoietin family of growth factors, which includes Ang-1 and Ang-2 [115]. Although there is no naturally known ligand for Tie-1, recombinant protein/Tie-1 interactions suggest that the Tie-1 receptor is functional [116]. Ang-1 and Ang-2 compete for Tie-2 receptor binding and serve as functional antagonists. Thus, Ang-1 binding to Tie-2 promotes vascular maturation by recruiting periendothelial support cells [117] while Ang-2 binding promotes destabilization of blood vessels allowing initiation of neovascularization [114]. Fluctuations in Ang-1/Ang-2 protein ratios can alter the angiogenic response [118]. Tie-2 signaling induced by Ang-1 may be necessary to maintain vessel integrity within the endometrium [119]. However, regulation is complex and may be tissue specific. Mice devoid of either Tie-1 or Tie-2 expression do not survive beyond approximately

midgestation with severe defects in capillary development and/or vessel stability [118, 120]. Ang-2 is a conditional angiogenic factor during early pregnancy, inhibiting Tie-2 signaling. A prolonged effect by Ang-2 in the absence of VEGF signaling factors, leads to vessel leakage [121]. However, in the presence of VEGF, the Ang-2 signaling loosens vascular cell–matrix and cell–cell contacts [119], which is thought to reveal nascent angiogenic factor recognition sites on the newly uncovered endothelial surface and initiates sprouting [122].

Tie-2 signaling directs angiogenesis in mice during decidualization following implantation [123], and inhibition of Tie-2 and Ang-1 results in disrupted vasculature and embryonic lethality [118, 120]. In early pregnancy of the marmoset, Ang-1 mRNA is expressed in the glandular uterine epithelium [124], and may play a key role in the progesterone-dependent growth of endometrial spiral arteries in both the marmoset and mouse [125].

Though the Ang-1, -2 factors and Tie-2 receptor mRNAs were all expressed in the endometrium during the menstrual cycle, only Ang-1 is up regulated during the secretory phase in human endometrium [119]. Ang-2 is selectively expressed in the ovaries, uterus, and placenta [119] and can be induced by hCG [126]. During early human pregnancy, Ang-1 mRNA and Ang-2 mRNA/protein are expressed in the syncytiotrophoblast [15], and there is evidence suggesting that Ang-2 mRNA and protein expression also occur in invasive cytotrophoblast [127]. Ang 2 mRNA expression localizes to the marmoset endothelia of large, luminal vessels [124]. Ang-1 and Ang-2 are also expressed by uterine NK cells, trophoblast, Hofbauer cells and hemoangiogenic cell cords in the mesenchymal villi [28, 128]. In addition to being expressed on almost all endothelial cells, the Tie receptors are expressed on trophoblast and Hofbauer cells of the early human placenta raising the possibility that the angiopoietins could regulate trophoblast function [129].

The importance of the angiopoietins and their receptors in vascularization in general has prompted studies into their aberrant expression levels in human miscarriage. Compared to gestational aged matched control tissues, there is reduced Tie-1 and Tie-2 receptor expression on endometrial vascular endothelia in recurrent aborters and lower expression of Tie-1 on trophoblast [130]. There is no correlation between Ang-2 polymorphisms and a history of recurrent miscarriage in humans [131]. With recent advances in the placental/uterine localization of the Ang/Tie-2 receptor axis, further clinical studies are necessary to determine its potential role to modulate vascularity associated with early implantation.

#### *Transforming growth factor-beta (TGF-β)*

Transforming growth factor β belongs to a large family of proteins with a wide variety of functions. Individual

functions of each member has not been clearly delineated but in reproduction, TGF $\beta$ -1 has been widely studied and is proposed to assist in spermatogenesis, ovulation, ductal branching in mammary glands, implantation, trophoblast differentiation, immunoregulation at the maternal–fetal interface and in angiogenesis [132].

The biological activity of TGF $\beta$ -1 is generally thought to have dual, cell specific functions in many situations. For example, TGF $\beta$ -1 has been shown to inhibit endothelial cell growth in vitro [133], yet it can promote angiogenesis in vivo [134]. TGF $\beta$ -1 can induce angiogenesis in chick chorioallantoic membrane bioassays either directly [135] or indirectly by increasing expression of pregnancy associated plasma protein-A [136]. Similarly, TGF $\beta$ -1 increases expression of VEGF in trophoblast [40, 137] suggesting an indirect function is likely.

Null mutations for TGF $\beta$ -1 in mice lead to significantly increased prenatal and post natal lethality. Litter sizes are 50% of expected and the mice tend to develop severe inflammatory disease that may be lethal during postnatal period [138, 139]. The prenatal mortality revealed that 25% of heterozygous mice and 50% of the homozygous TGF $\beta$ -1 knockout mice died at ~Ed10.5, although there are strain related differences [140]. The high mortality in these mice is attributed to defective yolk sac vasculogenesis with fewer number of developing plexuses, higher levels of delicate or disorganized vessels and in some cases absence of vasculogenesis and impaired endothelial differentiation [141]. Similarly, deletion of TF $\beta$ R-I gene in mice caused a death mid-gestation with severe defects in yolk sac and placental vasculature [142].

TGF $\beta$ -1 expression varies during the menstrual cycle and early pregnancy. In pregnancy, transcription of TGF $\beta$ -1 increases five fold in human uterine endometrium however, this increase is confined to the first trimester [143]. An excessive increase in TGF $\beta$ -1 may associated with higher incidence of spontaneous abortions in humans which could be mediated via the inhibitory action of TGF $\beta$ -1 on trophoblast invasion [144]. There may be a deficiency in uterine cells producing TGF $\beta$ -2 in subsets of RSA patients [145], although this is not reflected in peripheral plasma levels of TGF $\beta$ -1 [144, 146]. There does not seem to be a significant correlation between TGF $\beta$ -1 gene polymorphisms and RSA [147].

Although TGF $\beta$ -1 plays a role in angiogenesis and the temporal pattern of expression suggests that the role may be important in early pregnancy, the pleiotropic effects of TGF $\beta$ -1 precludes definitive associations with inadequate vascularization during implantation. Like many angiogenic growth factors, the contextual nature of the expression and/or biological activity is likely to be critical. Factors affecting the expression of TGF $\beta$ -1 at the materno fetal interface, the ability of TGF $\beta$ -1 to regulate angiogenic

gene expression and its role in RSA require further investigation.

### *Human chorionic gonadotropin (hCG)*

There are numerous effects of hCG on uterine receptivity [148]. Aside from the more classical effects of hCG within the female reproductive tract, there is a growing body of evidence that hCG can induce angiogenesis [149, 150]. Collectively, these studies show that hCG is able to induce neovascular activity in several classical in vivo assays [149, 150]. Uterine vascular endothelial cells express functional LH/hCG receptors [151] and recent evidence suggests that hCG can act directly on endothelial cells to induce proliferation in some [149], but not all [150], in vitro assays and can increase migration and in vitro tube formation [150]. In vivo, the mechanism of hCG-induced angiogenesis may be augmented by the induction of VEGF expression from endometrial epithelial cells [149] and/or from trophoblast themselves [152]. The high level expression of hCG from trophoblast early in embryogenesis suggests that hCG might be an important contributor to endometrial angiogenesis, either directly or indirectly, during implantation. Thus, aberrations in hCG production by early embryos may contribute to pregnancy failure by failing to augment local angiogenesis within the decidua.

### *Insulin-like growth factor-II (IGF II)*

Expression of placental specific IGF-II is required for appropriate placental growth and transport function [25] and is a well known autocrine mediator of trophoblast function (primarily migration) [153]. IGF-II is expressed by trophoblast in early murine implantation sites and receptors for IGF-II are expressed on developing vessels near the implantation site suggesting that it may facilitate decidua angiogenesis [9]. IGF-II can directly function as an angiogenic growth factor [154] in certain assays. It functionally augments angiogenic potentials at implantation sites by inducing expression of other well established angiogenic growth factors such as proliferin (in mice) [154] or VEGF [155]. Full biological activity of IGF-II can be regulated by the presence or absence of specific binding factors (IGFBP). Thus, increasing presence of IGFBP-1 within the human uterine lumen after day 10 of the cycle [156] may functionally inhibit embryo induced vascularization and contribute to poor implantation potentials.

### **Conclusions and future studies**

There is little doubt that angiogenesis is required for normal events of early implantation and placentation to

proceed [2]. The question remains however as to the extent that faulty angiogenesis contributes to implantation and/or early pregnancy failures in humans. Requirements for temporal and spatially discreet angiogenesis during implantation/placentation suggests that aberrations in these processes are likely to contribute to some forms of pregnancy loss. Although circumstantial evidence suggests that disruption in vascularity at the implant site is associated with poor reproductive performance in humans, ethical considerations preclude most of these studies from accurately defining cause and effect relationships. Certainly, advances in the vascular imaging of early stages of implantation are needed to help clarify the temporal aspects of angiogenesis and vessel permeability during normal and abnormal human pregnancy outcomes.

Gross abnormalities in placentation concomitant with defective angiogenesis occur with several single gene knock-outs in mice. Whether inadequate vascularity is the primary cause of the placental abnormalities or rather a reflection of the defective growth requirements of the placenta remains to be determined for many of the defects [20]. Either way, it remains to be characterized whether similar single gene defects contribute to aberrations in human pregnancy outcomes. The multitude of factors required for physiological angiogenesis and the complexity of regulating their temporal–spatial activities, suggest that more than one factor may be required for the robust angiogenesis associated with successful early pregnancy. Yet, it is also the complex orchestration of physiological angiogenesis that suggests defects in any single factor may inhibit angiogenesis, or temporally impede it long enough, such that the pregnancy will fail. In this review, we have focused on factors that seem particularly important in angiogenesis related to early implantation/placentation. Many of the factors also could play critical roles in developmental angiogenesis/vasculogenesis within the embryo proper. Clearly, the complexities of both of these systems are likely to contribute to the high embryo mortality that occurs in human pregnancy. Increased knowledge of the temporal expression patterns, functions, and regulatory mechanisms of angiogenic factors during early implantation/placentation will enable novel therapeutic advances to be made for some forms of human implantation failure and recurrent spontaneous abortion.

**Acknowledgments** Supported in part by the National Institute of Child Health and Human Development (2RO1 HD36830) (DST), the National Heart, Lung and Blood Institute (R15 HL072802) (RJT), and March of Dimes Birth Defects Foundation (DST).

## References

1. Torry RJ, Rongish BJ. Angiogenesis in the uterus: potential regulation and relation to tumor angiogenesis. *Am J Reprod Immunol* 1992;27:171–9.
2. Klauber N, Rohan RM, Flynn E, D'Amato RJ. Critical components of the female reproductive pathway are suppressed by the angiogenesis inhibitor AGM-1470. *Nat Med* 1997;3:443–6.
3. Reynolds LP, Caton JS, Redmer DA, Grazul-Bilska AT, Vonnahme KA, Borowicz PP, et al. Evidence for altered placental blood flow and vascularity in compromised pregnancies. *J Physiol* 2006;572:51–8.
4. Meegdes BH, Ingenhoes R, Peeters LL, Exalto N. Early pregnancy wastage: relationship between chorionic vascularization and embryonic development. *Fertil Steril* 1988;49:216–20.
5. Vuorela P, Carpen O, Tulppala M, Halmesmaki E. VEGF, its receptors and the tie receptors in recurrent miscarriage. *Mol Hum Reprod* 2000;6:276–82.
6. Jauniaux E, Johns J, Burton GJ. The role of ultrasound imaging in diagnosing and investigating early pregnancy failure. *Ultrasound Obstet Gynecol* 2005;25:613–24.
7. Reynolds LP, Redmer DA. Growth and microvascular development of the uterus during early pregnancy in ewes. *Biol Reprod* 1992;47:698–708.
8. Plaks V, Kalchenko V, Dekel N, Neeman M. MRI analysis of angiogenesis during mouse embryo implantation. *Magn Reson Med* 2006;55:1013–22.
9. Pringle KG, Roberts CT. New Light on Early Post-Implantation Pregnancy in the Mouse: Roles for Insulin-Like Growth Factor-II (IGF-II)? *Placenta* 2007;28:286–97.
10. Bany BM, Cross JC. Post-implantation mouse conceptuses produce paracrine signals that regulate the uterine endometrium undergoing decidualization. *Dev Biol* 2006;294:445–56.
11. Girling JE, Rogers PA. Recent advances in endometrial angiogenesis research. *Angiogenesis* 2005;8:89–99.
12. Pijnenborg R, Vercruyse L, Hanssens M. The uterine spiral arteries in human pregnancy: facts and controversies. *Placenta* 2006;27:939–58.
13. Cross JC, Hemberger M, Lu Y, Nozaki T, Whiteley K, Masutani M, et al. Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta. *Mol Cell Endocrinol* 2002;187:207–12.
14. Huppertz B, Peeters LL. Vascular biology in implantation and placentation. *Angiogenesis* 2005;8:157–67.
15. Geva E, Ginzinger DG, Zaloudek CJ, Moore DH, Byrne A, Jaffe RB. Human placental vascular development: vasculogenic and angiogenic (branching and nonbranching) transformation is regulated by vascular endothelial growth factor-A, angiopoietin-1, and angiopoietin-2. *J Clin Endocrinol Metab* 2002;87:4213–24.
16. Cervar M, Blaschitz A, Dohr G, Desoye G. Paracrine regulation of distinct trophoblast functions in vitro by placental macrophages. *Cell Tissue Res* 1999;295:297–305.
17. Demir R, Kayisli UA, Seval Y, Celik-Ozenci C, Korgun ET, mir-Weusten AY, et al. Sequential expression of VEGF and its receptors in human placental villi during very early pregnancy: differences between placental vasculogenesis and angiogenesis. *Placenta* 2004;25:560–72.
18. Wulff C, Weigand M, Kreienberg R, Fraser HM. Angiogenesis during primate placentation in health and disease. *Reproduction* 2003;126:569–77.
19. Cross JC, Simmons DG, Watson ED. Chorioallantoic morphogenesis and formation of the placental villous tree. *Ann N Y Acad Sci* 2003;995:84–93.
20. Cross JC, Nakano H, Natale DR, Simmons DG, Watson ED. Branching morphogenesis during development of placental villi. *Differentiation* 2006;74:393–401.
21. Cross JC. Genetic insights into trophoblast differentiation and placental morphogenesis. *Semin Cell Dev Biol* 2000;11:105–13.
22. Adamson SL, Lu Y, Whiteley KJ, Holmyard D, Hemberger M, Pfarrer C, et al. Interactions between trophoblast cells and the

- maternal and fetal circulation in the mouse placenta. *Dev Biol* 2002;250:358–73.
23. Hemberger M, Nozaki T, Masutani M, Cross JC. Differential expression of angiogenic and vasodilatory factors by invasive trophoblast giant cells depending on depth of invasion. *Dev Dyn* 2003;227:185–91.
  24. Georgiades P, Watkins M, Burton GJ, Ferguson-Smith AC. Roles for genomic imprinting and the zygotic genome in placental development. *Proc Natl Acad Sci U S A* 2001;98:4522–7.
  25. Constanca M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, et al. Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature* 2002;417:945–8.
  26. Watson ED, Cross JC. Development of structures and transport functions in the mouse placenta. *Physiology (Bethesda)* 2005;20:180–93.
  27. van den Heuvel MJ, Xie XM, Tayade C, Peralta C, Fang Y, Leonard S, et al. A review of trafficking and activation of uterine natural killer cells. *Am J Reprod Immunol* 2005;54:322–31.
  28. Leonard S, Murrant C, Tayade C, van den Heuvel M, Watering R, Croy BA. Mechanisms regulating immune cell contributions to spiral artery modification—Facts and hypotheses—a review. *Placenta* 2006;27:S40–6.
  29. Dosiou C, Giudice LC. Natural killer cells in pregnancy and recurrent pregnancy loss: endocrine and immunologic perspectives. *Endocr Rev* 2005;26:44–62.
  30. Ledee-Bataille N, Bonnet-Chea K, Hosny G, Dubanchet S, Frydman R, Chaouat G. Role of the endometrial tripod interleukin-18, -15, and -12 in inadequate uterine receptivity in patients with a history of repeated in vitro fertilization-embryo transfer failure. *Fertil Steril* 2005;83:598–605.
  31. Croy BA, van den Heuvel MJ, Borzychowski AM, Tayade C. Uterine natural killer cells: a specialized differentiation regulated by ovarian hormones. *Immunol Rev* 2006;214:161–85.
  32. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, et al. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 2006;12:1065–74.
  33. agaard-Tillery KM, Silver R, Dalton J. Immunology of normal pregnancy. *Seminars Fetal Neonatal Med* 2006;11:279–95.
  34. Ruddell A, Mezquita P, Brandvold KA, Farr A, Iritani BM. B lymphocyte-specific c-Myc expression stimulates early and functional expansion of the vasculature and lymphatics during lymphomagenesis. *Am J Pathol* 2003;163:2233–45.
  35. Quenby S, Bates M, Doig T, Brewster J, Lewis-Jones DI, Johnson PM, et al. Pre-implantation endometrial leukocytes in women with recurrent miscarriage. *Hum Reprod* 1999;14:2386–91.
  36. Thum MY, Bhaskaran S, Bansal AS, Shehata H, Ford B, Sumar N, et al. Simple enumerations of peripheral blood natural killer (CD56+ NK) cells, B cells and T cells have no predictive value in IVF treatment outcome. *Hum Reprod* 2005;20:1272–6.
  37. Naldini A, Carraro F. Role of inflammatory mediators in angiogenesis. *Curr Drug Targets Inflamm Allergy* 2005;4:3–8.
  38. Hayashi T, Matsuoka K, Saitoh M, Takeda S, Kimura M. Influence of alpha-tumor necrosis factor and beta-interleukin-1 on production of angiogenic factors and thymidine phosphorylase activity in immortalized human decidual fibroblasts in vitro. *J Obstet Gynaecol Res* 2006;32:15–22.
  39. van Nieuwenhoven ALV, Heineman MJ, Faas MM. The immunology of successful pregnancy. *Hum Reprod Updat* 2003;9:347–57.
  40. Chung IB, Yelian FD, Zaher FM, Gonik B, Evans MI, Diamond MP, et al. Expression and regulation of vascular endothelial growth factor in a first trimester trophoblast cell line. *Placenta* 2000;21:320–4.
  41. Naldini A, Carraro F. Role of inflammatory mediators in angiogenesis. *Curr Drug Targets Inflamm Allergy* 2005;4:3–8.
  42. Szukiewicz D, Szewczyk G, Watroba M, Kurowska E, Maslinski S. Isolated placental vessel response to vascular endothelial growth factor and placenta growth factor in normal and growth-restricted pregnancy. *Gynecol Obstet Investig* 2005;59:102–7.
  43. Croy BA, Esadeg S, Chantakru S, van den HM, Paffaro VA, He H, et al. Update on pathways regulating the activation of uterine Natural Killer cells, their interactions with decidual spiral arteries and homing of their precursors to the uterus. *J Reprod Immunol* 2003;59:175–91.
  44. Druckmann R, Druckmann MA. Progesterone and the immunology of pregnancy. *J Steroid Biochem Mol Biol* 2005;97:389–96.
  45. Naldini A, Pucci A, Bernini C, Carraro F. Regulation of angiogenesis by Th1- and Th2-type cytokines. *Curr Pharm Des* 2003;9:511–9.
  46. Laird SM, Tuckerman EM, Li TC. Cytokine expression in the endometrium of women with implantation failure and recurrent miscarriage. *Reprod Biomed Online* 2006; 13:13–23.
  47. Robertson SA. Seminal plasma and male factor signalling in the female reproductive tract. *Cell Tissue Res* 2005;322:43–52.
  48. Kabawat SE, Mostoufizadeh M, Driscoll SG, Bhan AK. Implantation site in normal-pregnancy—a study with monoclonal-antibodies. *Am J Pathol* 1985;118:76–84.
  49. Pollard JW, Hunt JS, Wiktor-Jedrzejczak W, Stanley ER. A pregnancy defect in the osteopetrotic (op/op) mouse demonstrates the requirement for CSF-1 in female fertility. *Dev Biol* 1991;148:273–83.
  50. Cooper JC, Sharkey AM, McLaren J, Charnock-Jones DS, Smith SK. Localization of vascular endothelial growth factor and its receptor, flt, in human placenta and decidua by immunohistochemistry. *J Reprod Fertil* 1995;105:205–13.
  51. Pongcharoen S, Somran J, Sritippayawan S, Niumsup P, Chanchan P, Butkhamchot P, et al. Interleukin-17 expression in the human placenta. *Placenta* 2007;28:59–63.
  52. Guilbert L, Robertson SA, Wegmann TG. The trophoblast as an integral component of a macrophage cytokine network. *Immunol Cell Biol* 1993;71:49–57.
  53. Girardi G, Yarilin D, Thurman JM, Holers VM, Salmon JE. Complement activation induces dysregulation of angiogenic factors and causes fetal rejection and growth restriction. *J Exp Med* 2006;203:2165–75.
  54. Vessella RL, Corey E. Targeting factors involved in bone remodeling as treatment strategies in prostate cancer bone metastasis. *Clin Cancer Res* 2006;12:6285s–90s.
  55. Lipinski MJ, Frias JC, Fayad ZA. Advances in detection and characterization of atherosclerosis using contrast agents targeting the macrophage. *J Nucl Cardiol* 2006;13:699–709.
  56. Siristatidis C, Nissotakis C, Chrelias C, Iacovidou H, Salamalekis E. Immunological factors and their role in the genesis and development of endometriosis. *J Obstet Gynaecol Res* 2006;32:162–70.
  57. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 2006;66:605–12.
  58. Takahashi H, Shibuya M. The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. *Clin Sci (Lond)* 2005;109:227–41.
  59. Tammela T, Enholm B, Alitalo K, Paavonen K. The biology of vascular endothelial growth factors. *Cardiovasc Res* 2005;65:550–63.
  60. Halder JB, Zhao X, Soker S, Paria BC, Klagsbrun M, Das SK, et al. Differential expression of VEGF isoforms and VEGF(164)-specific receptor neuropilin-1 in the mouse uterus suggests a role for VEGF(164) in vascular permeability and angiogenesis during implantation. *Genesis* 2000;26:213–24.

61. Gluzman-Poltorak Z, Cohen T, Herzog Y, Neufeld G. Neuropilin-2 and neuropilin-1 are receptors for the 165-amino acid form of vascular endothelial growth factor (VEGF) and of placenta growth factor-2, but only neuropilin-2 functions as a receptor for the 145-amino acid form of VEGF. *J Biol Chem* 2000;275:18040–5.
62. Carmeliet P, Ferreira V, Breier G, Pollefeijt S, Kieckens L, Gertsenstein M, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 1996;380:435–9.
63. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996;380:439–42.
64. Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* 1995;376:66–70.
65. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 1995;376:62–6.
66. Takashima S, Kitakaze M, Asakura M, Asanuma H, Sanada S, Tashiro F, et al. Targeting of both mouse neuropilin-1 and neuropilin-2 genes severely impairs developmental yolk sac and embryonic angiogenesis. *Proc Natl Acad Sci U S A* 2002;99:3657–62.
67. Chakraborty I, Das SK, Dey SK. Differential expression of vascular endothelial growth factor and its receptor mRNAs in the mouse uterus around the time of implantation. *J Endocrinol* 1995;147:339–52.
68. Das SK, Chakraborty I, Wang J, Dey SK, Hoffman LH. Expression of vascular endothelial growth factor (VEGF) and VEGF-receptor messenger ribonucleic acids in the peri-implantation rabbit uterus. *Biol Reprod* 1997;56:1390–9.
69. Kapiteijn K, Koolwijk P, van der Weiden RM, van Nieuw AG, Plaisier M, van HV, et al. Human embryo-conditioned medium stimulates in vitro endometrial angiogenesis. *Fertil Steril* 2006;85(Suppl 1):1232–9.
70. Kingdom JC, Kaufmann P. Oxygen and placental vascular development. *Adv Exp Med Biol* 1999;474:259–75.
71. Shore VH, Wang TH, Wang CL, Torry RJ, Caudle MR, Torry DS. Vascular endothelial growth factor, placenta growth factor and their receptors in isolated human trophoblast. *Placenta* 1997;18:657–65.
72. Ahmed A, Dunk C, Ahmad S, Khaliq A. Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) and soluble Flt-1 by oxygen—a review. *Placenta* 2000;21(Suppl A):S16–24.
73. Nagamatsu T, Fujii T, Kusumi M, Zou L, Yamashita T, Osuga Y, et al. Cytotrophoblasts up-regulate soluble fms-like tyrosine kinase-1 expression under reduced oxygen: an implication for the placental vascular development and the pathophysiology of preeclampsia. *Endocrinology* 2004;145:4838–45.
74. Kendall RL, Wang G, Thomas KA. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. *Biochem Biophys Res Commun* 1996;226:324–8.
75. Evans PW, Wheeler T, Anthony FW, Osmond C. A longitudinal study of maternal serum vascular endothelial growth factor in early pregnancy. *Hum Reprod* 1998;13:1057–62.
76. Licht P, Russu V, Wildt L. On the role of human chorionic gonadotropin (hCG) in the embryo-endometrial microenvironment: implications for differentiation and implantation. *Semin Reprod Med* 2001;19:37–47.
77. Papazoglou D, Galazios G, Papatheodorou K, Liberis V, Papanas N, Maltezos E, et al. Vascular endothelial growth factor gene polymorphisms and idiopathic recurrent pregnancy loss. *Fertil Steril* 2005;83:959–63.
78. Yamazaki Y, Morita T. Molecular and functional diversity of vascular endothelial growth factors. *Mol Divers* 2006.
79. Torry DS, Mukherjea D, Arroyo J, Torry RJ. Expression and function of placenta growth factor: implications for abnormal placentation. *J Soc Gynecol Investig* 2003;10:178–88.
80. Torry DS, Hinrichs M, Torry RJ. Determinants of placental vascularity. *Am J Reprod Immunol* 2004;51:257–68.
81. Ikai T, Miwa H, Shikami M, Hiramatsu A, Tajima E, Yamamoto H, et al. Placenta growth factor stimulates the growth of Philadelphia chromosome positive acute lymphoblastic leukemia cells by both autocrine and paracrine pathways. *Eur J Haematol* 2005;75:273–9.
82. Autiero M, Lutun A, Tjwa M, Carmeliet P. Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. *J Thromb Haemost* 2003;1:1356–70.
83. Carmeliet P, Moons L, Lutun A, Vincenti V, Comperolle V, De Mol M, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med* 2001;7:575–83.
84. Lijnen HR, Christiaens V, Scroyen I, Voros G, Tjwa M, Carmeliet P, et al. Impaired adipose tissue development in mice with inactivation of placental growth factor function. *Diabetes* 2006;55:2698–704.
85. Ziche M, Maglione D, Ribatti D, Morbidelli L, Lago CT, Battisti M, et al. Placenta growth factor-1 is chemotactic, mitogenic, and angiogenic. *Lab Invest* 1997;76:517–31.
86. Welch PC, Amankwah KS, Miller P, McAsey ME, Torry DS. Correlations of placental perfusion and PlGF protein expression in early human pregnancy. *Am J Obstet Gynecol* 2006;194:1625–9.
87. Demir R, Kayisli UA, Cayli S, Huppertz B. Sequential steps during vasculogenesis and angiogenesis in the very early human placenta. *Placenta* 2006;27:535–9.
88. Ghosh D, Sharkey AM, Charnock-Jones DS, Dhawan L, Dhara S, Smith SK, et al. Expression of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) in conceptus and endometrium during implantation in the rhesus monkey. *Mol Hum Reprod* 2000;6:935–41.
89. Levine RJ, Thadhani R, Qian C, Lam C, Lim KH, Yu KF, et al. Urinary placental growth factor and risk of preeclampsia. *JAMA* 2005;293:77–85.
90. Tidwell SC, Ho HN, Chiu WH, Torry RJ, Torry DS. Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia. *Am J Obstet Gynecol* 2001;184:1267–72.
91. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. *Am J Obstet Gynecol* 2003;188:177–82.
92. Ornitz DM, Itoh N. Fibroblast growth factors. *Genome Biol* 2001;2.
93. Rossant J, Cross JC. Placental development: lessons from mouse mutants. *Nat Rev Genet* 2001;2:538–48.
94. Moscatelli D, Joseph-Silverstein J, Presta M, Rifkin DB. Multiple forms of an angiogenesis factor: basic fibroblast growth factor. *Biochimie* 1988;70.
95. Hamai Y, Fujii T, Yamashita T, Kozuma S, Okai T, Taketani Y. Evidence for basic fibroblast growth factor as a crucial angiogenic growth factor, released from human trophoblasts during early gestation. *Placenta* 1998;19.
96. Hyder SM, Stancel GM. Regulation of angiogenic growth factors in the female reproductive tract by estrogens and progestins. *Mol Endocrinol* 1999;13.

97. Wei P, Chen XL, Song XX, Han CS, Liu YX. VEGF, bFGF, and their receptors in the endometrium of rhesus monkey during menstrual cycle and early pregnancy. *Mol Reprod Dev* 2004;68.
98. Wei P, Yu FQ, Chen XL, Tao SX, Han CS, Liu YX. VEGF, bFGF and their receptors at the fetal-maternal interface of the rhesus monkey. *Placenta* 2004;25.
99. Srivastava RK, Gu Y, Aylloo S, Zilberstein M, Gibori G. Developmental expression and regulation of basic fibroblast growth factor and vascular endothelial growth factor in rat decidua and in a decidual cell line. *J Mol Endocrinol* 1998;21.
100. Wordinger RJ, Smith KJ, Bell C, Chang IF. The immunolocalization of basic fibroblast growth factor in the mouse uterus during the initial stages of embryo implantation. *Growth Factors* 1994;11.
101. Shams M, Ahmed A. Localization of mRNA for basic fibroblast growth factor in human placenta. *Growth Factors* 1994;11:105–11.
102. Wollenhaupt K, Welter H, Einspanier R, Manabe N, Brussow KP. Expression of epidermal growth factor receptor (EGF-R), vascular endothelial growth factor receptor (VEGF-R) and fibroblast growth factor receptor (FGF-R) systems in porcine oviduct and endometrium during the time of implantation. *J Reprod Dev* 2004;50.
103. Buscaglia ML, Ong M, Fuller J, Gonzalez AM, Baird A. Inhibition of pregnancy in the passively and actively immunized mammals rabbit, rat, and mouse. *Ann N Y Acad Sci* 1991;638.
104. Sangha RK, Li XF, Shams M, Ahmed A. Fibroblast growth factor receptor-1 is a critical component for endometrial remodeling: localization and expression of basic fibroblast growth factor and FGF-R1 in human endometrium during the menstrual cycle and decreased FGF-R1 expression in menorrhagia. *Lab Invest* 1997;77.
105. Anteby EY, Greenfield C, Natanson-Yaron S, Goldman-Wohl D, Hamani Y, Khudyak V, et al. Vascular endothelial growth factor, epidermal growth factor and fibroblast growth factor-4 and -10 stimulate trophoblast plasminogen activator system and metalloproteinase-9. *Mol Hum Reprod* 2004;10.
106. Wollenhaupt K, Welter H, Brussow KP, Einspanier R. Regulation of endometrial fibroblast growth factor 7 (FGF-7) and its receptor FGFR2IIIb in gilts after sex steroid replacements, and during the estrous cycle and early gestation. *J Reprod Dev* 2005;51.
107. Massabba E, Parveen S, Weisoly DL, Nelson DM, Smith SD, Fant M. PLAC1 expression increases during trophoblast differentiation: evidence for regulatory interactions with the fibroblast growth factor-7 (FGF-7) axis. *Mol Reprod Dev* 2005;71.
108. Tsai SJ, Wu MH, Chen HM, Chuang PC, Wing LY. Fibroblast growth factor-9 is an endometrial stromal growth factor. *Endocrinology* 2002;143.
109. Wing LY, Chuang PC, Wu MH, Chen HM, Tsai SJ. Expression and mitogenic effect of fibroblast growth factor-9 in human endometrial implant is regulated by aberrant production of estrogen. *J Clin Endocrinol Metab* 2003;88.
110. Fujimoto J, Hori M, Ichigo S, Hirose R, Tamaya T. Ability of ovarian steroids to regulate the expression of the fibroblast growth factor family in fibroblasts derived from uterine endometrium. *J Biomed Sci* 1996;3.
111. Presta M. Sex hormones modulate the synthesis of basic fibroblast growth factor in human endometrial adenocarcinoma cells: implications for the neovascularization of normal and neoplastic endometrium. *J Cell Physiol* 1988;137.
112. Rider V, Carlone DL, Foster RT. Oestrogen and progesterone control basic fibroblast growth factor mRNA in the rat uterus. *J Endocrinol* 1997;154.
113. Matsui H, Taga M, Kurogi K, Minaguchi H. Gene expressions of keratinocyte growth factor and its receptor in the human endometrium/decidua and chorionic villi. *Endocr J* 1997;44.
114. Eklund L, Olsen BR. Tie receptors and their angiopoietin ligands are context-dependent regulators of vascular remodeling. *Exp Cell Res* 2006;312:630–41.
115. Lee HJ, Cho CH, Hwang SJ, Choi HH, Kim KT, Ahn SY, et al. Biological characterization of angiopoietin-3 and angiopoietin-4. *FASEB J* 2004;18:1200–8.
116. Saharinen P, Kerkela K, Ekman N, Marron M, Brindle N, Lee GM, et al. Multiple angiopoietin recombinant proteins activate the Tie1 receptor tyrosine kinase and promote its interaction with Tie2. *J Cell Biol* 2005;169:239–43.
117. Wakui S, Yokoo K, Muto T, Suzuki Y, Takahashi H, Furusato M, et al. Localization of Ang-1, -2, Tie-2, and VEGF expression at endothelial-pericyte interdigitation in rat angiogenesis1. *Lab Invest* 2006;86:1172–84.
118. Sato TN, Tozawa Y, Deutsch U, Wolburg-Buchholz K, Fujiwara Y, Gendron-Maguire M, et al. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation2. *Nature* 1995;376:70–4.
119. Hirchenhain J, Huse I, Hess A, Bielfeld P, De BF, Krussel JS. Differential expression of angiopoietins 1 and 2 and their receptor Tie-2 in human endometrium1. *Mol Hum Reprod* 2003;9:663–9.
120. Puri MC, Rossant J, Alitalo K, Bernstein A, Partanen J. The receptor tyrosine kinase TIE is required for integrity and survival of vascular endothelial cells4. *EMBO J* 1995;14:5884–91.
121. Roviezzo F, Tsigkos S, Kotanidou A, Bucci M, Brancaleone V, Cirino G, et al. Angiopoietin-2 causes inflammation in vivo by promoting vascular leakage1. *J Pharmacol Exp Ther* 2005;314:738–44.
122. Tait CR, Jones PF. Angiopoietins in tumours: the angiogenic switch. *J Pathol* 2004;204:1–10.
123. Matsumoto H, Ma WG, Daikoku T, Zhao X, Paria BC, Das SK, et al. Cyclooxygenase-2 differentially directs uterine angiogenesis during implantation in mice3. *J Biol Chem* 2002;277:29260–7.
124. Rowe AJ, Wulff C, Fraser HM. Localization of mRNA for vascular endothelial growth factor (VEGF), angiopoietins and their receptors during the peri-implantation period and early pregnancy in marmosets (*Callithrix jacchus*). *Reproduction* 2003;126:227–38.
125. Nayak NR, Kuo CJ, Desai TA, Wiegand SJ, Lasley BL, Giudice LC, et al. Expression, localization and hormonal control of angiopoietin-1 in the rhesus macaque endometrium: potential role in spiral artery growth1. *Mol Hum Reprod* 2005;11:791–9.
126. Wulff C, Wilson H, Lague P, Duncan WC, Armstrong DG, Fraser HM. Angiogenesis in the human corpus luteum: localization and changes in angiopoietins, tie-2, and vascular endothelial growth factor messenger ribonucleic acid. *J Clin Endocrinol Metab* 2000;85:4302–9.
127. Zhou Y, Bellingard V, Feng KT, McMaster M, Fisher SJ. Human cytotrophoblasts promote endothelial survival and vascular remodeling through secretion of Ang-2, PIGF, and VEGF-C9. *Dev Biol* 2003;263:114–25.
128. Li XF, Charnock-Jones DS, Zhang E, Hiby S, Malik S, Day K, et al. Angiogenic growth factor messenger ribonucleic acids in uterine natural killer cells. *J Clin Endocrinol Metab* 2001;86:1823–34.
129. Kayisli UA, Cayli S, Seval Y, Tertemiz F, Huppertz B, Demir R. Spatial and temporal distribution of Tie-1 and Tie-2 during very early development of the human placenta. *Placenta* 2006;27: 648–59.
130. Vuorela P, Carpen O, Tulppala M, Halmesmaki E. VEGF, its receptors and the tie receptors in recurrent miscarriage. *Mol Hum Reprod* 2000;6:276–82.
131. Pietrowski D, Tempfer C, Bettendorf H, Burkle B, Nagele F, Unfried G, et al. Angiopoietin-2 polymorphism in women with idiopathic recurrent miscarriage. *Fertil Steril* 2003;80:1026–9.

132. Ingman WV, Robertson SA. Defining the actions of transforming growth factor beta in reproduction. *Bioessays* 2002;24:904–14.
133. Pardali K, Moustakas A. Actions of TGF-beta as tumor suppressor and pro-metastatic factor in human cancer. *Biochim Biophys Acta* 2006.
134. Relf M, LeJeune S, Scott PAE, Fox S, Smith K, Leek R, et al. Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. *Cancer Res* 1997;57:963–9.
135. Lilli C, Marinucci L, Bellocchio S, Ribatti D, Balducci C, Baroni T, et al. Effects of transforming growth factor-beta1 and tumour necrosis factor-alpha on cultured fibroblasts from skin fibroma as modulated by toremifene. *Int J Cancer* 2002;98:824–32.
136. Jadlowiec J, Dongell D, Smith J, Conover C, Campbell P. Pregnancy-associated plasma protein-a is involved in matrix mineralization of human adult mesenchymal stem cells and angiogenesis in the chick chorioallantoic membrane. *Endocrinology* 2005;146:3765–72.
137. Qian D, Lin HY, Wang HM, Zhang X, Liu DL, Li QL, et al. Involvement of ERK1/2 pathway in TGF-beta1-induced VEGF secretion in normal human cytotrophoblast cells. *Mol Reprod Dev* 2004;68:198–204.
138. Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, et al. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci U S A* 1993;90:770–4.
139. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992;359:693–9.
140. Kallapur S, Ormsby I, Doetschman T. Strain dependency of TGFbeta1 function during embryogenesis. *Mol Reprod Dev* 1999;52:341–9.
141. Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karlsson S, Akhurst RJ. Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. *Development* 1995;121:1845–54.
142. Larsson J, Goumans MJ, Sjostrand LJ, van Rooijen MA, Ward D, Leveen P, et al. Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice. *EMBO J* 2001;20:1663–73.
143. Giudice LC. Growth-factors and growth modulators in human uterine endometrium - their potential relevance to reproductive medicine. *Fertil Steril* 1994;61:1–17.
144. Ogasawara MS, Aoki K, Aoyama T, Katano K, Iinuma Y, Ozaki Y, et al. Elevation of transforming growth factor-beta1 is associated with recurrent miscarriage. *J Clin Immunol* 2000;20:453–7.
145. Lea RG, Underwood J, Flanders KC, Hirte H, Banwatt D, Finotto S, et al. A subset of patients with recurrent spontaneous abortion is deficient in transforming growth factor beta-2-producing “suppressor cells” in uterine tissue near the placental attachment site. *Am J Reprod Immunol* 1995;34:52–64.
146. Hossein H, Mahroo M, Abbas A, Firouzeh A, Nadia H. Cytokine production by peripheral blood mononuclear cells in recurrent miscarriage. *Cytokine* 2004;28:83–6.
147. Amani D, Dehaghani AS, Zolghadri J, Ravangard F, Niikawa N, Yoshiura K, et al. Lack of association between the TGF-beta1 gene polymorphisms and recurrent spontaneous abortion. *J Reprod Immunol* 2005;68:91–103.
148. Filicori M, Fazleabas AT, Huhtaniemi I, Licht P, Rao C, Tesarik J, et al. Novel concepts of human chorionic gonadotropin: reproductive system interactions and potential in the management of infertility. *Fertil Steril* 2005;84:275–84.
149. Berndt S, d'Hauterive SP, Blacher S, Pequeux C, Lorquet S, Munaut C, et al. Angiogenic activity of human chorionic gonadotropin through LH receptor activation on endothelial and epithelial cells of the endometrium. *FASEB J* 2006.
150. Zygmunt M, Herr F, Keller-Schoenwetter S, Kunzi-Rapp K, Munstedt K, Rao CV, et al. Characterization of human chorionic gonadotropin as a novel angiogenic factor. *J Clin Endocrinol Metab* 2002;87:5290–6.
151. Toth P, Li X, Rao CV, Lincoln SR, Sanfilippo JS, Spinnato JA, et al. Expression of functional human chorionic gonadotropin/human luteinizing hormone receptor gene in human uterine arteries. *J Clin Endocrinol Metab* 1994;79:307–15.
152. Islami D, Bischof P, Chardonnens D. Modulation of placental vascular endothelial growth factor by leptin and hCG. *Mol Hum Reprod* 2003;9:395–8.
153. Herr F, Liang OD, Herrero J, Lang U, Preissner KT, Han VK, et al. Possible angiogenic roles of insulin-like growth factor II and its receptors in uterine vascular adaptation to pregnancy. *J Clin Endocrinol Metab* 2003;88:4811–7.
154. Volpert O, Jackson D, Bouck N, Linzer DI. The insulin-like growth factor II/mannose 6-phosphate receptor is required for proliferin-induced angiogenesis. *Endocrinology* 1996;137:3871–6.
155. Kwon YW, Kwon KS, Moon HE, Park JA, Choi KS, Kim YS, et al. Insulin-like growth factor-II regulates the expression of vascular endothelial growth factor by the human keratinocyte cell line HaCaT. *J Invest Dermatol* 2004;123:152–8.
156. Licht P, Russu V, Lehmeier S, Moll J, Siebzehnruhl E, Wildt L. Intrauterine microdialysis reveals cycle-dependent regulation of endometrial insulin-like growth factor binding protein-1 secretion by human chorionic gonadotropin. *Fertil Steril* 2002;78:252–8.