Regulatory proteins in placental angiogenesis

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ABSTRACT

The vasculature of the placenta plays a crucial role during the course of pregnancy in order to maintain the growing need of the fetus. Abnormal placental structure and function significantly increase the risk of stillbirth. Various growth factors and cytokines play an important role in the vasculogenesis and angiogenesis of placenta. These processes are stimulated by various pro-angiogenic factors. The activities of these factors are also stimulated by hypoxia. In some of the physiological phenomenon like ovulation, embryogenesis as well as in wound healing intense blood vessel growth can be seen similar to that seen in placenta. Therefore, factors that induce and maintain placental vascular growth and function are of considerable developmental and clinical significance. The total arterial architecture may also depend upon the pro-angiogenic factors. Hormones and other growth factors are other contributors of this vasculogenesis and angiogenesis. Any dysfunction of factors can lead to foetal hypoxia and related complications. This review describes the major growth factors and their significant role in vasculogenesis and angiogenesis of placenta.

Keywords: Angiogenesis; cytokines; growth factors; hypoxia; placenta; vasculogenesis.

INTRODUCTION

The placenta of a mammalian is a union or apposition of the foetal membranes to the uterine mucosa for physiological exchange. Placental angiogenesis is a critical mechanism that ensures successful materno-foetal exchanges by establishing foeto-maternal circulation. It also contributes to the overall development of the placental villous tree and plays a key regulatory role in its development (1). Placental pathologies such as pre-eclampsia (PE), preterm birth, and intrauterine growth restriction (IUGR) occur when these mechanisms are disrupted. As per recent research it is found that there is a close relation of embryonic development and the degree of placental angiogenesis. Placental angiogenesis is mainly mediated by novel angiogenic proteins like EG-VEGF, which is endocrine gland associated endothelial growth factor along with angiogenin. It has been discovered that the mechanism is also controlled by complex microRNAs (miRNAs), and that changes in it have been linked to pregnancy-related pathologies (2). This review focuses mainly on the angiogenic proteins associated with pregnancy and their critical role in physiological changes of placenta during pregnancy.

Physiological changes in pregnancy

Pregnancy and childbirth necessarily require considerable maternal adaptation, with every organ system changing due to biochemical, mechanical, and other factors. Physiological changes begin at conception and progress throughout pregnancy and the postnatal phase. Maternal problems such as gestational hypertension, diabetes, or an increased risk of thrombosis may also be revealed during pregnancy. Since any woman of reproductive age has the ability to become pregnant, her care must take place within the framework of knowledge of normal and abnormal physiology of pregnancy (3). Maternal physiological modifications in pregnancy are the modifications that a woman's body goes through during pregnancy to support the developing embryo. These physiological changes, which include cardiovascular, hematologic, biochemical, renal, postural, and respiratory changes, are completely common. Increases in blood sugar, breathing, and cardiac function are all expected modifications that enable a pregnant woman's body to support proper embryo growth and development during the pregnancy. Several other hormones are released by pregnant women and the placenta also have a broad range of effects during pregnancy. The uterine vasculature undergoes noticeable reformations during pregnancy in order to provide the adequate increase in blood supply to maintain the development and nutrition of the uterus, placenta, and developing foetus.

Pregnancy-associated uterine vascular changes are coordinated by a complex network of endocrine and
cellular mechanisms to cause structural changes at the maternal-foetal interface, which together contribute to the formation of the utero-placental circulation. The physiological and pathophysiological control of maternal blood flow through uterus can better understand through the intrinsic uterine vascular remodelling mechanism. Several pregnancy related disorders can be well explained through the uterine vascular remodelling as it plays a pivotal role in maintaining the uterine vascular anatomy, maternal vascular growth associated with decidualization, invasion of trophoblast, intervillous blood circulation as well as defective utero-placental circulation leading to serious clinical implications (4). In order to nurture and accommodate the developing foetus, during pregnancy, major anatomical and physiological changes occur in the mother to adapt the changes. It is critical to understand the natural physiological modifications that occur during pregnancy since this can help in distinguishing abnormal adaptations (5).

Hormones such as oestrogen and progesterone, and their roles in preparing the uterus for pregnancy, are also crucial. Oestrogen and progesterone are the key hormones that regulate pregnancy throughout. Maternal blood flow within the uterus and placenta is stimulated by oestrogen during pregnancy. Oestrogen further helps the uterine growth along with maintaining the lining of uterus and helps in regulating the key hormones which are vital for the foetal organogenesis. Progesterone levels in pregnant women can be extremely high, causing certain internal structures, like the uterus, to expand in size, allowing it to support a full-term baby (6).

Changes in the uterus during pregnancy

Reynolds et al., investigated the significance of placental circulation, its role, and the close relationship between foetal weight, placental size, uterine and umbilical blood flows during normal pregnancy (7). The uterine size grows from the size of a small peach in its non-pregnant state to support a full-term baby at 40 weeks of gestation. It provides a nutritive and protective environment for the foetal development and growth. According to recent research, Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factors (FGF) are two protein families that are responsible for the majority of ovarian and placental tissue angiogenic activity. Both VEGF, VEGFR mRNA and proteins are present during gestation in foetal as well as in placental tissues, further their expression correlates very well with neovascularisation of placental tissue and development in late pregnancy (6). The role of VEGF in foetal development and angiogenesis of placenta were extensively studied by using gene knock out models. A homzygous knockout of the VEGFR-1 gene or VEGFR-2 gene also known as flt-1 and KDR or flk-1 respectively in mice resulted serious impairments in foetal and placental angiogenesis, and subsequently embryonic death at eighth day of pregnancy (8). As a result, various studies have concluded that VEGF is dependent not only on foetal and placental angiogenesis, but also on VEGF threshold levels as a strong criterion for normal vascular development. FGFs are angiogenic growth factors that are pleiotropic, and they influence not only angiogenesis but also a variety of other developmental functions (6).

Cardiovascular changes during pregnancy

Changes in cardiovascular system usually begin by eighth week of pregnancy. This is initiated with peripheral vasodilation which leads to reduction in systemic vascular resistance. The endothelium-dependent factors like nitric oxide which is usually up-regulated by oestradiol and vasodilatory prostaglandins (PGII) mediate the peripheral vasodilatation. It also results in a 25–30% decrease in systemic vascular resistance and a 40% increase in cardiac output Cardiac output is found to be at its peak between 20-28 weeks of gestation. An increase in stroke volume may occur as a result of an early increase in ventricular wall muscle mass and end-diastolic volume. Blood pressure falls in the first and second trimesters, but rises to non-pregnant levels in the third trimester (9).

Central venous and pulmonary capillary wedge pressures are usually unaffected during pregnancy. A gravid uterus, which causes anatomical changes, may be one of the etiological factors for cephalic and lateral displacement. By 20 weeks of gestation, a mechanical compression may exert to the inferior vena cava (IVC) and descending aorta in supine position by this gravid uterus. Hence there may be a decrease in venous return as well as CO, which may result in foetal compromise and maternal hypotension (10). In order to compensate this aortocaval compression, there may be a rise in sympathetic tone and heart rate. Through the vertebral plexus and aygos veins, blood from the lower limbs is directed to the right side of the heart. These compensatory mechanisms frequently fail to keep blood pressure stable in the supine position, resulting in aortocaval compression syndrome (11,12). This is characterised by pallor, transient tachycardia and subsequently bradycardia, sweating, nausea, hypotension, as well as dizziness. In extreme conditions, it may lead to unconsciousness or maternal death. Early in pregnancy, low blood pressure is common.

Oedema in pregnancy and respiratory changes

Oedema is a frequent ailment among pregnant women, especially if they stand for lengthy periods of time during the day. The combination of a slight increase in the permeability of the smallest blood vessels (which allows more fluid to drain out into the tissues), the extra weight of the uterus, and the
downward pull of gravity slows the rate at which blood is pumped back to the heart from the lower half of the body. Instead of being absorbed into the blood circulation, after the first trimester, the fluid very often collects in the legs and feet of pregnant women. The swelling caused by this collection of fluid is called oedema (13). Many women experience shortness of breath during pregnancy as the developing baby crowds the mother’s lungs, leaving less room to breathe.

Placental angiogenesis in normal pregnancy

The placenta is the first foetal organ to develop, which makes it a valuable element in pregnancy. To function normally, the placenta must be highly vascularized. The fate of the foetus is controlled by the development of placental arterial architecture. The vascular development of a foetus takes place in two stages. The vasculogenesis is the first. The formation of vessels begins on the 21st day following conception. The formation of a vascular plexus from pluripotent mesenchymal progenitor cells, which eventually evolves into endothelial cells, is known as vasculogenesis (14). Then these first formed vessels connect and further expand to form the placental architecture. This continues from 31st day after conception till delivery and is the second step called angiogenesis (15).

A better awareness and understanding of the placenta’s physiological microenvironment will result in a better pregnancy outcome. Different growth factors released by the decidua regulate these two processes. There are three distinct phases of placental angiogenesis and vasculogenesis (16). In stage I, the haemangeogenic stem cells induces cytotrophoblast differentiation. In Stage II, activation and pre-vascular network formation occur and in Stage III, remodelling of the process of differentiation of perivascular cells occur to form contractive vessels (Figure 1). The above processes are mediated by a different type of regulatory proteins secreted from different type of cells in the placenta like trophoblast cells, Hoffbauer cells and pericytes (17).

Fig. 1: Three steps of vasculogenesis and angiogenesis of placental villous development. Adopted from Vascular Biology of the Placenta. Morgan & Claypool Life Sciences; 2010

Placental abnormalities in pregnancy

The placenta is an important organ that develops throughout the pregnancy with the implantation of the blastocyst. During pregnancy, the placenta plays an essential role. If we look at the normal placenta, it weighs about one sixth of the foetal birth weight and...
is round or oval in shape. It is attached to the uterine wall firmly. It has a diameter of around 22 cm and a thickness of 2-2.5 cm (18). As a result, the placental abnormalities can vary from anomalies in structure to function including a defect in site of implantation also. When the placental villi adhere to the myometrium rather than the decidua, this is referred to as placenta accrete; when the chorionic villi penetrate the myometrium, this is referred to as placenta increta (19). Placenta increta and percreta are uncommon diseases. These variants can result in more serious maternal complications. Placenta praevia is described as a placenta that completely or partially covers the internal cervical os of the uterus. Vasapraevia is a rare disorder, in which the foetal blood vessels cross the lower uterine segment in advance of the presenting part, unsupported by either the umbilical cord or placental tissue (20). This pathologic structure can result in foetal blood loss, significant neonatal morbidity, or death in the case of spontaneous membrane rupture or amniotomy (21). Placental morphological anomalies refer to a placenta that is divided into two lobes separated by membranes. If there are more than two lobes, the placenta is referred to as a multilobed placenta. The incidence is predicted to be 2-8 percent of placentas (22). Circumvallate placenta is an extra-chorial placenta that is characterised as an annularly shaped placenta with elevated borders composed of a double fold of chorion, amnion, degenerated decidua, and fibrin deposits. The basal plate is pathologically larger than the chorionfrondosum (23).

Another deformity is Placentamembranacea, a very rare variant in placental morphology in which the placenta develops as a thin structure that occupies the whole chorion (24). In Chronic intervillositis, also known as vastchronicintervillositis or chronic histiocycticintervillositis, a very uncommon placental abnormality characterised by inflammatory placental lesions, mainly diffuse histiocytic infiltration in the intervillous region. Placental mesenchymal dysplasia of the placenta is an unusual vascular abnormality characterised by mesenchymal stem villous hyperplasia. Yet another deformity of the placenta is chorioangioma, which is a benign vascular tumour caused by a deformation of the placental primitive angioblastic tissue, which is perfused by the foetal circulation.

Regulatory proteins and their role in pregnancy

Only a few normal physiological processes, such as ovulation embryogenesis and wound healing, display intensive blood vessel growth comparable to that seen in the placenta. Therefore, factors that induce and maintain placental vascular growth and function have significant developmental and clinical implications (25). So many factors have been implicated in the regulation and stimulation of angiogenesis in the placenta, with consistent action throughout the pregnancy. The major being VEGF and FGFAL, which act through specific cell receptors on the endothelium.

Many molecules have been identified as angiogenesis positive regulators including acidic Fibroblast Growth Factor (FGF), basic FGF, Transforming Growth Factor (TGF)-α, TGF-β, Hepatocyte Growth Factor (HGF, or scatter factor; 26; Table:1). For more than a decade, researchers have been focusing on the role of VEGF (also known as VEGFA) in the regulation of angiogenesis. According to new evidence, new vessel growth and maturation are highly complex and coordinated processes that require the sequential activation of a series of receptors by a variety of ligands. VEGF also appears to play a role in pathological angiogenesis, such as that seen in tumour growth (27). VEGF is a member of a gene family that includes placental growth factor. VEGFA is an important factor in the regulation of blood vessel growth. VEGFC and VEGFD control lymphatic angiogenesis, highlighting the family's unique role in controlling the growth and differentiation of multiple anatomic components of the vascular system (27).

Table 1: Positive Regulators of Angiogenesis

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<tr>
<th>Growth Factor</th>
<th>Abbreviation</th>
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<tr>
<td>Fibroblast Growth Factor</td>
<td>(FGF)</td>
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<tr>
<td>Transforming Growth Factor-α</td>
<td>(TGF)-α</td>
</tr>
<tr>
<td>Transforming Growth Factor- β</td>
<td>TGF-β</td>
</tr>
<tr>
<td>Hepatocyte Growth Factor</td>
<td>HGF</td>
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<tr>
<td>Vascular Endothelial Factor</td>
<td>VEGF</td>
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VEGF and its function

In vitro studies have shown that VEGF can promote the growth of the vascular endothelial cells (ECs) which are derived from arteries, veins, and lymphatics. Since VEGF is a secretary angiogenic mitogen, it can induce potent angiogenic responses as shown in a variety of in vivo models. Although ECs are the primary target of VEGF, several research has found that it has mitogenic effects on non-EC cell types. Recent research has also shown that VEGF stimulates surfactant production by alveolar type II cell. VEGF is a survival factor for ECs, both in vitro and in vivo. In vitro, VEGF prevents apoptosis caused by serum deficiency. Gerber et al. have shown that such activity is mediated by the phosphatidylinositol (PI)-3 kinase–Akt pathway (28). Further the expression of the anti-apoptotic proteins like Bcl-2 and A1 in endothelial cells are promoted by VEGF (Figure 2). Moreover, VEGF promotes the expression of vascular permeability factor. Now it is very clearly understood from studies that such permeability-increasing activity support this molecule to act as a mediator for inflammation as well to express in pathological conditions. Further studies have shown the effect of VEGF on isolated micro vessel hydraulic conductivity which may be
mediated by high calcium influx (29). In some vascular beds, VEGF induces endothelial fenestration and is consistent to involve in the regulation of permeability of vasculature.

**Fig. 2:** Antiapoptotic Proteins Bcl-2 and A1 in Vascular Endothelial Cells showing VEGF induced Expression. Adopted from Journal of Biological Chemistry

### VEGF family and their receptors

VEGF is an endothelial cell mitogen, and its expression in the placenta increases as pregnancy progresses. There are several isoforms of this growth factor, with VEGF being the most abundant in most tissues. The biological function of the various isoforms differs. VEGF is expressed in a variety of embryonic and foetal tissues during development. In the foetal heart and placenta, VEGF expression is inducible by hypoxia (30). As a result of alternative RNA splicing, different isoforms of VEGF and PLGF are expressed. Five VEGF isoforms and two PLGF isoforms are so far known with 121, 145, 165, 189, and 206 amino acid residues and with 131 and 152 amino acid residues respectively. The two isoforms of PLGF are PLGF-1 and-2, respectively (Figure 3). A heterodimer is formed in case of VEGF and PLGF which is found to be a potent endothelial cell mitogen. There are eight exons separated by seven introns in human VEGFA gene as it is organized. Four distinct isoforms are formed due to alternate splicing of exons named as VEGF121, VEGF165, VEGF189 and VEGF206, with 121, 165, 189 and 206 amino acids residues in the sequence once they are cleaved (31). The predominant isoform, VEGF165, lacks the exon 6-encoded residues, whereas VEGF121 lacks the exon7-encoded residues. Less frequent splice variants have been also reported, such as VEGF145 and VEGF183.

Studies indicating that, VEGF or VPF (Vascular permeability factor) is one of the chief regulators of normal angiogenesis as well as pathological

**Figure 3:** VEGF family members and their receptors at a glance.
angiogenesis due to many reasons. VEGF/VPF is a secreted growth factor that is mitogenic especially for endothelial cells in culture, angiogenic in vivo, and promotes vascular permeability, which leads to the development of vascular stroma; the expression of VEGF/VPF and its receptors correlates strongly with periods of vasculogenesis and angiogenesis during embryonic development and with programmed neovascularization that occurs in the female reproductive cycle (Fig. 4). Previous studies indicate the critical involvement of VEGF in vasculogenesis and angiogenesis during embryonic development. Poor placental vascular development, intrauterine growth restriction and an increase in the mitotic index of cytotrophoblast cells (32) results due to poor development of placental vasculature as indicated by morphological studies. There is distinct regulatory mechanism for the expression of VEGF as well as PIGF in embryonic trophoblast as suggested in a study were there was alterations in vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) those may restrict angiogenesis in the intervillous space in contact with IUGR placental villi. An eight-fold increase in VEGF expression was seen in trophoblast cultured under conditions of hypoxia (1 per cent O2) and PIGF expression was found to be decreased by 73 +/- 5.5 per cent in the same trophoblast (33).

CONCLUSION
Several physiological changes are associated with placenta during pregnancy. The regulatory proteins are critical in determining these placental changes during pregnancy. Among the various growth factors and cytokines, the VEGF play an important role in the vasculogenesis and angiogenesis of placenta along with PIGF. Though the mechanism is complicated, their involvement in the placental angiogenesis and vascular development are critical for the foetus to have a nourishing environment for growth and development. Hence the placenta with the angiogenic factors is a potent target for diagnosis as well as prognosis of foetal development and anomalies.

CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

REFERENCES