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Effects of Recombinant Endothelial Growth Factor 121 Therapy on Maternal Vascular Function and Vascular Fetal Programming in a Mouse Model of Preeclampsia

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by

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Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas Medical Branch

in Partial Fulfillment

of the Requirements

for the Degree of

Clinical Science Doctor of Philosophy Program

The University of Texas Medical Branch

July, 2011

Dedication

<I dedicate this achievement of my career to my family, specially my parents Aracely and Eduvino, my sister Claudia, my wife Clara, and my children for their unconditional support during the last four years.>

Effects of Recombinant Endothelial Growth Factor 121 Therapy on Maternal Vascular Function and Vascular Fetal Programming in a Mouse Model of Preeclampsia

Publication No._____

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The University of Texas Medical Branch, 2011

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Abstract: Preeclampsia is believed to be caused by an imbalance between placental perfusion and placental metabolic needs. The hypoxic placenta releases factors into the maternal circulation, causing dysfunctional maternal endothelium, and resulting in blood pressure increase and end organ damage. Pregnancies destined to develop preeclampsia have altered cardiovascular adaptations which results in endothelial dysfunction, and abnormal placental perfusion. Various angiogenic growth factors, such as the vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), have been shown to play an important role in the vascular physiologic changes occurring

during pregnancy; therefore, inhibition of their function is believed to be one of the mechanisms leading to preeclampsia. One of the inhibitors of these angiogenic factors is the circulating form of the VEGF receptor 1, or soluble fms-like tyrosine kinase1 (sFlt-1). The soluble Flt-1 is generated by differential mRNA splicing of VEGF receptor 1. Soluble Flt-1 acts as a potent antagonist of VEGF and PlGF by preventing them from binding to their endothelial cell receptor, VEGFR-1 and VEGFR-2. Recently, we validated a mouse model of preeclampsia induced by overexpression of sFlt-1 through injection of the adenovirus. Given its key role in preeclampsia, sFlt-1 has turned into a new target for drugs therapies. Clinical and animals studies have shown that exogenous VEGF is useful in vascular conditions with endothelial dysfunction and altered angiogenesis. Our study examined the efficacy of VEGF-121, a freely diffusible protein, in improving maternal vascular dysfunction and altered fetal vascular programming in the preeclampsia-like pregnant mice overexpressing sFlt-1. The therapy had beneficial effects including inhibition of circulating sFlt-1, reduction of maternal blood pressure, amelioration of endothelial function, and diminishment of placental hypoxia. In addition, VEGF-121 enhanced intrauterine fetal growth and prevented hypertension and endothelial dysfunction in the adult male offspring. Our findings underscore the importance of angiogenic factors in mediating vascular adaptations in pregnancy and in regulating fetal vascular development. Targeting the VEGF signaling pathway may play a role in the treatment of preeclampsia and thereby this innovative therapy modality needs further to be tested in clinical studies.

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CHAPTER 1: INTRODUCTION

1.1 EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS OF PREECLAMPSIA

Hypertensive disorders of pregnancy are a leading cause of maternal and perinatal morbidity and mortality. Pregnancy associated hypertension occurred in 39.1 per 1,000 live births in the United States (US) in 2007 (Martin, 2010). Its incidence has increased 38% since 1990. In a recent large cohort study of 1,461,270 births in US (Clark, 2008), preeclampsia-related complications were the leading cause of maternal death (16%). Abruption of placenta, hepatic rupture, eclampsia, and cerebrovascular disease were the most common causes of maternal death linked to preeclampsia.

Preeclampsia occurs in 2% to 7% of pregnancies (Sibai, 2003; Hauth, 2000). This multisystemic disorder is characterized by increased blood pressure and proteinuria. Elevated gestational blood pressure is defined as a blood pressure greater than 140 mm Hg systolic or greater than 90 mm Hg diastolic in a woman who was normotensive before 20 weeks of gestation. Proteinuria is defined as urinary protein excretion equal or greater than 300 mg in a 24-hour specimen (National High Blood Pressure Education Program (NHBPEP), 2000). In the absence of proteinuria, the disease is highly suspected when hypertension is accompanied by any of the following symptoms: headache, blurry vision, abdominal pain, and abnormal lab results, particularly low platelet count and elevated liver enzymes (NHBPEP, 2000).

At least two thirds of the cases of preeclampsia occur in first pregnancies that progress beyond the first trimester (Sibai, 2003). Other recognized risk factors are multifetal gestation, preeclampsia in a previous pregnancy, chronic hypertension, pregestational diabetes, vascular and connective tissue disorder, nephropathy, antiphospholipid antibody syndrome, obesity, age 35 years or older, and African

American race (Sibai, 2003). Obesity is an emerging risk factor of preeclampsia as the incidence of this condition is rapidly growing worldwide. It is estimated that the risk of preeclampsia is three-fold higher in obese women that normal weight women (Sibai, 1995).

Fetal and neonatal morbidity and mortality are also increased as preeclampsia is responsible of 15% of all preterm births and predisposes to intrauterine growth restriction (Roberts, 2003). A large cohort of 10,614,679 singleton pregnancies after 24 weeks in US from 1995 to 1997 showed that the relative risk for fetal death was 1.4 in infants born to mothers diagnosed with any of the gestational hypertensive disorders and 2.7 for those born to women with chronic hypertension compared with normotensive pregnancies (Basso, 2006). Causes of fetal death are attributed to abruption of placenta, placental insufficiency, and prematurity. The rate of mortality correlates linearity with the severity of the disease, being the highest in women with superimposed preeclampsia on underlying vascular disease. Intrauterine growth restriction is a common complication of preeclampsia and increases in relation with the severity of the disease and earlier diagnosis (Odegård, 2000).

Epidemiological data suggest that preeclampsia is associated with risk of subsequent cardiovascular disease later in life. Common risk factors of cardiovascular disease such as hypertension, dyslipidemia, and glucose intolerance have been linked to hypertensive disorders in pregnancy such as preeclampsia (Harskamp, 2007; Magnussen, 2009). Preeclampsia may act as a stress state that either provokes metabolic and systemic endothelial dysfunction or amplifies the effect of underlying cardiovascular risk factors (Harskamp, 2007). Women with a history of preeclampsia compared with those without it have two-three times higher risk for later diabetes. The effect persists after adjustment is made for potential confounders (Magnussen, 2009). A population-base study conducted

in Norway reported a modest 1.65 fold increased cardiovascular mortality among nulliparous women with preeclampsia at term and an eightfold increase risk when preeclampsia was severe enough to lead to preterm delivery (Irgens, 2001). Other reports support a link between preeclampsia and maternal ischemic heart disease that usually occurs twenty years after a preeclamptic pregnancy and coincides with the onset of menopause (Funai, 2005; Arnadottir, 2005). Recurrence of preeclampsia in consequent pregnancies has been associated with a stronger risk for later cardiovascular disease (Sibai, 1986). Women with preeclampsia in both the first a second pregnancy have substantially higher blood pressure several years after in life than nonpreeclamptic women and also higher than women with preeclampsia either in their first or second pregnancy (Magnussen, 2009).

1.2 PATHOGENESIS OF PREECLAMPSIA

Despite intense investigation and important progress in recent years, the etiology of preeclampsia remains to be elucidated. Recent data support the concept that placenta is centrally involved in the pathogenesis of this syndrome. The role of placenta in this syndrome is supported by the occurrence of preeclampsia in molar pregnancies where the fetus is absent and the usual regression of the disease shortly after delivery occurs.

The placenta is essential for exchange of nutrients, oxygen, and waste between the mother and developing fetus. Coordinated vascularization of the placenta is essential for proper placental development and involves the processes of vasculogenesis, defined as new blood vessel formation, and angiogenesis in which blood vessels growth from preexisting vessels (Cross, 1994).

Extensive research work has shown that placenta of women with preeclampsia is abnormally developed and relatively hypoxic. Preeclampsia appears to progress in two stages. The first stage arises from defective early trophoblast invasion and remodeling of

the spiral arteries which lead to insufficient blood supply to the placenta. In the second stage, progression of placental dysfunction results in clinical manifestations of the disease including hypertension, proteinuria as well as liver and clotting dysfunction (Redman, 2005) (Figure 1).

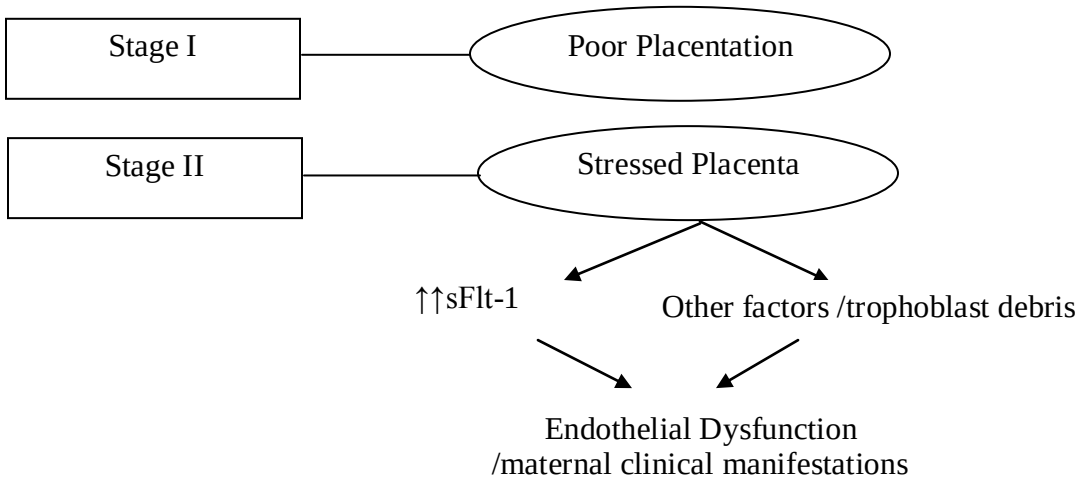


Figure 1: Pathogenesis of preeclampsia (Figure modified from Redman, 2005)

Failure of early trophoblastic invasion has been attributed to two overarching factors. One is characterized by a maternal-paternal immune maladaptation, whereas the other arises from a generalized systemic inflammatory response, of which endothelial dysfunction is a prominent component.

It has been suggested that trophoblast signaling to decidual immune cells in preeclamptic pregnancies is weak and fails to stimulate a normal maternal immunologic response, which is essential for placentation (Hiby, 2004; Parham, 2005). Some experts consider that preeclampsia occurs as result of a maternal immune rejection of the genetically foreign fetus (Renaud, 2008; Saito, 2007). Invasive cytotrophoblasts that infiltrate maternal territory during placentation express a unique combination of human

lymphocyte antigens (HLAs), namely HLA-C, -E, and –G (Moffett-King, 2002). Of these, HLA-C, polymorphic signaling paternal alloantigens, interacts with different maternal natural-killer (NK) cell receptors. There is great diversity of haplotypes of killer-cell immunoglobulin-like receptors (KIR) in humans, and because HLA-C is polymorphic, every pregnancy will have different combinations of paternally-derived fetal HLA-C and maternal KIRs. Some haplotypes inhibit NK cell function, whereas others are stimulatory. Mothers lacking most of all activating KIRs (AA genotype) when the fetus has HLA-C (belonging to the HLA-C2 group) are at substantial risk of preeclampsia (Parham, 2005).

The other theory supports the concept that a hypoxic placenta releases factors into the maternal circulation originating the clinical signs of preeclampsia. In healthy pregnancies, the appropriate interaction between endovascular trophoblast and decidual leukocytes, especially NK cells, results in substantial release of vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) (Dekker, 1998). Raised concentrations of free VEGF are important in maintaining the quiescent endothelial state under the existing increased shear and inflammatory stress typical of pregnancy (Maynard, 2003).Several factors have been proposed as inhibitors of VEGF and PlGF biological functions. The most prominent of those is the soluble receptor for vascular endothelial growth factor (VEGF-1), also known as sFlt-1 (soluble fms-like tyrosine kinase (sFlt-1). This placental-derived antiangiogenic factor binds to VEGF and PlGF and deprives the systemic endothelium from essential survival factors (Maynard, 2003). Human studies have shown that elevated levels of circulating sFlt-1 precedes the clinical manifestations of preeclampsia and the levels fall shortly after delivery (Levine, 2004). The magnitude of the sFlt-1 raise correlates with the severity of the disease (Chaiworapongsa, 2004; Levine, 2004).

1.1.2 Placental Vascular Formation and Function

During early normal placental development, the human placenta develops in specialized trophoblast types that differ in function. The multinucleated syncytiotrophoblast layer encases the floating villi of the placenta and provides the barrier to maternal blood, regulating oxygen and protein transport. Extravillous trophoblasts of fetal origin migrate from the villous tips in columns anchoring the placenta to the maternal deciduas (Cartwright, 2010). These cells play an active role in the remodeling events that occur in the uterine spiral arteries. Remodeling leads to both loss of vascular cells and alterations of the extracellular matrix proteins resulting in dilated arteries that allow greater transport of maternal blood to the intervillous space (Brosens, 1967). Trophoblast invasion into the uterine spiral arteries transform them from small, high resistant vessels into large-caliber capacitance vessels capable of providing adequate placental perfusion to nourish the fetus. Cytotrophoblasts undergo pseudovasculogenesis, or vascular mimicry, to assume an endothelial phenotype. Pseudovasculogenesis occurs through down regulation of adhesion molecules and adoption of an endothelial cell-surface adhesion phenotype (Zhou, 1993).

In the decidua, extravillous trophoblasts interact with decidual stromal, epithelial, and immune cells, all of which are able to regulate their differentiation and invasion. Trophoblasts exit the cell cycle during decidual invasion. The expression of several transcription factors have been implicated in the cell cycle arrest including the activator protein (AP-1) (Bamberger, 2004), STAT3 and peroxime proliferator-activated receptor-γ (Knofler, 2010). Up-regulation of proteases is required for the degradation the extracellular matrix. Urokinase plasminogen activator promotes the activation of several matrix methaloproteinases involved in the degradation process (Liu, 2003). The microenvironment into which trophoblast invades influence the expression of factors and

oxygen is known to alter this process. The physiologic hypoxic conditions of the first trimester (~2-3% oxygen) are thought to promote an invasive trophoblast phenotype (James, 2006).

Preeclampsia has long been suspected of being a placental disorder (Zuspan, 1988; Redman, 1991). The paucity of placental lobes, abnormal maturation of the terminal villi, reduced mass of syncytiotrophoblasts, relative excess of cytotrophoblasts and multiple placental infarcts all support a role for the placenta in the etiology of preeclampsia (Hustin, 1983; Khong, 1986; Shanklin & Sibai, 1989; Redman, 1993; Pijnenborg, 1996). Preeclampsia has been associated with inadequate spiral artery remodeling (Brosens, 1972; Pijnenborg, 1996) (Figure 2). Compared with healthy pregnancies, the diameter of the spiral arteries in third trimester of preeclamptic placentas is decreased (Brosens, 1972) and defective remodeling can be detected in both decidual and myometrial portions of the vessels. Impairment of the events involved in normal spiral artery remodeling has the potential to affect the blood supply to the placenta by altering the flow rate of blood flow leading to reduced placenta perfusion and oxidative stress.

Figure 2: Trophoblast invasion in normal and preeclamptic pregnancy

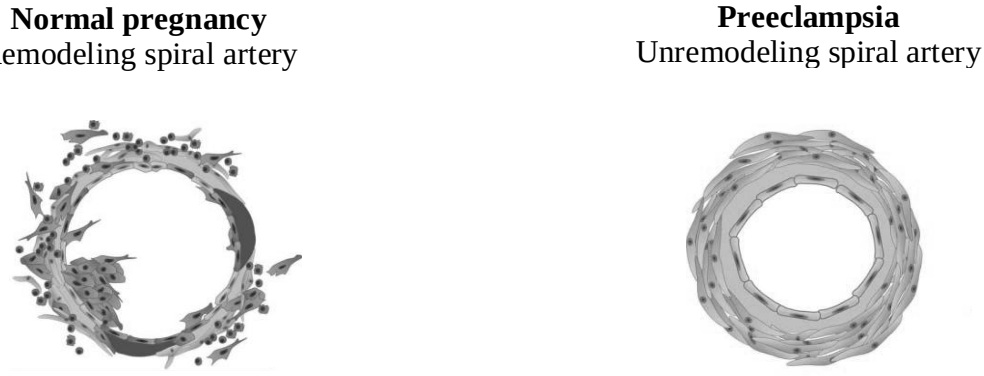


Figure 2: Trophoblast invasion into the spiral arteries in the placental bed of normal pregnancy, left image, and in preeclampsia, right image (Figure modified from Cartwright et al, 2010).

1.3. ANGIOGENIC IMBALANCE AND PREECLAMPSIA

Recent evidence suggests that changes in circulating angiogenic factors play an important role in the pathogenesis of preeclampsia. During normal pregnancy, the placenta undergoes high levels of vasculogenesis and angiogenesis. Angiogenesis is the process of neovascularization from preexisting blood vessels, whereas vasculogenesis is the process of blood vessel generation from de novo vessel formation. Placental perfusion is dependent on angiogenesis, a major mechanism responsible for the increase in placental blood flow throughout gestation. Numerous factors have been implicated in this process. Based on in vivo and in vitro studies, it has been shown that the VEGFs family is the main regulator of the angiogenesis process. The same factors are involved in normal and pathological processes including tumor growth and wound healing (Ferrara, 1997, Fraser, 2000). VEGF/VEGFR mRNA and their protein are abundantly present in the placenta and are essential for normal placental vascular development (Maglione, 1991, Cooper, 1995, Charnock-Jones, 1994; Bdolah, 2004). VEGF ligands and receptors are highly expressed by placental tissue in the first trimester. Proangiogenic factors are highly expressed in several organ systems during pregnancy. VEGF stabilizes endothelial cells and maintain endothelial function in the renal glomerulus, brain, and liver, organs severely affected in preeclampsia (Esser, 1998). VEGF is highly expressed by glomerular podocytes, and VEGF receptors are present on glomerular endothelial cells (Maharaj, 2006). Production of angiogenic factors vary throughout gestation. Circulating sFlt-1 levels are relatively low early in pregnancy and begin to rise in the third trimester. This rise in sFlt-1 is concurrent with decreases in VEGF and PlGF, changes that are thought to be a normal physiological mechanism design to rein in placental vascular growth.

Increased expression of sFlt-1, associated with decreased PlGF and VEGF signaling are abnormalities that have been seen in women with preeclampsia (Maynard,

2003; Ahmad, 2004). sFlt-1 is secreted rather than bound to the cell surface, and it has biological activity as an antagonist to both VEGF and PlGF, binding them in the circulation and preventing interaction with their receptors on the cell surface (Wathen, 2006). In vitro effects of sFlt-1 include vasoconstriction and endothelial dysfunction. Derangements in other angiogenic molecules have also been observed. Maternal serum levels of endostatin, an inhibitor of angiogenesis, are elevated in preeclampsia (Hirtenlehner, 2003). Soluble endoglin (sEng) is upregulated in preeclampsia in a similar pattern to that of sFlt-1. sEng is a truncated form of endoglin (CD105), a cell receptor for transforming growth factor-β (TGF-β), which binds and antagonizes TGF-β. sEng amplifies the vascular damage mediates by sFlt-1 in pregnant rats, inducing a severe preeclampsia-like syndrome with features of hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome (Venkatesha, 2006).

VEGF also plays an essential role in placental development and function in other species. Gene knockout studies provide convincing evidence for a key role of VEGF in fetal and placental angiogenesis. Mice embryonic stem cells lacking the VEGFR1 (Flt-1) show an increased endothelial cell mitotic index, indicating that aberrant endothelial cell division occurs in vivo due to lack of VEGFR1 (Flt-1) (Kearney, 2002). Homozygous knockout mice lacking the VEGFR2 gene have altered fetal and placental angiogenesis, characterized by the failure of blood-island formation, organization and patterning (Shalaby, 1995). Similarly, heterozygous gene knockouts for VEGF have an abnormal development of the embryo and placental vasculature (Carmeliet, 1996).

1.3.1 Role of sFlt-1 in the Pathogenesis of Preeclampsia

The soluble Flt-1 is generated by differential mRNA splicing of VEGF receptor 1 (VEGFR-1). However, sFlt-1 lacks the cytosolic domain compared with VEGFR-1. sFlt1 acts as a potent antagonist of VEGF and PlGF in two ways: firstly, by binding directly to

VEGF and PlGF thus decreasing their available free levels for their angiogenic action; secondly, sFlt-1 hetero-dimerizes with the extra-cellular ligand binding region of the membrane spanning VEGF receptors, thereby blocking the phosphorylation and activation of the downstream signal transduction pathways for endothelial cell proliferation (Kendall, 1993; Kendall, 1996).

The first study suggesting that sFlt-1was associated with preeclampsia showed that sFlt-1 levels are increased in the amniotic fluid of women with preeclampsia (Vuorela, 2000). High levels of circulating sFlt-1 detected in early pregnancy are associated with later onset of preeclampsia (Maynard, 2003; Thadhani, 2004; McKeeman, 2004). Moreover, a fall in sFlt-1 levels following delivery of the placenta correlates with improvement in clinical syndromes (Maynard, 2003). Increased sFlt-1 and decreased PlGF/VEGF levels have been proposed in several clinical studies as specific biomarkers for the diagnosis and prediction of preeclampsia (Salahuddin, 2007; Moore Simas, 2007).

The trigger event of sFlt-1 rise in preeclampsia is still not known. Placenta seems to be the main source of excess sFlt-1 production; however it is also produced in endothelial cells and activated mononuclear cells (Rajakumar, 2005). In vitro studies demonstrated that sFlt-1 inhibits tube formation of endothelial cells from human umbilical vein and trophoblast migration and differentiation (Maynard, 2003; Ahmad, 2004; Zhou, 2002). In primary cytotrophoblast cell culture, sFlt-1 production and mRNA expression is inversely related to oxygen saturation (Nagamatsu, 2004). A two-fold elevation in the level of sFlt-1 was also observed when normal villous explants were exposed to a hypoxic state (1% O_2) compared to the physiological 5% O_2 exposure (Ahmad, 2004). Hence, sFlt-1 is up-regulated in response to hypoxia in vitro. An in vivo study (Makris, 2007) had shown that increased circulating level of sFlt-1 correlates with

the onset of clinical syndromes and the severity of disease in an artificial model of preeclampsia induced by decreasing the blood flow to the utero-placental units, this has also been confirmed by other investigators in clinical studies (Wathen, 2006; McKeeman, 2004; Hertig, 2004).

Accumulating evidence suggests that sFlt-1 may act as a mediator in inducing maternal endothelial dysfunction, hypertension and proteinuria in preeclampsia, and therefore operate as a key part of the mechanism linking the placenta with maternal endothelial dysfunction. Although the above studies indicate that excess sFlt-1 production noted in preeclampsia may be secondary to placental hypoxia, it remains unclear whether impaired placental perfusion initiates preeclamptic symptoms such as hypertension, endothelial dysfunction, and increased sFlt-1 or whether inadequate placental development occurs initially and is followed by a pathological rise in sFlt-1 expression and secretion. Genetic factors, metabolic derangements, and inflammatory factors could lead to increased sFlt-1 level and decreased free VEGF and PlGF levels which contribute to abnormal placentation, resulting in placental hypoxia (Figure 3). Pathogenic effects caused by sFlt-1 have been reproduced in rodent animal models. Exogenous sFlt-1 administrated to pregnant rats and mice produces a syndrome resembling preeclampsia, including hypertension and renal damage (Maynard, 2003; Lu, 2007).

Figure 3: Interactions of imbalanced angiogenesis and maternal/placental factors

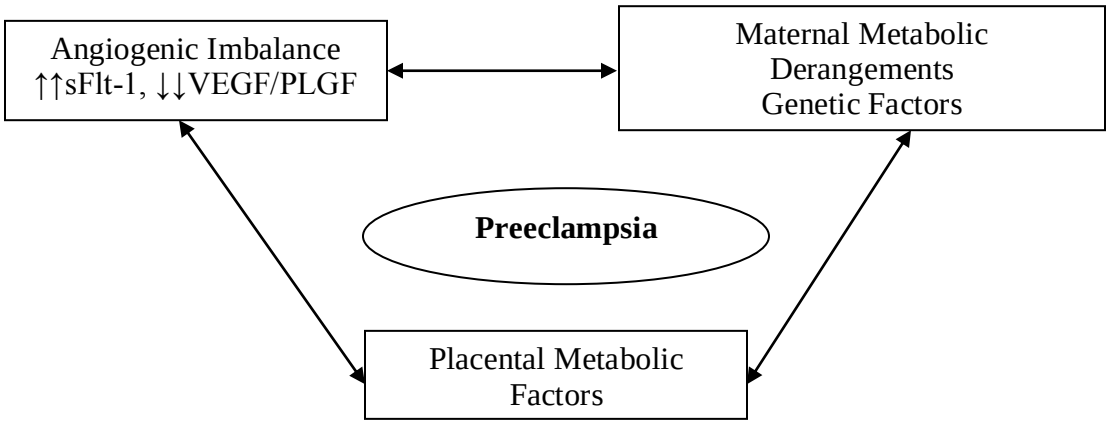


Figure 3 Possible interactions between angiogenic imbalance and maternal and placental factors in the pathogenesis of preeclampsia (Figure modified from Shenoy et al, 2010)

1.3.1 Animal Models of Preeclampsia

Numerous animal models have been developed in an attempt to determine causative factors, pathogenesis of the disease, and development of therapeutic options. Several species have been used for this purpose including dogs, rats, rabbits, mice, sheep and non-human primates. The common theme of these animal models is the altered placental vascular function. The attempted methods include surgical, pharmacological, and genetic manipulations. Specifically, the broad group of mechanisms used in modeling preeclampsia fall into the following general categories: mechanical interference of uterine blood flow and reduced uterine perfusion pressure (RUPP) by surgical means such as ligation or banding of either the abdominal aorta below the renal arteries, the internal iliac arteries or the uterine arteries (Ogden, 1940; Lunell, 1984; Woods, 1989); alteration of vascular function via impairment in the nitric oxide system by knocking out the endothelial NOS (nitric oxide synthase) gene (Zatz, 1998; Hefler, 2001), chronic blockade of the endothelial derived relaxing factor (Baylis, 1992) or using a NOS

inhibitor such as N (G)-nitro-L- arginine methyl ester (L-NAME)(Greenberg, 1997), over-activity of the renin-angiotensin systems (Kanayama, 1996; Takimoto, 1996; Kananyama, 1997; Bohlender, 2000; Takiuti, 2002), or activation of systemic inflammatory responses (Faas, 1994; Hung, 2004). Although, these animal models share some features of preeclampsia such as hypertension, they have not reproduced an acceptable model of the human disease.

The recognized role of imbalance in angiogenic factors in the pathogenesis of preeclampsia has promoted investigators to develop animal models of preeclampsia mimicking the overproduction of antiangiogenic factors. Maynard et al administrated either sFlt-1 to pregnant rats by use of adenovirus vector (Maynard, 2003). The pregnant rats developed hypertension, proteinuria, and glomerular endotheliosis. Chronic administration of sFlt-1 by infusion to rats also leads to intrauterine growth restriction (Bridges, 2009). Administration of sEng in adenovirus vector to pregnant rats also produced hypertension and mild proteinuria, whereas co-administration of both sFlt-1 and sEngl led to a HELLP-like syndrome including decreased platelets counts, elevated liver enzymes and signs of hemolysis (Venkatesha, 2006). Recently, we validated a mouse model of preeclampsia induced by sFlt-1 overexpression. Pregnant mice injected in the tail vein tail with adenovirus vector carrying sFlt-1 at day 8 of gestation developed hypertension in the late gestation, endothelial dysfunction, and pups showed signs of intrauterine growth restriction (Lu, 2007).

1.3.2 Gene Expression in Response to Hypoxia

Shallow trophoblastic invasion along with insufficient spiral artery remodeling are pathologic changes in the placental development that are believed lead to preeclampsia. These changes result in decreased uteroplacental blood flow and placental hypoxia, recognized risk factors of diminished oxygen and energy supply to the fetus,

which predisposes ultimately to intrauterine growth restriction and other developmental fetal abnormalities (Mayhew, 2004). The hypoxic placenta releases several factors to the maternal circulation which are major contributors to endothelial dysfunction. The predominant factor is sFlt-1. In primary cytotrophoblast cell culture, sFlt-1 production and mRNA expression is inversely related to oxygen saturation (Nagamatsu, 2004). A two-fold elevation in the level of sFlt-1 has been observed when normal villous explants were exposed to a hypoxic state (1% O_2) compared to the physiological 5% O_2 exposure (Ahmad, 2004).

A variety of genes are activated in target organs as placenta and kidney in response to hypoxic conditions. The hypoxic-inducible factor-1 (HIF-1) is a crucial regulator of adaptive processes to hypoxia. It is composed of two subunits, the constitutively-expressed HIF-1β and the inducible HIF-1α. Under low oxygen conditions, HIF-1α dimerizes with HIF-1β to form the active HIF complex, which can bind the promoters of oxygen-responsive genes and activate their transcription. Therefore, it has been suggested that HIF-1α plays an important role in signaling hypoxia. HIF-1 is believed to be involved in the pathogenesis of several human diseases such as ischemic cardiovascular disorders, pulmonary hypertension and preeclampsia (Semenza, 2000**)**. HIF-1α is rapidly ubiquitinated and degraded via the proteosomal pathway when normoxia is reestablished (Cockman, 2000). HIF-1α also regulates trophoblast differentiation by up-regulating the expression of transforming growth factor β3 (TGFβ3). TGFβ3 is involved in oxygen-dependent differentiation processes during placental development as well as in various pregnancy disorders (Hernandez-Valencia, 2001). Decrease in TGFβ3 expression occurs immediately prior to the peak of trophoblast invasion, suggesting a role for TGFβ3 as an inhibitor of early trophoblast differentiation (Caniggia, 2000a; Caniggia, 2000b; Caniggia, 1999). HIF-1α and TGFβ3 are over-

expressed in placental tissue from preeclamptic women (Caniggia 2000a). Therefore, expression of TGFβ3 and HIF-1α seems to be a good indicator of integrity of placental function.

Glial Cells Missing (GCM) gene is the primary regulator of glial cell determination in the nervous system of *Drosophilia* (Chen, 2004). There are two isoforms of GCM genes, GCM1 and GCM2. GCM1 is selectively expressed in the placentas of human and mouse, and is a key transcription factor in placental development. Genetic ablation of mouse GCM1 is embryological lethal due to failure of the formation of the labyrinth layer and the fusion of trophoblasts to syncytiotrophoblasts (Anson-Cartwright, 2000). Decreased placental expression of GCM1 gene expression as well as its target genes, such as syncytin 1, has been observed in preeclampsia (Chen, 2008; Munaut, 2008). This gene induces trophoblast placental growth supporting its role in trophoblast differentiation and placental vascular function (Chen, 2004; Chang, 2008). There is no evidence that HIF-1α poses any direct effect in GCM1 expression. In summary, hypoxic conditions activate HIF-1α expression and upregulates TGFβ3 expression. This mechanism, together with a decrease in GCM1 expression inhibits throphoblastic differentiation, which is one of the putative causes of preeclampsia (Figure 4). Maternal endothelial dysfunction generated secondary to sFlt-1 overproduction can cause glomerular endotheliosis in the kidney leading to renal injury. As response to this injury genes related to ischemia/hypoxia can be activated.

Figure 4: Gene activation in response to placental hypoxia

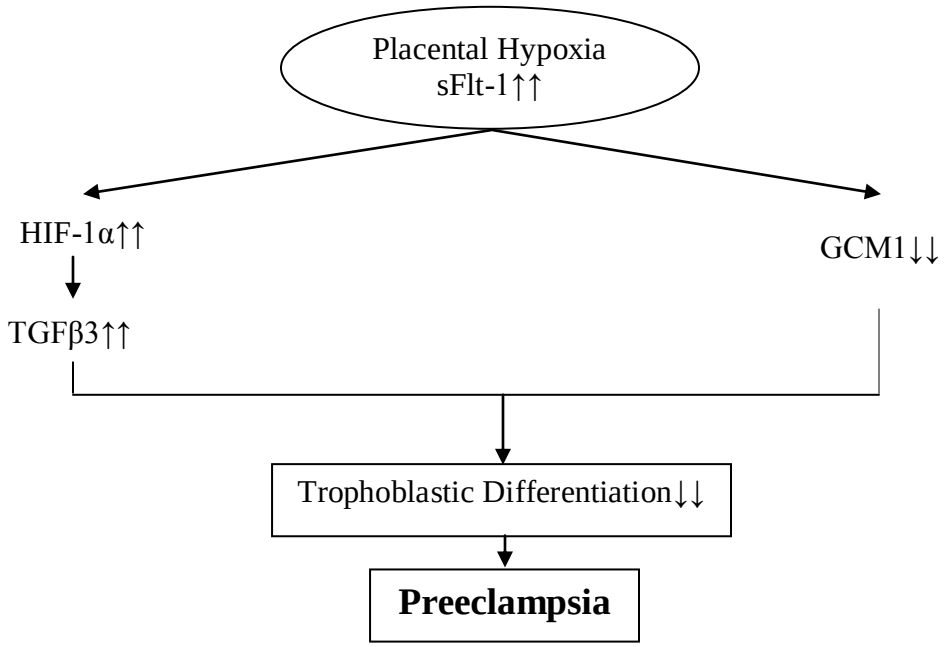


Figure 4 Activation of genes in response to placental hypoxia leading to impaired throphoblast function, a putative cause of preeclampsia

1.3 REGULATION OF VASCULAR FUNCTION AND TONE

1.3.1 Endothelial Function

The endothelium lines the blood vessels of the vascular tree. In humans, it is a pervasive layer which extension is about four times greater than the earth circumference (Wolinsky, 1980). Endothelial cell participates in several physiological processes including the control of the vasomotor tone, allowance of cells and nutrients passage, the maintenance of blood fluids, the regulation of permeability, and the formation of new blood vessels (Cines, 1998). The biology of the endothelial cell can be assumed as an adaptive input-output device. The input is originated from the extracellular milieu and may include biochemical or biomechanical signals. The output constitutes the cellular phenotype in which structural and functional properties are expressed (Aird, 2005). Some of these

properties are expressed at the individual cell level or cell culture including protein, mRNA, proliferation, apoptosis, migration, and permeability. Other properties are expressed at higher levels of organization such as the blood vessel or organism (Aird, 2005). Epigenetic and environmental factors influence the intrinsic properties in each endothelial cell. Epigenetic modification occurs during development and/or in the postnatal period.

The endothelium expresses a number of vasodilators and vasoconstrictors that regulate vascular tone. An increase in free cytoplasmic Ca^{2+} levels, which is achieved by various mechanisms including influx of extracellular Ca^{2+}, Na^{+}-Ca^{2+} exchange and liberation of Ca^{2+} from intracellular stores (e.g. sarcoplasmic reticulum) (Singer, 1982; Winquist, 1985, Luckhoff and Busse, 1986), triggers the release of endothelial relaxing factors in the endothelial cells (Furchgott, 1983). A number of factors such as nitric oxide (NO) (Palmer et al., 1987), prostacyclin (Moncada and Vane, 1979), and endothelium derived hyperpolarizing factor (EDHF) (Rubanyi and Vanhoutte, 1987) have been involved in the endothelium-dependent relaxation of the vasculature; while vasoconstrictors, namely endothelin-1, thromboxane A_2 and angiotensin II, have been identifies as being involving in the endothelium-dependent vasoconstriction (Hurairah H, 2004).

Early understanding of the role of the endothelium in disease comes from studies of inflammation. Studies in ear chamber model demonstrated that leukocytes adhere to the damaged site of a blood vessel, suggesting that the blood vessel is primarily responsible for mediating adhesion (Allison, 1955). The mechanisms underlying inflammation-induced leukocyte remained unknown for several decades. Pober et al identified the first inducible endothelial cell-specific leukocyte adhesion molecule (ELAM-1) (Pober, 1982). This group of investigators demonstrated in consequent years

that numerous inflammatory mediators, including endotoxin, TNF-α, and IL-1 induced the expression of antigens on the surface of human umbilical vein endothelial cell (HUVEC), an effect correlated with the expression of proadhesive, antigen-presenting, and procoagulant activities (Schleimer, 1986; Gamble, 1985). Endothelial dysfunction was first introduced by Gimbrone in 1980 to describe hyper-adhesiveness of the endothelium to platelets (Gimbrone, 1980). A paradoxical vasoconstriction of coronary arteries to acetylcholine has been demonstrated in early and advanced human atherosclerosis suggesting that abnormal vascular response to acetylcholine may represent a defect in endothelial vasodilator function (Ludmer, 1986). The term of endothelial dysfunction may be applied to states in which the endothelial phenotype poses a net liability to the host. It can manifest either by decreased secretion of vasodilatory mediators, increased production of vasoconstrictors, increased sensitivity to vasoconstrictors and/or resistance of vascular smooth muscle to endothelial vasodilators (Vapaatalo, 2001). Endothelial cell dysfunction is not restricted anatomically to the heart; instead arteries, capillaries, and veins of every tissue are prone to dysfunction.

Vascular adaptations during a normal pregnancy in both humans and animals include physiological changes such as increased blood volume, increased cardiac output, and decreased vascular resistance. The endothelium appears to govern some of these adaptations maintaining a low vascular tone by either an increased release of vasodilators or a decreased vasoconstrictor output (Poston, 1995). There is an increased production of the endogenous nitric oxide (NO) as a consequence of increased endothelial shear stress and hormones-related to pregnancy such as estrogen and gonadotropins (Wieczorek, 1995; Hayashi, 1997). There is a critical balance between endothelium-derived vasoconstrictors and vasodilators in normal pregnancies. In contrast, preeclampsia is

characterized by an imbalance of these factors predisposing the vasculature to vasoconstriction (VanWijk, 2000).

1.3.2 Nitric Oxide and Other Vasodilators in Pregnancy

NO acts as a central signal transduction pathway in the endothelium (Ignarro, 1999). NO regulates hemostasis of fibrinolysis, regulation of vascular tone, proliferation of vascular smooth muscle cells (VSMC), and blood pressure (BP) (Napoli, 2001; Rabelink, 2006). Disturbances in the NO pathway can cause endothelial dysfunction leading to vascular diseases such as atherosclerosis, hypercholesterolemia, hypertension, diabetes mellitus, and cerebrovascular disease (Napoli, 2001).

NO is produced by a family of NO synthase (NOS) enzymes, of which three isoforms have been identified in humans and other species: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3) (Forstermann, 1995; Wu, 1995). nNOS is the constitutive isoform mainly expressed in neural tissues, while iNOS becomes active in the presence of inflammation. In the vasculature, eNOS is the main isoform responsible for the production of NO (Wu, 1995). Similar enzymatic mechanisms accompanied the three isoforms in the electron transfer for oxidation of the terminal guanidine nitrogen of L-arginine (Bredt, 1990). These enzymes require several cofactors for proper function, including the tetrahydrobiopterin, nicotinamide-adenine-dinucleotide (NADPH), flavin adenine dinucleotide, and flavin mononucleotide. Disruption in the levels of these cofactors predisposes to endothelial dysfunction. After synthesis, NO diffuses from its original production site to the target cells where it binds and interacts with specific molecules containing iron in either a heme or iron-sulfur complex (Sato, 1995). NO increases cGMP levels by activating guanylate cyclase through heme moiety binding. In the vasculature, cGMP mediates NO-dependent

relaxation of VSMC, resulting in vasodilation via activating an intracellular molecular cascade which results in a reduction of intracellular Ca^{2+} concentration and an reduction of the sensitivity of the contractile system to the Ca^{2+} (Holzmann, 1982; Carvajal, 2000). Many agents produce the effect of vascular smooth muscle relaxation via NO-cGMP pathway such as acetylcholine (Pussard, 1995; Iranami, 1996), estrogens (White, 1995; Rosenfeld, 1996) and insulin (Trovati 1995 and 1996). NO also plays an important role in vascular remodeling in response to injury (Rudic, 1998; Ledingham, 2001).

The state of vasodilatation during pregnancy is partly mediated by nitric oxide which is important for the maintenance of adequate utero-placental perfusion (Langille, 1986; Yallampalli, 1993). Findings in pregnant guinea pigs confirm that NO is responsible for the physiological dilation of the uteroplacental arteries (Nanaev, 1995). Blockade of NO synthesis in animal models induces a preeclampsia-like syndrome (Molnar, 1994; Yallampalli, 1993). Therefore, it is thought that a decrease in NO could contribute to the pathogenesis of hypertension in preeclampsia and intrauterine growth restriction (Diket, 1994; Molnar, 1994; Sankaralingam, 2006). Increased oxygen free radicals can scavenge NO or increased plasma levels of S-nitrosoalbumin in preeclampsia can result in a decrease in the release of NO. The decreased release of NO also has been attributed to decreased vitamin C levels (Tyurin, 2001; Gandley, 2005). Other study has shown that elevation of the plasma dimethylarginine, an endogenous inhibitor of NO, in the second trimester leads to endothelial dysfunction, impaired uterine artery Doppler and the subsequent development of preeclampsia (Sawidou, 2003).

Prostacyclin (PGI_2) is an anti-platelet aggregator and a vasodilator produced from the metabolism of arachnoid acid by cyclooxygenases. Its biosynthesis is stimulated by numerous agonists that make free arachidonic acid available as a substrate for cyclooxygenase by increasing the activity of phospholipase enzymes. PGI_2 is mainly

biosynthesized in the endothelium of large blood vessels (Moncada and Vane, 1979) and in human glomeruli (Sraer, 1982), and may be increased locally in the uterine and renal circulations or in placenta during pregnancy possibly in response to increased circulating levels of angiotensin II (Magness, 1995; Glance, 1985). PGI_2 exerts its vasodilatory effects via the stimulation a G-protein-coupled, cell-surface receptor termed IP, which in turn activates the intracellular enzyme adenylyl cyclase, leading to an increase in intracellular cyclic AMP levels, cAMP then activates protein kinase A which decreases myosin light-chain kinase (MLCK) activity, hence inhibiting contraction and causing vasodilatation (Nakagawa, 1994; Hebert, 1998). In addition, a nuclear receptor, the peroxisomal proliferator-activated receptor α (PPARα) is also activated by PGI_2, and together with IP receptor activation may mediate the anti-platelet and vasodilatation effects of PGI_2 in the vasculatures (Marx, 2003; Vu-Dac, 1995). Cyclooxygenase is down-regulated and endothelial production of PGI_2 is altered in preeclampsia.

1.3.2 Role of Vasoconstrictors in Preeclampsia

Thromboxane A_2 (TxA_2) is a potent vasoconstrictor and platelet aggregator produced by platelets. It has a half-life of approximately 30 sec under physiological conditions. It increases three to five folds during pregnancy and remains elevated throughout (Fitzgerald, 1987a), which is consistent with evidence of the enhanced platelet activation associate with pregnancy (McKay, 1981). It diffuses into vascular smooth muscles where TxA_2 can stimulate endoperoxide/thromboxane receptors to induce an increase in cytosolic Ca^{++} and hence vasoconstriction (Mayeux, 1989; Morinelli, 1989; Smith, 1994).

One probable cause of preeclampsia is thought to be caused by underproduction of PGI_2 from the endothelium coupled with overproduction of TxA_2. Reduced PGI_2 production in preeclampsia is possibly due to chronic damage to the maternal vascular

endothelium (Roberts, 1989; Roberts and Redman, 1993), which is reflected indirectly by increased concentrations of serum fibronectin and factor VIII antigen/coagulant ratio. Reduced PGI_2 itself could contribute to over-production of TxA_2 because of insufficient PGI_2 anti-aggregatory activity (Fitzgerald, 1987b; Walsh, 1990). Increased TxA_2 production is reflected in increased excretion of its major urinary metabolites; also, it has been shown that placenta from preeclampsia biosynthesize more TxA_2 compared with healthy placenta (Fitzgerald, 1990; Walsh, 1985).

Endothelin-1 (ET-1) is produced by endothelial cells and induces vasoconstriction. ET-1 production through activation of angiotensin receptor-1 (AT_1R) appears to be increased in women with preeclampsia (Clark, 1992; Bernardi, 2008). ET-1 secretion is 4 –to 8- fold higher in umbilical cord endothelial cells of preeclampsia compared to normal pregnancy (Kourembana, 1993). In addition, elevated serum levels of ET-1 in women with preeclampsia returns to baseline values within 48 hours of delivery (Taylor, 1990). ET-1 interacts with endothelial cells receptors leading to vasoconstriction (Weigand, 2006), but also can induce vasodilation by releasing NO, PGI_2, and EDHF (Giardina, 2001). Because ET-1 causes a dual effect on the vascular tone, the role of ET-1 in the vascular dysfunction seen in preeclampsia remains to be elucidated.

Angiotensin II (AngII) mediates BP and electrolyte homeostasis. Activation of this protein results in vasoconstriction, vascular growth and inflammation (Wesselman, 2002). Angiotensinase activity, the enzyme that degradates AngII, is elevated in healthy pregnancies. In contrast, its activity is lower in women with preeclampsia leading to increased sensitivity and enhanced vascular response to AngII (Mizutani, 1996). AT_1R mediated the vasoconstrictor effects induced by AngII. Furthermore, AngII promotes vasoconstriction by increasing the production of ET-1 (Cingolani, 2006). Activation of

circulating IgG antibodies that prompts AT_1R production has been linked to the pathogenesis of preeclampsia and its associated vascular complications (Sheppard, 2010).

1.4 PREVENTION OF PREECLAMPSIA

Numerous clinical trials have examined the role of various therapies for the prevention of preeclampsia. The observation that preeclampsia is associated with increased platelet turnover and increased TxA_2 levels promoted investigators to design randomized controlled trials that examine the efficacy of low-dose aspirin in reducing the onset of preeclampsia in high-risk women for the disease (Benigni, 1989; Schiff, 1989; Wallenburg, 1986; Dekker, 1993). Low-dose aspirin as 60 to 150 mg diminishes platelet TxA_2 synthesis while maintaining vascular wall prostacyclin synthesis (Dekker, 1993; Patrono, 1994). This drug has been studied as primary and secondary preventive agent. Existing data indicate that low-dose aspirin as compared to placebo improves pregnancy outcomes and reduces in about 10% to 15% the risk for the disease in pregnant women at moderate to high risk for preeclampsia.

Calcium supplementation has been evaluated for prevention of hypertensive disorders associated with pregnancy. In a systematic review of 13 randomized trials including over 15,000 women, calcium supplementation appeared to reduce the risk for preeclampsia by almost 50% and also reduces the rate of preterm birth and maternal major morbidity and mortality (Hofmeyr, 2010). The effect was more marked in high-risk women. In contrast, no beneficial effects of calcium supplementation were demonstrated in the largest randomized trial involving women with a low baseline calcium intake (Villar, 2006). In agreement, the Calcium for Preeclampsia Prevention study in the US did not show reduction of the incidence of preeclampsia neither diminished the rate of adverse fetal outcomes in nulliparous women assigned to calcium supplementation as compared with those who received placebo (Levine, 1997). Based in these data, it seems

that calcium supplementation has a modest effect in the reduction of preeclampsia and its associated adverse pregnancy outcomes in women who are at high-risk for the disease.

Oxidative stress is one of the several mechanisms that have been proposed to cause the manifestations of the disease (Hubel, 1999). This concept is supported by studies showing oxidative modifications of proteins (Roggensak, 1999; Zusterzeel, 2001), lipids, (Hubel, 1996 and 1999; Zhang, 2008) and DNA (Wiktor, 2004) in the blood of women with preeclampsia and their infant. In addition, concentrations of antioxidants, such as ascorbate, seem to be reduced early in pregnancy in women destined to develop preeclampsia (Chappell, 2002). A randomized study of women considered at high risk for preeclampsia, supplementation with vitamins C and E was effective in reducing the occurrence of preeclampsia by 60% (Chappell, 1999). However, the results of this study were not replicated in consequent larger multicenter randomized studies involving women at high risk and women at low risk for the disorder (Villar, 2009; Spinnato, 2007; Rumbold, 2006; Poston, 2006). In the most recent multicenter randomized study involving nulliparous women who were at low risk for preeclampsia, supplementation of vitamins C and E as compared with placebo did not reduce the rate of adverse maternal or perinatal outcomes related to pregnancy-associated hypertension (Roberts, 2010).

Other investigators have proposed that fish oil supplements may have a variety of protective vascular effects, including reductions in systemic blood pressure and in the incidence of preeclampsia and pregnancy-induced hypertension (Secher, 1990; Sørensen, 1993). A clinical trial involving over three hundred pregnant women with a history of pregnancy-induced hypertension in a previous pregnancy and assigned to either fish oil or placebo did not have a significant reduction of hypertensive disorders. Consequent studies confirmed that the use of fish oil does not prevent the incidence of preeclampsia and other hypertensive disorders in pregnancy (Olsen, 2000; Villar, 2004).

1.4.1 VEGF for Prevention and Treatment of Preeclampsia

VEGF regulates important biological functions in the endothelial cell. This native protein promotes the formation of new vessels (angiogenesis) and maintains their integrity and function by activating endothelial cell survival or anti-apoptic signaling. VEGF also induces expression of the antiapoptic proteins (Bcl-2, A1) (Gerber, 1998), and mediates cell survival by activating tyrosine phosphorylation and focal adhesion kinase (FAK) (Abedi, 1998; Zachari, 1998). Furthermore, it stimulates VEGFR-2 mediated DNA synthesis and proliferation in endothelial cells. Another essential function is the induction of endothelial cell proliferation by activating signaling pathways including the extracellular signal-regulated kinases (ERKs) 1 and 2 and mitogen-activated protein (MAP) kinase pathways leading to activation of c-Jun N-terminal protein kinase (JNK). It is already known that VEGF stimulates endothelial cell production of NO and PGI_2, which are implicated in mediating diverse biological effects of VEGF including angiogenesis, increased vasopermeability, inhibition of neointimal vascular smooth cell hyperplasia, increased expression of thrombolytic pathways and vasorelaxation (Zachary, 2000).

At least five truncated forms of human VEGF mRNA have been described including isoforms of 121, 145, 165, 189 and 206 amino acid residues (Neufeld, 1996). VEGF-121 and VEGF-165 are characterized by absence of exon 6, whereas exon 7 is absent only in VEGF-121. Indeed, VEGF-121 and VEGF-165 are the only two freely diffusible isoforms, being biologically active in endothelial cells in the human vascular system. Thus, these two truncated forms have been detected in the majority of cells and tissues expressing the VEGF gene. These proangiogenic actions support the view that VEGF poses therapeutic benefits promoting angiogenesis and endothelium repair in several vascular conditions. VEGF-121 is one of the commonest isoforms that has been

studied as a therapeutic agent in vascular diseases given its biological actions and wide tissue distribution. Administration of VEGF-121 in an adenovirus vector into an ischemic myocardium of porcine heart resulted in significant improvement of both myocardial perfusion and function (Mack, 1998). Similarly, VEGF-121 therapy resulted in improvement of endothelial function and increase in lower-extremity flow reserve in patients with peripheral arterial disease (Rajagopalan, 2001). In a randomized, double blinded, placebo-controlled trial, intracoronary administration of recombinant human VEGF-121 to patients with stable exertional angina showed significant improvement of angina (Henry, 2003). Animal studies have also shown beneficial effects with the administration of VEGF-121. Thus, administration of recombinant human VEGF-121 to pregnant rats overexpressing sFlt-1 reduced arterial blood pressure and improved renal injury (Li, 2007). Our preliminary studies showed that a short course of VEGF-121 SC administered to pregnant mice in mid-gestation decreases temporally maternal blood pressure and reduces plasma levels of sFlt-1. Coronary artery disease, peripheral vascular disease, and preeclampsia are conditions that have in common insufficient angiogenesis and impaired vascular function. Based on extensive evidence and our preliminary data, we elucidated the role of VEGF-121 in ameliorating vascular endothelial dysfunction and improving maternal and fetal outcomes in pregnancies complicated by high circulating levels of sFlt-1 using a validated mouse model of preeclampsia.

1.5 FETAL VASCULAR PROGRAMMING

Cardiovascular disease (CVD) is a leading cause of major morbidity and mortality in the US and several other countries around the world. The American Heart Association estimated that 81,100,000 people in the US had one or more forms of CVD in 2006, and in the same year 34% of all deaths were attributed to it. The etiology, prevention, and treatment of CVD are very complex and are under intense investigation. Hereditary

factors and lifestyle habits are important contributing elements, but alone don't account for all cases. It has been long known that intrauterine and postnatal environments can significantly influence subsequent health in adult life. In 1986, Barker proposed the "fetal origins of adult diseases" based on the observed association between low birth weight and cardiovascular disease, hypertension and diabetes later in life (Barker, 1986). Fetal programming is then defined as a process in which the fetus adapts in order to survive stimuli or insults occurring during a critical period of fetal development. The resulting fetal adaptations may have permanent specific short and long term effects on the development of various organ systems that include the cardiovascular and metabolic systems (Barker, 1986; Barker, 1989). Although genetic factors, including the maternal and paternal genes as well as the phenomenon of genomic imprinting that influence the fetal phenotype and contribute to the occurrence of fetal origin of adult disease via the intrauterine environment, these stimuli or insults are usually maternal factors which affect the fetus via the altered the intrauterine environment. Maternal hypertension, either chronic or pregnancy-related, leads to inadequate vascular adaptations during pregnancy, alteration in the maternal circulation at the utero-placental interface with consequently poor perfusion of the placental-fetal unit (Rotmensch, 1994; Henriksen, 2002). These mal-adaptations lead to fetal hypoxemia and reduction in fetal perfusion, which can convey long lasting physiological and structural alterations that predispose the fetus to diseases in later life (Henriksen, 2002).

In the past few years, significant research effort has been directed towards unraveling the underlying mechanisms of the fetal origin of adult disease. Experimental methods used to produce an unfavorable uterine environment and induce fetal growth restriction have included dietary, pharmacological, and surgical manipulations (Benediktsson, 1993; Block, 1989 and 1990; Murotsuki, 1997; Langley-Evans, 1999).

Protein restriction in rats during pregnancy, such as low protein diet supplemented with methionine, has been found to result in hypertension in the offspring later in life (Petrie, 2002). Reduction of the uterine perfusion by placing a silver clip around the aorta below the renal arteries during mid-late gestation in pregnant rats caused low-birth weight offspring predisposed to development of hypertension (Alexander, 2003). Another example is the transgenic animal model used by Longo et al (Longo, 2005) in which female eNOS knockout mice ($NOS3^{-/-KO}$) were bred with wild-type mice ($NOS3^{+/+WT}$) to produce maternally-derived heterozygous ($NOS3^{+/-mat}$) and paternally-derived heterozygous ($NOS3^{+/-pat}$) litters that are genomically similar. Impaired vascular function has been seen in the $NOS3^{+/-mat}$ offspring that developed in a NOS3 deficient maternal/uterine environment compared with the normal vascular function seen in $NOS3^{+/-pat}$ offspring born to wild-type mothers. Embryo transfer experiments confirmed the predominance of the uterine environment over the genetic background in determining fetal programming (Longo, 2004). In our validated animal model of preeclampsia-like condition induced by over-expression of sFlt-1, birth weight was significantly lower in the pups born to mothers treated with sFlt-1 compared with the controls (Lu, 2007). The adult male offspring at 6 months of age from sFlt-1 treated mothers compared with controls had significantly higher arterial blood pressure (Lu, 2007). These studies provide direct evidence in support of the role of the uterine environment in determining the risk of CVD disease later in life.

1.6 RESEARCH PROJECT OBJECTIVES

Given the strong amount of evidence indicating the role of sFlt-1 in the pathogenesis of preeclampsia, the next step is to focus on reversing the angiogenic imbalance leading to preeclampsia. Antihypertensives are frequently used in preeclamptic patients; however, medications like clonidine, diazoxide, and hydralazine do not inhibit

sFlt-1 production in placental explant cultures (Xu, 2009). Furthermore, as today none of the known antihypertensive medications have been shown to prevent preeclampsia. Other therapies tested to prevent preeclampsia have provided disappointing results. New approaches for prevention are therefore needed. VEGF-121 has proved to be therapeutically effective in vascular conditions characterized by angiogenesis deficiency, the landmark of preeclampsia. We hypothesized that administration of VEGF-121 would reverse the preeclampsia-like syndrome induced by overexpression of sFlt-1 in our validated mouse model. Improving maternal vascular function and placental perfusion will diminish the adverse intrauterine environment caused by sFlt-1 and thereby prevent altered fetal vascular programming and long lasting effects in the adult life.

Our overall hypothesis is that VEGF-121 administration ameliorates abnormal utero-placental adaptation and endothelial dysfunction caused by imbalance of angiogenic factors which leads to preeclampsia and altered fetal vascular programming. As a step toward the overall goal, we propose to test the hypothesis that continuous infusion of VEGF-121, a soluble isoform of VEGF, inhibits circulating sFlt-1, repairs abnormal angiogenesis in the placenta, and improves fetal vascular programming in pregnant mice that exhibit manifestations of preeclampsia-like condition due to overexpression of sFlt-1. This general hypothesis was tested in a mouse model by examining the following specific hypotheses:

Specific Hypothesis 1: VEGF-121 therapy normalizes maternal blood pressure and prevents from hypertension in pregnant mice that over-express sFlt-1.

Specific Hypothesis 2: Inhibition of elevated levels of sFlt-1 during pregnancy by recombinant VEGF-121 improves maternal vascular endothelial dysfunction and decreases structural changes in target organs such as maternal kidney and placenta.

Specific Hypothesis 3: VEGF-121 decreases hypoxic changes and thereby regulates the expression of hypoxia-related genes in the placenta and kidney of pregnancies with elevated circulating levels of sFlt-1.

Specific Hypothesis 4: VEGF-121 therapy reduces altered fetal vascular programming caused by excess circulating sFlt-1 and by that mechanism diminishes the risk for vascular disease in the offspring later in life.

The following aims are proposed to test the corresponding hypotheses:

Specific Aim 1: To determine the effect of VEGF-121 on maternal blood pressure in pregnancies transfected with adenovirus carrying sFlt-1.

Specific Aim 2: To evaluate vascular function of the carotid artery by vascular reactivity studies and examine histology characteristics in the kidney and the placenta of pregnant mice transfected with adenovirus carrying sFlt-1 that were treated with VEGF-121 or placebo.

Specific Aim 3: To compare mRNA expression level of the hypoxic induced factors in pregnant mice over-expressing sFlt-1 and treated with VEGF-121 or placebo.

Specific Aim 4: To evaluate fetal vascular programming by determining blood pressure and in vitro vascular function in the carotid artery of adult offspring born to pregnant mice over-expressing sFlt-1 that were treated with VEGF-121 or placebo.

1.7 SIGNIFICANCE

The dramatic rise in the prevalence of risk factors for preeclampsia such as obesity, diabetes and chronic hypertension in women attempting pregnancies in the United States and worldwide has resulted in a progressive increase in the incidence of this disease. In turn, this phenomenon increases the occurrence of major maternal and perinatal morbidity and mortality associated with preeclampsia. Recent evidence suggests that preeclampsia also increases the risk for adverse events such as cardiovascular disease, diabetes, metabolic syndrome and cerebrovascular disease in both the mother and the fetus later in life. The clinical consequences summed to the elevated health costs generated for this disease are major public health concerns. As today there is not effective prevention or treatment for this maternal disease. Altered angiogenesis during placental formation is considered as one of the most plausible mechanisms that lead to preeclampsia. Data from both clinical and animal studies support this theory. Therefore, this study proposes a novel approach that aims to improve maternal vascular function, placental perfusion, and optimize intrauterine environment in a preeclampsia model induced by the anti-angiogenic sFlt-1 factor. The findings of this study will provide a new scientific avenue, and will enhance our understanding of this condition.

CHAPTER 2: MATERIAL AND METHODS

ANIMAL CARE

Pregnant CD-1 mice were purchased from Charles River Laboratories (Wilmington, MA). The animals were maintained in our animal care facility at the University of Texas Medical Branch. All performed procedures were approved by the Animal Care and Use Committee (IACUC) of our university. The animals were housed separately in temperature- and humidity-controlled quarters with constant light: dark cycles of 12 h: 12 h. They were provided with food and water ad libitum. Surgical procedures were performed following the IACUC guidelines. Anesthesia protocol consisted of ketamine (Ketalar; Parke-Davis, Morris Plains, NJ) and xylazine (Gemini; Rugby, Rockville Center, NY). The animals were sacrificed by CO2 inhalation according to the guidelines of the American Veterinary Medical Association.

AMPLIFICATION OF VIRUS VECTORS

Ad-Flt-1 (1-3) (first generation, E1 and E3 deleted) as the active vector, and Ad-mFc as the adenovirus control were prepared and tittered by the Research Vector Core, Harvard Medical School. The 293 cell line was used to grow and transfect the virus. The cells were cultured in 150 mm plates and seeded for 3 days. Cells reaching 70%-80% of confluence were considered ready for transfection. Then, the transfection medium containing the virus (2×10^7 PFU; transfection medium: 1% P/S and 2% FBS) was added to each plate. Approximately 20 hours later, almost 50% of the cells have started to detach from the plates showing the typical cytopathic effect. The cells were collected and spun at 2,000 rpm for 15 minutes at 4^oC. The supernatant was removed and the cells were re-suspended in 10 ml cold PBS again and centrifuged at 2,000rpm/4^oC/15min. The supernatant was removed and the cells were stored at -80^oC for purification. For the

purification process, 2mL of sterile 10mM Tris (pH=8) was added to the cell pellet which was then placed in a water bath to thaw, then vortexed briefly, and finally placed on the dry slurry to be frozen. The cycle was repeated 6 times. The cells were lysed after spinning them at 2,000rpm/15min/4^{o}C and the supernatant was collected. The supernatant containing virus was added into a CsCl step gradient (d=1.43 and d=1.34). This preparation was centrifuged at 30,000 rpm for 3 hours at 4^{o}C in a SW40 rotor. The adenovirus band was apparent at this point and easily collected using an 18 gauge needle and 5mL syringe, and added to a tube containing 1.34g/ml CsCl. The sample was again centrifuged at 35,000 rpm for 16 hours at 4^{o}C in a VTi90 rotor. The adenovirus band, which was now more evident, was collected in a clean dialysis tube containing the dialysis buffer to isolate the virus. The concentration of adenovirus particles was determined by spectrophotometric analysis of an appropriate dilution of the test sample (typically 1/10) in a solution of 10mM Tris/1mM EDTA/0.1% SDS.

RECOMBINANT VEGF-121

VEGF-121 was produced in E. coli. It is a polypeptide of 121 amino acids with a molecular mass of 28423 Dalton. The purity was > 98% using SDS-Page analysis. The lyophilized product was obtained from the biology vascular laboratory at Harvard Medical School Boston, Massachusetts, US. The solution concentration was 1.7 mg per ml and it was stored in working aliquots at -20 ºC.

VEGF-121 DOSAGE

Previous studies have demonstrated that VEGF-121 has a short half life. A study in non-pregnant rats showed that peak plasma concentrations were achieved 100 minutes after 100 µg of VEGF-121 subcutaneous (SC) (Suga, 2001). In non-pregnant rats, the time required to attain maximum VEGF-121 plasma concentrations is 2 hours after 400

µg/kg SC injection (Li, 2007). Angiogenesis can be achieved with VEGF-121 50 µg SC daily, while higher dosages are required (200-400 µg/kg daily) for reduction of BP (Suga, 2001; Li, 2007). In our previous studies, we found that VEGF-121 100 µg/kg SC twice a day decreased significantly maternal BP. The effect, however, was transitory. Based on previous reports and our experience we selected VEGF-121 dosage of 400 µg/kg daily from day 8 to day 18 of gestation. We used an osmotic pump instead of scheduled injection for medication delivery as the former system rapidly achieves a steady-state delivery rate and maintains a constant rate throughout the duration of treatment (Theeuwes, 1976). Other advantages of this system are elimination of the need for nighttime or weekend dosing, reduction of handling and stress to animals, and it minimizes unwanted experimental variables and ensures reproducible and consistent results.

INSERTION OF OSMOTIC MINIPUMP AND DOSING CALCULATION

We used the osmotic pump model 2002, manufactured by ALZET ®, Cupertino, CA. This pump has a length of 3 cm, a diameter of 0.7 cm, and weighs 1.1 grams (Figure 5). It delivers the dispended medication at 0.5 µl/hr at a maximum duration of 2 weeks. The pump functions in basis of an osmotic pressure difference system. The high osmolality of the pump's sleeve causes water influx through a semipermeable membrane. This effect results in compression of the flexible reservoir, which displaces the dispended medication from the pump at a steady rate. The rate of delivery by the pump is controlled by the water permeability of the pump's outer membrane. To calculate the amount of dispended medication, we used the formula provided by the manufacturer (ALZET ®, Cupertino, CA).

Sterile phosphate buffered saline (PBS) was the selected solvent as it is compatible with both the pump and VEGF-121. The osmotic pump was implanted SC at day 8 of gestation. Mice were anesthetized using the aforementioned protocol. We used the same skin incision made for the insertion of the BP transducer to create a SC space in the lower dorsal aspect of the animal. The pump was placed in this space and the incision was closed using 2-0 silk (Figures 5 and 6).

Figure 5: In vivo photograph of osmotic pump

Figure 6: Osmotic pump in the SC space

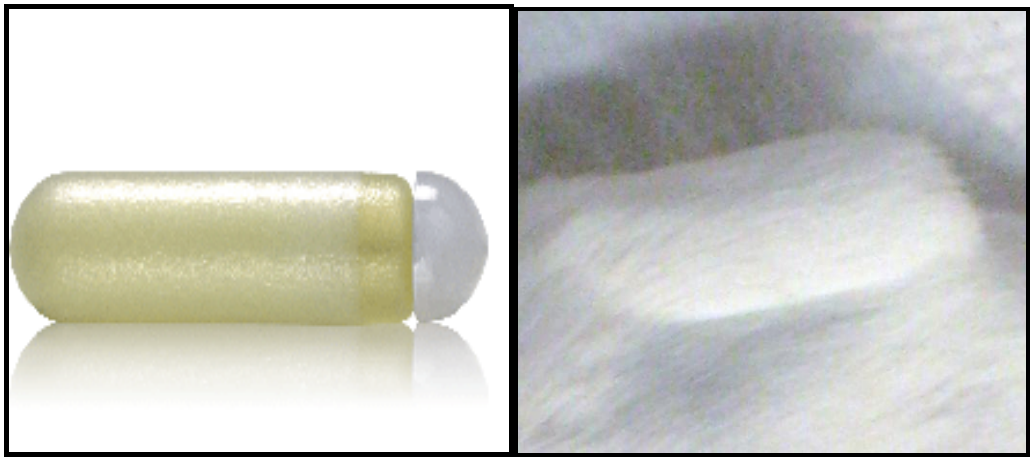


Figure 5 *Left:* in-vivo photograph of the osmotic minipump model 2002 (ALZET ®, Cupertino, CA) used to deliver VEGF-121 or placebo. Figure 6 *Right:* in-vivo photograph of the minipump implanted in the SC tissue of the mice's lower back at day 8 of gestation.

IN VIVO BLOOD PRESSURE MEASUREMENT

A telemetric method was used for an in vivo BP recording. This system requires the insertion of a 0.4 mm catheter into the carotid artery and tunneled into aortic arch. In this system (PA-C20, Data Sciences International, St. Paul, MN), the catheter was connected to a combination pressure transducer, transmitter and battery, all encased in a miniature capsule. The capsule transmits to a pad under the cage which can be turned on

and off magnetically. The system allows arterial BP monitoring in unrestrained conscious mice.

The protocol for BP measurement was as follows. Mice were anesthetized following the same protocol described for the insertion of the osmotic minipump. A vertical midline skin incision was made along the neck and the left common carotid artery was isolated. The catheter was inserted into the carotid artery through a small incision in the vessel wall and tunneled to the aortic arch (Figure 7). The transducer (PA-C20 model, Data Sciences, St. Paul, MN) was secured in a subcutaneous pouch along the animal's right flank through the same ventral neck incision. The incision was closed using 6–0 silk suture. Mice were kept warm on a heating pad and monitored closely for several hours until full recovery from anesthesia and proper mobilization were observed.

BP data were transmitted to RLA 1020 telemetry receivers (Data Sciences), multiplexed by BCM consolidation matrix (Data Sciences), and sent to an adapter, where the signal was de-multiplexed, sampled at 1024 Hz, and converted to analog output (UA-10 universal adapter, Data Sciences). This output was subsequently band-pass filtered and amplified and the information fed to a recording system (MacLab 16/s, AD Instruments, Castle Hill, Australia).

POWER ANALYSIS

Our overall hypothesis was that continuous VEGF treatment would reduce BP in pregnant mice overexpressing sFlt-1. Along with this effect, we hypothesized that VEGF therapy improves maternal vascular function and altered fetal vascular programming. We expected that VEGF-sFlt-1 pregnant mice as compared with sFlt-1 animals will have a 20% reduction in the mean BP with a standard deviation of 10 mm Hg. Using an alpha level of 0.05 and a beta level of 80%, we calculated a simple size of 4 animals for each group. All analyses were performed using SAS version 9.2 (Cary, NC).

Figure 7: In vivo photograph of insertion of blood pressure catheter

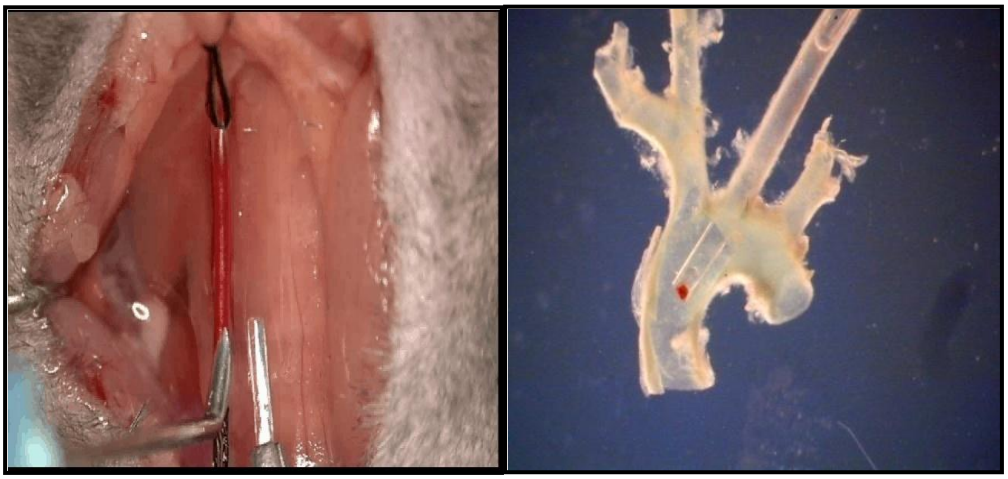


Figure 7 *Left:* in-vivo photograph demonstrating the exposure of the left common carotid artery which has been ligated proximally and distally to the incision site. *Right:* ex-vivo aortic arch and main arteries showing the catheter insertion through the left carotid artery and placement of the catheter tip within the lumen of the aortic arch. (Pictures courtesy of Esther H. Tamayo, University of Texas Medical Branch, Galveston, Texas).

Data Analysis

Systolic, mean, and diastolic BP was evaluated in pregnant mice and their offspring when reached adult age (N = 4/6 per group). Dataquest A.R.T.3.1 was used for data acquisition and analysis. Mean BP in intervals of 12 hours was calculated in animal. Data were analyzed using repeated measures mixed models. BP mean differences among treatment groups. BP recorded each day across the treatment groups was analyzed using one-way ANOVA test. Results were adjusted with the post hoc Tukey test. Multiple comparison data are presented by difference between means and 95% confidence intervals (95% CIs). Adjusted p values are reported as the significant level among the treatment groups.

ELISA ASSAY FOR MEASUREMENT OF PLASMA SFLT-1 AND VEGF LEVELS

ELISA for sFlt-1 and free VEGF mouse plasma concentrations were performed according to the manufacturer's instructions (R&D systems, Minneapolis, MN). The samples were diluted 2-fold by Calibrator Diluent RD5-3 and incubated in a 96-well plate pre-coated with a capture antibody directed against sFlt-1 and VEGF for 2 hours at room temperature. The wells were washed and incubated with a secondary antibody against sFlt-1 and VEGF conjugated to horseradish peroxidase for 2 hours. Then, the plates were washed and the substrate solution was added to each well and incubated for additional 30 minutes at room temperature. The plate was read at an optical density of 450nm. All assays were run in duplicate, and the protein levels were calculated using a standard curve derived from the known concentrations of the respective recombinant proteins.

Data Analysis

Data were calculated using a standard curve derived from known concentrations of the recombinant protein and showed as mean ± SEM. Data were analyzed by one-way ANOVA test and results were adjusted using Tukey test. Multiple comparison data are presented by difference between means and 95% confidence intervals (95% CIs). Adjusted p values are reported as appropriate.

IN VITRO VASCULAR STUDIES

Pregnant CD1 mice treated with adenovirus carrying sFlt-1-PBS, VEGF-sFlt-1 or VEGF- mFc were used for these studies (N=4/6 per group). The carotid artery was isolated preserving the endothelium layer. The artery was removed just proximal to their bifurcation into internal and external carotid artery. The vessel was immersed in physiological salt solution. Two-millimeter segments of the vessel were mounted between the two jaws of a wire myograph (Model 410A, J.P. Trading I/S, Aarhus,

Denmark) using 25 μm tungsten wires. One jaw in these myographs is attached to a micromanipulator for adjustment of tension in the vessel while the other jaw is fixed to an isometric force transducer (Figure 8). The preparations were bathed in physiological salt solution maintained at a temperature of 37°C and a pH of 7.4. A mixture of 95% O_2 and 5% CO_2 was bubbled continuously through the solution. The force was continuously recorded by an isometric force transducer and analyzed using computerized software: Power Lab system and Chart 5 data acquisition and playback software (AD Instruments, Castle Hill, Australia).

Drugs and Solutions

The drugs used in the in-vitro experiments were acetylcholine hydrochloride (Ach), phenylephrine hydrochloride (PE), and sodium nitroprusside (SNP) and L-NAME (Sigma, St. Louis, MO). TxA_2 was purchased from another company (Cayman chemical, Ann Arbor, MI). Stock solutions of the drugs (10^{-2} mol/L) were prepared in deionized water and stored at -20°C (Table 1). The composition of physiological salt solution was: NaCl 115 mmol/L, KCl 5 mmol/L, NaH_2PO_4 1.2 mmol/L, $NaHCO_3$ 25 mmol/L, $MgCl_2$ 1.2 mmol/L, $CaCl_2$ 2.5 mmol/L, ethylene diamine tetra acetic acid (EDTA) 0.026 mmol/L, glucose 11 mmol/L.

Vascular Reactivity Protocol

The vessels were allowed to stabilize for one hour. After stabilization of the tone, and in order to enhance reproducibility of responses, the vessels were given two successive stimulations of 10 min duration with 60 mM KCl separated by 30 min equilibration. The second response to KCl was used as the reference contraction in the final calculations. The presence or absence of endothelium in the preparations was

confirmed by contracting with α_1-adrenergic agonist phenylephrine PE (10^{-6} M) and testing for relaxation with endothelium-dependent vasodilator ACh (10^{-6} M).

After equilibration in physiological salt solution, contractile responses to PE (10^{-10}-10^{-5} mol/L) and TxA_2 were assessed. In addition, vessels were pre-contracted with PE (10^{-7}-10^{-6} M) to produce matching contractions in the different study groups and relaxant responses to cumulative concentrations of ACh and SNP were assessed.

Figure 8 Diagrham of the myograph used for the experiments

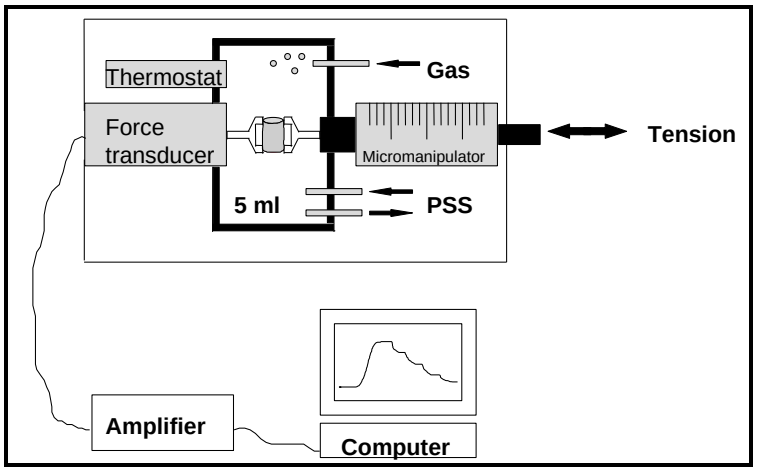


Figure 8: Methods: in vitro contractility of carotid/uterine artery. Vascular segments of the arteries were mounted in a small vessel myograph in 5 ml chambers filled with PSS and bubbled with gas mixture (95% O_2 and 5% CO_2). The chambers were maintained at 37°C by a thermostat. The preparations were mounted between two jaws of the myograph with 25 µm tungsten wires. One jaw was fixed with a micromanipulator which allowed change in the distance between the wires and hence the tension in the vessel while the other was connected to the force transducer. The signal was amplified and recorded with a data acquisition system.

Data Analysis

Response to KCl was used as a reference to calculate the percent of contraction achieved by PE and TxA_2. Responses to ACh and SNP were expressed as

percentage relaxation of the PE-induced precontraction. The area under the concentration response curves (AUC) and the maximal effect (Emax) were calculated. Data were analyzed by one-way ANOVA test and results were adjusted using Tukey test. Multiple comparison data are presented by difference between means and 95% confidence intervals (95% CIs). Adjusted p values are reported as appropriate.

Table 1: Compounds used to investigate selected pathways involved in the regulation of vascular function

Agents used in dose-response curves			
Compound	*Type*	*Action*	*Dose*
PE	α_1-adrenergic agonist	smooth muscle contraction	10^{-10}-10^{-5} M
Ach	Muscarinic-receptor agonist	endothelium-dependent-vasorelaxation	10^{-10}-10^{-5} M
TxA_2	anti-platelet aggregation	smooth muscle contraction	10^{-10}-10^{-5} M
SNP	smooth muscle relaxant	Endothelium-independent vasorelaxation	10^{-10}-10^{-5} M
L-NAME	NO antagonist	Nonselective NOS inhibitor	10^{-4} M

HISTOLOGICAL EXAMINATION

Kidney and liver tissues were fixed in 10% phosphate-buffered formalin (pH 7.0) and embedded in paraffin. Paraffin sections (4–6 μm) were obtained and mounted on super frost-plus slides (Allegience Health Care, IL) and melted at 58°C in an oven for 1 h. The slides were then de-paraffinized with xylene and re-hydrated using a decreasing ethanol series of baths. While the sections are in water, the surface was skimmed with a Kimwipe tip to remove oxidized particles and blot excess water from slide holder. The

staining was performed using hematoxilin & eosin which is ideal for evaluating tissue and vascular morphology and structure.

GENE EXPRESSION STUDIES

Gene expression in the placenta and kidney from the various groups was analyzed using real time RT-PCR analysis. Total RNA was isolated using RNAqueous- small scale phenol-free total RNA isolation kit (Ambion, Co). 10μg of RNA for target genes and endogenous control was used. RNA concentrations were measured by ultraviolet absorbance at 260 nm, using a spectrophotometer (DU-64, Beckman Instruments, Palo Alto, CA). RT-PCR involves the use of specifically-designed primers (forward and reverse) and a probe that binds the gene of interest. During the reverse transcription (RT) step, the reverse primer synthesizes the cDNA. Then in the PCR cycles, the forward primer and the probe anneal to the cDNA. During primer extension, the Taq DNA polymerase, that has a fork-like structure-dependent 5' nuclease activity, cuts the TaqMan probe. The probe contains a reporter dye (FAM for the gene-specific probes, and VIC for the 18s rRNA probe) at its 5'-end and a quencher dye (MGB) at its 3'-end. When the two dyes are close to each other, the quencher dye quenches the signal of the reporter dye. However, the DNA polymerase action and the resulting strand cutting separate the reporter dye from the quencher dye resulting in the release of fluorescence from the reporter. Additional reporter dye molecules are cleaved from their respective probes with each cycle, resulting in an increase in fluorescence intensity proportional to the amount of amplicon produced. This technique was used to evaluate mRNA expression level of HIF1α and TGFβ3 and GCM1.

Probes and Primes

Specific primers and probes for the genes of interest (HIF-1α, TGFβ3 and GCM1) and for the 18s rRNA (internal control) were obtained from Applied Biosystems (Foster City, CA) (Table 2). The Assays-by-Design 20× assay mix contained the specific primers as well as the TaqMan MGB probes. The probes 5'-end FAM^{TM} dye-labeled for the target genes and 5'-end VIC^{TM} dye-labeled for the for18S rRNA and 3'-end MGB^{TM} dye-labeled. These assays were designed to span exon-exon junctions so as not to detect genomic DNA.

Table 2: Probe used for the Assays-on-DemandTM

HIF1α	NM_010431.1	Mm00468869_m1
TGFβ3	NM_009368.2	Mm00436960_m1
GCM1	NM_008103.3	Mm00492310_m1

Two-step Real-Time RT-PCR Reactions

The reactions were conducted with 1μg RNA for both target genes and endogenous control (18s rRNA). The cycling parameters for reverse transcription (Perkin-Elmer, Applied Biosystems, CA, 94404), 25 ^{o}C for 10 min, extension at 37 ^{o}C for 120 min and inhibition at 85 ^{o}C for 5 sec, keep at 4 ^{o}C after finish. The cycling parameters for real-time PCR were: stage 1 included one step for initial denaturation at 95 ^{o}C for 20 sec; stage 2 included two steps, step one for denaturation at 95 ^{o}C for 3 sec and step two annealing/extension 60 ^{o}C for 30 sec (repeated 40 times) on 7500 Fast Real Time PCR (Applied Biosystems)

Duplicate of the threshold cycle or C_t values were used and analyzed in Microsoft Excel using the comparative C_T ($\Delta\Delta C_T$) method as described by the manufacturer (Applied Biosystems).

Data Analysis

The C_t was determined for each sample; The results were expressed as mean $\pm$ SEM of HIF1α, TGFβ3 and GCM1 mRNA expression relative to a reference sample (one wild-type control sample) and normalized to 18s rRNA (internal control). The comparative C_t ($\Delta\Delta C_T$) was used for data analysis. Data were analyzed by one-way ANOVA test and results were adjusted using Tukey test. Multiple comparison data are presented by difference between means and 95% confidence intervals (95% CIs). Adjusted p values are reported as appropriate.

CHAPTER 3: RESULTS

SPECIFIC HYPOTHESIS 1:

VEGF-121 therapy decreases maternal blood pressure and prevents hypertension development in pregnant mice that over-express sFlt-1.

SPECIFIC AIM 1:

To determine the effect of VEGF-121 on maternal blood pressure and examine its ability to inhibit circulating sFlt-1 in pregnancies transfected with adenovirus carrying sFlt-1.

INTRODUCTION

Clinical studies support the role of sFlt-1 in the pathogenesis of preeclampsia. This factor is significantly elevated in maternal circulation weeks prior to the onset of the clinical disease (Levine, 2004). Indeed, the magnitude of the sFlt-1 rise is associated with the severity of preeclampsia and the presence of intrauterine growth restriction (Chaiworapongsa, 2004). Replicating what is seen in pregnancies complicated with preeclampsia, induction of sFlt-1 overexpression in pregnant rodents produces a preeclampsia-like syndrome (Maynard, 2003, Lu, 2007, Li, 2007). In our validated mouse model of preeclampsia, pregnant mice transfected with adenovirus carrying sFlt-1 in the mid gestation leads to significant elevation of sFl-t1 plasma concentrations later in pregnancy (Lu, 2007). This effect is associated with maternal vascular dysfunction and hypertension in late pregnancy, as well as low weight of term pups, which suggests intrauterine growth restriction (Lu, 2007). Our model is unique as BP is recording longitudinally in unrestricted animals and that minimizes the effect of potential confounding factors related to animal handling and manipulation.

Overwhelming evidence supports that imbalance of angiogenic factors is a prominent pathologic process in women destined to develop preeclampsia. Therefore, sFl-t1 turns into an attractive target to treat this disease. Inhibition of sFlt-1 would probably ameliorate maternal endothelial damage, increase placental perfusion and improve fetal outcomes. We propose a novel therapy that aims to antagonize the adverse effects caused by sFlt-1. Biologically, VEGF-121 has the capacity to bind sFlt-1 and enhances proangiogenic actions including angiogenesis, vascular permeability and vasodilation. VEGF-121 has shown to be an effective therapy of several human vascular diseases characterized by impaired angiogenesis and vascular dysfunction (Rajagopalan, 2001; Henry, 2003). The use of this truncated form of native VEGF has not been associated with significant adverse effects. Our preliminary results showed that a short course of VEGF-121 in pregnant mice overexpressing sFlt-1 leads to reduction of BP. We hypothesize that continuous infusion of VEGF-121 inhibits circulating sFlt-1 and normalizes maternal BP of unrestrained pregnant mice.

SPECIFIC AIM 1: STUDY DESIGN

Anesthetized pregnant CD-1 mice at day 8 of gestation underwent insertion of blood pressure catheters and subcutaneous implantation of osmotic mini pumps (N = 4/6 per group). The pumps were randomly filled with either VEGF-121 to deliver 400 µg/kg per day or 200 µL of PBS) used as vehicle. On day 9 of gestation, animals treated with VEGF-121 were divided randomly into two groups and injected with either Adv-sFlt-1, 10^9 PFU in 100 µL or adenovirus carrying the murine immunoglobulin G2α Fc fragment, Adv-mFc, 10^9 PFU in 100 µL used as a control for the virus. Animals allocated to PBS treatment were injected in the tail vein with Adv-sFlt-1, 10^9 PFU in 100 µL also on day 9 of gestation. BP was measured continuously from day 8 to day 18 of gestation. SBP, MBP, and DBP were averaged over 12 hour intervals. Maternal blood (100 µL) was

collected on days 8, 14 and 18 of gestation for measurement of sFlt-1 and free-VEGF plasma concentrations using enzyme-linked immunosorbent immunoassay (ELISA). Animals were sacrificed at day 18 of gestation, and pups and placentas were counted and weighed.

SPECIFIC AIM 1: RESULTS

Plasma sFlt-1 and free-VEGF levels

sFlt-1 plasma levels were low and similar in the mice at day 8 of gestation prior to be randomly allocated to PBS-sFlt-1, VEGF-sFlt-1, or VEGF-mFc treatments. Significant increase in the sFlt-1 plasma levels was noted in the mice treated with PBS-sFlt-1 as compared to the levels in the animals assigned to VEGF-sFlt-1 and VEGF-mFc. Difference between means was -3.50 (95% CI, -6.26, -0.724) and -8.75 (95% CI; -11.284, -6.216), respectively. sFlt-1 levels were also significantly higher in the VEGF-sFlt-1 mice than in the VEGF-mFc mice at day 14 (difference between means: -5.25 (95% CI; -7.78, -2.72)). A similar pattern remained across the groups at day 18 of gestation, except that sFlt-1 levels were not significantly higher in the VEGF-sFlt-1 than in the VEGF-mFc mice. Figure 9 illustrates the mean plasma sFlt-1 concentrations across the groups during three points in gestation.

Equivalent to sFlt-1 levels, plasma levels of free VEGF at day 8 of gestation were low and similar across the groups. On day 14, free VEGF plasma levels were significantly higher in the VEGF-mFc group as compared with the PBS-sFlt-1 mice (difference between means: -6.67 (95% CI; -12.23, -1.10)). VEGF-sFlt-1 mice VEGF concentrations did not vary significantly respecting to the PBS-sFlt-1 and the VEGF-mFc groups. On day 18 of gestation, the free VEGF plasma levels dropped in all mice groups. The levels remained significantly higher in the VEGF-mFc than in the PBS-sFl-t1 mice,

but also at this gestational age free VEGF concentrations became to be considerably higher in the VEGF-sFl-t-1 than in the PBS-sFl-t1 animals (Figure 10).

Figure 9: Plasma concentrations of sFlt-1

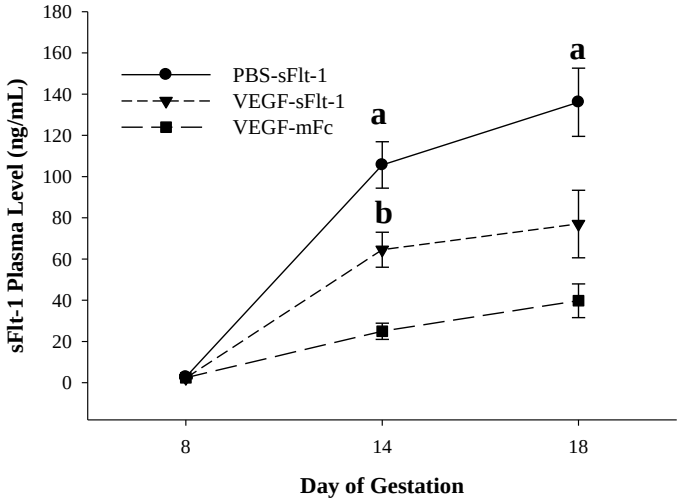


Figure 9 Plasma levels of sFlt-1 at different times in gestation. Data are shown as mean ± SEM. a denoted an adjusted probability level < 0.05 for PBS-sFlt-1 versus VEGF-sFlt-1 and VEGF-mFc. b denoted an adjusted probability level < 0.05 between VEGF-sFlt-1 and VEGF-mFc (N = 4-6 per group).

Longitudinal BP Measurements

Repeated measures mixed models for Systolic BP (SBP), mean BP (MBP), and diastolic BP (DBP) were created. These included the following independent variables: therapy (PBS-sFlt-1, VEGF-sFlt-1 and VEGF-mFc), days of treatment (day 8 to day 18 of gestation), and BP intervals (averaged BP every 12 hours). Therapy had a significant effect on DBP, MBP, and SBP (F-values 20.12, 24.79, and 33.10, respectively; $p < 0.0001$ in all). Similarly, days of treatment had a significant effect on maternal diastolic, mean, and systolic BP (F-values 11.00, 13.23, and 20.30, respectively; $p < 0.0001$ in all). Significant interaction was, however, noticed between the aforementioned independent variables. In a further analysis, we compared BP across the groups in each of the

recording days. Significant level was adjusted through the use of Tukey test. SBP, MBP and DBP between day 8 and 9 of gestation were not significantly different between the 3 groups. SBP and DBP were significantly higher in the PBS-sFlt-1 than in the VEGF-sFlt-1 and VEGF-mFc mice from day 10 to day 18 of gestation (Figures 11 and 12). During the same period, MBP was also significantly lower in the VEGF-treated mice than in the PBS-sFlt-1 animals. Hypertension was observed in the PBS-sFlt-1 mice in late gestation, days 17 and 18. On these days, the range of SBP and DBP were 141.47-146.71 mmHg and 102.8-107.2 mmHg, respectively. The median drop of BP between the PBS-sFlt-1 and VEGF-sFlt-1 mice was 16.2 mm Hg from days 8 to 13 of gestation and 22.7 mm Hg from days 14 to 18 of gestation (Figure 13).

Figure 10: Free VEGF plasma concentrations

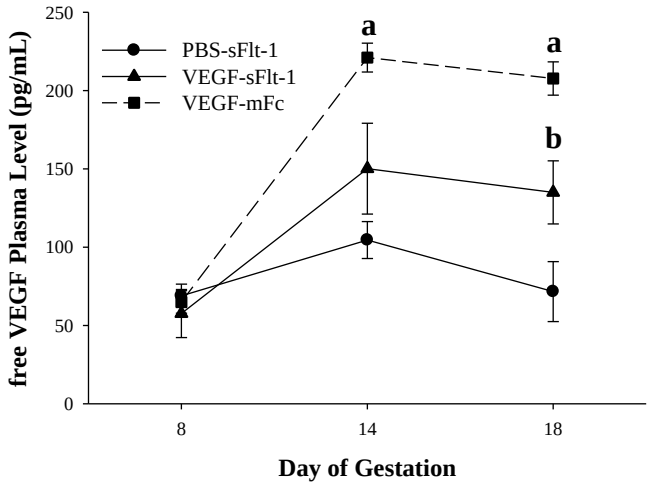


Figure 10 Free VEGF plasma concentrations at different points in gestation. Data are presented as mean ± SEM. a denoted an adjusted probability value < 0.05 between VEGF-mFc and PBS-sFlt-1 mice. b denoted an adjusted probability value < 0.05 between VEGF-sFlt-1 and PBS-sFlt-1 mice (N = 4-6 per group).

Figure 11: Systolic blood pressure in pregnant mice

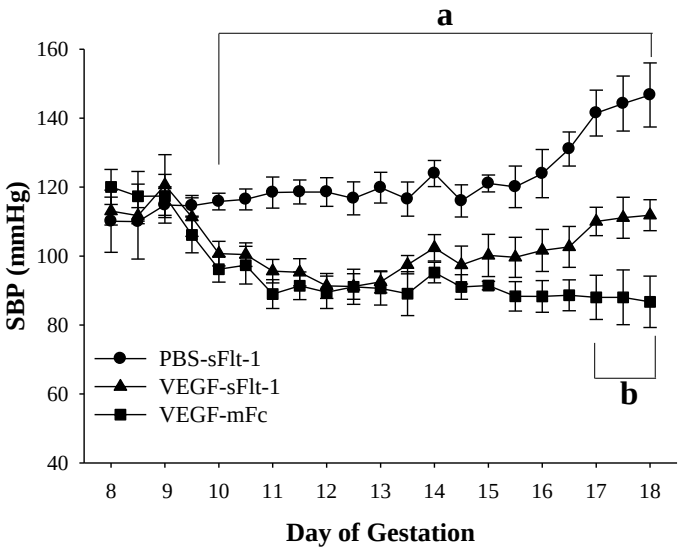


Figure 11 Systolic BP averaged over 12-hour periods from day 8 to day 18 of gestation. Data are illustrated as mean ± SEM. a denoted an adjusted *p* value < 0.05 of PBS-sFlt-1 mice compared with VEGF-sFlt-1 and VEGF-mFc mice. b denotes an adjusted *p* value < 0.05 between VEGF-sFlt-1 and VEGF-mFc mice (N = 4/6 per group).

Figure 12: Diastolic blood pressure in pregnant mice

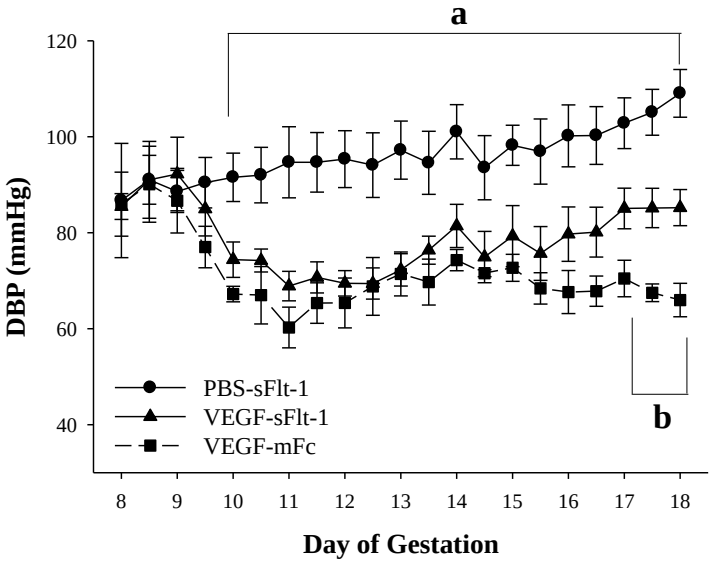


Figure 12 Diastolic BP averaged over 12-hour periods in pregnant mice from day 8 to day 18 of gestation. Data are presented as mean ± SEM. a denoted an adjusted *p* value < 0.05 of PBS-sFlt-1 mice compared with VEGF-sFlt-1 and VEGF-mFc. b denotes an adjusted *p* value < 0.05 among VEGF-sFlt-1 and VEGF-mFc mice (N = 4/6 per group).

Figure 13: Reduction of mean BP in pregnant mice

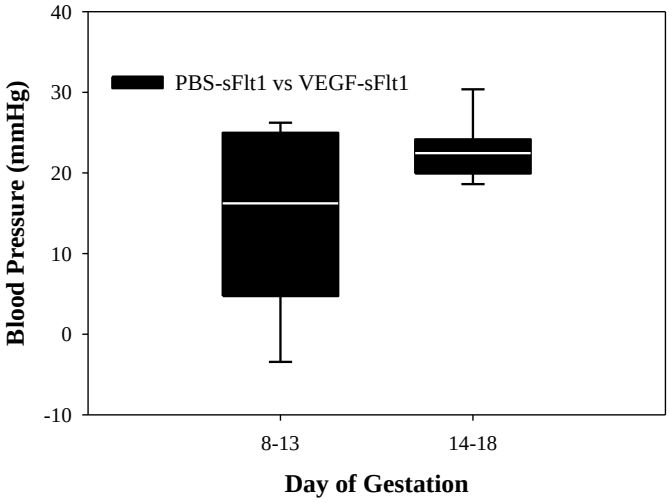


Figure 13 Reduction of mean BP of VEGF-sFlt-1 treated mice compared with PBS-sFl-t1 mice during two equivalent gestational age periods. Box plots data represented median, upper and lower quartiles, as well as maximum and minimum BP value differences among these two groups at the described time periods.

Litter Characteristics

At day 18 of gestation, the average pup weight per litter was significantly lower in the animals treated with PBS-sFlt-1 (1.01 ± 0.03 g) than in the VEGF-sFlt-1 (1.30 ± 0.12 g) and the VEGF-mFc mice (1.33 ± 0.07 g) (Figure 14). The difference between means for PBS-sFlt-1 compared with VEGF-sFlt-1 and VEGF-mFc were 7.0 (95% CI; 1.90, 12.09) and 7.50 (95% CI; 1.81, 13.19). Placental weight per litter trended towards lower in the PBS-sFlt-1 mice compared with the VEGF-sFlt-1 and VEF-mFc animals, but the difference did not reach statistical significance (Figure 15). Neither the number of pups nor the number of placentas differed significantly among the groups. There was one case of fetal resorption identified in a pregnancy treated with VEGF-sFlt-1.

Figure 14: Pup weights per litter

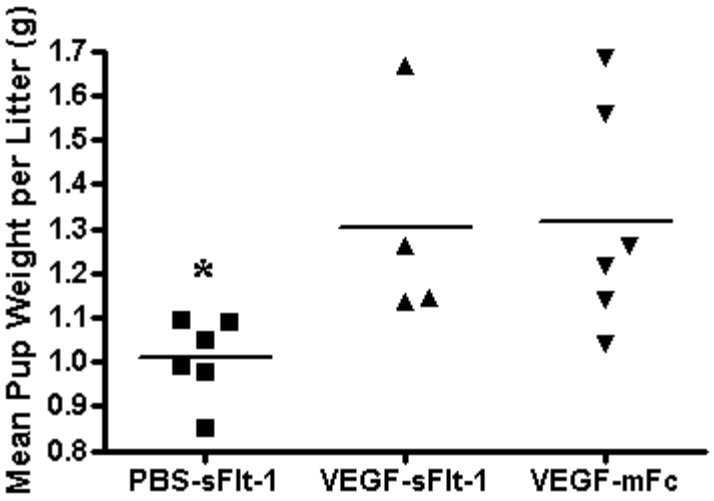


Figure 14 Mean pup weights per litter at day 18 of gestation from pregnant mice treated with either PBS-sFlt-1, VEGF-sFlt-1, or VEGF-mFc. Asterisk denoted an adjusted *p* value < 0.05 (N = 4/6 per group).

Figure 15: Placenta weights per litter

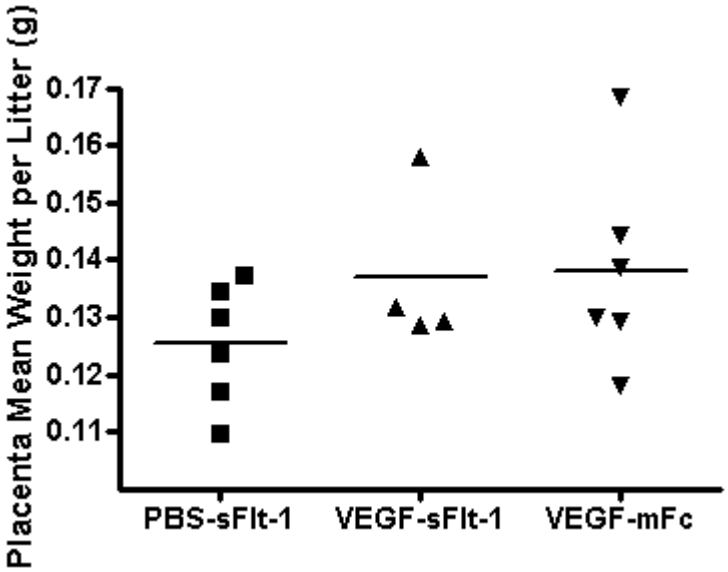


Figure 15 Mean placenta weights per litter at day 18 of gestation from pregnant mice treated with either PBS-sFlt-1, VEGF-sFlt-1, or VEGF-mFc (N= 4/6 per group).

SPECIFIC AIM 1: CONCLUSION

The results showed that VEGF-121 therapy inhibits plasma sFlt-1 concentrations of pregnant mice transfected with adenovirus carrying sFlt-1 in mid-gestation. Simultaneously, exogenous administration of this proangiogenic factor contributed to increasing plasma concentrations of free-VEGF. Linked to these effects, VEGF- sFlt-1 treated mice compared with PBS-sFlt-1 mice had significant reduction of BP and did not develop hypertension in late gestation. BP reduction was first noticed at 24-48 hours after therapy was initiated (day 10 of gestation) and the effect lasted through out pregnancy. Drop of BP in the PBS-sFlt-1 group as compared with the VEGF-sFlt1 group was most pronounced from day 14 to day 18. BP control in the VEGF-sFlt-1 mice likely resulted in improvement of utero-placental blood flow minimizing intrauterine growth restriction. The number of pups carrying in each pregnancy and the number of pregnancy resorptions were not modified by VEGF-121 therapy.

SPECIFIC HYPOTHESIS 2:

VEGF-121 ameliorates maternal vascular endothelial dysfunction and decreases structural abnormalities in target organs such as kidney and placenta.

SPECIFIC AIM 2:

To examine vascular function in the carotid artery by invitro vascular reactivity studies and to define renal and placental histological characteristics of pregnant mice transfected with adenovirus carrying sFlt-1 and treated with VEGF-121 or placebo.

INTRODUCTION

A multitude of throphoblast-secreted factors are present in different quantities in the circulation of women with preeclampsia compared with healthy pregnancies. These factors may act directly or indirectly on the endothelium of vulnerable maternal organs, resulting in pathophysiologic changes including vasoconstriction, enhanced capillary leak with plasma volume reduction, and platelet activation (Brown, 1995). Elevated plasma sFl-t1 levels released by throphoblast cells contribute to endothelial dysfunction in preeclamptic pregnancies. This effect is primordially caused through antagonism of VEGF (Maynard, 2003; Levine 2004).

The kidney seems to be one of the most vulnerable organs in preeclampsia. The characteristic lesion in women with preeclampsia is 'glomerular endotheliosis'. This lesion is characterized by endothelial cell swelling, obliteration fenestrae, and occlusion of capillary lumen (Stillman, 2007). The presence of excess of sFl-t1, in addition to decreased VEGF production disrupts the normal function of the glomerular endothelium thus inducing proteinacious deposits in the glomeruli and proteinuria (Henao, 2010). Hepatic dysfunction, secondary to endothelium dysfunction and ischemia is one of the hallmarks of HELLP syndrome, a severe variant of preeclampsia. Hepatic sinusoids may be blocked by intravascular fibrin deposition leading to blood flow obstruction (Barton, 2009). Inflammatory mediators and sFl-t1 seem to be involved in the development of hepatic dysfunction.

Preliminary data from our lab showed that pregnant mice that over-express sFlt-1 have signs of endothelial dysfunction and typical renal and hepatic pathological changes. Thus, these animals compared to controls had a significantly increased response to the endothelium dependent vasoconstrictor PE in invitro vascular reactivity studies of the carotid artery. Renal and hepatic histology examination from pregnant mice at day 18 of

gestation revealed that sFlt-1 overexpression leads to liver inflammatory changes, glomerular sclerosis, and peri-glomerular proteinacious deposition. We hypothesize that VEGF-121 therapy would contribute to repair the damaged endothelium and reverse partially or completely pathological changes in the vascular endothelium, kidney, and liver of pregnant mice with high circulating levels of sFlt-1.

SPECIFIC AIM 2: STUDY DESIGN

Pregnant CD-1 mice at day 8 of gestation were randomly assigned to insertion of osmotic minipumps (ALZET ® model 2002; Cupertino, CA) medicated with either VEGF-121 400µg/kg per day or PBS used as placebo. At day 9, VEGF-121 treated mice were injected in the tail vein with either Adv-sFlt-1 or mFc, as virus control (10^9 PFU). PBS treated-mice were injected with Adv-sFlt-1 10^9 PFU also in the tail vein. Pregnant mice (N=4 per group) were sacrificed at day 18 of gestation and carotid artery was isolated and 2 mm segments were mounted in wires for invitro vascular reactivity studies. Liver and kidney were isolated and fixed in 10% phosphate-buffered formalin (pH 7.0) and embedded in paraffin for histological examination. The tissues were stained with hematoxilin & eosin.

SPECIFIC AIM 2: RESULTS

In vitro vascular activity of the carotid artery

The contractile responses to KCl were similar between the groups (Figure 16). The AUC and the E_{max} responses to PE were significantly lower in the VEGF-mFc compared with the VEGF-sFlt-1 and PBS-sFlt-1 mice (Figures 17a and 17b). The difference was abolished after incubation with L-NAME (Figure 18). The AUC and the E_{max} to TxA_2 were significantly higher in the PBS-sFlt-1 compared with the VEGF-sFlt-1

and VEGF-mFc mice (Figures 19a and 19b). The E_{max} response to Ach did not show a significant difference between the three groups (Figure 20a). Vasorelaxation to SNP was significantly higher in the VEGF-sFlt-1 and VEGF-mFc compared with the PBS-sFlt-1 mice (Figure 20b).

Figure 16: Contractile Response to KCl in the Carotid Artery

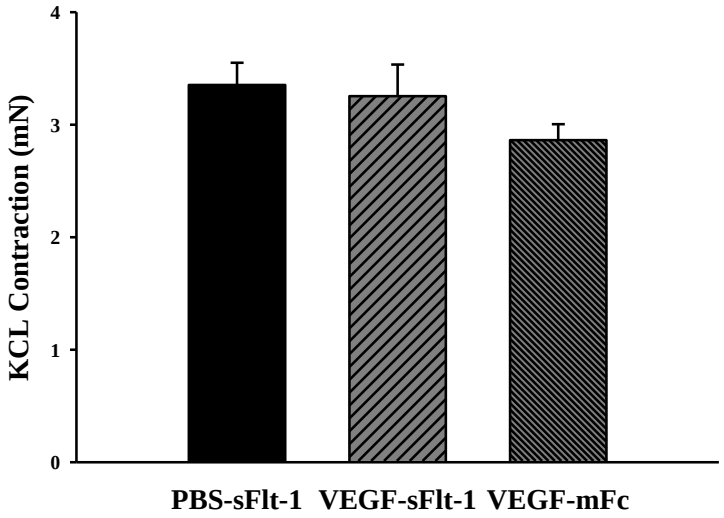


Figure 16 KCl contraction in the carotid artery at day 18 of gestation of pregnant mice treated with PBS-sFlt-1, VEGF-sFlt-1, and VEGF-mFc.

Figure 17a PE response curve

Figure 17b: PE Area under the curve

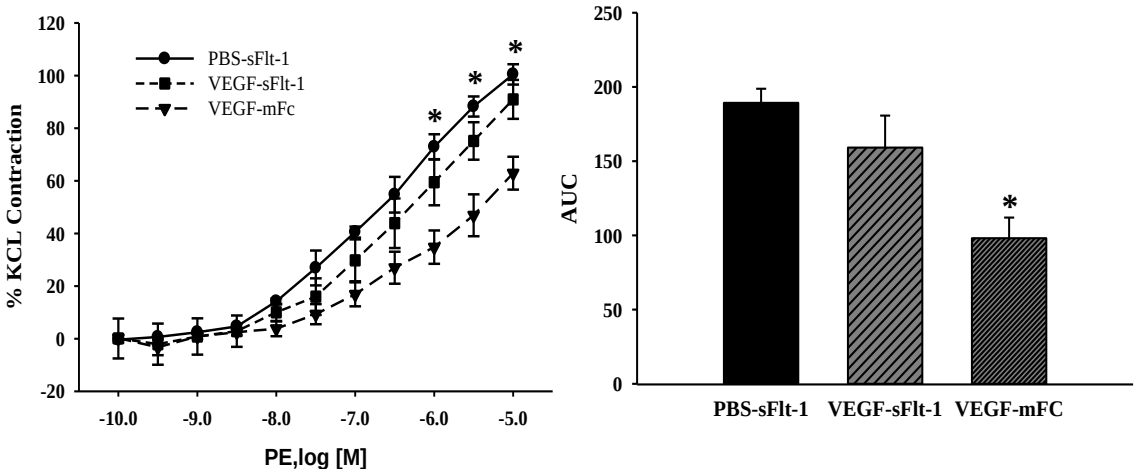


Figure 17 Contractile responses to Phenylephrine (PE). Figure 17a PE concentration-response curve in the carotid artery at day 18 of gestation of pregnant mice treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc in the mid-gestation. Figure 17b PE area under the concentration curve in the carotid artery at day 18 for the same treatment groups. Asterisk denoted an adjusted probability value < 0.05 of VEGF-mFc compared with VEGF-sFlt-1 and PBS-sFlt-1 mice (N = 6-8 vessels per group).

Figure 18: PE in presence of L-Name response curve

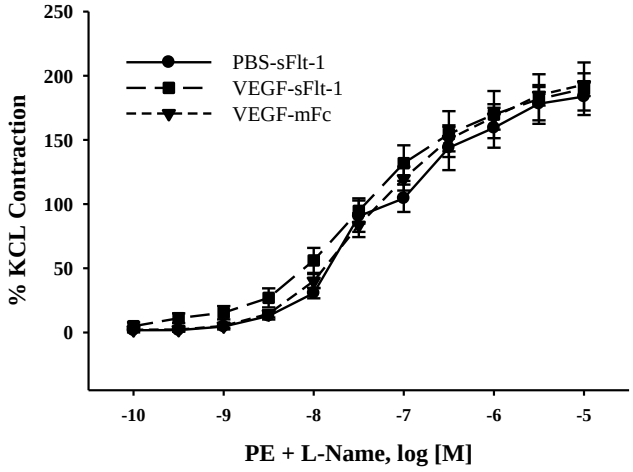


Figure 18 Phenylephrine (PE) in presence of L-NAME concentration- response curve in the carotid artery at day 18 of gestation of pregnant mice treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc in the mid-gestation (N = 6-7 vessels per group).

Figure 19a: TxA_2 response curve

Figure 19b: TxA_2 area under the curve

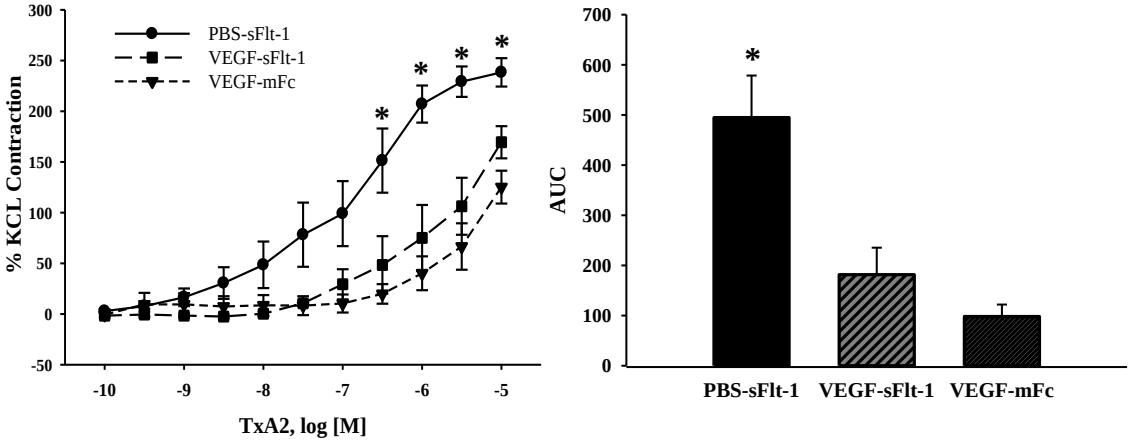


Figure 19 Contractile responses to Thromboxane (TxA_2). Figure 19a TxA_2 concentration-response curve in the carotid artery at day 18 of gestation of pregnant mice treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc in the mid-gestation. Figure 19b TxA_2 area under the concentration curve for the same treatment groups. Asterisk denoted a probability value < 0.05 of PBS-sFlt-1 compared with VEGF-sFlt-1 and VEGF-mFc mice (N = 6-8 vessels per group).

Figure 20a: Ach response curve

Figure 20b: SNP response curve

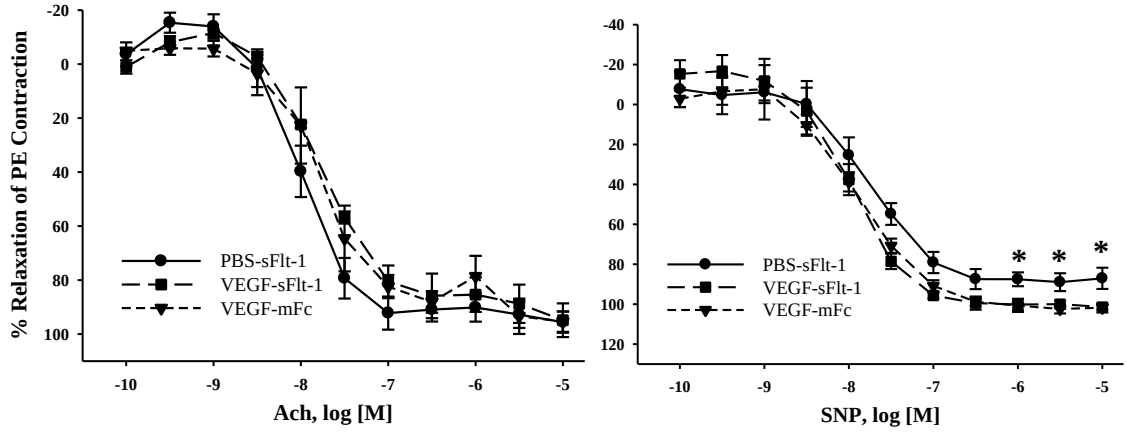


Figure 20 Vasorelaxation responses to Acetylcholine (Ach) and Sodium Nitroprusside (SNP). Figure 20a Ach concentration- response curve in the carotid artery at day 18 of gestation of pregnant mice treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc in the mid-gestation. Figure 20b SNP concentration-response curve for the same treatment groups. Asterisk denoted an adjusted probability value < 0.05 of PBS-sFlt-1 compared with VEGF-sFlt-1 and VEGF-mFc mice (N = 7-11 vessels per group).

Renal and liver histopathological studies

Microscopic examination of liver and kidney sections from the various studies groups revealed a few pathologic changes. The liver sections of the PBS-sFlt-1 group showed collections of neutrophils in the sinusoids. Several apoptotic bodies were observed, specially surrounding the terminal hepatic venules and in the periportal area. Mitotic figures were prominent and could be observed in almost every high-power field (Figure 21a). Figure 21b shows representative photomicrographs from the liver tissue sections of the VEGF-sFlt1 pregnant mice. Tissue section appeared mostly unremarkable in this animal group. Occasional neutrophils and apoptotic bodies were identified. Most kidney sections were unremarkable. In the PBS-sFlt-1 group, rare intertubular cluster of polymorphonuclear cells and monocytes were observed (Figure 21c). The kidney sections of the VEGF-sFlt-1 mice did not revealed morphologic abnormalities (Figure 21d). Liver and kidney histology examination of the VEGF-mFc and VEGF-sFlt-1 mice were similar.

Figure 21: Liver and kidney histology pictures

Figure 21a Liver, PBS-sFlt-1 Group

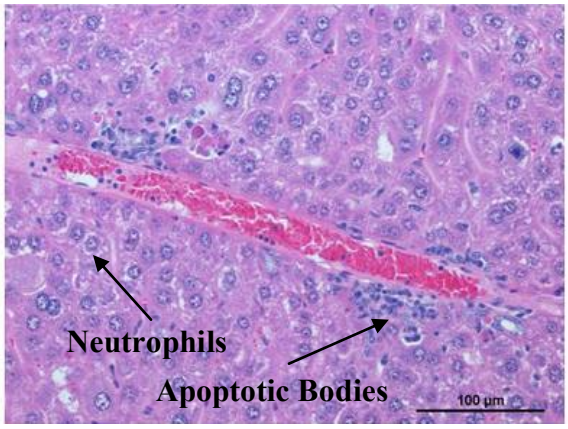


Figure 21b Liver, VEGF-sFlt-1 Group

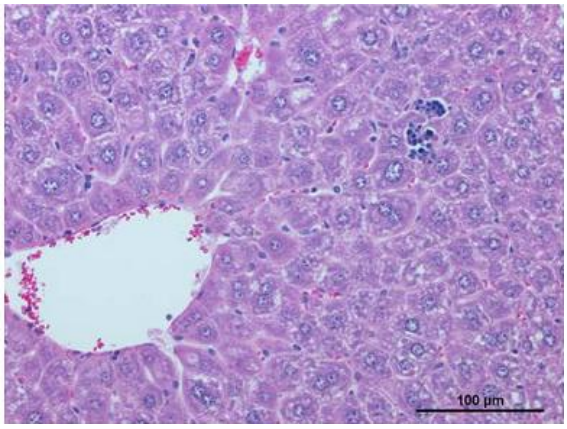


Figure 21c Kidney, PBS-sFlt-1 Group

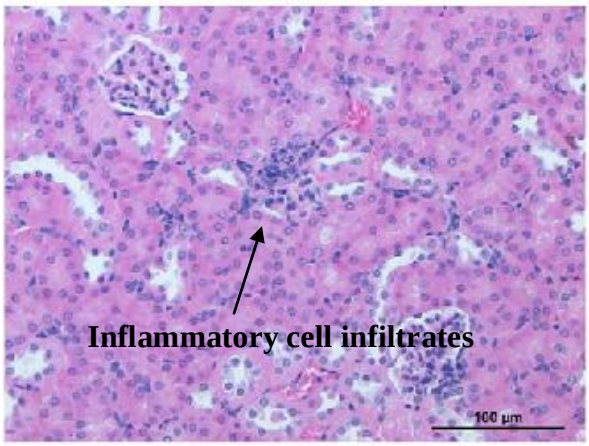
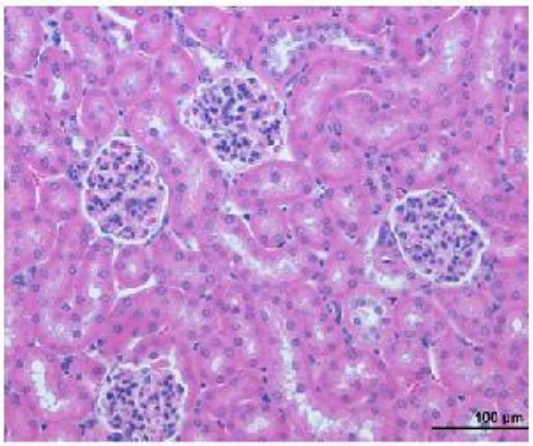


Figure 21d Kidney, VEGF-sFlt-1 Group



SPECIFIC AIM 2: CONCLUSION

Continuous infusion of VEGF-121 ameliorated vascular dysfunction in pregnant mice transfected with adenovirus vector carrying at day 8 of gestation. Improved vascular function was evidenced by decreased contractile response to TxA_2 and increased vasorelaxant response to the NO donor SNP in the carotid artery of term pregnant mice treated with VEGF-sFl-t1 as compared with the PBS-sFlt-1 mice. These improvements in the carotid artery may have significance in relation to brain and other target organs blood flow in preeclampsia No significantly difference was seen in smooth muscle contractile responses to membrane depolarization by high-KCl solution, which indicates no structural changes in the carotid artery. Although, the response to PE was lower in the VEGF-sFlt-1 than in the PBS-sFlt-1 mice the difference did not reach the significant level. It was surprising that vasorelaxation response to Ach did not vary considerably between the three treatment groups. We were also surprised for the lack of major histopathologic changes in the liver and kidney of sFlt-1 treated pregnant mice. This may be secondary to differences in the vulnerability of the endothelium and structure of

maternal organs to the biological actions of sFlt-1. The amount of sFlt-1 injected and the replication capacity of the adenovirus in the host liver are other plausible explanations of these findings. In contrast, VEGF treatment did not cause structural variation of the liver or kidney architecture. Histology exam of these organs in both of the VEGF treatment groups was unremarkable.

SPECIFIC HYPOTHESIS 3:

VEGF-121 decreases hypoxic changes and thereby regulates the expression of hypoxia-related genes in the placenta and kidney of pregnancies with elevated circulating levels of sFlt-1.

SPECIFIC AIM 3:

To compare mRNA expression level of the hypoxic induced factors in pregnant mice over-expressing sFlt-1 and treated with VEGF-121 or placebo.

INTRODUCTION

The results in the specific aims 1 and 2 have demonstrated the impaired maternal vascular function and signs of intrauterine growth restriction in the pups of sFlt-1 treated mice. We also evidenced decrease in endothelial dysfunction and acceleration of fetal growth in pregnancies with high levels of sFlt1 that were treated with VEGF-121. The development and function of the utero-placental unit depends primordially on a balanced production of angiogenic factors (Lunell, 1984).

sFlt-1 is up-regulated in response to hypoxia. An in vivo study showed that rise in sFlt-1 levels induced artificially by decreasing the utero-placental blood flow correlates with the onset of preeclampsia and its severity (Makris, 2007). We have shown in our mouse model of preeclampsia indirect evidence of the relationship between high levels of

sFlt-1 and decreased placental blood flow (Lu, 2007). Therefore, placental hypoxia may be the main source of excess sFlt-1 production noted in preeclampsia. In turn, this factor can activate hypoxia signaling pathways in the placenta and other target organs such as the kidney.

HIF is a key regulator of oxygen hemostasis. HIF-1α, one of the HIF isoforms, seems to be involved in the pathogenesis of several human ischemic diseases including preeclampsia (Semenza, 2000). This transcriptor factor regulates trophoblast differentiation by up-regulating the expression of TGFβ3. HIF-1α and TGFβ3 are over-expressed in placental tissue from preeclamptic women (Caniggia 2000). The altered trophoblast differentiation could also be affected by GCM1, another gene which seems to be down-regulated in the preeclamptic placentas. We test the hypothesis that sFlt-1 induces hypoxia changes in the placenta and the kidney by activating hypoxia-related genes and that can be reversed by the use of VEGF-121.

SPECIFIC AIM 3: STUDY DESIGN

CD1 mice at day 8 of gestation were randomly allocated to VEGF-121 400 µg/kg/day or PBS in an osmotic minipump. At day 9, VEGF-treated mice were injected with adenovirus carrying sFlt-1 or mFc. PBS-treated mice were injected with adenovirus carrying sFlt-1. At day 18, placentas and kidneys were collected from these pregnant for detection of hypoxic genes expression levels.

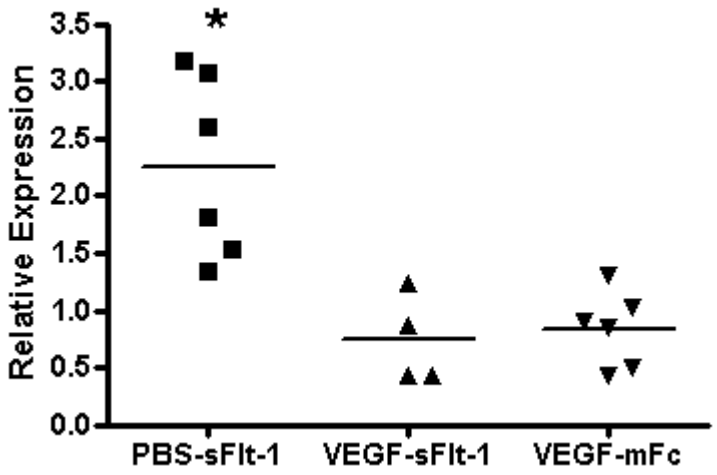
SPECIFIC AIM 3: RESULTS

Gene expression of hypoxia-related genes in placenta and kidney

The relative expression of HIF-1 alpha in the placenta was significantly higher in the sFlt-1 versus the VEGF-mFc treated animals. The difference between means of sFlt-1

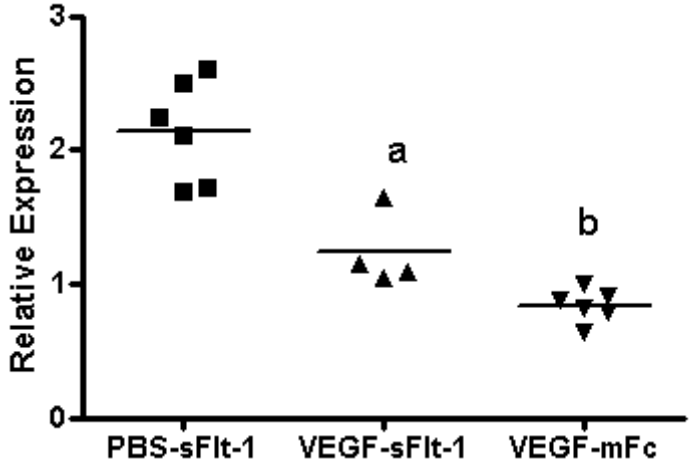
mice respecting VEGF-sFlt-1 and VEGF-mFc mice were -8.5 (95% CI; -12.32,-4.67) and -7.67 (95% CI; -11.08,-4.25), respectively (Figure 22). In the kidney, HIF1-α expression was significantly lower in the VEGF-mFc mice compared to VEGF-sFlt-1 and PBS-sFlt-1 animals (difference between means: 5.0 (95% CI; 2.55, 7.47) and 10.0 (95% CI; 7.8, 12.19), respectively). Renal HIF-1α expression was also significantly lower in the VEGF-sFlt-1 in reference to the PBS-sFlt-1 mice (Figure 23). Placental TGFβ3 expression level was significantly higher in the PBS-sFlt-1 versus the VEGF-mFc (difference between means: -8.5(95% CI; -11.89, -5.10) and the VEGF-sFlt-1 mice (difference between means: -7.25(95% CI; -11.04, -3.45); Figure 24). In the kidney, although TGFβ3 level was also higher in the PBS-sFlt-1 mice compared with the other groups, the difference was not significant (Figure 25). Placental GCM1 expression level was lower in the PBS-sFlt-1 compared with the VEGF-treated groups. Difference between means compared with VEGF-sFlt-1 mice was 5.41(95% CI; 0.62, 10.21), whereas compared with VEGF-mFc it was 8.83 (95% CI, 4.54-13.12; Figure 26). GCM1 expression was significantly higher in the VEGF-mFc mice compared with VEGF-sFlt-1 and PBS-sFlt-1 (difference between means: -6.58 (95% CI; -10.58, -2.58) and -8.5 (95% CI; -12.07, -4.92). Its expression did not vary significantly among the two latest groups (Figure 27).

Figure 22: Placental HIF-1α expression



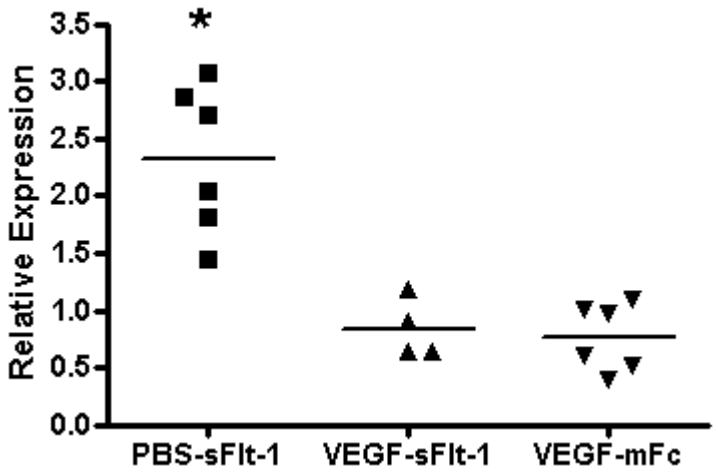
Fold expressions of HIF1α mRNA shown by $\Delta\Delta C_t$ (RQ) values in the placenta at day 18 of gestation in mice treated with sFl-t1, VEGF-sFlt-1, and VEGF-mFc. The asterisk denotes an adjusted *p* value of <0.05 for sFlt-1-treated pregnant mice versus VEGF-sFlt-1 and VEGF-mFc mice.

Figure 23: Renal HIF-1α expression



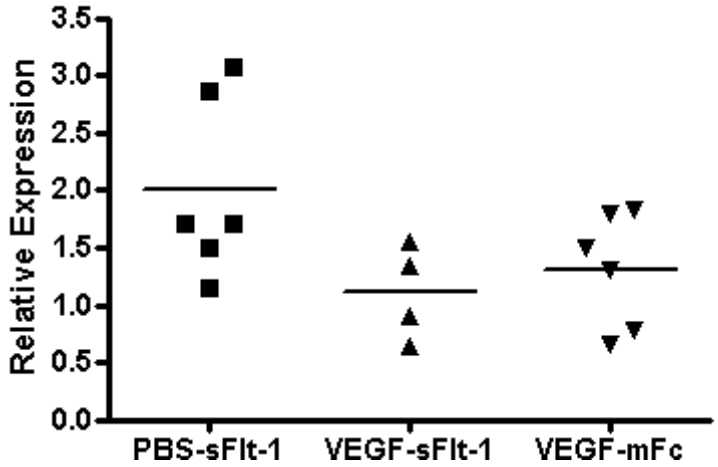
Fold expressions of HIF1α mRNA shown by $\Delta\Delta C_t$ (RQ) values in the kidney at day 18 of gestation in mice treated with sFl-t1, VEGF-sFlt-1, and VEGF-mFc. a denotes an adjusted *p* value of <0.05 for VEGF-sFlt-1-treated pregnant mice versus PBS-sFlt-1 mice. b denotes an adjusted *p* value of <0.05 for VEGF-mFc-treated pregnant mice versus the other two groups.

Figure 24: Placental TGFβ3 expression



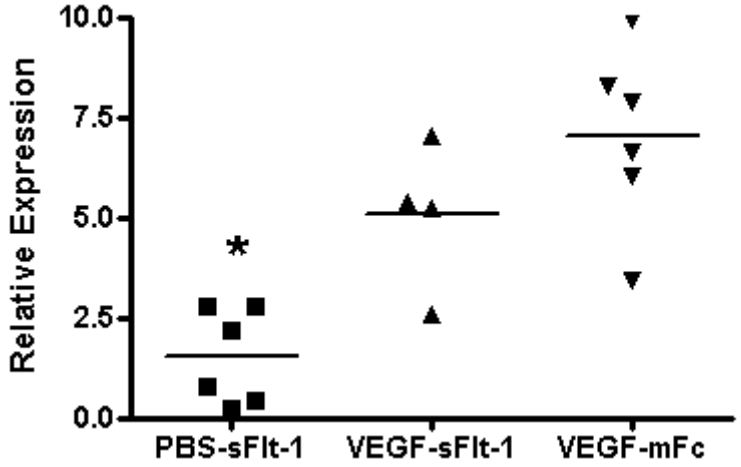
Fold expressions of TGFβ3 mRNA shown by $\Delta\Delta C_t$ (RQ) values in the placenta at day 18 of gestation in mice treated with sFl-t1, VEGF-sFlt-1, and VEGF-mFc. The asterisk denotes an adjusted *p* value of <0.05 for PBS-sFlt-1-treated pregnant mice versus the other groups.

Figure 25: Renal TGFβ3 expression



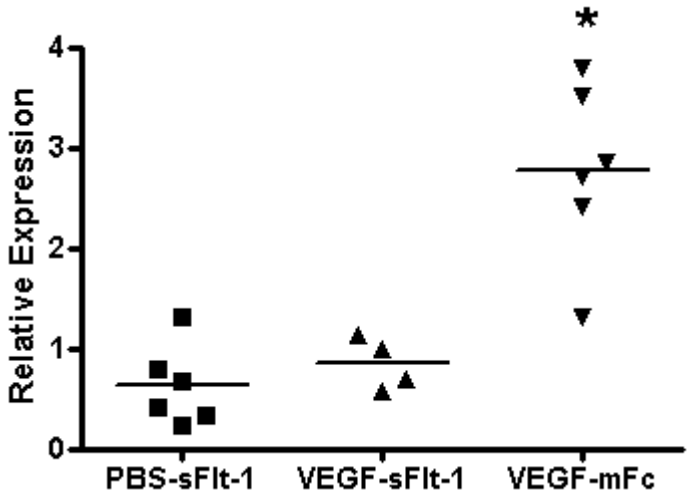
Fold expressions of TGFβ3 mRNA shown by $\Delta\Delta C_t$ (RQ) values in the kidney at day 18 of gestation in mice treated with sFl-t1, VEGF-sFlt-1, and VEGF-mFc.

Figure 26: Placental GCM1 expression



Fold expressions of GCM1 mRNA shown by $\Delta\Delta C_t$ (RQ) values in the placenta at day 18 of gestation in mice treated with sFl-t1, VEGF-sFlt-1, and VEGF-mFc. The asterisk denotes an adjusted *p* value of <0.05 for PBS-sFlt-1-treated pregnant mice versus the other groups.

Figure 27: Renal GCM1 expression



Fold expressions of GCM1 mRNA shown by $\Delta\Delta C_t$ (RQ) values in the kidney at day 18 of gestation in mice treated with sFl-t1, VEGF-sFlt-1, and VEGF-mFc. The asterisk denotes an adjusted *p* value of <0.05 for VEGF-mFc-treated pregnant mice versus the other groups.

SPECIFIC AIM 3: CONCLUSION

Results showing in aim 1 suggest that sFlt-1 over-expression diminishes utero-placental blood flow as the placenta trends to be smaller and the pups weight are significantly lower in sFlt-1 treated mice that received PBS versus VEGF-121. These findings are correlated with indirect signs of placental hypoxia evidenced in aim 3. Placental relative expression of HIF-1α and TGFβ3 was significantly elevated, whereas GCM1 expression was lower in PBS-sFlt-1-treated mice compared to VEGF-sFlt-1 animals. These findings suggest the presence of hypoxic placenta in this mouse model of preeclampsia, in which sFlt-1 is the main trigger factor. VEGF-121 therapy modulates the expression of these genes by reducing HIF-1α and TGFβ3 and increasing GCM1. Furthermore, the expression levels in the VEGF-sFlt-1 mice were equivalent to that seen in the VEGF-mFc mice. In conclusion, this therapy seems to facilitate an increase in placental blood flow and placental oxygenation leading to a rise in fetal blood supply and consequently an acceleration in intrauterine growth as was demonstrated in aim 1.

sFlt-1 elicited a higher expression of HIF-1α, but did not pose a major effect in the TGFβ3 expression in the kidney. In addition, GCM1 expression in this organ was minimally modified by sFlt-1 overexpression. The endothelium that covers renal vessels seems to be vulnerable to sFlt-1 actions in a similar fashion to that in the placental vascular bed. We showed that sFlt-1 expression was higher in the PBS-sFlt-1 mice than in the VEGF-sFlt-1 mice. However, we did not find major changes in the expression of TGFβ3 across the treatment groups suggesting that it is not activated under renal hypoxia or ischemia. The lack of activation of this gene makes us believe that different genes may be involved in response to excess circulating sFlt-1. TGFβ1, an isoform of the TGFβ gene, plays an important role in the formation of fenestrated glomerular endothelium (Liu, 1999; Ballermann, 2007). It is plausible that induction of renal ischemia by sFlt-1

results in altered expression of TGFβ1 instead of TGFβ3 and that deserves further investigation. The expression level of the third gene, GCM1, did not differ significantly as result of VEGF-121 therapy. Possible explanations of this finding are either GCM1 is not down-regulated in response to renal ischemia/hypoxia or the plasma levels of sFlt-1 reached in our study are insufficient to alter its expression.

SPECIFIC HYPOTHESIS 4:

VEGF-121 therapy prevents altered fetal vascular programming in pregnancies overexpressing sFlt-1 reducing the risk for vascular disease in the offspring later in life.

SPECIFIC AIM 4:

To evaluate fetal vascular programming by determining blood pressure and in vitro vascular function in the carotid artery of adult offspring born to pregnant mice over-expressing sFlt-1 that were treated with VEGF-121 or placebo.

INTRODUCTION

In fetal life the tissues and organs go through critical periods of development, which may coincide with periods of rapid cell division (Widdowson, 1975). The "Barker's Hypothesis" states that the intrauterine environment where the fetus develops can be altered by insults or stimulus occurring during critical periods leading to lifelong effects, a process known as fetal programming (Barker, 1986; 1989). Persisting changes in the structure may have permanent specific disruptions in the development of various organ systems, including the cardiovascular and metabolic systems (Barker, 1986; 1989). Under nutrition in utero may be one of the influences that program the human body. Epidemiological studies have demonstrated that low birth weight predisposes

cardiovascular diseases later in life such as coronary artery disease, stroke and hypertension (Barker, 1989; Hoy, 1999).

The environment in which the fetus develops is created by interactions between the fetal genome and the intrauterine environment, and that may be operational in pregnancy complicated by vascular diseases such as diabetes, hypertension and preeclampsia. However, the observed association between an unfavorable/hostile fetal environment and diseases in later life may be confounded by the presence of a genetic predisposition for a particular disease. This is more applicable to cardiovascular and/metabolic disorders since genetic predispositions for these disorders may affect the fetal environment, as well as the risk for adult diseases when they occur in both mother and offspring. In these cases, epidemiological studies may not be able to differentiate or quantify the risk of a specific disease in later life caused between a hostile intrauterine environment versus a hereditary predisposition (Dunger, 2006). Animal models can be very useful to discern the role of intrauterine environment and genetic predisposition for systemic disorders like preeclampsia.

The elevated incidence of fetal growth restriction in pregnancies complicated by preeclampsia is associated with placental dysfunction resulting in decreased fetal nutritional supply and adverse intrauterine environment (Zusterzeel, 2001; Naicker, 2003). In this regard, the utero-placental insufficiency that occurs in preeclampsia can potentially lead to altered fetal vascular programming and risk of cardiovascular disease later in life. Epidemiological data have found that SBP is higher in the offspring born to preeclamptic mothers compared to those who were normotensive and the BP difference started to be noticed as early as the adolescence age (Øglaend, 2009). These findings have been confirmed in animal models of preeclampsia. Previous data from our lab showed that BP of male offspring born to mothers that were injected with adenovirus

carrying sFlt-1 was significantly higher that the similarly aged male offspring born from mFc-treated mothers (Lu, 2007b). These differences were not present in adult female offspring suggesting a gender-sensitive fetal programming in our animal model of preeclampsia. Based on the strong evidence from human and animal studies that supports fetal programming in response to adverse intrauterine environment, we hypothesize that adult offspring born to mothers treated with sFlt-1 develop vascular dysfunction and high BP as early as 3 months of age and those changes are prevented with VEGF-121 therapy during gestation.

SPECIFIC AIM 4: STUDY DESIGN

A subset of pregnant mice from each of the three experimental groups (N= 4-6 per group) were allowed to deliver. The offspring were weaned at day 21 and a subset of female and male offspring were randomly separated (N = 10-12 males and 10-12 females per group). Offspring were weighed weekly from week 4 to week 9 of age. At 3 months of age, a subset of male (N = 4-6 per group) and female (N = 4-6 per group) offspring were randomly assigned to insertion of BP catheters through the left carotid artery into the aortic arch and tunneled to a telemetric transmitter. BP was recorded continuously for 1 week. SBP, MBP, and DBP were averaged over 12 hour intervals. Consequently, animals were sacrificed and 2 mm segments of carotid arteries were mounted in a small-vessel myograph for vascular reactivity studies. Four to six female and male offspring for each group underwent same experimental procedures at 6 months of age.

SPECIFIC AIM 4: RESULTS

Post weaning weight

Female and male offspring from sFlt-1, VEGF-sFlt-1, and VEGF-mFc-treated pregnant mice were followed towards adulthood. Using repeated measure mixed models, we found that weight from weaning towards adulthood in the female offspring was significantly predicted by in-utero treatment exposure (PBS-sFlt-1, VEGF-sFlt-1, or VEGF-mFc) and post-weaning age (weeks), being their F values were 29.58 and 63.23, respectively ($p < .0001$). There was no significant interaction between these two independent variables in the model ($F = 1.64$; $p = 0.10$). At four-weeks of age, the weight of PBS-sFlt-1 female offspring was significantly lower than that in the VEGF-sFlt-1 and VEGF-mFc female mice (Figure 28). By the fifth week of postnatal age, weight difference was still in favor of the VEGF-mFc mice compared with the sFlt-1 mice, but it was similar between the VEGF-sFlt-1 and sFlt-1 mice. The weight was not significantly distinct from week 6 onward across the groups (Figure 28).

Similar to female offspring, in-utero treatment and postnatal age were significant predictors of the male offspring's weight from week 4 to week 9 of age ($F = 49.49$ and 119.82, respectively; $p < .0001$). However, there was significant interaction between treatment and postnatal age among this offspring group ($F = 2.35$; $p = 0.01$). Weight of offspring born to VEGF-mFc mothers were consistently higher than the weight of those born to PBS-sFlt-1 pregnancies. Furthermore, VEGF-sFlt-1 male offspring's weight was significantly higher than that of PBS-sFlt-1 mice, except on week 6 and week 9 (Figure 29). VEGF-sFlt-1 and VEGF-mFc offspring had a similar weight during the post weaning observation time.

Figure 28: Female post-weaning weights

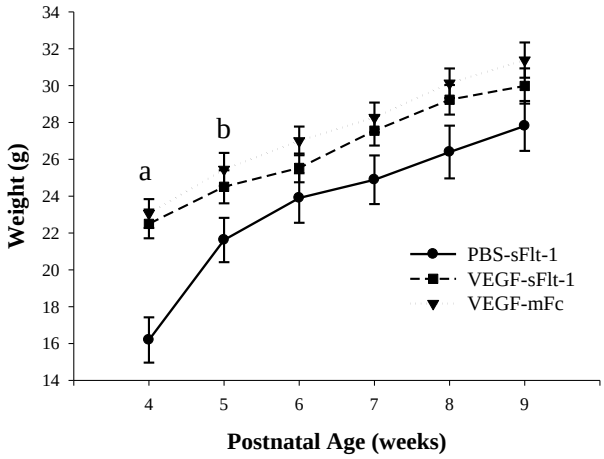


Figure 28 Post weaning mean weight of female offspring, born to pregnancies treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF-mFc. Data are shown as mean ± SEM. a denotes an adjusted probability value of < 0.05 for PBS-sFlt-1 versus VEGF-sFlt-1 and VEGF-mFc female offspring. b denotes an adjusted probability value of < 0.05 for PBS-sFlt-1 versus VEGF-mFc female offspring.

Figure 29: Male post-weaning weights

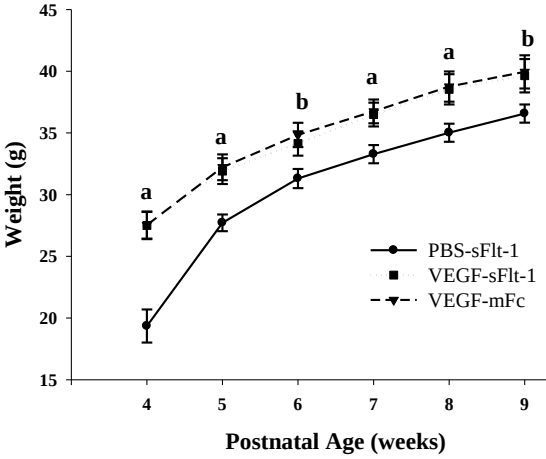


Figure 29 Post weaning weight of male offspring born to pregnant mothers treated with PBS-sFlt-1, VEGF-sFlt-1, and VEGF-mFc. Data are shown as mean ± SEM. a denotes an adjusted probability value of <0.05 for PBS-sFlt-1 versus VEGF-sFlt-1 and VEGF-mFc male offspring. b denotes an adjusted probability value of <0.05 for PBS-sFlt-1 versus VEGF-sFlt-1mice.

Blood pressure in female offspring

We created repeated measures mixed models to analyze BP in the offspring using the same variables used in the models for maternal BP. In the 3 month-old female offspring, treatment did not predict significantly MBP (F = 0.51; p = 0.60). In contrast, observation time (days) was a significant predictor of MBP (F = 10.30; $p < .0001$). MBP averaged over 12 hour periods is illustrated in Figure 30. Overall, MBP was slightly lower in the female offspring born to pregnancies treated with VEGF-sFlt-1 and VEGF-mFc compared with those treated with PBS–sFlt-1 (106.03 ± 1.20 mmHg and 108.34 ± 0.91 mmHg versus 111.45 ± 0.88 mmHg,). A similar pattern was seen for SBP and DBP across the groups. At 6 months of age, time of BP recording (day-time versus night-time) predicted significantly MBP (F = 37.23; $p < .0001$), being night-time lower than day-time BP. For this group, type of treatment in utero did not have any significant effect in MBP (F = 0.51; p = 0.60). No significant interactions were evidenced among the independent variables. Figure 31 illustrated MBP in the 6-month of age female offspring.

Figure 30: Blood pressure in 3 month-old female offspring

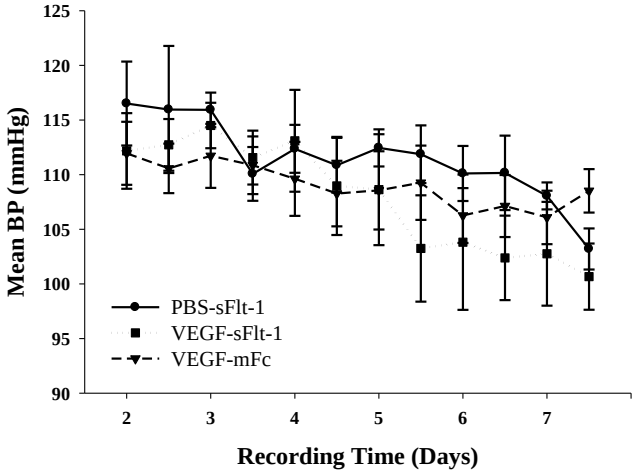


Figure 30 MBP pressure, averaged over 12-hour periods in 3 month-old female offspring born to pregnant mothers treated with PBS-sFlt-1, VEGF-sFlt-1 and VEGF-mFc recorded from day 2 to day 7 after transducer insertion. Data are shown as mean ± SEM.

Figure 31: Blood pressure in 6 month-old female offspring

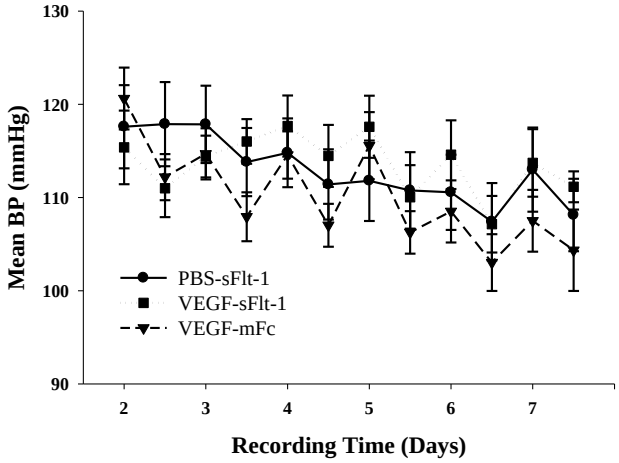


Figure 31 MBP, averaged over 12-hour periods in 6 month-old female offspring born to pregnant mothers treated with PBS-sFlt-1, VEGF-sFlt-1 and VEGF-mFc recorded from day 2 to day 7 after transducer insertion. Data are shown as mean ± SEM.

Blood pressure in male offspring

Type of treatment in-utero (PBS-sFlt-1, VEGF-sFlt-1, or VEGF-mFc) and the recording period (days) had a significant effect on MBP in the 3 month-old male offspring ($F = 5.03$; $p = .007$ and $F = 10.90$; $p < .0001$, respectively). At this age, MBP was higher in the male offspring born to PBS-sFlt-1 mothers than the MBP in the offspring from VEGF-sFlt-1 and VEGF-mFc pregnancies (Figure 32). The BP did not differ significantly among the VEGF-sFlt-1 and VEG-mFc mice. In the six-months of age male offspring, all of the independent variables included in the model as they were treatment, recording period, and night vs day BP recording were significant predictors of MBP ($F = 7.62$ ($p = .0007$), $F = 20.97$ ($p < .0001$), and $F = 18.08$ ($p < .0001$), respectively). Notable interactions were seen between these variables. Similar to the 3 month-old male offspring, overall MBP was higher in the male offspring born to PBS-sFlt-1 mothers as compared to the VEGF-sFlt-1 and VEGF-mFc offspring as shown in Figure 33. VEGF-sFlt-1 and VEGF-mFc offspring had similar BP.

Figure 32 Blood pressure in 3 month-old male offspring

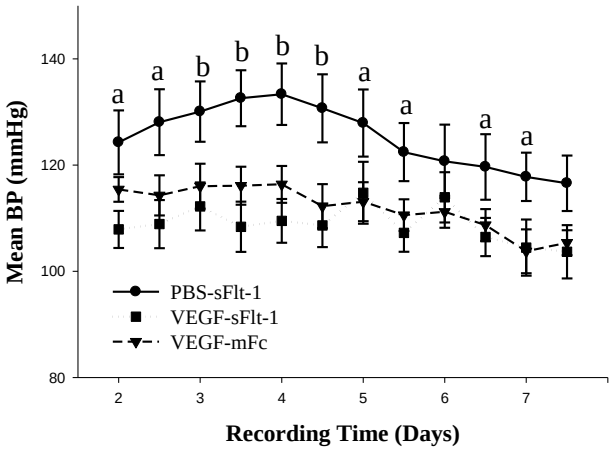


Figure 32 MBP pressure, averaged over 12-hour periods in 3 month-old male offspring born to pregnant mothers treated with PBS-sFlt-1, VEGF-sFlt-1 and VEGF-mFc recorded from day 2 to day 7 after transducer insertion. a denotes an adjusted p value < 0.05 for PBS-sFlt-1 compared with VEGF-sFlt-1 mice. b denotes an adjusted a p value < 0.05 for PBS-sFlt-1 versus VEGF-sFlt-1 and VEGF-mFc mice. Data are shown as mean ± SEM.

Figure 33 Blood pressure in 6 month-old male offspring

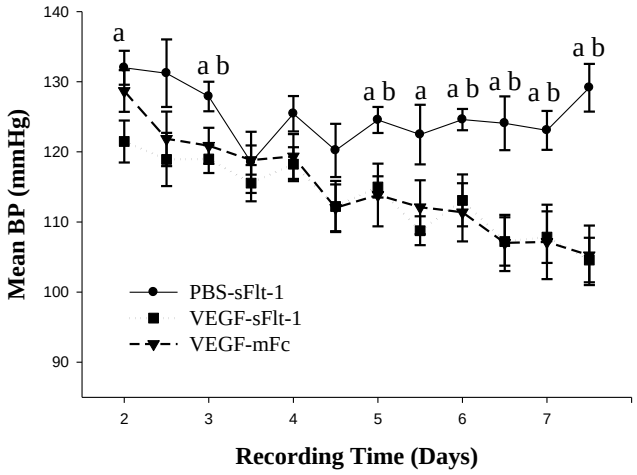


Figure 33 MBP averaged over 12-hour periods in 6 month-old male offspring born to pregnant mothers treated with PBS-sFlt-1, VEGF-sFlt-1 and VEGF-mFc recorded from day 2 to day 7 after transducer insertion. a denotes an adjusted p value < 0.05 for PBS-sFlt-1 compared with VEGF-sFlt-1 mice. b denotes an adjusted a p value < 0.05 between PBS-sFlt-1 and VEGF-mFc mice. Data are shown as mean ± SEM.

Carotid artery vascular activity in female offspring

Vascular studies were performed in 3 and 6 month old female offspring born to pregnancies treated with PBS-sFlt-1, VEGF-sFlt-1, and VEGF-mFc. The contractile responses to KCl did not differ significantly between the 3 month-old female groups (2.97± 0.51 mN, 2.92 ± mN, and 3.45 ± 0.43 mN for PBS-sFlt-1, VEGF-sFlt-1, and VEG-mFc treatments, respectively). There was no significant difference in the contractile responses to PE and TxA_2 between these female offspring groups (Figures 34 and 35). A similar pattern was seen with the vasorelaxant response to Ach and SNP across the groups (Figures 36 and 37).

Figure 34 PE response in the 3-month old female offspring

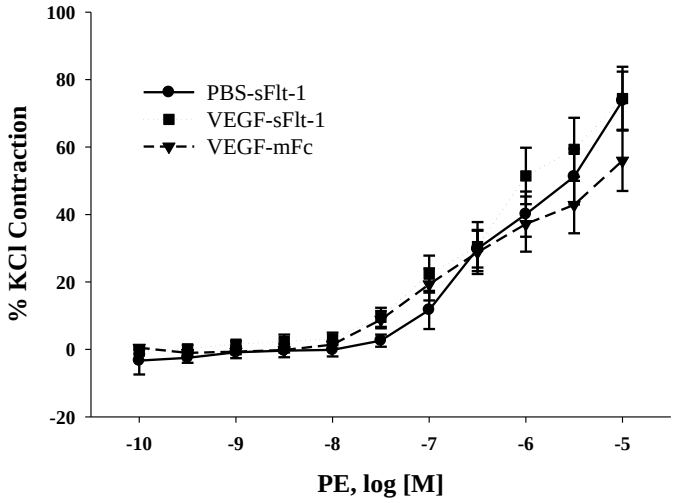


Figure 34 Phenylephrine (PE) concentration curve in the carotid artery of offspring from mothers treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc (N = 10-12 per group).

Figure 35 TxA_2 response curve in the 3-month old female offspring

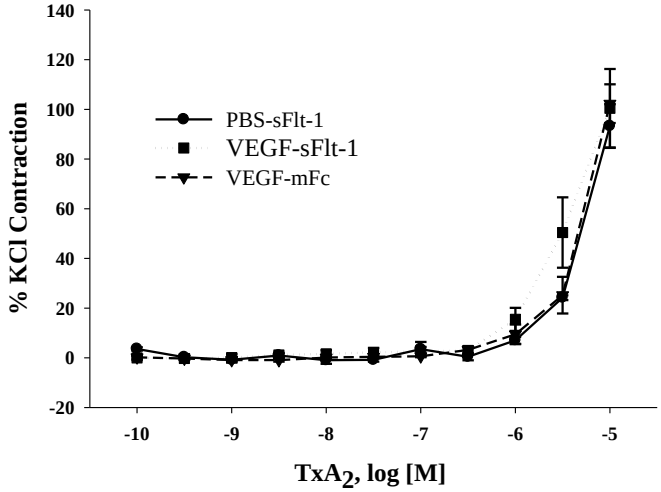


Figure 35 Thromboxane (TxA_2) concentration- response curve in the carotid artery of the offspring from mothers belonging to distinct treatment groups (N = 9-12 per group).

Figure 36 Ach response curve in the 3-month old female offspring

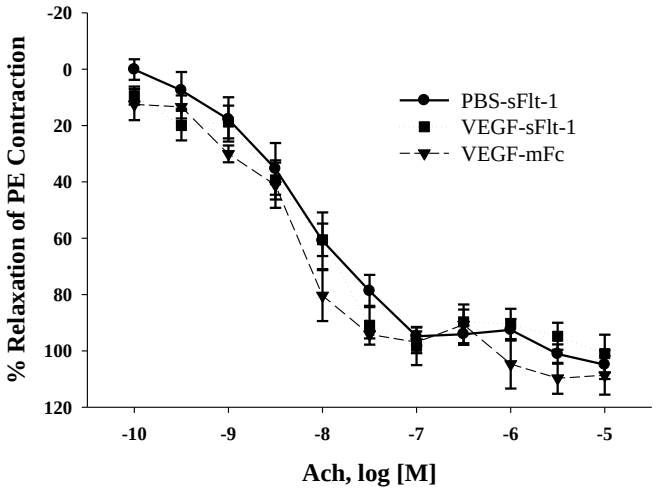


Figure 36 Ach concentration- response curve in the carotid artery of offspring born to pregnancies treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc (N = 9-11 per group).

Figure 37 SNP response curve in the 3-month old female offspring

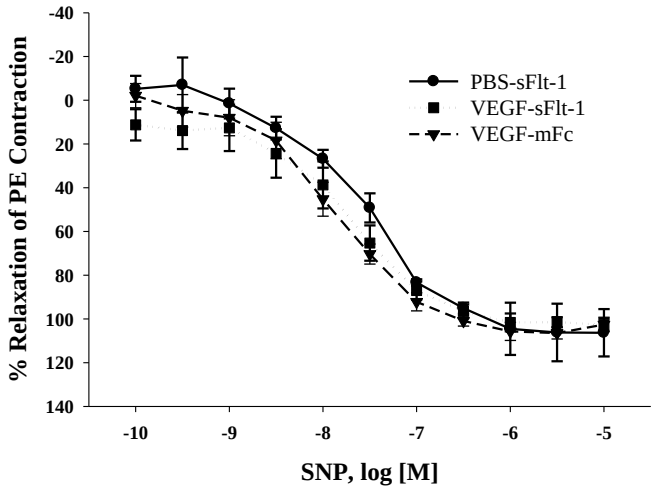


Figure 37 Sodium Nitroprusside (SNP) concentration-response curve in the carotid artery of offspring from the distinct treatment group mothers (N = 9-12 per group).

KCl response was no significantly different among the 6 month female offspring born to mothers treated with PBS-sFlt-1, VEGF-sFlt-1, and VEGF-mFc (3.28 ± 0.19 mN, 3.72 ± 3.72 ± mN, respectively). The response to the highest concentrations of PE was significantly higher in the female offspring born to VEGF-sFlt-1 versus PBS-sFlt-1 prenatal treatments (Figure 38). The E_{max} PE contractile response was higher in the VEGF-mFc than in the PBS-sFlt-1 female offspring. The presence of L-Name abolished the aforementioned PE contractile response differences. There was no significant difference on the PE responses among VEG-sFlt-1 and VEGF-mFc mice. Similarly, contractile response to TxA_2 did not vary significantly across the groups (Figure 39). Vasorelaxation to Ach was achieved faster in the VEGF-mFc offspring than in the VEGF-sFlt-1 and PBS-sFlt-1 mice (Figure 40). However, the E_{max} response to Ach did not vary significantly across the groups. The response to the agent SNP was similar between the 6 month old female offspring (Figures 41).

Figure 38: PE response curve in the 6-month old female offspring

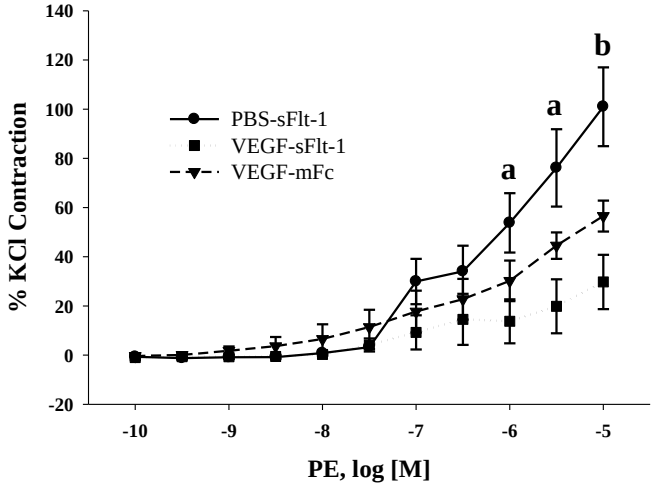


Figure 38 Phenylephrine (PE) concentration curve in the carotid artery of offspring from mothers with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc (N = 8-10 per group). a denotes an adjusted *p* value < 0.05 for PBS-sFlt-1 versus VEGF-sFlt-1 group. b denotes an adjusted *p* value < 0.05 for PBS-sFlt-1 versus VEGF-mFc and VEGF-sFlt-1 group.

Figure 39: TxA_2 response curve in the 6-month old female offspring

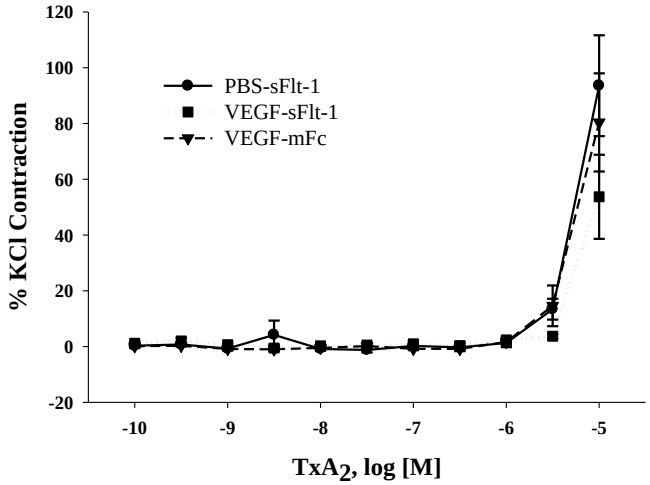


Figure 39 Thromboxane (TxA_2) concentration- response curve in the carotid artery of the offspring born to mothers treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF-mFc (N = 8-10 per group).

Figure 40: Ach Response curve in the 6-month old offspring

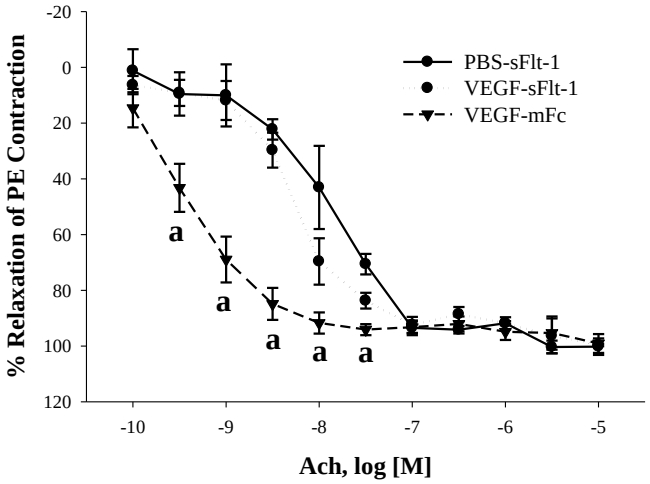


Figure 40 Acethylcoline (Ach) concentration- response curve in the carotid artery of offspring born to pregnancies treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc (N = 8-10 per group). a denotes an adjusted *p* value < 0.05 for VEGF-mFc mice versus VEGF-sFlt-1 and PBS-sFlt-1 mice.

Figure 41: SNP Response curve in the 6-month old female offspring

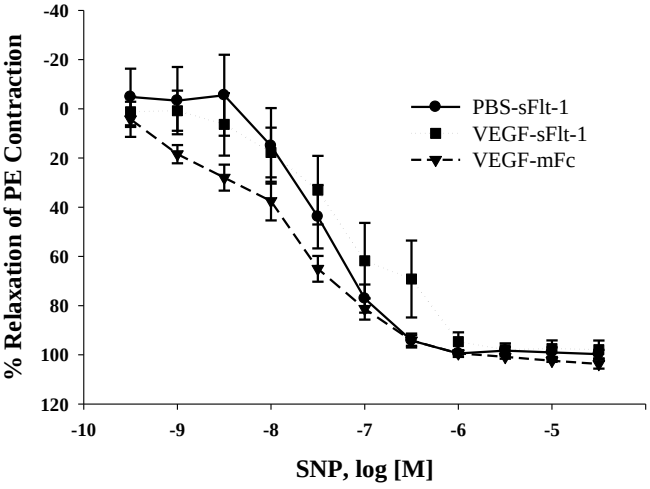


Figure 41 Sodium Nitroprusside (SNP) concentration-response curve in the offspring born to mothers treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF-mFc (N = 7-10 per group).

Carotid artery vascular activity in male offspring

Male offspring underwent vascular reactivity studies in the carotid artery following the same protocol used for the female offspring. KCl responses did not differ significantly among the 3 month male offspring born to mothers treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF-mFc (3.82 ± 0.32 mN, 4.12 ± 0.19 mN, and 3.97 ± 0.20 mN, respectively). Contractile responses to PE and TxA_2 were similar among the 3 month old male offspring (Figures 42 and 43). A similar pattern was seen with the relaxant responses to Ach and SNP across the groups (Figures 44 and 45).

Figure 42: PE Response curve in the 3 month-old male offspring

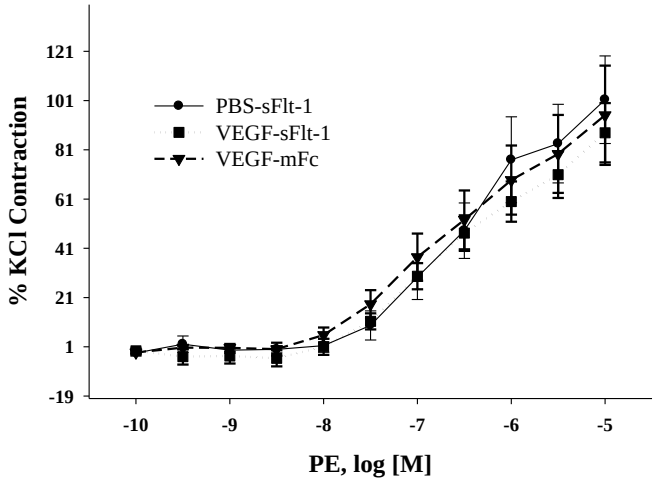


Figure 42 Phenylephrine (PE) concentration curve in the carotid artery of offspring from mothers with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc (N = 7-10 per group).

Figure 43: TxA_2 response curve in the 3-month old male offspring

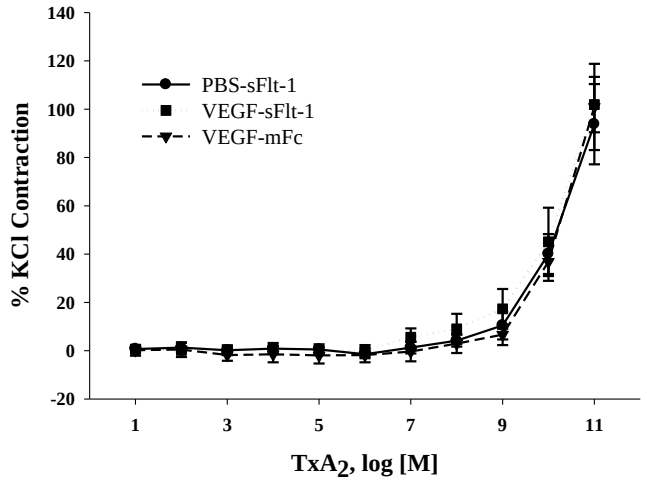


Figure 43 Thromboxane (TxA_2) concentration- response curve in the carotid artery of the offspring born to mothers treated with PBS-sFlt-1, VEGF-sFlt-1 or VEGF-mFc (N = 7-10 per group).

Figure 44: Ach response curve in the 3-month old male offspring

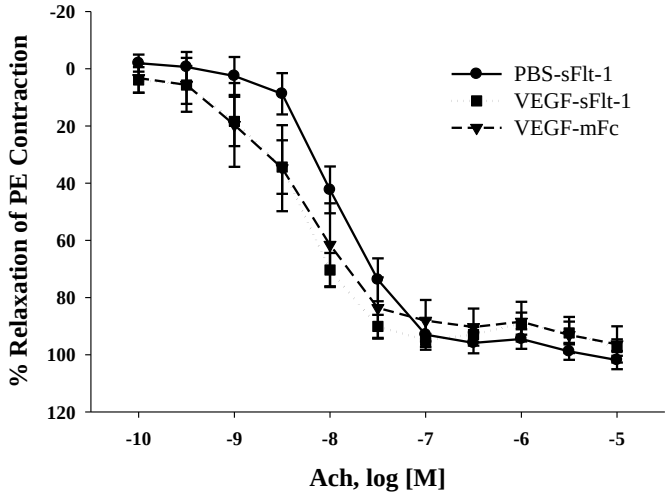


Figure 44 Acethylcoline (Ach) concentration- response curve in the carotid artery of offspring born to pregnancies treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc (N = 8-10 per group).

Figure 45: SNP Response curve in the 3-month old male offspring

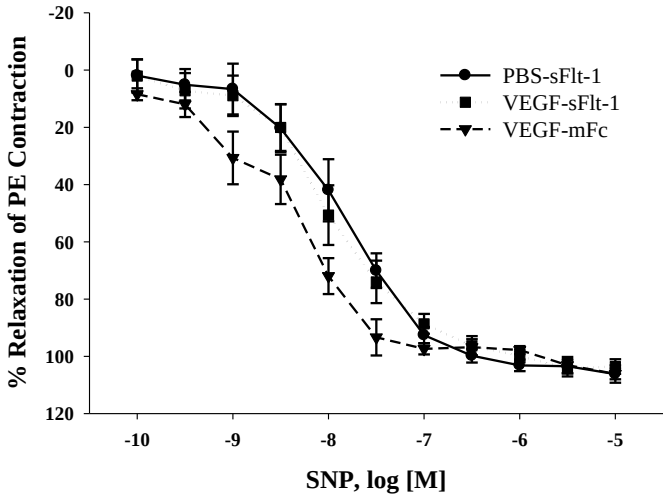


Figure 45 Sodium Nitroprusside (SNP) concentration-response curve in the offspring born to mothers treated with PBS-sFlt-1, VEGF-sFlt-1, and VEGF-mFc (N = 8-10 per group).

There was no significant difference on the KCl response between the 6 month old PBS-sFlt-1, VEGF-sFlt-1, and VEGF-mFc male offspring (3.93 ± 0.29 mN, 4.41 ± 0.26 mN, and 3.76 mN ± 0.31 mN, respectively). The E_{max} response to the contractile agent PE was significantly higher in the 6 month PBS-sFlt-1 male offspring than in their counterpart VEGF-sFlt-1 and VEGF-mFc offspring (Figure 46). In a similar fashion, response to TxA_2 in these PBS-sFlt-1 offspring was significantly higher than in the offspring born to mothers that received prenatal VEGF treatment (Figure 47). The presence of L-Name abolished the PE contractile response differences among the offspring groups. The response to the endothelium dependent agent Ach was significantly higher in the 6 month old VEGF-mFc male offspring than in the 6 month old male offspring born to VEGF-sFlt-1 and PBS-sFlt-1 mothers (Figure 48). The response to the NO donor SNP was significantly higher in the offspring from VEGF-sFlt-1 and VEGF-mFc than in those born to PBS-sFlt-1 pregnancies (Figure 49).

Figure 46: PE response curve in the 6-month old male offspring

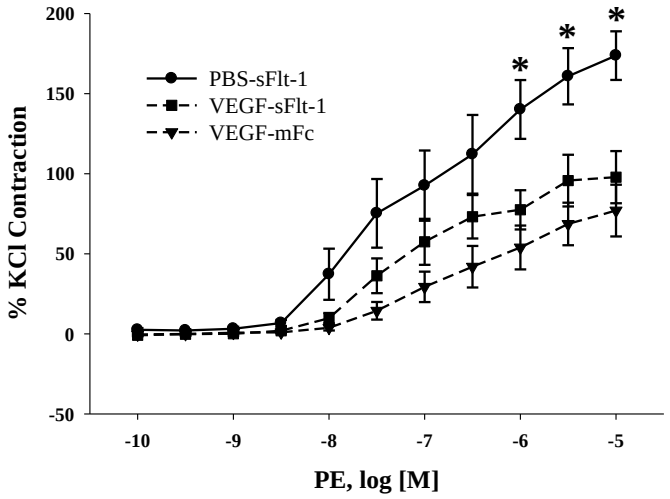


Figure 46 Phenylephrine (PE) concentration curve in the carotid artery of offspring from mothers with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc (N = 6-9 per group). The asterisk denotes an adjusted *p* value < 0.05 for PBS-sFlt-1 versus VEGF-sFlt-1 and VEGF-mFc offspring.

Figure 47: TxA_2 response in the 6-month old male offspring

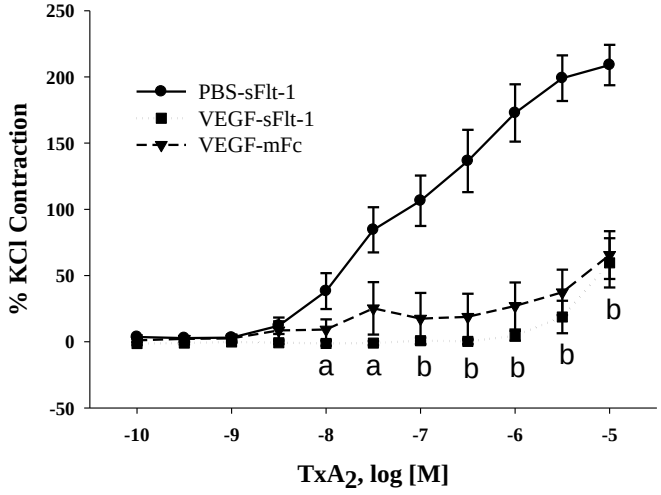


Figure 47 Thromboxane (TxA_2) concentration- response curve in the carotid artery of the offspring from the mother-treatment groups (N = 6-9 per group). a denotes an adjusted *p* value < 0.05 for PBS-sflt-1 versus VEGF-sflt-1 mice. b denotes an adjusted *p* value < 0.05 for PBS-sFlt-1 versus VEGF-sFlt-1 and VEGF-mFc mice.

Figure 48: Ach response curve in the 6-month old male offspring

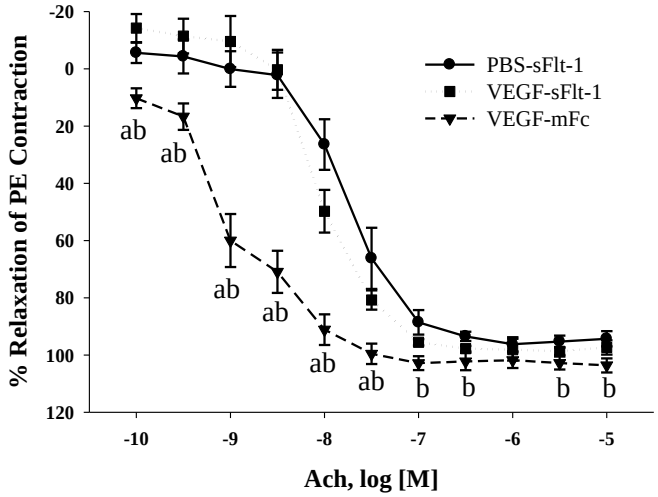


Figure 48 Acethylcoline (Ach) concentration- response curve in the carotid artery of offspring born to pregnancies treated with PBS-sFlt-1, VEGF-sFlt-1, or VEGF- mFc (N = 8-11 per group). a denotes an adjusted *p* value for VEGF-sFlt-1 versus PBS-sFlt-1 mice. b denotes an adjusted *p* value < 0.05 for VEGF-mFc versus PBS-sFlt-1 mice.

Figure 49: SNP response curve in the 6-month old male offspring

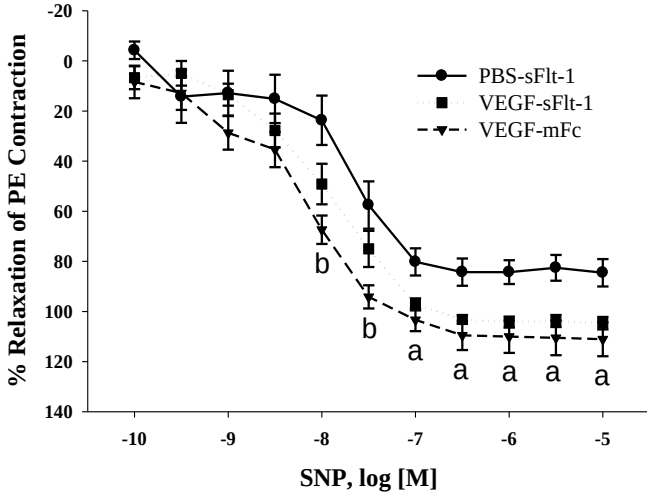


Figure 49 Sodium Nitroprusside (SNP) concentration-response curve for the mother's treatment groups (N = 8-10 per group). a denotes an adjusted *p* value for PBS-sFlt-1 versus VEGF-sFlt-1 and VEGF-mFc. b denotes an adjusted *p* value < 0.05 for VEGF-mFc versus PBS-sFlt-1 mice.

SPECIFIC AIM 4: CONCLUSION

Our results showed that the intrauterine growth abnormality associated with sFlt-1 overexpression as was described in aim 1 persisted postnatally. By week 4 of life both male and female offspring born to mothers treated with sFlt-1 in the mid-gestation had a significantly lower weight than those born to VEGF-treated mothers. From there onward, the growth accelerated in both male and female offspring being more marked in the latest group. Thus, the males remained smaller for most of the postnatal development. Their weight was comparable to their counterpart males born to VEGF-treated mothers only by 9 weeks of postnatal life. These growth abnormalities may be associated with the consequent findings of hypertension and vascular dysfunction seen in the adult male offspring born to sFlt-1 mothers. Hypertension was exclusively seen in the male sFlt-1 offspring since 3 months of age. Importantly, prenatal VEGF treatment prevented hypertension in these mice. The protective effect was persistent as the BP in the VEGF-sFlt-1 male offspring at 3 and 6 months of age was equivalent to the BP of those born to mothers treated with VEGF and mFc, the control of the virus. The high BP in the adult male offspring from sFlt-1 treated pregnancies was associated with vascular dysfunction, which was seen in invitro vascular studies of the carotid artery. However, these pathologic changes were only noted at 6 months of age suggesting that endothelial dysfunction has a slow progression during adulthood. Prenatal VEGF infusion also prevented endothelial dysfunction highlighting the role of this prenatal therapy in improving intrauterine environment and thereby in reducing the risk of vascular diseases later in life. Our results confirm a gender-sensitive developmental programming of BP and vascular function in this particular animal model of adverse uterine environment. Hypertension and vascular dysfunction in the 6 month old male offspring in association with altered post-weaning growth support the developmental origin of the hypertension.

CHAPTER 4: DISCUSSION

Strong evidence from human and animal studies support the role of imbalance of angiogenic factors in the pathogenesis of preeclampsia. sFlt-1, or soluble VEGFR-1 receptor of native VEGF inhibits the proangiogenic actions of VEGF and PlGF in the maternal endothelium and placental vascular bed leading to the clinical signs of preeclampsia. In recent years, we validated an animal model of preeclampsia by transfecting pregnant mice with adenovirus carrying sFlt-1 mice in mid-gestation. Remarkable pathological changes in this model include maternal hypertension, endothelial dysfunction, and signs of renal injury. We also demonstrated signs of placental hypoxia, intrauterine growth restriction and elevated BP in the adult male offspring born to sFlt-1-treated pregnancies. Considering that these findings resemble closely preeclampsia, the next step was to test the efficacy of a novel therapy that would reverse the effects of sFlt-1. We hypothesized, that the use of exogenous VEGF in pregnant mice overexpressing sFlt-1 inhibits the circulating levels of this antiangiogenic factor, promotes proangiogenic actions in the maternal endothelium and placenta and thus improves the aforementioned maternal and fetal abnormalities.

EFFECTS OF VEGF-121 IN MATERNAL VASCULAR FUNCTION

We showed that VEGF-121 therapy in an animal model of preeclampsia induced by overexpression of sFlt1 improves maternal vascular function. This therapy decreased systemic BP and thus prevented hypertension in pregnant mice that overexpressed sFlt-1. Reduction of BP was persistent throughout the duration of therapy, but was more pronounced in late gestation. It is important to note, that treatment period had a significant effect on the maternal BP and that interacted with the above-described effect exerted by VEGF. Infusion of VEGF-121 also was associated with improved vascular

endothelial cell function as the VEGF-sFlt-1 compared with the PBS-sFlt-1 pregnant mice had a lower contractile response to TxA_2 and higher vasorelaxation to the NO donor SNP in invitro vascular reactivity studies using vascular rings of the carotid artery. Surprisingly, the response to PE and Ach did not vary significantly among the treatment groups.

In Similarity to our findings, other investigators have observed beneficial effects of VEGF-121 in pregnancies overexppressing sFlt-1. An animal study observed that recombinant human VEGF-121 reduced BP and ameliorated renal damage in pregnant mice transfected with adenovirus carrying sFlt-1 (Li, 2007). Others showed that co-administration of $3.5x10^9$ PFU of Adv-sFlt-1 and $1x10^8$ VEGF-encoding virus particles to Balb/c mice also resulted in significant reduction of BP and improvement of the glomeruli pathogenic changes (Bergmann, 2010). Our study, however, differed from these studies on the employed drug delivery method. Instead of scheduled injections, we used a continuous osmotic delivery system that releases the drug at a constant rate leading to a steady drug bioavailability in target organs. This method also poses the advantage of minimizing animal handling and pain attributed to scheduled injections, two recognized influences on arterial BP. The use of osmotic minipumps medicated with VEGF-121 also decreased BP in a rat model of preeclampsia induced by chronic RUPP (Gilbert, 2010). In this study, VEGF-121 also normalized glomerular filtration rate and endothelial function in the RUPP rats.

We noticed a slight increase in BP in the VEGF-sFlt-1 mice in late gestation which contrasted with the physiologic drop in BP in the VEGF-mFc group. It is plausible that late in gestation, the constant release of sFlt-1 into systemic circulation by the transduced hepatocytes (Lu, 2007a) may have overcame the binding capacity of VEGF-121 limiting its biological actions including vasodilatation. Alternatively, VEGF-121

may partially reduce the heterodimerization of sFlt-1 with the extra-cellular ligand binding region of the membrane spanning VEGF receptors that blocks the phosphorylation and activation of the downstream signal transduction pathways for endothelial cell proliferation (Kendall, 1996). The mechanisms involved in the reduction of BP and improvement of endothelial cell dysfunction are probably inhibition of circulating sFlt-1 allowing natural VEGF and PlGF to bind the endothelial surface receptors VEGFR-1 and VEGFR-2 as well as a direct vasodilatory effect of the exogenous VEGF (Ferrara, 2003). It is also plausible that VEGF-121 binds the endothelial cell membrane receptors VEGFR-1 and VEGFR-2 and thereby activates cell signaling pathways that induce endothelial cell proliferation, endothelial survival, angiogenesis, vasopermeability, and vasodilatation. Activation of the PGI_2 signaling pathway through VEGFR-2 may play a major role in the vasodilatory effects seen by VEGF-121 therapy.

VEGF signaling is inducible under physiological and pathological processes. Angiogenesis is a key process for the formation of the placental vascular bed and is fundamental in the development of the fetal vascular system. In contrast, abnormal angiogenesis occurs in pathological disorders like neoplasia. Thus, inhibition or activation of the VEGF signaling has turned into an effective therapy modality for certain conditions. Inhibition of angiogenesis is now an effective approach to cancer therapy tested in multiple clinical trials. Several VEGF inhibitors have received FDA approval including bevacizumb (Avastin), sorafenib (Nexavar), sunitinib (Sutent), and pazopanib (Votrient). These medications are, however, associated with cardiovascular toxic effects such as hypertension and proteinuria, the predominant signs of preeclampsia. It has been suggested that these drugs raise BP by increasing the vascular tone because of reduction of NO production and by increasing vascular peripheral resistance because of endothelial

cell damage and dysfunction (Fernando, 2003; Posey, 2003). Inhibition of VEGF through the use of the above-mentioned therapeutic agents or induced by elevated circulating sFlt-1 levels in pregnancy share similar vascular phenotypes. Therefore, targeting sFlt-1 may be a potentially efficacious therapy for pregnancies affected by overproduction of this antiangiogenic factor. We successfully achieved increased circulating levels of sFlt-1 using an adenovirus to introduce the gene encoding sFlt-1 into the host DNA, likely in the hepatocytes. Administration of VEGF-121 decreased sFlt-1 levels by 61% and 56% on days 14 and 18 of gestation, respectively. This reduction is likely responsible for the improvement in maternal vascular function seen in our study.

VEGF-121 THERAPY AND PLACENTAL FUNCTION

We have shown that VEGF-121 therapy reduces placental hypoxia in pregnancies overexpressing sFlt-1. Along with this effect, weight of term pups from VEGF-sFlt-1 treated mothers was significantly higher than the pup's weight of sFlt-1 treated pregnancies. Placenta weight also trended to be higher in pregnancies treated with VEGF. All together, our results suggest that the use of VEGF-121 increases placental blood flow facilitating oxygen and micronutrient exchange at the maternal-fetal interface and thus prevents intrauterine growth restriction.

Angiogenic factors play an essential role in the regulation of placental vascular development and function (Redman, 2005; Young, 2010; Maynard, 2003). Placental hypoxia seems to be the insult leading to an excessive placental production of sFlt-1 (Maynard, 2003). This factor binds with high affinity the proangiogenic factors VEGF and PlGF in the maternal circulation, acting as a potent antagonist of their biological function. Our study provided the evidence that an increase in sFlt-1 levels during pregnancy was a primary event leading to the abnormal placentation and hypoxic

placenta. The etiology of increased sFlt-1 level in preeclampsia remains unknown; however there is evidence that lowered oxygen tension in cytothrophoblast culture and villous explants causes increased sFlt-1 expression suggesting that sFlt-1 expression may be stimulated by hypoxia (Ahmad, 2004). Furthermore, VEGF expression increases, but free VEGF and PlGF are low, which suggests that sFlt-1 exceeds VEGF production resulting in a net antiangiogenic effect.

In our study, excess of circulating sFlt-1 elicited the activation placental signaling pathways involved in oxygen hemostasis at the transcriptional level. Simultaneously, free VEGF plasma concentrations were depleted. Placental mRNA levels of HIF-1α and TGFβ3 were elevated, while GCM1 levels were decreased in sFlt-1 pregnant mice as compared with controls. These results agree with data reported in clinical studies. HIF-1α and TGFβ3 are over-expressed (Caniggia 2000), whereas placental GCM1 gene expression is decreased in villous explants from preeclamptic pregnancies (Chen, 2004; Chang, 2008). Thus, upregulation of HIF-1α and TGFβ3 as well as low GCM1 expression could be responsible of pathologic placental changes seen in preeclampsia including poor trophoblast differentiation and growth, and lack of remodelation of the spiral arteries. These factors also play an important role in placental development and function in mice. For example, the placenta from GCM1-deficient mice has a complete block of vessel branching in the chorioallantoic interface and chorionic trophoblast cells which do not fuse to form the syncytiotrophoblast (Anson-Cartwright, 2000).

VEGF-121 therapy not only reduced circulating sFlt-1 levels, but also replete plasma levels of free VEGF, enhancing proangiogenic actions of native VEGF in maternal endothelium and placental vascular bed. Transcriptional level of the study genes in sFlt-1 mice that received VEGF-121 was equivalent to the levels seen in the control group suggesting that this therapy may induce placental reoxygenation and repair

of damaged endothelium in placental blood vessels. This will be further study by examining the expression of these genes at the protein level using western blot and immunohistochemistry techniques. The aforementioned effects of VEGF-121 may have important clinical implications as it alleviates placental dysfunction and that can lead to improvement of oxygen and nutrient supply to the fetus reducing substantially perinatal and neonatal morbidity and mortality related to preeclampsia.

KIDNEY AND LIVER HISTOLOGY AND VEGF-121 THERAPY

High levels of circulating antiangiogenic substances like sFlt-1 seem to cause renal injury by depriving the glomerular endothelium of essential growth factors (Maynard, 2003). The classic renal lesion in preeclampsia is glomerular endotheliosis, which is demonstrated by the loss of glomerular endothelial cell fenestrae and glomerular endothelial swelling and proteinuria (Ballermann, 2007; Stillman, 2007). It has been known that VEGF plays an important role in the glomerular endothelial cell fenestrae formation and the reduced VEGF production by 50% in a mouse glomerulus could lead to glomerular endotheliosis and the loss of glomerular endothelial fenestrae (Eremina, 2003). Therefore, inhibitions of VEGF by sFlt-1 or neutralizing VEGF antibodies could result in glomerular injury (Sugimoto, 2003; Karumanchi, 2006). In previous studies of our lab, we showed findings of glomerular injury through histology examination of pregnancies that over-express sFlt-1. However, this effect has not seen consistently in all pregnancies injected with Adv-sFlt-1. Thus, Adv-sFlt-1 dose seems to be critical for induction of kidney injury. When we developed our model the optimal dose of Adv-sFlt1 was set at 10^9 PFU as it induced more closely signs of preeclampsia. Higher doses resulted in pregnancies losses and were lethal for the mother in many circumstances. Others agree with our observations. In a rat model of preeclampsia, Adv-sFlt-1 $\leq 1x10^9$

PFU had no effect on the kidney histology, whereas injection of Adv-sFlt-1 between 2.5-3.5x10^9 PFU induced moderate histochemical changes in the glomeruli (Bergmann, 2010). Doses between 5-7.5x10^9 PFU resulted in severe histological changes including severe endotheliosis and occlusion of capillary lumens. VEGF-121 therapy seems to have protective effects in the renal endothelium as histology exams of sFlt-1 pregnancies treated with VEGF-121 were unremarkable and comparable to controls. The variability seen in renal histology exams in our study may be related to the results obtained in the mRNA expression of the study genes in this organ. Although HIF-1α expression was higher and GCM1 was lower in the sFlt-1 mice than in the controls and VEGF-treated mice, no differences were noted on the TGFβ3 expression across the groups.

Abnormal liver function tests and hemolysis are common complications of preeclampsia. Thrombocytopenia, signs of hemolysis, and abnormal liver function tests characterize HELLP syndrome, a severe variant of preeclampsia, which was described for the first time in 1982 (Weinstein, 1982). Liver histological findings in women with HELLP syndrome include periportal hemorrhage and necrosis, fibrin microthrombi and fibrinogen deposits in the sinusoids deposition, and steatosis (Aarnoudse, 1986; Arias, 1995). The severity of the histological findings is associated with the degree of lab abnormalities and severe signs of the disease. We observed mild inflammatory changes and apoptotic bodies in the liver of sFlt-1 mice. These findings were no longer observed after treatment VEGF-121 suggesting that this therapy normalizes endothelial function and reduces the activation of inflammatory pathways in the maternal liver. We were, however, unable to induce severe pathology in the liver. Factors already discussed such as Adv-sFlt-1 dose, virus replication in the host liver, and availability of Adv-sFlt-1 in the circulation after the intravenous injection may explain our findings. However, other factors no examined in this study may play a role in the degree of liver damage induced

by antiangiogenic factors. In pregnant rats, overexpression of sEng amplifies the vascular damage mediated by sFlt-1 inducing a HELLP-like syndrome with severe liver damage and abnormal liver function tests (Venkatesha, 2006). Although, the combination of these two antiangiogenic factors may cause more drastic effects in animal models, the influence of sEng in preeclampsia and HELLP syndrome remains to be elucidated.

VEGF-121 AND FETAL VASCULAR PROGRAMMING

In recent years, we validated a mouse model of preeclampsia induced by overexpression of sFlt-1. In addition to the maternal effects, we demonstrated intrauterine growth abnormality and hypertension in the adult offspring born to pregnancies that over-express sFlt-1 (Lu, 2007b). Inhibition of VEGF and PlGF by sFlt-1 leads to inadequate vascular adaptations during pregnancy, alters the circulation at the utero-placental interface, and consequently causes poor perfusion of the placenta-fetal unit. These mal-adaptations lead to fetal hypoxemia and reduction in fetal perfusion, which can convey long lasting physiological and structural alterations that predispose the fetus to diseases in later life.

Our results provide new information on the role of sFlt-1 in inducing altered fetal vascular programming. We demonstrated that vascular programming is gender-selective affecting exclusively male offspring. Hypertension appeared early at 3 months of age and by 6 months male offspring had vascular dysfunction in addition to hypertension suggesting that vascular disease if a progressive process, in which clinical manifestations became to be evident early in the adulthood. In females, only mild signs of endothelial dysfunction were seen in the 6 months PBS-sFlt-1 offspring. Our findings are consistent with prior studies of altered uterine environment induced by manipulation of maternal diet. In these studies, fetal vascular programming and hypertension later in life were more

pronounced in male versus female offspring (Kwong, 2000; Ozaki, 2001). Thus, increased systolic blood pressure and abnormal organ/body mass ratios was noted in male rat offspring born to pregnant mothers on a low protein diet regimen during the pre-implantation period of development (Kwong, 2000). These investigators speculated that male pre-implantation embryos have a greater susceptibility to respond to the maternal environment and lower possibility to adapt and may consequently exhibit increased sensitivity to specific fetal programming effects. Gender-related hypertension was also demonstrated in another rat model in which dietary intake was manipulated to induce maternal under-nutrition. In this model, increased systolic, diastolic and mean arterial blood pressures were more pronounced in male rat offspring, and these abnormalities increased with age (Ozaki, 2001). In addition, prenatal glucocorticoids can program adulthood cardiovascular and metabolic physiology in a gender-specific pattern (O'Regan, 2004; McMullen, 2005; Roghair, 2009). Interestingly, hepatic LDL-receptor-mediated programming of altered lipid metabolism and aortic vascular dysfunction was found in the female offspring of mothers fed a high saturated fatty acid, while male offspring had only dyslipidemia (Chechi, 2009). Although, there are growing evidences to support gender sensitivity to fetal programming, the mechanisms are not completely understood. We speculate that this selective process may be related to protective effect by ovarian hormones, which are known to play a protective effect against cardiovascular diseases during the reproductive ages.

This study demonstrated that inhibition of sFlt-1 by VEGF-121 therapy reestablishes maternal vascular function, reduces placental hypoxia, and thereby improves intrauterine fetal growth. All of these effects combined probably resulted in a more optimal intrauterine environment for fetal development. The acute beneficial effects of VEGF-121 therapy are magnified by the fact that postnatal growth as well as BP and

vascular function during the adulthood remained normal and were comparable to parameters seen in the offspring born from non-preeclamptic pregnancies. These findings underscore the influence that intrauterine environment has in the development of fetal organs including the cardiovascular system which can be affected permanently and lead to hypertension and other cardiovascular diseases later in life.

In epidemiological studies low birth weight relates to with adult BP and the incidence of cardiovascular–related morbidity and mortality (Barker, 1986 and 1989). However, low birth may be the result of a series of pathologic events during fetal development that are manifested as altered organ structure and function or both later in life. Therefore, the mechanisms involved in fetal vascular programming may involved multiple pathways and affect critical organs that mediate vascular function and BP. The kidney is one of the most sensitive organs to the hostile intrauterine environment during critical periods of early fetal development (Bagby, 2007). In animal models of fetal programming as well as in humans, low birth weight is directly related with a reduction in nephron number (Hoy, 2005; Moritz, 2009). Nephron loss that occurs as a consequence of fetal insult is probably mediated by altered gene and protein function essential for a normal nephrogenesis (Abdel-Hakeem, 2008; Moritz, 2008). In the other hand, there is strong evidence from animal studies that inhibition of VEGF signaling pathway during pregnancy alter vascular structure development of several fetal organs including skin, pancreas, kidney, and lung (Wada, 2010). We believe that VEGF-121 therapy contributed to enhance normal fetal development in both organ function and structure.

Overall our findings highlight the role of VEGF therapy during pregnancy in ameliorating the adverse/hostile intrauterine environment that predispose to adult vascular disease using a mice model over-expressing sFlt-1 in the mother during

pregnancy. This therapy inhibits sFlt-1 and mediates proangiogenic actions in both maternal endothelium and placental vascular bed. Thereby, it may prevent altered fetal vascular programming as vascular disease and hypertension later in life were prevented in the male offspring born to VEGF-sFlt-1 mothers. This study also confirms the role played by the gender in the fetal programming of adult hypertension and vascular disease later in life. Importantly, signs of vascular dysfunction that became to be evident by 6 months of age in the female offspring born to preeclampsia-like pregnancies were also attenuated by prenatal VEGF-121 therapy.

CONCLUSIONS

In conclusion, our study demonstrated that exogenous VEGF reverses deleterious effects caused by sFlt-1, a key pathogenic factor in preeclampsia. This study provides new insights on the potential therapeutic effects of proangiogenic factors in preeclampsia. Therapeutic angiogenesis is a new and exciting development that promises relief from vascular diseases with impaired angiogenesis such as occlusive arterial disease. Interest in the area has been stimulated by recent encouraging results with antiangiogenic therapies in the treatment of cancers and advancing knowledge of the biology vascular growth. There is a growing body of evidence suggesting a potential clinical utility of VEGF therapy in prevention or treatment of preeeclampsia. The fact that VEGF therapy attenuated altered fetal vascular programming preventing vascular disease and hypertension during adulthood underscores the need for further studies to elucidate the signaling pathways that regulate endothelial function in pregnancy and fetal vascular development under physiological and pathological conditions. Further studies are also needed to elucidate the mechanisms that lead to selective fetal programming in our animal model of preeclampsia.

Appendix: List of Abbreviations

US	United States
NHBPEP	National high blood pressure education program
HLAs	Human lymphocyte antigens
NK	Natural killer
KIR	Killer-cell immunoglobulin-like
VEGF	Vascular endothelium growth factor
PlGF	Placental growth factor
sFlt-1	Soluble fms-like tyrosine kinase
AP-1	Activator protein-1
sEng	Soluble endoglin
TGFβ	Transforming growth factor-β
HELLP	Hemolysis, elevated liver enzymes, low platelets
HIF1α	Hypoxic-inducible factor 1alpha
VEGF-R1	Vascular endothelial growth factor receptor-1
RUPP	Reduced uterine perfusion pressure
NOS	Nitric oxide synthase
L-NAME	Nω-nitro-L-arginine methyl ester
HIF	Hypoxia inducible factor
TGFβ3	Transforming growth factor-beta 3
GCM1	Glial cells missing1
EDHF	Endothelium derived hyperpolarizing factor
ECM-1	Endothelial cell-specific leukocyte adhesion molecule
HUVEC	Human umbilical vein endothelial cell

VSMC	Vascular muscle cells
BP	Blood pressure
nNOS	Neural nitric oxide synthase
iNOS	Inducible nitric oxide synthase
eNOS	Endothelial nitric oxide synthase
NADPH	Nicotinamide-adenine-dinucleotide- oxidase-synthase
PGI_2	Prostacyclin
MLCK	Myosin light-chain kinase
TNFα	Tumor necrosis factor alpha
PPARα	Peroxisomal proliferator-activated receptor α
TxA2	Thromboxane
ET-1	Endothelin-1
AT_1R	Angiotensin receptor-1
AngII	Angiotensin II
FAK	Focal adhesion kinase
ERKs	Extracellular signal-regulated kinases
MAP	Mitogen activated protein
JNK	c-Jun-N-terminal protein kinase
CVD	Cardiovascular disease
IACUC	Animal care and use committee
SC	Subcutaneous
PBS	Phosphate buffered saline
Ach	Acetylcholine
PE	Phenylephrine
SNP	Sodium nitroprusside

EDTA	Ethylene diamine tetra acetic acid
AUC	Area Under the concentration curve
CI	Confidence interval
cAMP	cyclic adenosine monophosphate
SBP	Systolic blood pressure
MBP	Mean blood pressure
DBP	Diastolic blood pressure
cGMP	cyclic guanosine monophosphate
RT-PCR	reverse transcription polymerase chain reaction
Emax	Maximal effect

REFERENCES

Aarnoudse JG, Houthoff HJ, Weits J, Vellenga E, Huisies HJ. A syndrome of liver damage and intravascular coagulation in the last trimester of normotensive pregnancy. A clinical and histopathological study. *Br J Obstet Gynaecol.* 1986, 93(2):145-155.

Abdel-Hakeem AK, Henry TQ, Magee TR, Desai M, Ross MG, Mansano RZ, Torday JS, Nast CC. Mechanisms of impaired nephrogenesis with fetal growth restriction: altered renal transcription and growth factor expression. *Am J Obstet Gynecol.* 2008,199(3):252.e 1-7.

Abedi H, Zachary I. Cytochalasin D stimulation of tyrosine phosphorylation and phosphotyrosine-associated kinase activity in vascular smooth muscle cells. *Biochem Biophys Res Commun.* 1998, 245(3):646-650.

Ahmad S, Ahmed A. Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. *Circ Res.* 2004, 95(9):884-891.

Aird WC. 2005. Endothelial Cell in Health and Disease. Boca Raton: Taylor & Francis Group.

Alexander, BT., Placental insufficiency leads to development of hypertension in growth-restricted offspring. *Hypertension.* 2003, 41(3):457-462.

Allison F Jr, Smith MR, Wood WB Jr. Studies on the pathogenesis of acute inflammation. The inflammatory reaction to thermal injury as observed in the rabbit ear chamber. *J Exp Med.* 1955, 102(6):655-668.

Arias F, Mancilla-Jimenez R. Hepatic fibrinogen deposits in pre-eclampsia. Immunoflourescent evidence. *N Engl J Med.* 1976, 295(11):578-582.

Arnadottir GA, Geirsson RT, Arngrimsson R, Jonsdottir LS, Olafsson O. Cardiovascular death in women who had hypertension in pregnancy: a case-control study. *BJOG*.2005, 112(3):286-292.

Bagby SP. Maternal nutrition, low nephron number, and hypertension in later life: pathways of nutritional programming. *J Nutr.* 2007,137:1066-1072.

Ballermann, BJ., Contribution of the endothelium to the glomerular permselectivity barrier in health and disease**.** *Nephron Physiol.* 2007, 106(2):19-25.

Bamberger AM, Bamberger CM, Aupers S, Milde-Langosch K, Loning T, Makrigiannakis A. Expression patter of the activating protein-1 family of transcription factors in the human placenta. *Mol Hum Reprod.* 2004, 10(4):223-228.

Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet.* 1986, 1(8489):1077-1081.

Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ.* 1989, 298(6673):564-567.

Barton JR, Sibai BM. Gastrointestinal complications of pre-eclampsia. *Semin Perinatol.* 2009, 33(3): 179-188.

Basso O, Rasmussen S, Weinberg CR, Wilcox AJ, Irgens LM, Skjaerven R. Trends in fetal and infant survival following preeclampsia. *Jama*.2006, 296(11):1357-1362.
Bdolah Y, Sukhatme VP, Karumanchi SA. Angiogenic imbalance in the pathophysiology of preeclampsia: newer insights. *Semin Nephrol.* 2004, 24: 548-556.

Benediktsson R, Lindsay RS, Noble J, Seckl JR, Edwards CR. Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet.* 1993, 341: 339–341.
Benigni A, Gregorini G, Frusca T, Chiabrando C, Ballerini S, Valcamonico A, Orisio S, Pinciroli V, Fanelli R et al. Effect of low-dose aspirin on fetal and maternal generation of thromboxane by platelets in women at risk for pregnancy-induced hypertension. *N Engl J Med*. 1989, 321(6):357-362.
Bergmann A, Ahmad, Cudmore M, Gruber AD, Wittschen P, Lindenmaier W, Christofori G, Gross V, Gonzalves Ach, Gröne HJ, Ahmed A, Weich HA. Reduction of circulating soluble Flt-1 alleviates preeclampsia-like symptoms in a mouse model. *J Cell Mol Med*. 2010, 14(6B):1857-1867.
Bernardi F, Constantino L, Machado R, Petronilho F, Dal-Pizzol F. Plasma nitric oxide, endothelin-1, arginase and superoxide dismutase in pre-eclamptic women. *J Obstet Gynaecol Res.* 2008, 34(6):957-963.
Block BS, Schlafer DH, Wentworth RA, Kreitzer LA, Nathanielsz PW. Intrauterine growth retardation and the circulatory responses to acute hypoxemia in fetal sheep. *Am J Obstet Gynecol.* 1989, 161(6 Pt 1):1576-1579.
Block BS, Schlafer DH, Wentworth RA, Kreitzer LA, Nathanielsz PW. Regional blood flow distribution in fetal sheep with intrauterine growth retardation produced by decreased umbilical placental perfusion. *J Dev Physiol.* 1990, 13(2):81-85.

Bredt, DS, Snyder, SH. Isolation of nitric oxide synthase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci.* 1990, 87(2): 682–685.
Brosens IA, Robertson WB, Dixon HG. The physiological response of the vessels of the placental bed to normal pregnancy. *J Pathol Bacteriol.* 1967, 93(2):569-579.
Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. *Obstet Gynecol Annu*.1972;1:177-191.
Brown MA. The physiology of pre-eclampsia. *Clin Exp Pharmacol Physiol.* 1995, 22(11):781-791.

Caniggia I, Winter J, Lye SJ, Post M. Oxygen and placental development during the first trimester: implications for the pathophysiology of pre-eclampsia. *Placenta.* 2000, 21(Suppl A): S25–30.

Carvajal JA, Germain AM, Huidobro-Toro JP, Weiner CP. Molecular mechanism of cGMP-mediated smooth muscle relaxation. *J Cell Physiol.* 2000, 184(3):409-420.
Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature.* 1996, 380(6573):435-439.
Cartwright JE, Fraser R, Leslie K, Wallace AE, James JL. Remodeling at the maternal-fetal interface: relevance to human pregnancy disorders. *Reproduction.*2010, 140:803-813.

Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Mee Kim Y, Goncalves LF, Gomez R, Edwin S. *Am J Obstet Gynecol.* 2004, 190(6):1541-1547.
Charnock-Jones DS, Sharkey AM, Boocock CA, Ahmed A, Plevin R, Ferrara N, Smith SK. Vascular endothelial growth factor receptor localization and activation in human trophoblast and choriocarcinoma cells *Biol Reprod*. 1994, 51(3):524-530.
Chappell LC, Seed PT, Briley AL, Kelly FJ, Lee R, Hunt BJ, Parmar K, Bewley SJ, Shennan AH, Steer PJ, Poston L. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomized trial. *Lancet.* 1999, 354(9181):810-816.
Chappell LC, Seed PT, Briley A, Kelly FJ, Hunt BJ, Charnock-Jones DS, Mallet AI, Poston L. A longitudinal study of biochemical variables in women at risk of preeclampsia. *Am J Obstet Gynecol.* 2002, 187(1):127-136.
Chechi K, McGuire JJ, Cheema SK. Developmental programming of lipid metabolism and aortic vascular function in C57BL/6 mice: a novel study suggesting an involvement of LDL-receptor. *Am J Physiol Regul Integr Comp Physiol.* 2009,296(4):R1029-R1040.
Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver JS, Wick TM, Konkle BA, Schwartz BS, Bernathan ES, McCrae KR, Schmidt A, Stern DM. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood.* 1998, 91(10):3527-3561.
Cingolani HE, Villa-Abrille MC, Cornelli M, Nolly A, Ennis IL, Garciarena C, Suburo AM, Torbidoni V, Correa MV, Camilionde Hurtado MC, Aiello EA. The positive inotropic effect of angiotensin II: role of endothelin-1 and reactive oxygen species. *Hypertension*. 2006, 47(4):727-734.
Clark BA, Halvorson L, Sachs B, Epstein FH. Plasma endothelin levels in preeclampsia: elevation and correlation with uric acid levels and renal impairment. *Am J Obstet Gynecol.* 1992, 166(3):962-968.
Clark SL, Belfort MA, Dildy GA, Herbst MA, Meyers JA, Hankins GD. Maternal death in the 12 st century: causes, prevention, and relationship to cesarean delivery. *Am J Obstet Gynecol.* 2008, 199 (1):36.e1-5.
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(2):205-213.
Cross JC, Werb Z, Fisher SJ. Implantation and the placenta: key pieces of the development puzzle. *Science.* 1994, 266(5190):1508-1518.
Dekker GA, Sibai BM. Low-dose aspirin in the prevention of preeclampsia and fetal growth retardation rationale. *Am J Obstet Gynecol.* 1993, 168 (1 Pt 1):214-227.
Dekker GA, Sibai BM. Etiology and pathogenesis of preeclampsia: current concepts. *Am J Obstet Gynecol.* 1998, 179(5):1359-1375.

Dunger DB, Petry CJ, Ong KK. Genetic variations and normal fetal growth. *Horm Res*. 2006, 65 Suppl 3:34-40.

Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest.* 2003, 111(5):707-716.

Esser S, Wolburg K, Wolburg H, Breier G, Kurzchalia T, Risau W. Vacular endothelial growth factor induces endothelial fenestrations in vitro. *J Cell Biol*. 1998, 140(4):947-959.
Fernando NH, Hurwitz HI. Inhibition of vascular endothelial growth factor in the treatment of colorectal cancer. *Semin Oncol.* 2003,30(Suppl6):39-50.
Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Re.* 1997, 18(1):4-25.
Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med.* 2003, 9(6):669-676
Forstermann U, Kleinert H. Nitric oxide synthase: expression and expressional control of the three isoforms. *Naunyn-Schmiedeberg's Arch Pharmac.*1995, 352(4): 351–364.
Fraser HM, Dickson SE, Lunn SF, Wulff C, Morris KD, Carroll VA, Bicknell R. Suppression of luteal angiogenesis in the primate after neutralization of vascular endothelial growth factor. *Endocrinology*. 2000, 141(3):995-1000.
Funai EF, Friedlander Y, Paltiel O, Tiram E, Xue X, Deutsch L, Harlap S. Long-term mortality after preeclampsia. *Epidemiology*.2005:16(2):206-215.
Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc Natl Acad Sci USA.* 1985, 82(24):8667-8671.
Gerber HP, Dixit V, Ferrara N. Vascular endothelial growth factor induces expression of the antiapototic proteins Bcl-2 and A1 in vascular endothelial cells. *J Biol Chem.* 1998, 273(21):13313-13316.
Giardina JB, Green GM, Rinewalt AN, Granger JP, Khalil RA. Role of endothelin B receptors in enhancing endothelium-dependent nitric oxide-mediated vascular relaxation during high salt diet. *Hypertension.* 2001, 37:516-523.
Gilbert JS, Verzwyvelt J, Colson D, Arany M, Karumanchi SA, Granger JP. Recombinant vascular endothelial growth factor 121 infusion lowers blood pressure and improves renal function in rats with placental ischemia-induced hypertension. *Hypertension.* 2010, 55(2):380-385.
Gimbrone MA. 1980. Endothelial dysfunction and the pathogenesis of atherosclerosis. New York: Springer-Verlag.
Harskamp RE, Zeeman GG. Preeclampsia: at risk for remote cardiovascular disease. *Am J Med Sci.*2007, 334(4):291-295.
Hauth JC, Ewell MG, Levine RL, Esterlitz JR, Sibai BM, Curet LB. Pregnancy outcomes in healthy nulliparous women who subsequently developed hypertension. *Obstet Gynecol.* 2000, 95(1):24-28.
Hayashi T, Yamada K, Esaki T, Mutoh E, Iguchi A. Effect of estrogen on isoforms of nitric oxyde synthase: Possibel mechanism of anti-atherosclerotic effect of estrogen. *Gerontology.* 1997, 43 (suppl1):24-34.
Henao DE, Saleem MA, Cadavid AP. Glomerular disturbances in preeclampsia: disruption between glomerular endothelium and podocyte symbiosis. *Hypertens Pregnancy.* 2010, 29(1):10-20.

Henriksen T, Clausen T. The fetal origins hypothesis: placental insufficiency and inheretence versus maternal malnutrition in well-nourished populations. *Acta Obstet Gynecol Scand*. 2002, 81(2):112-114.

Henry TD, Annex BH, McKendall GR, Azrin MA, Lopez JJ, Giordano FJ, Shah PK, Willerson JT, Benza RL, Berman DS, Gibson CM, Bajamonde A, Rundle AC, Fine J, McCluskey ER; VIVA investigators. The VIVA trial: Vascular endothelial growth factor in ischemia for vascular angiogenesis. *Circulation*. 2003, 107(10):1359-1365.

Hertig A, Berkane N, Lefevre G, Toumi K, Marti HP, Capeau J, Uzan S, Rondeau E Maternal serum sFlt1 concentration is an early and reliable predictive marker of preeclampsia. *Clin Chem*. 2004, 50(9):1702-1703.

Hiby SE, Walker JJ, O'shaughnessy KM, Redman CW, Carrington M, Trowsdale J, Moffett A. Combinantions of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reporductive success. *J Exp Med*. 2004, 200(8):957-965.

Hirtenlehner K, Pollheimer J, Lichtenberger C, Wolschek MF, Zeisler H, Husslein P, Knofler M. Elevated serum concentrations of the angiogenesis inhibitor endostatin in preeclamptic women. *J Soc Gynecol Investig*. 2003, 10(7):417-417.

Hofmeyr GJ, Lawrie TA, Atallah AN, Duley L. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *Cochrane Database Syst Rev*. 2010, (8):CD001059.

Holzmann S. Endothelium-induced relaxation by acetylcholine associated with larger rises in cyclic GMP in coronary arterial strips. *J Cyclic Nucl Res*. 1982, 8(6): 409-419.

Hoy WE, Rees M, Kile E, Mathews JD, Wang Z. A new dimension to the Barker hypothesis: how birth weight and susceptibility to renal disease. *Kidney Int*. 1999, 56: 1072-1077.

Hoy WE, Hughson MD, Bertram JF, Douglas-Denton R, Amann K. Nephron number, hypertension, renal disease, and renal failure. *J Am Soc Nephrol*. 2005,16(9):2557-2564.

Hubel CA, McLaughlin MK, Evans RW, Hauth BA, Sims CJ, Roberts JM. Fasting serum triglycerides, free fatty acids, and malondialdehyde are increased in preeclampsia, are positively correlated, and decrease within 48 hours post partum. *Am J Obstet Gynecol*. 1996, 174(3):975-982.

Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. *Proc Soc Exp Biol Med*. 1999, 222:222-235

Hustin J, Foidart JM, Lambotte R. Maternal vascular lesions in pre-eclampsia and intrauterine growth retardation: light microscopy and immunofluorescence. *Placenta* 1983, 4 Spec No: 489-498.

Hurairah H, Ferro A. The role of the endothelium in the control of vascular function. *Int J Clin Pract*. 2004, 58(2):173-183.

Ignarro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharmacol*. 1999, 34(6):879-886.

Iranami H, Hatano Y, Tsukiyama Y, Maeda H, Mizumoto K. A beta-adrenoceptor agonist evokes a nitric oxide-cGMP relaxation mechanism modulated by adenylyl cyclase in rat aorta. Halothane does not inhibit this mechanism. *Anesthesiology.* 1996, 85(5):1129-1138.

Irgens HU, Reisaeter L, Irgens LM, Rie RT. Long term mortality of mothers and fathers after preeclampsia: Population based cohort study. *BMJ*.2001, 323(7323):1213-1217.

James JL, Stone PR, Chamley LW. The regulation of trophoblast differentiation by oxygen in the first trimester of pregnancy. *Hum Reprod Update*.2006, 12(2):137-144.

Karumanchi SA, Stillman IE. In vivo rat model of preeclampsia. *Methods Mol Med.* 2006, 122: 393-399.

Kearney JB, Ambler CA, Monaco KA, Johnson N, Rapoport RG, Bautch VL. Vascular endothelial growth factor receptor Flt-1 negatively regulates developmental blood vessel formation by modulating endothelial cell division. *Blood.* 2002, 99(7):2397-2407.

Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc. Natl. Acad. Sci. USA* 1993, 90(22)*:* 10705-10709.

Kendall R L, Wang G, Thomas K A. 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(2)*:* 324-328.

Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *Br J Obstet Gynaecol.* 1986, 93(10):1049-1059.

Knofler M. Critical growth factors and signaling pathways controlling human trophoblast invasion. *Int J Dev Biol.* 2010, 54 (2-3): 269-280.

Kourembanas S, McQuillan LP, Leung GK, Faller DV. Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia. *J Clin Invest.* 1993;92:99-104.

Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development.* 2000, 127(19): 4195-4202.

Langille BL, O'Donnell F. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science.* 1986, 231(4736):405–407.

Langley-Evans SC, Welham SJ, Jackson AA. Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. *Life Sci.* 1999,64(11):965-974.

Ledingham JM, Laverty R. Effects of nitric oxide synthase inhibition and low-salt diet on blood pressure and mesenteric resistance artery remodeling in genetically hypertensive rats. *Clin Exp Pharmacol Physiol.* 2001, 28: 761–763.

Levine RJ, Hauth JC, Curet LB, Sibai BM, Catalano PM, Morris CD, DerSimonian R, Esterlitz JR, Raymond EG, Bild DE, Clemens JD, Cutler JA. Trial of calcium to prevent preeclampsia. *N Engl J Med.* 1997, 337(2):69-76.

Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*. 2004, 350(7):672-683.

Li Z, Zhang Y, Ying Ma J, Kapoun AM, Shao Q, Kerr I, Lam A, O'Young G, Sannajust F, Stathis P, Schreiner G, Karumanchi SA, Protter AA, Politt NS. Recombinant vascular endothelial growth factor 121 attenuates hypertension and improves kidney damage in a rat model of preeclampsia. *Hypertension*. 2007, 50(4):686-692.

Liu A, Dardik A, Ballermann BJ. Neutralizing TGF-beta1 antibody infusion in neonatal rat delays in vivo glomerular capillary formation 1. *Kidney Int.* 1999, 56(4):1334-1348.

Liu J, Chakraborty C, Graham CH, Barbin YP, Dixon SJ, Lala PK. Noncatalytic domain of uPA stimulates human extravillous trophoblast migration by using phospholipase C, phosphatidylinositol 3-kinase and mitogen-activate protein kinase. *Exp Cell Res*. 2003, 286(1):138-151.

Longo M, Jain V, Langenveld J, Vedernikov YP, Bukowski R, Anderson GD, Garfield RE, Saade GR. Embryo transfer prevents development of abnormal vascular function in offspring of endothelial nitric oxide synthase knockout mice. *J Soc Gynecol Investig*. 2004, 11(2 Suppl 1): 294A.Abstract.

Longo M, Jain V, Vedernikov YP, Bukowski R, Garfield RE, Hankins GD, Anderson GD, Saade GR. Fetal origins of adult vascular dysfunction in mice lacking endothelial nitric oxide synthase. *Am J Physiol Regul Integr Comp Physiol.* 2005, 288(5):R1114-1121.

Lu F, Longo M, Tamayo E, Maner W, Al-Hendy A, Anderson GD, Hankins GDV, Saade GR. The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. *Am J Obstet Gynecol.* 2007a, 196(4):396.e1-7.

Lu F, Bytautiene E, Tamayo E, Gamble P, Anderson GD, Hankins GDV, Longo M, Saade GR. Gender-specific effect of overexpression of sFlt-1 in pregnant mice on fetal programming of blood pressure in the offspring later in life. *Am J Obstet Gynecol.* 2007b, 197:418.e1-481.e5.

Lückhoff A, Busse R. Increased free calcium in endothelial cells under stimulation with adenine nucleotides. *J Cell Physiol*. 1986, 126(3):414-420.

Lunell NO, Lewander R, Mamoun I, Nylund L, Sarby S & Thornstrom S. Uteroplacental blood flow in pregnancy induced hypertension. *Scand J Clin Lab Invest Suppl*. 1984, 169: 28-35.

Mack CA, Magovern CJ, Budenbender KT, Patel SR, Schwarz EA, Zanzonico P, Perris B, Sanborn T, Isom P, Ferris B, Sanborn T, Isom OW, Crystal RG, Rosengart TK. Salvage angiogenesis induced by adenovirus-mediated gene transfer of vascular endothelial growth factor protects against ischemic vascular occlusion. *J Vasc Surg*. 1998, 27(4):699-709.

Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG. Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. *Proc Natl Acad Sci U S A.* 1991, 88 (20):9267-9271.
Magnussen EB, Vatten LJ, Smith GD, Romundstad PR. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. *Obstet Gynecol.*2009, 114:961-970.
Maharaj AS, Saint-Geniez M, Maldonado AE, D'Amore PA. Vascular endothelial growth factor localization in the adult. *Am J Pathol*. 2006, 168(2):639-648.

Makris A, Thornton C, Thompson J, Thomson S, Martin R, Ogle R, Waugh R, McKenzie P, Kirwan P, Hennessy A. Uteroplacental ischemia results in proteinuric hypertension and elevated sFlt-1. *Kidney Int.* 2007, 71(10):977-984.
Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Mathews TJ, Kirmeyer S, Osterman MJ. *Natl Vital Stat Rep*.2010, 58(24):1-85.
Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest.* 2003, 111(5):649-658.

McKeeman GC, Ardill JE, Caldwell CM, Hunter AJ, McClure N. Soluble vascular endothelial growth factor receptor-1 (sFlt-1) is increased throughout gestation in patients who have preeclampsia develop. *Am J Obstet Gynecol*. 2004,191 (4):1240-1246.

McMullen S, Langley-Evans SC. Sex-specific effects of prenatal low-protein and carbenoxolone exposure on renal angiotensin receptor expression in rat *Hypertension.* 2005, 46(6):1374-1380.

Mizutani S, Tomodak Y. Effects of placental proteases on maternal and fetal blood pressure in normal pregnancy and preeclampsia. *Am J Hypertens.* 1996,9(6):591-597.
Moffett-King A. Natural killer cells and pregnancy. *Nat Rev Immunol.* 2002, 2(9):656-663.
Molnar M, Suto T, Toth T, Hertelendy F. Prolonged blockade of nitric oxide synthesis in gravid rats produces sustained hypertension, proteinuria, thrombocytopenia, and intrauterine growth retardation. *Am J Obstet Gynecol.* 1994, 170(5 Pt 1):1458-1466.

Moncada S, Vane JR. Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls. *N Engl J Med.* 1979, 300(20):1142-1147.
Moore Simas TA, Crawford SL, Solitro MJ, Frost SC, Meyer BA, Maynard SE. Angiogenic factors for the prediction of preeclampsia in high-risk women. *Am J Obstet Gynecol.*2007;197(3):244.e1-8.
Moritz KM, Singh RR, Probyn ME, Denton KM. Developmental programming of a reduced nephron endowment: more than just a baby's birth weight. *Am J Physiol Renal Physiol.* 2009,296(1):F1-9.
Moritz KM, Wintour EM, Black MJ, Bertram JF, Caruana G. Factors influencing mammalian kidney development: implications for health in adult life. *Adv Anat Embyol Cell Biol*. 2008,196:1-78.

Murotsuki J, Challis JR, Han VK, Fraher LJ, Gagnon R. Chronic fetal placental embolization and hypoxemia cause hypertension and myocardial hypertrophy in fetal sheep. *Am J Physiol*. 1997, 272(1 Pt 2):R201-207.

Nagamatsu T, Fujii T, Kusumi M, Zou L, Yamashita T, Osuga Y, Momoeda M, Kozuma S, Taketani Y. 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(11):4838-4845.

Naicker T, Khedun SM, Moodley J, Pijnenborg R. Quantitative analysis of trophoblast invasion in preeclampsia. *Acta Obstet Gynecol Scand*. 2003, 82:722-729.

Nanaev A, Chwalisz K, Frank HG, Kohnen G, Hegele-Hartung C, Kaufmann P. Physiological dilation of uteroplacental arteries in the guinea pig depends on nitric oxide synthase activity of extravillous trophoblast. *Cell Tissue Res*. 1995, 282(3): 407–421.

Napoli C, Ignarro LJ. Nitric oxide and atherosclerosis. *Nitric Oxide*. 2001, 5(2):88-97.

Neufeld G, Cohen T, Gitay-Goren H, Poltorak Z, Tessler S, Sharon R, Gengrinovitch S, Levi BZ. Similarities and differences between the vascular endothelial growth factor (VEGF) splice variants. *Cancer Metastasis Rev*. 1996, 15(2):153-158.

Odegård RA, Vatten LJ, Nilsen ST, Salvasen KA, Austgulen R. Preeclampsia and fetal growth. *Obstet Gynecol.*2000, 96(6):950-955.

Øglaend B, Forman M, Romundstad PR, Nilsen ST, Vatten L. Blood pressure in early adolescence in the offspring of preeclamptic and normotensive pregnancies. *J Hypertens*. 2009, 27:2051-2054.

Olsen SF, Secher NJ, Tabor A, Weber T, Walker JJ, Gluud C. Randomised clinical trials of fish oil supplementation in high risk pregnancies. Fish Oil Trials In Pregnancy (FOTIP) Team. *BJOG*. 2000, 107(3):382-395.

O'Regan D, Kenyon CJ, Seckl JR, Holmes MC Glucocorticoid exposure in late gestation in the rat permanently programs gender-specific differences in adult cardiovascular and metabolic physiology. *Am J Physiol Endocrinol Metab*. 2004, 287(5):E863-870.

Ozaki T, Nishina H, Hanson MA, Poston L. Dietary restriction in pregnant rats causes gender-related hypertension and vascular dysfunction in offspring. *J Physiol*. 2001, 530(Pt 1):141-152.

Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 1987, 327(6122):524–526.

Parham P. Influence of KIR diversity on human immunity. *Adv Exp Med Biol.*2005, 560:47-50.

Patrono C. Aspirin as an antiplatelet drug. *N Engl J Med*. 1994, 330(18):1287-1294.

Petrie L, Duthie SJ, Rees WD, McConnell JM. Serum concentrations of homocysteine are elevated during early pregnancy in rodent models of fetal programming. *Br J Nutr*. 2002, 88(5):471-477.

Pijnenborg R, Luyten C, Vercruysse L, Van Assche FA. Attachment and differentiation in vitro of trophoblast from normal and preeclamptic human placentas. *Am J Obstet Gynecol*. 1996, 175(1):30-36.

Pober JS, Bevilacqua MP, Mendrick DL, Lapierre LA, Fiers W, Gimbrone MA Jr,. Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human endothelial cells. *J Immunol*. 1986, 136(5):1680-1687.

Posey JA, Ng TC, Yang B, Khazaeli MB, Carpenter MD, Fox F, Needle M, Waksal H, LoBuglio AF. A phase I study of anti-kinase insert domain-containing receptor antibody, IMC-1C11, in patients with live metastases from colorectal carcinoma. *Clin Cancer Res.* 2003, 9(4):1323-1332.

Poston L, McCarthy AL, Ritter JM. Control of vascular resistance in the maternal and feto-placental arterial beds. *Pharmacol Ther*. 1995, 65(2):215-239.

Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH; Vitamins in pre-eclampsia (VIP) trial consortium. Vitamins C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomized placebo-controlled trial. *Lancet.* 2006, 367(9517):1145-1154.

Pussard G, Gascard JP, Gorenne I, Labat C, Norel X, Dulmet E, Brink C. Endothelin-1 modulates cyclic GMP production and relaxation in human pulmonary vessels. *J Pharmacol Exp Ther*. 1995, 274(2):969-975.

Rabelink TJ, Luscher TF. Endothelial nitric oxide synthase: host defense enzyme of the endothelium. *Arterioscler Thromb Vasc Biol*. 2006;26(2):267-271.

Rajagopalan S, Shah M, Luciano A, Crystal R, Nabel EG. Adenovirus-mediated gene transfer of VEGF(121) improves lower-extremity endothelial function and flow reserve. *Circulation*.2001, 104(7):753-755.

Rajakumar A, Whitelock KA, Weissfeld LA, Daftary AR, Markovic N, Conrad, KP. Selective overexpression of the hypoxia-inducible transcription factor, HIF-2alpha, in placentas from women with preeclampsia. *Biol Reprod*. 2001, 64(2):499–506.

Redman CW, Roberts JM. Management of pre-eclampsia. *Lancet.* 1993, 341 (8858):1451-1454.

Redman CW. Current topic: pre-eclampsia and the placenta. *Placenta*. 1991, 12(4):301-308.

Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science.* 2005, 308 (5728):1592-1594.

Renaud SJ, Graham CH. The role of macrophage in utero-placental interactions during normal and pathological pregnancy. *Immunol Invest.*2008, 37(5):535-564.

Roghair RD, Segar JL, Volk KA, Chapleau MW, Dallas LM, Sorenson AR, Scholz TD, Lamb FS. Vascular nitric oxide and superoxide anion contribute to sex-specific programmed cardiovascular physiology in mice. *Am J Physiol Regul Integr Comp Physiol.* 2009,296(3):R651-R662.

Roberts JM, Pearson G, Cutler J, Lindheimer M; NHLBI Working Group on Research on Hypertension During Pregnancy. Summary of the NHLBI Working Group on Research on Hypertension During Pregnancy. *Hypertension.*2003, 41(3):437-445.

Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol.*2000, 183:S1-S22.

RobertsJM, Myath L, Spong CY, Thom EA, Hauth JC, Leveno KJ, Pearson GD, Wapner RJ, Varner MW, Thorp Jr JM, Mercer BM, Peaceman AM, Ramin SM, Carpenter MW, Samuels P, Sciscione A, Harper M, Smith WJ, Saade G, Sorokin Y, Anderson G; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *N Engl J Med*. 2010;362:1281-1291.

Roggensack AM, Zhang Y, Davidge ST. Evidence for peroxynitrite formation in the vasculature of women with preeclampsia. *Hypertension.* 1999,33:83-89.

Rosenfeld CR, Cox BE, Roy T, Magness RR. Nitric oxide contributes to estrogen-induced vasodilation of the ovine uterine circulation. *J Clin Invest.* 1996, 98(9):2158-2166.

Rotmensch S, Liberati M, Luo JS, Kliman HJ, Gollin Y, Bellati U, Hobbins JC, Copel JA. Color Doppler flow patterns and flow velocity waveforms of the intraplacental fetal circulation in growth-retarded fetuses. *Am J Obstet Gynecol.* 1994, 171(5):1257-1264.

Rubanyi GM, Vanhoutte PM. Nature of endothelium-derived relaxing factor: are there two relaxing mediators? *Circ Res*. 1987, **61**(5 Pt 2):II61-67.

Rudic RD, Shesely EG, Maeda N, Smithies O, Segal SS, Sessa C. Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. *J Clin Invest* 1998, 101: 731–736.

Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS;ACTS Study Group. *N Engl J Med*. 2006, 354(17):1796-1806.

Sato I, Murota S. Paracrine function of endothelium-derived nitric oxide. *Life Sci* 1995, 56(13):1079-1087.

Saito S, Sakai M, Sasaki Y, Nakashima A, Shiozaki A. Inadequate tolerance induction may induce preeclampsia. *J Reprod Immunol.* 2007, 76(1-2):30-39.

Salahuddin S, Lee Y, Vadnais M, Sachs BP, Karumanchi SA, Lim KH. Diagnostic utility of soluble fms-like tyrosine kinase 1 and soluble endogling in hypertensive diseases of pregnancy. *Am J Obstet Gynecol* 2007;197(1):28.e1-6.

Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. *Lancet.* 2003, 361(9368):1511-1517.

Schleimer RP, Rutledge BK. Cultured human vascular endothelial cells acquire adhesiveness for neutrophils after stimulation with interlukin 1, endotoxin, and tumor-promoting phorbol diesters. *J Immunol.* 1986, 136(2):649-654.

Secher NJ, Olsen SF. Fish-oil and pre-eclampsia. *Br J Obstet Gynaecol.* 1990, 97(12):1077-1079.

Semenza GL. HIF-1 and human disease: one highly involved factor. *Genes and development.* 2000, 14(16):1983-1991.

Shanklin DR, Sibai BM. Ultrastructural aspects of preeclampsia. I. Placental bed and uterine boundary vessels. *Am J Obstet Gynecol.* 1989, 161(3):735-741.

Sibai BM, el-Nazer A, Gonzalez-Ruiz A. Severe preeclampsia-eclampsia in Young primigravid women: subsequent pregnancy outcome and remote prognosis. *Am J Obst Gynecol.*1986, 155(5):1011-1016

Sibai BM. Diagnosis and management of gestational hypertension and preeclampsia. *Obstet Gynecol.*2003, 102(1):181-92.

Sibai BM, Gordon T, Thom E, Caritis SN, Klebanoff M, McNellis D, Paul RH. Risk factors for preeclampsia in healthy nulliparous women: a prospective multicenter study, The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *Am J Obstet Gynecol.*1995:172(3 Pt 1):642-648.

Schiff E, Peleg E, Goldenberg M, Rosenthal T, Ruppin E, Tamarkin M, Barkai G, Ben-Baruch G, Yahal I, Blankstein J, et al. The use of aspirin to prevent pregnancy-induced hypertension and lower the ration of thromboxane A2 to prostacyclin in relatively high risk pregnancies. *N Engl J Med.* 1989, 321(6):351-356.

Singer HA, Peach MJ. Calcium- and endothelial-mediated vascular smooth muscle relaxation in rabbit aorta. *Hypertension.* 1982, 4(3 Pt 2):19-25.

Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 1995, 376(6535):62-66.

Sheppard SJ, Khalil RA. Risk factors and mediators of the vascular dysfunction associated with hypertension in pregnancy. *Cardiovasc Hematol Disord Drug Targets.* 2010, 10(1):33-52.

Sørensen JD, Olsen SF, Pedersen AK, Boris J, Secher NJ, Fitzgerald GA. Effects of fish oil supplementation in the third trimester of pregnancy on prostacyclin and thromboxane production. *Am J Obstet Gynecol.* 1993, 168(3 Pt 1):915-922.

Spinnato JA 2nd, Freire S, Pinto E Silva JL, Cunha Rudge MV, Martins-Costa S, Koch MA, Goco N, Santos Cde B, Cecatti JG, Costa R, Ramos JG, Moss N, Sibai BM. *Obstet Gynecol.* 2007, 110(6):1311-1318.

Stillman IE, Karumanchi SA. The glomerular injury of preeclampsia. *L Am Soc Nephrol.* 2007, 18(8):2281-2284.

Sugimoto H, Hamano Y, Charytan D, Cosgrove D, Kieran M, Sudhakar A, Kalluri R. Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. *J Biol Chem.* 2003, 278(15):12605-12608.

Taylor RN, Varma M, Teng NN, Roberts JM. Women with preeclampsia have higher plasma endothelin levels than women with normal pregnancies. *J Endocrinol Metab.* 1990, 71:1675-1677.

Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, Ecker J, Karumanchi SA.. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *J Clin Endocrinol Metab.* 2004, 89(2):770-775.

Trovati M, Massucco P, Mattiello L, Cavalot F, Mularoni E, Hahn A, Anfossi G. Insulin increases cyclic nucleotide content in human vascular smooth muscle cells: a mechanism potentially involved in insulin-induced modulation of vascular tone. *Diabetologia.* 1995, 38(8):936-941.

Trovati M, Massucco P, Mattiello L, Cavalot F, Mularoni EM, Hahn AW, Anfossi G. Studies on the influence of insulin on cyclic adenosine monophosphate in human vascular smooth muscle cells: dependence on cyclic guanosine monophosphate and modulation of catecholamine effects. *Diabetologia*. 1996, 39(10):1156-1164.

Vappatalo H, Mervaala E. Clinically important factors influencing endothelial function. *Med Sci Monit*.2001, 7)5):1075-1085.

VanWijk MJ, Kublickiene K, Boer K, VanBavel E. Vascular function in preeclampsia. *Cardiovasc Res*. 2000, 47(1):38-48.

Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Selke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med*. 2006, 12(6):642-649.

Villar J, Abalos E, Nardin JM, Merialdi M, Carroli G. Strategies to prevent and treat preeclampsia: evidence from randomized controlled trials. *Semin Nephrol.* 2004, 24(6):607-615.

Villar J, Abdel-Aleem H, Merialdi M, Mathai M, Ali MM, Zavaleta N, Purwar M, Hofmeyr J, Nguven TN, Compodonico L, Landoulsi S, Carroli G, Lindheimer M: World Health Organization Calcium Supplementation for the Prevention of Preeclampsia Trial Group. *Am J Obstet Gynecol.* 2006, 194(3):639-649.

Villar J, Purwar M, Merialdi M, Zavaleta N, Thi Nhu Ngoc N, Anthony J, De Greeff A, Poston L, Shennan A; Who Vitamin C and Vitamin E trial group. World health organization multicentre randomized trial of supplementation with vitamins C and E among pregnant women at high risk for pre-eclampsia in populations of low nutritional status from developing countries. *BJOG*. 2009, 116(6):780-800.

Vuorela P, Helske S, Hornig C, Alitalo K, Weich H, Halmesmaki E. Amniotic fluid—soluble vascular endothelial growth factor receptor-1 in preeclampsia. *Obstet Gynecol*. 2000, 95(3):353-357.

Wallenburg HC, Dekker GA, Makovitz JW, Rotmans P. Low-dose aspirin prevents pregnancy-induced hypertension and pre-eclampsia in angiotensin-sensitive primigravidae. *Lancet.* 1986, 1(8471):1-3.

Wathén KA, Tuutti E, Stenman UH, Alfthan H, Halmesmäki E, Finne P, Ylikorkala O, Vuorela P. Maternal serum-soluble vascular endothelial growth factor receptor-1 in early pregnancy ending in preeclampsia or intrauterine growth retardation. *J Clin Endocrinol Metab*. 2006, 91(1):180-184.

Weigand L, Sylvester JT, Shimoda LA. Mechanisms of endothelin-1 induced contraction in pulmonary arteries from chronically hypoxic rats. *Am J Physiol Lung Cell Mol Physiol.* 2006, 290(2):L284-L290.

Weinstein L. Syndrome of hemolysis, elevated liver enzymes, and low platelet count: a severe consequence of hypertension in pregnancy. *Am J Obstet Gynecol.* 1982, 142(2):159-167.

Wesselman JP, De Mey JG. Angiotensin and cytoskeletal proteins: role in vascular remodeling. *Curr Hypertens Rep.* 2002,4:63-70.

Widdowson EM, McCance RA. A review: new thoughts on growth. *Pediatr Res.* 1975, 9:154-156.

Wieczorek KM, Brewer AS, Myatt L. Shear stress may stimulate release and action of nitric oxide in the human-placental vasculature. *Am J Obstet Gynecol.* 1995, 173 (3):708-713.

Wiktor H, Kankofer M, Schmerold I, Dadak A, Lopucki M, Niedermuller H. Oxidative DNA damage in placentas from normal and pre-eclamptic pregnancies. *Virchows Arch.* 2004, 445(1):74-78.

White RE, Darkow DJ, Lang JL. Estrogen relaxes coronary arteries by opening BKCa channels through a cGMP-dependent mechanism. *Circ Res.* 1995, 77(5):936-942.

Winquist RJ, Bunting PB, Schofield TL. Blockade of endothelium-dependent relaxation by the amiloride analog dichlorobenzamil: possible role of Na+/Ca++ exchange in the release of endothelium-derived relaxant factor. *J Pharmacol Exp Ther.* 1985, 235(3):644-650.

Wolinsky H. A proposal linking clearance of circulating lipoproteins to tissue metabolic activity as a basis for understanding atherogenesis. *Circ Res.* 1980, 47(3):301-311.

Wu KK. Inducible cyclooxygenase and nitric oxide synthase. *Adv Pharmacol.* 1995, 33:179–207.

Xu B, Thornton C, Tooher J, Ogle R, Lim S, Makris A, Hennessy A. Effects of anti-hypertensive drugs on production of soluble fms-like tyrosine kinase 1 and soluble endoglin from human normal and pre-eclamptic placentas in vitro. *Clin Exp Pharmacol Physiol.* 2009,36(8):839-842.

Yallampalli C, Garfield RE. Inhibition of nitric oxide synthesis in rats during pregnancy produces signs similar to those of preeclampsia. *Am J Obstet Gynecol.* 1993, 169 (5):1316–1320.

Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. *Annu Rev Pathol.* 2010, 5:173-192.

Zachary I. Vascular endothelial growth factor: how it transmits its signal. *Exp Nephrol.* 1998, 6(6):480-487.

Zachary I, Mathur A, Yla-Herttuala S, Martin J. Vascular protection: A novel nonangiogenic cardiovascular role for vascular endothelial growth factor. *Arterioscler Thromb Vasc Biol.* 2000, 20 (6): 1512-1520.

Zhang J, Masciochi M, Lewis D, Sun W, Liu A, Wang Y. Placental anti-oxidant gene polymorphisms, enzyme activity, and oxidative stress in preeclampsia. *Placenta.* 2008, 29(5):439-443.

Zhou Y, McMaster M, Woo K, Janatpour M, Perry J, Karpanen T, Alitalo K, Damsky C, Fisher SJ. Vascular endothelial growth factor ligands and receptors that regulate human

cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *Am J Pathol.* 2002, 160(4):1405-1423.
Zuspan FP The role of the uteroplacental bed in pregnancy-related hypertension. *Am J Hypertens.* 1988, 1(3 Pt 3):186S-188S.
Zusterzeel PL, Rutten H, Roelofs HM, Peters WH, Steegers EA. Protein carbonyls in decidua and placenta of pre-eclamptic women as markers for oxidative stress. *Placenta.* 2001, 22:213-219.

Vita

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Publications

1. **Mateus, J**. Diverticular disease of the colon. ILADIBA, 1991
2. **Mateus J**, Pereira L, Baxter J, Berghella V, Tolosa JE. Effectiveness of fetal fibronectin testing compared with digital cervical assessment of women with preterm contractions. Am J Perinatol. 2007;24(6):381-5.
3. Byers BD, Goharkhay N, **Mateus J**, Ward KK, Munn MB, Wen TS. Pregnancy outcome after the ultrasound diagnosis of fetal intra-abdominal vein varix. Ultrasound Obstet Gynecol. 2009;33(3):282-6.
4. Costantine M, Fox F, Byers BD, **Mateus J**, Ghulmiyyah LM, Blackwell S, Hankins GD, Grobman WA, Saade G. Validation of the prediction model for success of vaginal birth after cesarean delivery. Obstet Gynecol. 2009;114 (5):1023-1028.
5. **Mateus J**, Fox K, Jain, S, Jain SK, Cohen J, Latta R. Preterm premature rupture of membranes: clinical outcomes of late preterm infants. Clin Pediatr (Phila). 2010;49 (1):60-5.
6. Costantine MM, Fox KA, Pacheco LD, **Mateus J**, Hankins GD, Grobman WA, Saade GR. Does information available at delivery improve the accuracy of predicting vaginal birth after cesarean? Validation of the published models in an independent patient cohort. Am J Perinatol. 2011;28(4) :293-8.
7. **Mateus J**. Clinical Management of the short cervix. Obstet Gynecol Clin North Am. 2011 ;38(2) :367-85

ABSTRACTS :

Oral Presentations

1. Lu F, **Mateus J**, Goharkhay N, Yin H, Tamayo E, Anderson GD, Longo M, Saade G. Placental and renal expression of hypoxic genes in a mouse model of preeclampsia induced by over-expression SFLT-1. Abstract # 7. Plenary oral presentation at the 28th Annual Meeting Society for Maternal Fetal Medicine, Dallas, TX, 2008
2. **Mateus J**, Byers B, Bytautiene E, Tamayo E H, Betancourt A, Longo M, Saade G. Effect of a Short-Course of recombinant human vascular endothelial growth factor on vascular dysfunction in a mouse model of preeclampsia induced by sFlt-

1 over-expression. Oral concurrent presentation at the 29th Annual Meeting Society for Maternal Fetal Medicine, San Diego, CA, 2009

3. Costantine M, Yin H, Tamayo E, Makhlouf M, Ghulmiyyah L, **Mateus J**, Saade G, Longo M. Fetal Origin of Adult diseases: Genetic Imprinting versus Developmental Programming. Oral concurrent presentation at the 29th Annual Meeting Society for Maternal Fetal Medicine, San Diego, CA, 2009
4. **Mateus J**, Lu F, Bytautiene E, Tamayo E H, Betancourt A, Hankins G D V, Longo M, , Saade G. Abstract # 18. Effect of continuous infusion of vascular endothelial growth factor on blood pressure in a mouse model of preeclampsia induced by sFlt-1 overexpression. Oral concurrent presentation at the 30th Annual Meeting Society for Maternal Fetal Medicine, Chicago, IL, 2010
5. **Mateus J**, Lu F, Tamayo E H, Yin H, Hankins G D V, Longo M, Saade G. Abstract # 23. Effect of continuous delivery of recombinant vascular endothelial growth factor on vascular reactivity in a Sflt-1 induced animal model of preeclampsia. Oral concurrent presentation at the 30th Annual Meeting Society for Maternal Fetal Medicine, Chicago, IL, 2010
6. **Mateus J**, Yin H, Tamayo E H, Betancourt A, Hankins G D V, Longo M, Saade G. Abstract # 18. Regulation of placental and renal hypoxia gene expression by VEGF 121 therapy in a mouse model of preeclampsia induced by sFlt-1 overexpression. Oral concurrent presentation at the 31th Annual Meeting Society for Maternal Fetal Medicine, San Francisco, CA, 2011

Poster Presentations

1. **Mateus J**, Velasquez A. Correlation between cervical cytology and colposcopic biopsy. Resident oral presentation at the Colombian Congress of Obstetrics and Gynecology, Cali, Colombia, 1997
2. **Mateus J**, Narne S, Weiner S, Tolosa JE, Herman A, Cheku B, Filmer B, Figueroa E, Berghella V. What is the Accuracy of Prenatal ultrasound Diagnosis in Fetal Cystic Kidney Disease? Poster # 564. Presented at the 24th Annual Meeting Society for Maternal Fetal Medicine, New Orleans, LA, 2004
3. **Mateus J**, Pereira L, Baxter J, Berghella V, Cheku B. Fetal Fibronectin Compared to Cervical Dilation in Clinical Practice. Poster # 383. Presented at the 24th Annual Meeting Society for Maternal-Fetal Medicine, New Orleans, LA, 2004
4. Tolosa JE, **Mateus J**, Butani VK, Romero R, Wood DC, Abassi S, Silvieri S, Gomez R, Huhta J. Fetal Echocardiography in Twin-to-Twin Transfusion Syndrome can Identify Risk Factors for Neonatal Systemic Hypertension. Poster # 06.09. Presented at the 15th World Congress on Ultrasound in Obstetrics and Gynecology, Vancouver, Canada, 2005
5. **Mateus J**, DiVenti C, Cohen J, Latta R. Preterm Premature of Membranes: Optimal Timing of Delivery in a Community Hospital. Poster #162. Presented at the 26th Annual Meeting Society for Maternal Fetal Medicine, Miami, FL, 2006
6. **Mateus J**, Cohen J, Latta R. Preterm Premature Rupture of Membranes: Neonatal Outcome Comparison of Twins versus Singletons. Presented at the American

College of Obstetricians and Gynecologists 55th Annual Meeting, San Diego, CA, 2007

7. Goharkhay N, **Mateus J**, Egbert K, Smith O, Gamble P, Yin H, Anderson G, Saade GR, Longo M. Lack of activation of cholesterol synthesis and hypercholesterolemia during infancy in a mouse model of developmental programming of atherosclerosis. Poster #396. Presented at the 28th Annual Meeting Society for Maternal-Fetal Medicine, Dallas, TX, 2008
8. **Mateus J**, Moss J, Mateo R, Longo M, Saade G, Sbrana E. Association of fetal visceromegaly, large for gestational age, and congenital abnormalities with maternal hemoglobin A1c levels in an autopsy case series. Center of Interdisciplinary Research in Women's Health. Poster presentation session, The University of Texas Medical Branch at Galveston, TX, 2008
9. **Mateus J**, Lu F, Bytautine E, Costantine E, Tamayo EH, Longo M, Saade G. Effect of recombinant human vascular endothelial growth factor 121 on blood pressure in a mouse model of preeclampsia induced by sflt-1 over-expression. Poster #238. Presented at the 29th Annual Meeting Society for Maternal Fetal Medicine, San Diego, CA, 2009
10. Shrestha R, Latta R, **Mateus J**, Cohen J. Near-Term Premature Rupture of Membranes: Expectant Management Compared to Delivery in a Community Hospital. Poster #157. Presented at the 29th Annual Meeting Society for Maternal Fetal Medicine, San Diego, CA, 2009
11. Costantine M, Fox K, Byers B, **Mateus J**, Ghulmiyyah L, Grobman W, Blackwell S, Hankins GDV, Saade G. Validation of the vaginal birth after cesarean (VBAC) nomogram. Poster #222. Presented at the 29th Annual Meeting Society for Maternal Fetal Medicine, San Diego, CA, 2009
12. **Mateus J**, Goharkhay N, Longo M, Saade G, Moss J, Sbrana E. Placental histopathology in mild versus severe hypertensive disorders in pregnancy: Implications for feto-placental perfusion. Poster #756. Presented at the 29th Annual Meeting Society for Maternal Fetal Medicine, San Diego, CA, 2009
13. **Mateus J**, Fox K A, Hankins G D V, Saade G R, Jain S. Perinatal outcome in treated vs untreated maternal cardiac arrhythmias. Poster # 827. Presented at the 30th Annual Meeting Society for Maternal Fetal Medicine, Chicago, IL, 2010
14. **Mateus J**, Fox K A, Hankins G D V, Saade G R, Jain S. Effect of previous history of cardiac arrhythmias on the clinical course and pregnancy outcome of women who develop arrhythmias during pregnancy. Poster # 828. Presented at the 30th Annual Meeting Society for Maternal Fetal Medicine, Chicago, IL, 2010
15. Jain S, Basraon S, Maner W, **Mateus J**, Fox K, Wen T, Garfield R. Transabdominal uterine electromyography- a better predictor of preterm birth compared to advanced cervical dilation. Abstract # 298. Presented at the 30th Annual Meeting Society for Maternal Fetal medicine, Chicago, IL, 2010
16. Basraon S, Jain S, Fox K, **Mateus J**, Wen T, Maner W Garfield R. Comparing vaginal probe uterine electromyography to transabdominal & tocodynamometer in morbidly obese women. Abstract # 316. Presented at the 30th Annual Meeting Society for Maternal Fetal medicine, Chicago, IL, 2010

17. Costantine M, Fox K, Byers B, **Mateus J**, Pacheco L D, Gary D V Hankins, Grobman W, Saade G. Does information available at delivery improve the prediction of the vaginal birth after cesarean (VBAC) models? Validation in an independent patient cohort. Abstract # 687. Presented at the 30th Annual Meeting Society for Maternal Fetal medicine, Chicago, IL, 2010
18. **Mateus J**, Vincent K, Bytautiene E, Esenaliev R, Motamedi M, Longo M, Saade G. Determination of intrauterine fetal growth and prediction of birth weight using microultrasonography in mice. Poster # 352. Presented at the 31th Annual Meeting Society for Maternal Fetal Medicine, San Francisco, CA, 2011
19. **Mateus J**, Kechichian T, Tamayo E, Betancourt A, Longo M, Saade G. Signaling of endothelial nitric oxide synthase and activation of heomxygenase- 1 by VEGF121 therapy in a mouse model of preeclampsia induced by overexpression of sFlt-1. Poster # 762. Presented at the 31th Annual Meeting Society for Maternal Fetal Medicine, San Francisco, CA, 2011
20. Chiossi G, Bytautiene E, Kechichian T, Tamayo E, Saade ML, **Mateus J**, Longo M. The role of the renin angiotensin system in fetal programming of adult hypertension. Poster # F-117. Presented at the 58th Annual Meeting of the Society for Gynecologic Investigation, Miami, FL, 2011.
21. Basraon S, **Mateus J**, Dhari A, Kuo YF, Pacheco L, Saade G. Cervical change and duration of labor in full term pregnancies undergoing pre-induction cervical ripening. Poster # S-024. Presented at the 58th Annual Meeting of the Society for Gynecologic Investigation, Miami, FL, 2011.
22. **Mateus J**, Kechichian T, Tamayo E, Betancourt A, Longo M, Saade G. Activation of the placental nitric oxide signaling pathway by VEGF121 therapy in a mouse model of maternal endothelial dysfunction induced by sFlt-1 overexpression. Poster # T-018. Presented at the 58th Annual Meeting of the Society for Gynecologic Investigation, Miami, FL, 2011.

This dissertation was typed by Julio F Mateus Nino