J Assist Reprod Genet (2007) 24:303–315
DOI 10.1007/s10815-007-9152-7

Angiogenesis in implantation

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Published online: 7 July 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 summerize 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.
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.

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.

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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-γ [28]. Mature mouse uNK cells, abundant in the decidua basalis, are enhanced by IL-18 to modify spiral arteries through increased IFN-γ 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-α, a Th1 pro-inflammatory cytokine that influences angiogenesis in a context dependent manner [37]. TNF-α stimulates production of angiogenic factors from decidua fibroblasts yet suppresses their proliferation [38]. TNF-α has been associated with inhibiting embryonic and fetal development [39]; however, TNF-α 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-α 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-α in regulating angiogenic potentials at the maternal–fetal interface.

IFN-γ and IL-12, also pro-inflammatory Th1 cytokines, act as negative regulators of angiogenesis in vitro. IFN-γ 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-γ 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-γ 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 feto-placental border [17, 50].

Decidual macrophages secrete various factors such as epidermal growth factor, transforming growth factor (TGF)-β, 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 PlGF) 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/PlGF 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 (PlGF) is a constituent of the VEGF family of proangiogenic growth factors [78, 79]. PlGF is predominantly produced by trophoblast, exists as four different isoforms produced from a single PlGF primary transcript [80], and functions in both autocrine and paracrine manners [79, 81]. Receptors for PlGF include NRP-1 and NRP-2 and VEGFR-1 [82].

PlGF 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 PlGF expression in trophoblast [71] and induce sVEGFR-1 expression [72].

Gene knockout studies show that loss of PlGF impairs pathological angiogenesis in adult mice [78, 84] and PlGF is angiogenic in vivo and in vitro [85]. PlGF has been correlated with increased placental perfusion at the maternal/fetal interface [86] and it induces relaxation of placental vessels ex vivo [42]. Although PlGF null mice are viable [83], PlGF −/− pups exhibit lower fetal and placental weights when compared to wild types [84]. PlGF 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 PlGF expression during the first stages of human implantation. However, rhesus monkey trophoblast express VEGF initially and its expression decreases with a concurrent increase in PlGF expression as placentation advances [88]. This induction of PlGF 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 PlGF expression coupled with high sVEGFR-1 in maternal sera correlate with on-set of preeclampsia [89, 90]. Low PlGF results in deficiencies in placental angiogenesis [91], although the mechanisms behind this correlation are unclear. Aberrations in PlGF 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 17B-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- Bestradiol induces FGF9 expression in human endometriotic 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.

Angiopoetins (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 destablilzation 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 hemangiogenic 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 angiopoetins could regulate trophoblast function [129].

The importance of the angiopoetins 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, TGFβ−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 TGFβ-1 is generally thought to have dual, cell specific functions in many situations. For example, TGFβ−1 has been shown to inhibit endothelial cell growth in vitro [133], yet it can promote angiogenesis in vivo [134]. TGFβ-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, TGFβ-1 increases expression of VEGF in trophoblast [40, 137] suggesting an indirect function is likely.

Null mutations for TGFβ-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 TGFβ-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 TFβR-I gene in mice caused a death mid-gestation with severe defects in yolk sac and placental vasculature [142].

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

Although TGFβ-1 plays a role in angiogenesis and the temporal pattern of expression suggests that the role may be important in early pregnancy, the pleotrophic effects of TGFβ-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 TGFβ-1 at the materno fetal interface, the ability of TGFβ-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 knockouts 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).

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