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Therapies for preeclampsia and fetal growth restriction: restoration of uterine blood flow tested in mouse models

by

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DEDICATION

For my lovely wife, parents and brother who offered me unconditional love and support throughout the course of this thesis.

ABSTRACT

Preeclampsia (PE) and fetal growth restriction (FGR) complicate over 10% of human pregnancies and contribute significantly to fetal and maternal morbidity and mortality. Although the causes of PE and FGR are not well understood, they are known to be associated with impaired uterine artery blood flow. Endothelial nitric oxide synthase knockout mice ($eNOS^{-/-}$) and catechol-O-methyltransferase knockout mice ($COMT^{-/-}$) exhibit many signs of PE during pregnancy and deliver growth-restricted pups. We tested the ability of two potential treatments; resveratrol and 2-methoxyestradiol (2-ME) to increase uteroplacental blood flow and therefore ameliorate signs of PE and rescue FGR in relevant mouse models. 2-ME administration led to normalization of umbilical artery blood flow velocity and proteinuria in $COMT^{-/-}$ mice. Resveratrol supplementation increased uterine artery blood flow velocity and pup weight in $COMT^{-/-}$ but not in $eNOS^{-/-}$ mice. Our results indicate that resveratrol and 2-ME may have therapeutic potential in cases of PE and FGR.

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LIST OF ABBREVIATIONS

° C	Degrees Celsius
μ	Micro
2-ME	2-methoxyestradiol
AGT	Angiotensinogen
ANOVA	Analysis of variance
BP	Blood pressure
BPM	Beats per minute
Ca^{2+}	Calcium
cGMP	Cyclic guanosine monophosphate
COMT	Catechol-O-methyltransferase
$COMT^{-/-}$	Catechol-O-methyltransferase knockout
DBP	Diastolic blood pressure
EDHFs	Endothelial derived hyperpolarizing factors
EDV	End diastolic velocity
eNOS	Endothelial nitric oxide synthase
$eNOS^{-/-}$	Endothelial nitric oxide synthase knockout
ET-1	Endothelin-1
FGR	Fetal growth restriction
g	Gram
GD	Gestational day
Gen	Genotype
HR	Heart rate
iNOS	Inducible nitric oxide synthase
Int	Interaction
L-NAME	L-NG-Nitroarginine methyl ester

MCh	Methacholine
mol/L	Mole per litre
Na^+	Sodium
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
PE	Preeclampsia
PGI_2	Prostacyclin
Phe	Phenylephrine
PSS	Physiological salt solution
PSV	Peak systolic velocity
REN	Renin
ROS	Reactive oxygen species
RES	Resveratrol
RI	Resistance index
SBP	Systolic blood pressure
SEM	Standard error of mean
s-Flt-1	Soluble fms-like tyrosine kinase-1
SOD	Superoxide dismutase
SNP	Sodium nitroprusside
vs.	Versus

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.0 Prologue

The maternal cardiovascular system undergoes profound hemodynamic changes during pregnancy to ensure an adequate delivery of oxygen and nutrients to the developing fetus. Failure of these adaptations is associated with pregnancy complications such as preeclampsia (PE) and fetal growth restriction (FGR). PE and FGR are two of the most common complications of pregnancy worldwide and constitute major health threats for the mother and the newborn. Together, these conditions continue to be a leading cause of maternal and perinatal morbidity and mortality causing the deaths of over 100,000 women and 500,000 neonates worldwide every year. (Bujold et al., 2009a)

Despite intensive research, there are no curative therapies available for PE and FGR, and it is still not understood why some women develop these complications. However, several studies have shown that reduced uteroplacental blood flow plays a fundamental role in the development of these conditions. In normal pregnancies adequate trophoblast (placental cells) invasion of spiral arteries (terminal branches of uterine artery) leads to dilation of uteroplacental arteries. This hemodynamic change enables the increase of blood flow to the placental bed and therefore fulfills the increased demand for oxygen and nutrients for the developing fetus. In PE and FGR, decreased trophoblast invasion of the maternal spiral arteries leads to increased resistance, and therefore decreased uterine artery blood flow, leading to placental under-perfusion and ischemia. The study of pathophysiological mechanisms of PE and FGR in humans is limited given the ethical implications associated with these kinds of studies. Therefore,

my project aims to utilize relevant mouse models of PE and FGR to test two treatments (2-methoxyestradiol and resveratrol) that have the potential to increase uteroplacental blood flow. My studies will determine if these treatments can increase uteroplacental blood flow and whether this can successfully ameliorate the signs of PE and rescue fetal growth in relevant murine models. These studies are a crucial first step in identifying possible new treatments for these pathologies.

1.1 Pregnancy and the cardiovascular system

The cardiovascular system undergoes a number of structural and functional changes during pregnancy to fulfill the needs of the mother and developing fetus. The major hemodynamic changes in response to pregnancy include an increase in cardiac output, a blood volume expansion and a reduction in systemic blood pressure. Together, these adaptations maintain uteroplacental perfusion and allow the fetus to receive an adequate supply of oxygen and nutrients. (Abbas et al., 2005) A large increase in blood flow is observed in both renal and uteroplacental circulations and a significant quantity of total blood volume is circulated within the uterine and intervillous circulations. (Campbell et al., 1983, Collins et al., 2012) In normal pregnancy, these alterations ultimately contribute to the optimal growth and development of the fetus and protect the mother from cardiovascular complications. If these adaptations do not take place, fetal growth and development can be impaired.

The total blood volume during pregnancy increases, as there is an increase in red cell mass and plasma volume. The plasma volume starts to rise as early as 6-8

weeks of gestation and increases to a maximum of 45-55% (compared to nonpregnant women) at around 34 weeks. (Pirani et al., 1973, Dieckmann and Wegner, 1934) This increase in blood volume has important clinical implications. There is a direct correlation between the increase in blood volume during pregnancy and fetal growth and birth weight. (Hytten and Paintin, 1963, Goodlin et al., 1983) In addition to the blood volume expansion, pregnancy is associated with a 30-40% increase in cardiac output from early in the first trimester. (Grollman, 1932, Mabie et al., 1994) The initial increase in cardiac output is due to an increase in stroke volume but later it is accompanied by an increase in heart rate as well. Despite the increase in blood volume and cardiac output, systemic vascular resistance has been shown to decrease by 20% compared to vascular resistance in the same woman in a nonpregnant state. (Abbas et al., 2005, Clark et al., 1989) Studies in mesenteric arteries of pregnant rats also show a reduction in vascular stiffness and a decrease in vasoconstriction; together these changes lead to reduced vascular resistance. (Ralevic and Burnstock, 2012) The reduction in vascular resistance occurs as a result of a range of adaptations in the maternal vasculature, including structural adaptations and remodeling of the uterine artery and its downstream vessels and the extensive structural remodeling of the maternal spiral arteries that supply the placenta. (Brosens et al., 1967)

During pregnancy, a reduction in blood pressure despite an increase in plasma volume and cardiac output occurs as a result of reduction in maternal peripheral vascular resistance. Mechanisms accountable for decreased vascular resistance comprise an increased response to vasodilators and a blunted response to

vasoconstrictors. (O'Day, 1997) The mechanisms that produce these changes are not fully understood, however, in part they are known to occur due to alterations in endothelial regulation of maternal vascular tone. It is important to understand the maternal regulation of vascular tone during pregnancy because failure to instigate or to maintain these changes can lead to conditions such as PE and FGR, which are associated with increased vascular resistance and impaired blood flow in the uteroplacental and fetoplacental circulations. (Takata et al., 2002)

1.1.1 Regulation of vascular function and tone

1.1.1.1 Endothelial function

The endothelium is a continuous monolayer of simple squamous epithelial cells that lines the lumen of entire vascular system. The endothelium is involved in a number of important functions including the control of 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 et al., 1998) Furchgott and Zawadzki (1980) discovered that the relaxation response to acetylcholine in the rabbit aorta was evoked only in the presence of endothelium. Since then endothelial cells have been identified to produce a number of vasodilators and vasoconstrictors that regulate vascular tone.

Vascular tone is regulated by the contractile activity of the smooth muscle cells, which in turn rely on the action of vasoconstrictor or vasodilator molecules. The endothelium is constantly in contact with an array of constricting and/or relaxing signals. It integrates these signals and regulates vascular smooth muscle to

determine a vascular tone at any given time. An increase in cytosolic concentration of Ca^{2+} mediated through a number of mechanisms including influx of extracellular Ca^{2+}, Na^{+} -Ca^{2+} exchange and liberation of Ca^{2+} from intracellular stores such as endoplasmic reticulum, (Winquist et al., 1985, Singer and Peach, 1982, Lückhoff and Busse, 1986) stimulates the release of endothelial relaxing factors in the endothelial cells. (Furchgott, 1983) Endothelial derived vasorelaxation compounds include nitric oxide (NO), (Ignarro et al., 1987) prostacyclin, (Moncada and Vane, 1979) and endothelium derived hyperpolarizing factors (EDHFs), (Rubanyi and Vanhoutte, 1987) while vasoconstrictors secreted from endothelial cells include endothelin-1, thromboxane A_2 and angiotensin II. (Hurairah and Ferro, 2004) Ultimately the vasodilators and vasoconstrictors released by endothelial cells contribute to the regulation of blood pressure and blood flow. Endothelial cells possess cell surface receptors for circulating hormones such as catecholamines, angiotensin, vasopressin, bradykinin, serotonin, natiuretic peptide, substance P, adrenomedullin and acetylcholine, which act as stimulants for the endothelial-derived relaxing factors. (Yannopoulos and Nadkarni, 2012) Even though each of the endothelial derived factors play a role in maintaining vascular tone, their contribution differs according to vessel size, vascular bed, species, endothelial agonist, and the physiological state of the organism (for example pregnancy, aging). (Weiner et al., 1991, Aird, 2007)

1.1.1.2 Role of endothelium in pregnancy

Cardiovascular adaptations such as increased blood volume, increased cardiac output and decreased blood pressure in both animals and human pregnancies are in part governed by endothelium. The endothelium regulates these adaptations by maintaining a low vascular tone by either increasing the release of vasodilators or decreasing the vasoconstrictors release. (Poston et al., 1995) Vasodilators such as prostacyclin and EDHFs play an important role in pregnancy and are explored in a number of excellent reviews. (Lyall and Greer, 1996, Ozkor et al., 2010) For the purpose of this thesis, however, the role of NO will be discussed in detail, as it is known to play an important role in regulation of uteroplacental blood flow. (Bird et al., 2000, Magness et al., 1997, Nelson et al., 2000, Xiao et al., 2001)

1.1.1.3 Role of nitric oxide in pregnancy

Furchgott and Zawadzki discovered a molecule produced by endothelium that induced vascular smooth muscle relaxation and named it endothelial derived relaxing factor. (Furchgott and Zawadzki, 1980) Ignarro et al., (1987) later identified this molecule to be a free radical NO and since then it has been established as a principal molecule through which endothelium regulates vascular tone. NO is a free radical gas produced in the vascular endothelial cells. It is generated through the oxidation of amino acid L-arginine to L-citrulline (Palmer et al., 1988) by one of the three isoform of NO synthase (NOS) enzymes: nNOS (a neuronal form), iNOS (an inducible form) and eNOS (a constitutive form), which is the most commonly expressed form by the endothelium. (Kuboki et al.,

2000) NO induces vascular relaxation by diffusing through cell membrane into the underlying vascular smooth muscle cells, where it binds to the heme portion of guanylyl cyclase resulting in upregulation of cyclic guanosine monophosphate (cGMP). The increase in cGMP leads to the activation of cGMP dependent kinase followed by decrease in intracellular calcium, stimulation of calcium re-uptake, and membrane hyperpolarization due to opening of potassium channels. (Wolin et al., 1998) Together this leads to diminished actin-myosin binding and ultimately reduction in vascular tone. (Surks et al., 1999)

In response to pregnancy, expression of eNOS in uterine artery is increased in animals (Weiner et al., 1989) and humans. (Nelson et al., 2000) The state of vasodilatation during pregnancy is thought to be partly mediated by NO, which is important for maintenance of adequate uteroplacental blood flow. (Langille and O'Donnell, 1986, Yallampalli and Garfield, 1993) Infusion of a NOS inhibitor into umbilical artery in chronically catheterized sheep leads to increase in fetoplacental vascular resistance and decrease in umbilical artery blood flow. (Chang et al., 1992) Studies in rat, rabbit, and guinea pig uterine arteries showed increase in NO mediated vasodilation during pregnancy. (Ni et al., 1997, Brooks et al., 2001) Further, evidence to support the role of NO in uterine artery comes from studies in eNOS knockout mice, which show impaired uterine artery remodeling (van der Heijden et al., 2005b) and decreased uterine artery blood flow velocity during pregnancy. (Kulandavelu et al., 2012) Blockade of NO synthesis in animal models induces PE like phenotype and FGR, suggesting

impaired NO bioavailability could contribute to pathogenesis of these conditions. (Yallampalli and Garfield, 1993, Kaya et al., 2011)

Kopp and colleagues reported 140% increase in urinary cGMP in the first trimester of human pregnancy suggesting that NO synthesis increases early in gestation. (Kopp et al., 1977) This observation was confirmed in pregnant rats by Conrad and group, as they reported a reduction in urinary cGMP following NOS inhibition. (Conrad et al., 1993) Similarly, eNOS expression in uterine arteries is significantly increased at 37 weeks of gestation compared to non-pregnant women. (Nelson et al., 2000) Furthermore, serum concentration of nitrite (NO metabolite) in healthy pregnant women is increased compared to non-pregnant women. (Shaamash et al., 2000) Taken together, these studies suggest increased eNOS activity and NO production during pregnancy.

Although increased eNOS activity and NO production are consistently observed in animal pregnancies, findings in human pregnancies are somewhat inconsistent. Seligman et al., (1994) reported that the serum nitrite concentrations in pregnant women were slightly raised but were not significantly different to non-pregnant women. Other studies have failed to show the difference in urinary nitrite excretion (Brown et al., 1995, Curtis et al., 1995) or in exhaled NO in pregnant women. (Morris et al., 2008)

Differences between the human and the animal pregnancy data regarding eNOS and NO production may be in part explained by effect of diet on excretion of metabolites. Increased maternal intake of nitrites is associated with its increase

excretion in urine. (Ellis et al., 1998) In experimental conditions, diet of the animals is tightly regulated and as a result less variation in metabolite can be observed. In contrast, dietary intake in the human experimental subjects can be much more difficult to control and may account for confounding results in metabolite measurements in humans and animals.

1.1.2 Vascular remodeling of maternal spiral arteries during pregnancy

Along with the increase in endothelial-derived vasodilators and reduced vasoconstrictor response in pregnancy, vascular remodeling during pregnancy is essential in order to support the increased blood volume and therefore provide an adequate amount of nutrients and oxygen to the developing fetus. In humans, spiral uterine arteries that supply blood to the placenta undergo significant alterations during the first half of pregnancy. (Lyall, 2005) Early in pregnancy human placenta develops specialized trophoblast cell types that differ in function. The multinucleated syncytiotrophoblast layer encases the floating villi of the placenta and provides the barrier to maternal blood. The extravillous trophoblasts migrate out of the placenta to the maternal decidua (uterine lining or endometrium) and finally reach spiral arteries. These cells then differentiate into endovascular and interstitial cells. The endovascular cells, which proliferate and line the uterine cavity invade through the uterine decidua and initiate vascular remodeling of uterine spiral arterioles. Interstitial trophoblast cells migrate through the endometrial stroma and penetrate the myometrium to form placental bed giant cells. These trophoblasts also surround spiral arteries. Both

endovascular and interstitial invasion are associated with spiral arteries remodeling that involves vessel dilatation, the loss of endothelial cells, and loss of smooth muscle cells followed by breakdown of elastin fibers within the internal elastic lamina and tunica media. (Pijnenborg et al., 2006) The invading trophoblast and fibrinoid layer replaces the lost structural layer. Ultimately this process leads to the loss of vaso-active properties of the spiral arteries leading to increased dilation to accommodate the up regulated blood volume in pregnancy. (Pijnenborg et al., 1980) Furthermore, this process leads to increased diameters in uterine arteries. (Mazzuca et al., 2012) In addition, the remodeling of spiral arteries is also in part responsible for decreased systemic vascular resistance (Verkeste et al., 1998) and increases uteroplacental perfusion to meet the requirement of the fetus. (Moll et al., 1988) In normal pregnancy, trophoblast invasion is observed from implantation to up to twentieth week of gestation. (El-Hamedi et al., 2005) Although the mechanisms of trophoblast invasion remain unclear, the physiologic hypoxic conditions of the first trimester (~2-3 % oxygen) are thought to promote trophoblast invasion. (James et al., 2006)

1.1.3 Uteroplacental blood flow during pregnancy

The uterine arteries are the main source of blood supply to the uterus. Consequently, the uteroplacental circulation is a main determinant of oxygen and nutrient delivery to the developing infant. As discussed in section 1.1.2, remodeling of the spiral arteries in early pregnancy is an important step in regulating and maintaining uteroplacental blood flow. Evidence of a decrease in

resistance of the spiral arteries as a result of remodeling in pregnancy is supported by uteroplacental blood flow studies. Early human studies using electromagnetic flow probes reported that total uteroplacental blood flow increases from 20-50 ml/min in the non-pregnant state to as much as 450/800 ml/min at the end of gestation. (Assali et al., 1953, Assali et al., 1960) Subsequent assessment of uterine artery blood flow using radioactive xenon (133Xe) (Jouppila et al., 1978) have confirmed these findings.

The introduction of Doppler ultrasound in obstetrics in the last 50 years has provided further useful information, allowing non-invasive assessment of the feto-maternal unit. Doppler studies in non-pregnant women show a continuous peak systolic uterine artery blood flow velocity and a "notch" in the descending waveform in early diastole. At 20-24 weeks of gestation (in normal pregnancies) uterine artery Doppler scans show a high diastolic velocity with continuous flow throughout diastole and the diastolic "notch" is no longer present. (Collins et al., 2012, Campbell et al., 1983) These changes also correspond to a decrease in the resistance index (RI, maximum – minimum velocity/maximum velocity) in the uterine artery between 20 to 24 weeks of gestation. (Groom et al., 2009)

The uteroplacental blood flow in experimental animals during pregnancy is comparable to human pregnancies. In both women and experimental animals such as rodents and sheep, uteroplacental blood flow increases significantly compared to the non-pregnant state. Moreover, as pregnancy advances, there is a progressive increase in the proportion of blood directed to the placenta. (Dowell and Kauer, 1997, Rosenfeld et al., 1974)

Studies have shown that a number of exogenous factors can modulate maternal perfusion to the placenta but little is known about how the uteroplacental circulation is regulated in normal pregnancy. A lot of the current understanding of the factors able to alter uterine artery blood flow comes from *in vitro* studies of isolated maternal uterine arteries and arterioles. Skajaa et al., (2008) demonstrated that Mg^{+2} ion was able to induce relaxation of uterine artery, while other investigators have shown endothelin-1 to be a potent vasoconstrictor of uterine arteries. (Dechanet et al., 2011) However, whether these factors play a role in the *in vivo* regulation of uteroplacental flow is not known. Neri et al., (1996) infused L-arginine (the substrate of NO) intravenously into pregnant women, followed by an assessment of uterine artery resistance index (using ultrasound), and they showed a significant decrease in vascular resistance in uterine arteries in women with preexisting FGR. Similarly, Amit et al., (1998)showed that isosorbide dinitrate a nitric oxide donor, significantly decreased uterine artery resistance index in the first trimester of pregnancy. Collectively, these two studies suggest that NO may play an important role in modulating uteroplacental circulation during pregnancy.

1.1.4 Umbilical blood flow during pregnancy

Blood flow to the placenta through the umbilical blood vessels plays a vital role in ensuring a stable supply of oxygen and nutrients to the fetus. Umbilical blood flow velocity waveforms were first measured using Doppler ultrasound in 1977. (Fitzgerald and Drumm, 1977) In humans, the umbilical circulation is comprised

of two umbilical arteries and an umbilical vein. The umbilical arteries carry deoxygenated blood to the placenta, whereas the umbilical vein carries oxygenated and nutrient rich blood from the placenta to the fetus. The umbilical vein flow in humans increases as the gestation progresses. It increases from 35 mL/min at 20 weeks to 240 mL/min at 40 weeks of gestation. The peripheral vascular bed of the placenta mainly determines umbilical flow. The placental vasculature vasomotor tone is not regulated by catecholamines and does not have neural innervation. The chief regulators of vasomotor tone in the placenta are NO, prostacyclin and endothelin. (Sand et al., 2002) Placental vascularization in both humans and animals is known to increase during the late gestation and therefore accounts for low resistance and the corresponding high end diastolic blood flow velocity in the umbilical arteries. Hence the waveform recorded by Doppler measurement in the umbilical artery reflects the downstream impedance and is extensively utilized to identify placental insufficiency, a major cause of FGR. (Trudinger et al., 1985, Mu and Adamson, 2006)

1.2 PE and FGR

1.2.1 Definition and incidence

PE and FGR are the leading global causes for maternal and fetal morbidity and mortality. (Lockwood, 2002) PE is a multi-systemic disorder characterized by de-novo hypertension (> 140/90 mm Hg) and proteinuria (> 300 mg/L or more in a 24 hour urine sample) after 20^{th} week of gestation. (Khan et al., 2006, Schutte et al., 2010) PE can also progress to its severe form eclampsia, which is

characterized by seizures and blindness. According to the World Health Organization, PE is estimated to occur among 3.2% of live births worldwide, resulting in about 4 million cases per year, of which 72,000 are fatal to the offspring. (AbouZahr, 2003) Recently a large cohort study of 1,461,270 births in United States of America (USA) showed that PE related complications were associated with 16% of maternal deaths. (Clark et al., 2008)

FGR is widely defined as the inability of a fetus to grow to its genetically determined potential size. (Owens et al., 1989) This definition is very broad and is clinically impractical as the specific growth potential for a fetus in pregnancy is difficult to measure. (Cogswell and Yip, 1995) Therefore, in clinical and a research setting, the surrogate 'small-for-gestational age' (SGA) is often used, defined as birth weight below the $3^{rd}/5^{th}/10^{th}$ percentiles. (Rosenberg, 2008) A recent population based study in USA reported that 16.18% of babies born from 1995 to 2004 (excluding births in State of California) had the birth weights below the 10^{th} percentile. (Ananth and Vintzileos, 2009)

1.2.2 Immediate and long term consequences of PE and FGR

The immediate consequences for PE and FGR include complications during birth, increased susceptibility to infection, and an increased risk of neonatal complications and death. (Sibai, 2006, McMillen et al., 2001) FGR fetuses have five to six times higher perinatal mortality rates than those of normally grown infants and approximately 5-10% of all pregnancies complicated by FGR will result in either stillbirth or neonatal death. (McIntire et al., 1999, Thornton et al.,

2004, Lackman et al., 2001) PE is also associated with increased fetal and neonatal morbidity and mortality as it is responsible for 15% of all preterm births and predisposes to FGR. (Roberts et al., 2003) Furthermore, several studies suggest that women with PE have at least two fold increased risk of delivering a growth-restricted fetus compared to normotensive women. (Xiong et al., 1999, Chappell et al., 2008, Rasmussen and Irgens, 2006)

Moreover, studies suggest that these conditions are associated with worse health outcomes throughout later life. The infants born following pregnancies complicated by PE and FGR are known to have increased risk of diseases such as type 2 diabetes, central obesity, metabolic syndrome, hypertension and coronary heart disease in adult life. (McMillen et al., 2001, Magnussen et al., 2009) In addition women with history of PE have a higher risk of developing hypertension, coronary heart disease, stroke and type 2 diabetes in their later life. (Bellamy et al., 2007)

Development of therapies for these conditions would significantly reduce the number of fetal and maternal deaths. In addition, future health outcomes for women and children would be improved. Hence, testing of potential therapies for PE and FGR in relevant mouse models of these conditions takes a central role in this thesis.

1.3 Pathogenesis of PE and FGR

Despite intensive research and important advances in recent years, the etiology of PE and FGR remains unclear. Although maternal manifestations of these diseases are profoundly different, accumulating evidence suggests that placenta is centrally involved in the pathogenesis of both syndromes. Extensive research has shown that there is an insufficient blood supply to the placenta of women with PE and FGR. The impairment in blood flow to the placenta is thought to occur due to decrease in trophoblast invasion (Figure 1-1).

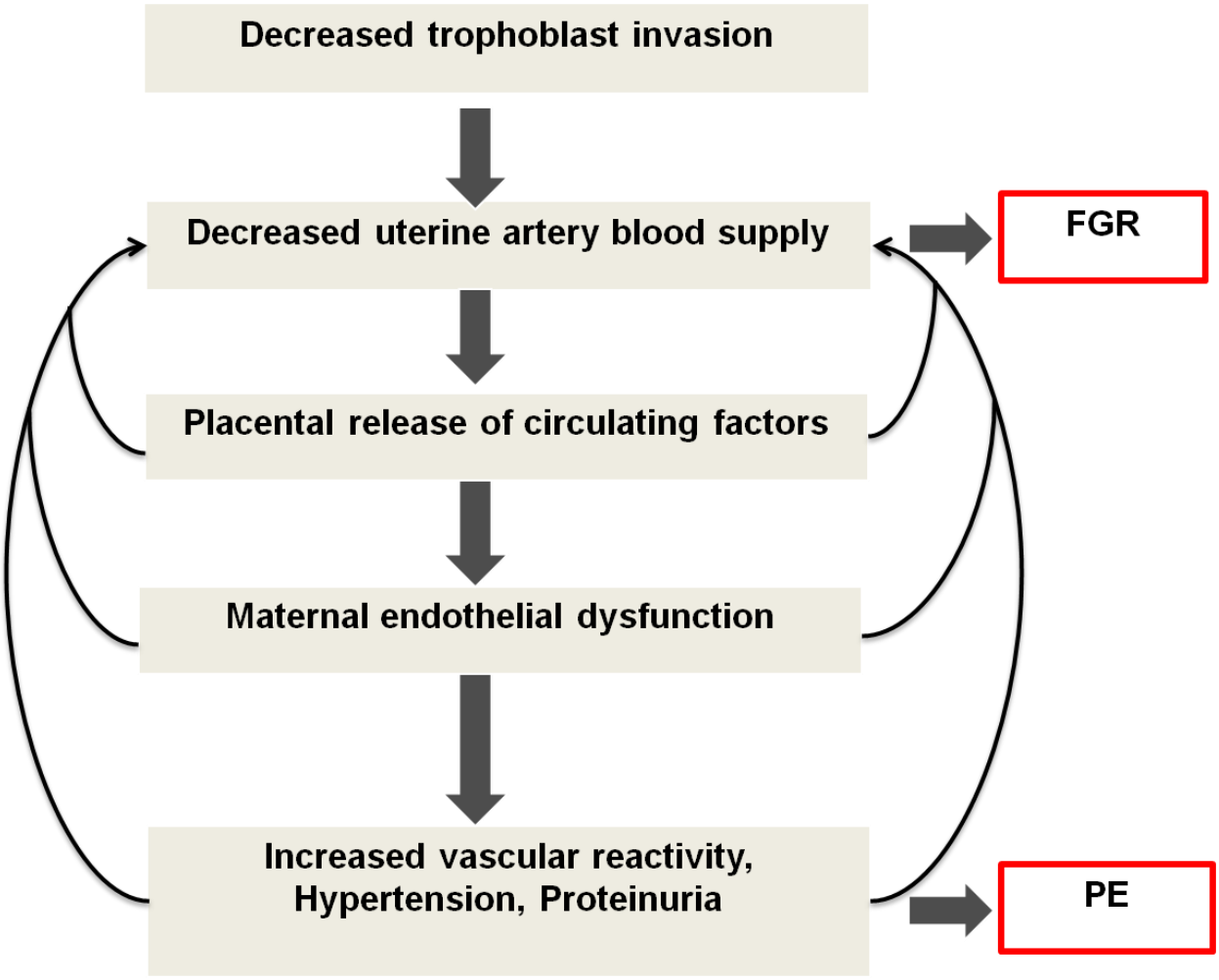


Figure 1-1: Potential pathophysiological mechanisms in PE and FGR.

Decreased trophoblast invasion leading to decrease in uterine artery blood flow is a common pathophysiological mechanism in PE and FGR

1.3.1 Decreased trophoblast invasion in PE and FGR

Brosens and colleagues first reported reduced trophoblast invasion and absence of pregnancy specific changes of uteroplacental arteries in placental bed specimens associated with PE and FGR. (Brosens et al., 1972) Since then, trophoblast invasion has been a major focus of placental research. It is now well accepted that

PE and FGR are associated with decreased trophoblast invasion of the maternal spiral arteries, which leads to increased resistance and therefore impaired uterine artery blood flow, leading to placental under-perfusion. (Aardema et al., 2001, Khong et al., 1986) It has been well documented that spiral arteries in PE and FGR do undergo minimal remodeling; these changes do not extend any further than the mucosal layer (not into the decidua) (Figure 1-2). (Pijnenborg et al., 1991) Placental biopsies obtained from pregnancies associated with PE and FGR have revealed the failure of trophoblast invasion of spiral arteries. (van Asselt et al., 1998, Antsaklis et al., 2000) In addition relatively few trophoblasts are found in the vessels and those that are present do not express an endothelial phenotype. This adaptive failure leads to impedance in the uteroplacental circulation followed by reduction in the flow volume to the placenta. In normal pregnancy, the spiral artery remodeling is completed by the 21st week of pregnancy, thus although PE is not typically diagnosed until the third trimester of pregnancy, its roots are likely early in gestation. (Pijnenborg et al., 1991)

Normal pregnancy
Spiral artery remodeling

Preeclampsia and FGR
Failure of spiral artery remodeling

Figure 1-2: Spiral artery remodeling in normal pregnancy and in pregnancy associated with PE and FGR.

Left image: Trophoblast invasion into the maternal spiral arteries in the placental bed of normal pregnancy leading to dilatation of these arteries. Right image: Impairment of trophoblast invasion in cases of PE and FGR. Adapted and modified from Cartwright et al., (2010).

1.3.2 Increased uterine artery resistance and impaired uterine artery blood flow

The increase in uterine artery resistance featured in both PE and FGR (Harrington et al., 1996, Clark et al., 1989) is associated with irregular blood flow and ischemia-reperfusion injury. In a case control study, Olofsson et al., (1993) examined placental biopsies taken during caesarean section in pregnancies complicated by PE and FGR and uncomplicated pregnancies; they linked increased uterine artery resistance to impaired trophoblastic invasion. Normally impedance to flow in the uterine arteries decreases as the pregnancy progresses. Increased impedance in the uterine artery early in pregnancy (usually 2nd trimester) is associated with PE and FGR. (Cnossen et al., 2008b) Women that exhibit abnormal uterine artery Doppler waveforms in the second trimester are

likely to have more than six-fold increase in rate of PE. (Harrington et al., 1996, Clark et al., 1989) In addition, it has been demonstrated that reduced uteroplacental blood flow, (Moore et al., 2000, Moore et al., 2007) compromises fetoplacental vascular development and function, and generates high resistance in the umbilical circulation. (Alfirevic and Neilson, 2010) A number of studies have shown FGR to be associated with reduction in both uterine and umbilical arteries blood flow velocities. (Groom et al., 2009, Lunell et al., 1979, Nylund et al., 1983) In contrast, systematic reviews (Chien et al., 2005, Papageorghiou et al., 2002, Conde-Agudelo et al., 2004) have reported that abnormal uterine artery Doppler indices are of only limited accuracy in predicting PE and FGR. However, these systematic reviews were restricted in terms of the methodology used. For instance two reviews only reported adverse outcomes associated with PE and FGR based on articles through MEDLINE only, while the other was limited to articles published before 1997. (Papageorghiou et al., 2002) Uterine arteries Doppler studies for prediction of PE and FGR are difficult to compare since investigators have used varying definitions of abnormal Doppler waveforms, populations and gestational age at examination. In addition the severity of the disease in patients may be different, which can confound the overall result. A recent study evaluated the use of uterine artery Doppler waveforms for prediction of PE in a systematic review of 74 studies that included approximately 80,000 women. The study concluded that the abnormal uterine artery waveforms during second trimester to be good predictors of PE. (Cnossen et al., 2008b) In line with human PE cases, studies in animal models have successfully demonstrated that

PE-like phenotype can be generated mechanically by reducing uteroplacental blood flow. (Makris et al., 2007, Casper and Seufert, 1995) A causal relationship between poor placental perfusion and PE has thus been established.

In FGR, the main consequence of impaired uterine artery flow is the malperfusion of the placenta, hence diminished oxygen and nutrient delivery to the fetus resulting in growth restriction. (Gagnon, 2003) In PE, however, reduced placental perfusion alone is unlikely to be the only factor that leads to the disease. Abnormal implantation and perfusion defects are also associated with both FGR and preterm births and occur in many pregnancies without the maternal manifestations associated with PE. (Lain and Roberts, 2002) In addition, FGR cases demonstrate inadequate trophoblast invasion of spiral arteries and reduced uterine artery perfusion. Interestingly, only one third of the babies born to women with PE exhibit FGR in presence of failed spiral artery remodeling. (Arias et al., 1993) Although reduced placental perfusion may be important in the development of PE, it may not be sufficient to cause PE. Hence it has been suggested that decreased placental perfusion must interact with maternal factors stemming from genetic, behavioral and environmental conditions, in order to cause PE. Indeed, PE appears to progress in two stages. (Redman and Sargent, 2005) The first stage initiates as a result of defective early trophoblast invasion and remodeling of spiral arteries leading to impairment in blood flow to the placenta and placental dysfunction. The under perfused placenta is believed to release various factors, which results in clinical manifestations of the disease including hypertension and proteinuria (2^{nd} stage). (Roberts and Hubel, 2009)

1.3.3 Endothelial dysfunction in PE and FGR

The vascular endothelium plays a significant role in cardiovascular adaptation to pregnancy and in the pathogenesis of PE and FGR. Endothelial dysfunction is associated with an imbalance in vasodilatory and vasoconstrictory compounds produced by the endothelium. Along with many other clinical manifestations, PE is also associated with intense vasoconstriction. (Brown, 2007) Originally, it was thought that the enhanced vasoconstrictor response in women with PE suggested an up-regulation of the vasoconstrictor pathways, but it progressively became clear that impaired vasodilatory function was also a contributing factor. (Mishra et al., 2011) Given the central role of vascular endothelium in regulation of vascular tone, vascular endothelial cells may play an important role in pathogenesis of PE and FGR. A series of experiments measured plasma biomarkers of endothelial cell activation including von Willebrand factor, (Calvin et al., 1988) plasma cellular fibronectin, (Friedman et al., 1995) thrombomodulin,(Boffa et al., 1998) VCAM-1 (soluble adhesion molecule), (Higgins et al., 1998) endothelin-1 (ET-1), (Schiff et al., 1993) which showed increase in both PE and FGR, although endothelial activator levels in PE exceed those observed in FGR. (Ness and Sibai, 2006) The indication of endothelial dysfunction is observed months before the onset of these conditions. For example, impaired uterine artery Doppler waveforms are reported at 23 to 25 weeks of pregnancy in pregnancies complicated by PE and FGR. (Collins et al., 2012, Cnossen et al., 2008b) The morphological evidence of endothelial damage in women with PE has been demonstrated in the glomerular

capillaries (endotheliosis) and in uteroplacental arteries (atherosis). (Poston and Williams, 2002)

The endothelium dependent dilatory function is compromised in the women with PE and FGR compared to healthy pregnant women. Knock and Poston investigated the bradykinin-mediated vasodilatation in small subcutaneous arteries from normal pregnant women and in women with PE, using myography (described in section 2.9). (Knock and Poston, 1996) They found that the preconstricted arteries from normal pregnancies had a greater relaxation response to bradykinin compared to arteries from women with PE. These results indicate that there is an increase in bradykinin-mediated NO generation in normotensive pregnancies while there may be a reduction in pregnancies complicated by PE. In addition impairment in vasodilation of myometrial arteries from women with PE and FGR has been demonstrated *ex vivo* using wire myography. (Wareing et al., 2005) Furthermore, Ashworth and coworkers reported that the endothelium-dependent relaxation to bradykinin was reduced in the myometrial arteries from women with PE compared to myometrial arteries from normotensive pregnant women. (Ashworth et al., 1997) Similarly, Kublickiene et al., (1998) reported an absence in flow-mediated dilatation (*in vitro*) in small myometrial arteries from women with PE, whereas arteries from healthy pregnant women showed increase dilatation in response to increased flow. They further examined the arteries from both groups in presence of L-NAME (an inhibitor of NOS) and found that flow mediated dilatation was abolished in the arteries from healthy pregnant women while arteries from women with PE showed no difference in response to flow

mediated dilatation. (Kublickiene et al., 1998) Taken together, these results suggest that shear stress mediated release of NO is impaired in PE in the myometrial arteries. Recently, Brandao and colleagues demonstrated that flow-mediated dilation in brachial artery *in vivo* was decreased during second half of gestation in women with PE compared to women with normotensive pregnancies. In the same study, the authors demonstrated that uterine artery perfusion was compromised in women with PE. (Brandão et al., 2012) These results support the evidence of impaired endothelial function secondary to poor uterine artery perfusion.

In summary, in PE and to a lesser degree in FGR, markers of endothelial dysfunction are apparent, and therefore this dysfunction may further contribute to decreased uteroplacental blood flow observed in these conditions. Therefore study of uterine artery endothelial function will play a central role in this thesis.

1.4 Current management of PE and FGR

Although PE and FGR share many aspects in terms of pathophysiology, maternal manifestations of these conditions are different. Hence the management of these conditions varies in the clinic and will be discussed separately.

1.4.1 Management of PE

The optimal management of a PE case depends on gestational age and the severity of the disease. Since delivery is the only cure for PE, clinicians try to minimize maternal risk while maximizing fetal maturity. (Dekker and Sibai, 2001, Wagner,

2004) The primary objective is the safety of the mother and then the delivery of a healthy newborn. (Sibai, 2006) Delivery is appropriate for the mother but may not be the best option for a premature fetus. Therefore severity of maternal disease needs to be assessed. (Sibai, 2006) Although PE has been recognised for centuries, there is no cure for this disease and treatment is mainly directed to the potential complications of the condition; anticonvulsants are administered to prevent eclampsia and antihypertensive drugs to control blood pressure. (Sibai, 2006) Magnesium sulfate is the first-line treatment for the prevention of primary and recurrent eclamptic seizures. Magnesium sulfate opposes calcium dependent arterial constriction and reduces cerebral vasospasm present in PE/eclampsia. (Sibai, 2004) Hydralazine (acts on the smooth muscle, limits calcium release and induces NO release to induce relaxation of the arteries) and Labetalol (acts on the receptors in blood vessels to induce relaxation) are used as anti-hypertensive agents. (Roccella, 2000, Magee et al., 2003) Although the above medications suppress the blood pressure and somewhat prevent the progression of the disease, they do not cure the condition. (Maynard et al., 2003) Therefore there is a need for a therapy that ameliorates the signs of PE and addresses the underlying disease.

1.4.2 Management of FGR

Since the FGR fetus is at increased risk of mortality and hypoxia and metabolic acidosis during labor, it is crucial that surveillance of fetal growth and well-being is thorough. (Resnik, 2002) The timing of the delivery is determined based on the fetal condition and gestational age. Currently the recommended methods for

management of FGR include anthropometric parameters such as fetal abdominal circumference, head circumference, biparietal diameter and femur length. These measurements are used to determine the fetal weight estimates using a standard equation and compared with population based fetal growth curves at specific gestational age. (Resnik, 2002) The gestational age is determined by using ultrasound. (Campbell et al., 1985) Although the biometric tests are useful measures for determining the well-being of a FGR fetus, umbilical Doppler velocimetry is a better predictor of fetal condition. For instance, absence or reversal of end diastolic flow in the umbilical artery is indicative of poor fetal condition. Umbilical Doppler velocimetry is especially useful in identifying fetuses that are constitutionally small from those that are growth restricted. (Ott, 2000)

1.5 Animal models of PE and FGR

Despite intensive research for decades, the current understanding of etiology and pathogenesis of PE and FGR is inadequate and research in women with PE is limited due to the lack of specimens and ethical issues. As a result, much of the current understanding about these conditions comes from animal studies. In addition, therapeutic advances for PE and FGR have been limited by the lack of appropriate animal models. A number of animal models have been developed in order to replicate the human disease to study etiologic factors, pathogenesis and treatment options for these conditions. Animal models of PE range from dogs, rabbits, sheep, baboons and murine. (Venuto and Lindheimer, 2009) The majority

of studies to date have been conducted in mice, since they have a short gestational age and are convenient models for conducting long-term follow up studies in a short time. They are also advantageous as information about their genome is readily accessible, and readily available for designing molecular studies. While rodent studies have provided important insights into the greater understanding of PE and FGR, it is important to recognize the limitations of rodent models. For instance, PE has been reported to occur spontaneously only in humans and higher apes. (Thornton and Onwude, 1992) Hence, the data obtained from the murine models of these conditions should be extrapolated into human setting with caution as there are important differences between human and mouse pregnancy.

1.5.1 Pregnancy in mouse and human

A number of differences exist between mouse and human pregnancy including length of gestation, placental size and placental classification. Furthermore, rodents differ from humans in that they bear litters, rather than singletons. Despite these obvious differences, signs of PE or FGR can be induced in a mouse through variety of methods, suggesting there may be more similarities between the species than is generally accepted. A number of pregnancy features in human and rodents are compared in Table 1-1.

Table 1-1: Comparison between human and mouse pregnancy

	Human	Mouse
Length of gestation	9 months	20 days
Stage of implantation (post fertilization)	7 days	4.5 days
Vascularization of placental villi	25 days	10 days
Placental classification	Monochorial & Villous	Labyrinthine
Hemochorial blood flow through placenta	Yes	Yes
Chorionic villi lined by syncytiotrophoblast	Yes	Yes
Invasive trophoblast	Extravillous cytotrophoblast	Trophoblast giant cells
Spiral artery invasion	Deep	Shallow

Although there are considerable differences in placental development between the two species, some components of placental development are analogous. The trophoblast cell lineage appears to follow the same pathway in both species; an invasive pathway involving extravillous trophoblasts in humans and giant cells and trophoblastic glycogen cells in mice, and an exchange pathway involving the synctiotrophoblast in both mouse and human. (Malassine et al., 2003) The mouse trophoblast giant cells, which are analogous to extravillous cytotrophoblast cells in humans, invade into the maternal spiral arteries and replace their endothelial linings, promoting the transition from endothelial lined artery to trophoblast-lined (hemochorial) blood space. Despite the aforementioned similarity, it is important to recognize that trophoblast invasion in mice is shallow compared to humans. (Pijnenborg et al., 2006) In addition, Adamson and co-workers reported that

mouse blood vessels in the decidua are lined by endothelium instead of trophoblast. (Adamson et al., 2002)

The blood flow velocity waveforms (in mice) in the uterine artery and the fetoplacental circulations are similar in shape and show similar changes during gestation when compared to human pregnancy. Similar to humans, uterine artery blood flow velocity in mice increases significantly in full-term versus non-pregnant females. In addition, there is a steady increase in blood flow velocities in the umbilical arteries from mid-pregnancy to term, analogous to human pregnancies. (Mu and Adamson, 2006)

In summary, there are considerable advantages in studying the pregnancy complications such as PE and FGR in mouse because investigators have the ability to carefully regulate both genetic and environmental influences. There are number of studies, in which modification of gene function using transgenic and knockout mouse approaches results in the induction of PE and FGR. Furthermore, by controlling the way in which these mice are bred, it is feasible to control and detect whether the altered gene is expressed in the fetoplacental unit, the mother or both.

1.5.2 Mouse models of PE and FGR

Over the past decades, a number of mouse models of PE have been developed, in which pregnant females develop hypertension, proteinuria and renal glomerular endotheliosis, which are classic characteristics of the disease. Several other models have been published which exhibit FGR (Table 1-2). The majority of

mouse models were primarily designed on the basis of perceived causes and mediators of these conditions. Therefore, these models have limitations and the results must be interpreted carefully, particularly when translating the findings into a clinical scenario. Nevertheless, various mouse models of these conditions have provided insights into factors responsible for inducing the pathogenesis of PE and FGR.

1.5.2.1 Hypertension as a risk factor for PE and FGR: BPH/5 mouse

A multivariate analysis of risk factors for PE in humans by Eskenazi and colleagues reported that the risk factors for essential hypertension and PE overlap significantly. (Eskenazi et al., 1991) Sibai et al., (1995) showed that increased systolic blood pressure before pregnancy increased the risk of PE. These observations were supported by the fact that some genes that have been associated as risk factors for PE, such as AGT (encoding angiotensinogen, a precursor of the vasoconstrictor angiotensin) (Ward et al., 1993) and eNOS, (ArngrÌmsson et al., 1995) which are also implicated to be risk factors for essential hypertension. It is important to note that the analysis of AGT and eNOS genes in the affected families revealed that they are not present in all cases of the disease, (Lachmeijer et al., 2002, Roberts and Cooper, 2001) indicating that AGT and eNOS mutations do not explain all cases of PE. Using a borderline hypertensive mouse strain; the BPH/5 mouse, Davisson and colleagues demonstrated that this mouse developed high blood pressure during pregnancy. (Davisson et al., 2002) In contrast to the normal mouse (C57BL/6J), the BPH/5 mouse showed an increase in blood pressure during mid to late pregnancy. The BPH/5 mouse also developed other

characteristics of PE including glomerular endotheliosis, proteinuria, endothelial dysfunction, abnormal maternal decidual arteries, and poor placental development. The results from this study suggest that hypertension during pregnancy is a risk factor for PE. Additionally, these mice were reported to exhibit FGR. (Davisson et al., 2002)

One of the limitations of the BPH/5 mouse model is that most PE cases occur in women who are normotensive prior to pregnancy and up to second trimester.

1.5.2.2 Placental influence in maternal hypertension: REN_AGT transgenic mouse

In pregnancy, the decrease in blood pressure is observed in both humans and mice. Therefore the fetoplacental unit may play a role in regulation of maternal blood pressure during pregnancy. As reported by Cross and colleagues, (Cross, 1996, Cross et al., 2002) the placenta is a principal source of vasoactive compounds such as NO, adrenomedullin, prostaglandins and prostacyclin as well as renin, (Cooper et al., 1999) an enzyme responsible for cleaving angiotensinogen to generate vasoconstrictor angiotensin II. As a result, placenta may in part be responsible for regulating cardiovascular adaptation observed during pregnancy.

Takimoto et al., (1996) showed that placentally derived renin plays an important role in blood pressure change during pregnancy. The female mouse carrying AGT transgene was mated with males that were transgenic for the renin gene. The females developed hypertension during pregnancy due to placental expression of renin and also proteinuria and glomerular endotheliosis, indicating that

hypertension can induce PE as observed in BPH/5 mouse model. The occurrence of FGR in this model is not known.

1.5.2.3 Defects in feto-placental development leading to signs of PE: P57 Kip2 mutant mouse

Kanayama et al., (2002) and Takahashi et al., (2000) reported that mice deficient for the cyclin-dependent kinase inhibitor, p57Kip2 (which regulates the cell cycle in trophoblasts), were an interesting model of PE as these mice exhibited placental lesions and maternal disease that was secondary to the placental defects. They discovered that the females that carried p57Kip2 deficient pups developed PE, even though they had normal p57Kip2 function themselves. It was concluded that the maternal disease was due to the mutation impacting fetoplacental development. Furthermore, Takahashi et al., (2000) reported that the mutant pups were growth restricted with a significantly reduced villous surface area in the placenta.

In contrast to Takahashi et al., (2000), Falcao et al., (2009) failed to demonstrate features of PE in the p57Kip2 knock out mouse. They investigated if the difference in diet played a factor in dichotomy between the results, however, they showed that feeding the p57Kip2 KO mouse with the same diet as Takahasi et al., (2000) resulted in endothelial dysfunction, left ventricular hypertrophy and placental pathology consistent with Takahashi and co-workers (Takahashi et al., 2000) but did not show difference in blood pressure or proteinuria. They attributed their results to differences in environmental factors.

1.5.2.4 Immunological mouse models of PE

Immune factors are involved in almost every stage of pregnancy, from implantation to placentation to labor. Accumulating evidence suggests that altered immune status, especially increased inflammation during pregnancy, may play a role in the development of PE.

Recently, Zhou et al., (2011) injected purified IgGs isolated from women with PE into C57BL/6J mice on day 13 and day 14 gestation. The aim of the study was to investigate how elevated endothelin-1 is associated with the disease. They discovered that IgG from diseased women led to increase in IL-6, TNF alpha and endothelin-1 production and these mice exhibited clinical manifestations of PE including hypertension, proteinuria and renal damage. Furthermore, the investigators discovered IL-6 to be a key cytokine that is responsible for induction of ET-1 as well as for increased levels of anti-angiogenic factors: FMS-like tyrosine kinase (sFlt-1) and soluble endoglin (sEng). Although this finding is important in terms of understanding the role of ET-1 in PE, therapeutically it may not be possible to use endothelin receptor blockers, as they are known to have teratogenic effects. (Battistini et al., 2006)

1.5.2.5 Model of endothelial dysfunction

sFlt-1, a splice variant of VEGF receptor, is an anti-angiogenic factor and an antagonist of VEGF and placental growth factor. sFLT-1 levels have been shown to be increased in women with PE. Recently Kumasawa et al., (2011) induced trophoblast-specific expression of sFLT1 using lenti virus vector and showed that

a PE-like disease can be specifically induced in mouse by placentally derived sFLT1. These mice demonstrated hypertension, proteinuria and FGR during pregnancy. The methods described by Kumasawa et al., (2011) result in expression of sFLT1 in all layers of murine trophoblast, and this pattern of expression is not applicable to human pregnancy and therefore this model may not completely recapitulate the human disease. (Clark et al., 1996)

1.5.2.6 Placental impairments and intrauterine growth restriction: Esx1 mutants and Igf 2 KO

A number of mouse models have been developed that exhibit FGR as a result of impaired placental function. For instance, Esx 1 (placental homeobox gene) mutants show impairment in vascularization of the labyrinth layer of the placenta and are known to deliver growth-restricted pups. (Li and Behringer, 1998) Constancia et al., (2002) demonstrated that deletion of paternally expressed placental specific Igf2 in mouse results in FGR. None of the aforementioned models have been investigated to determine if females develop PE.

Table 1-2: Mouse models of PE and FGR.

Model	Primary Defect	High BP	Proteinuria	Renal lesions	Placental changes	FGR	Reference
BPH/5 Strain	Hypertension	Y	Y	Y	Increased ROS	Y	(Davisson et al., 2002)
REN_AGT transgenic	Gestational hypertension	Y	Y	Y	?	?	(Takimoto et al., 1996)
P57 Kip2 mutant	Placental development	Y	Y	Y	Labyrinth, giant cells	N	(Kanayama et al., 2002)
Soluble Flt-1 administration	Endothelial dysfunction	Y	Y	Y	?	?	(Kumasawa et al., 2011)
Esx1 Mutant	Placental development	?	?	?	Vascularization of labyrinth	Y	(Li and Behringer, 1998)
IgF 2 Mutant	Placental transport	?	?	?	Reduced nutrient transport	Y	(Constancia et al., 2002)
COMT$^{-/-}$	COMT deficient	Y	Y	Y	Placental hypoxia, decreased placental eNOS	N	(Kanasaki et al., 2008)
eNOS$^{-/-}$	eNOS deficient	Y	Y	?	Increased ROS	Y	(Hefler et al., 2001b)

1.5.3 Mouse models of PE and FGR used in the current study

As discussed in the preceding sections, there is a range of murine models available to study PE and FGR. As none of the murine models exhibit every facet of these pregnancy complications it was decided to use two different models for this study: the endothelial NO synthase knock out mouse ($eNOS^{-/-}$) and the Catechol-O-methyltransferase deficient mouse ($COMT^{-/-}$) were utilized. These models were chosen as they exhibit evidence for impaired uteroplacental vascular development (discussed below).

1.5.3.1 Endothelial NO synthase (eNOS) knockout ($eNOS^{-/-}$) mouse

As discussed in section 1.1.1.3, endothelial NO synthase (eNOS) catalyses the cellular conversion of arginine to the potent vasodilator NO, which plays a crucial role in the cardiovascular adaptations of pregnancy and contributes to adequate uteroplacental perfusion. It has been previously observed that the activity of eNOS in umbilical artery endothelial cells is reduced in pregnancies complicated by FGR. (Casanello and Sobrevia, 2002) The $eNOS^{-/-}$ mouse demonstrates an increase in systolic blood pressure both when non-pregnant (Huang et al., 1995) as well as during pregnancy. (Hefler et al., 2001a) $eNOS^{-/-}$ mice have also been shown to exhibit abnormal uterine artery remodeling, such as reduced uterine artery diameter, uterine artery blood flow (Kulandavelu et al., 2012) and spiral artery length, (van der Heijden et al., 2005a) suggesting abnormal uterine artery remodeling may impact uterine artery blood flow. There are no differences in litter size compared with wild type controls, but this model deliver growth-restricted fetuses (Hefler et al.,

2001a) and our laboratory has verified this finding in a previous study. (Stanley et al., 2012a)

It has been shown that, *ex vivo,* uterine arteries from eNOS$^{-/-}$ mice exhibit reduction in methacholine-induced relaxation (Stanley et al., 2012a); this may be due to a reduction in NO production or bioavailability. It is possible that the decreased relaxation of uterine artery vasodilation observed in these mice leads to increased uterine artery resistance, which can further lead to irregular blood flow and under-perfusion of the placenta. This in turn can lead to oxidative stress, namely an increased production of superoxide anions. Indeed, our group has observed an increase in superoxide production in placentas from eNOS$^{-/-}$ mice. (Stanley et al., 2012a) NO, that is produced by either iNOS or nNOS, can be rapidly inactivated by increased superoxide observed in eNOS$^{-/-}$ mouse (Stanley et al., 2012a); as a result vasodilation can be reduced and therefore uteroplacental perfusion further reduced. In addition, reduced impaired uteroplacental perfusion can contribute to abnormal feto-placental vascular development leading to increased resistance in the umbilical circulation and hence reduced substrate supply to the fetus resulting in FGR.

1.5.3.2 Catechol-O-methyltransferase deficient (COMT$^{-/-}$) mouse

2-Methoxyestradiol (2-ME) is an endogenous metabolite of estrogen, synthesized by catechol-O-methyl transferase (COMT). Plasma concentrations of COMT and 2-ME are elevated in the third trimester of normal pregnancy but reduced in women with severe PE. (Barnea et al., 1988, Kanasaki et al., 2008) In addition, a recent study in Norwegian women reported that a low COMT activity haplotype was associated with recurrent PE. (Roten et al.,

2011) Therefore alteration in COMT gene may lead to reduced COMT enzyme activity and account for low levels of 2-ME observed in women with PE. (Roten et al., 2011) In fact, placental COMT levels are reduced in placentas from women with severe PE. (Barnea et al., 1988) Reduced COMT activity has also been linked to FGR. (Sata et al., 2006)

Pregnant mice that lack COMT ($COMT^{-/-}$) do not produce 2-ME and exhibit many features of PE including raised maternal blood pressure, proteinuria, placental abnormalities, endothelial cell activation and premature delivery. Kanasaki et al., (2008) reported that administration of 2-ME reversed all the features of PE in these mice. Recently we demonstrated that pups from $COMT^{-/-}$ mice featured FGR. In addition, these mice exhibited abnormal umbilical artery waveform, characterized by reduced umbilical artery blood flow velocity and reversed or absent end-diastolic umbilical flow velocity, suggesting that there was increased resistance in the placenta. Furthermore, we showed that uterine arteries from pregnant $COMT^{-/-}$ mice *ex vivo* exhibited increased constriction in response to phenylephrine compared to arteries from control mice. (Stanley et al., 2012b)

1.5.4 Summary

Consistent with the human disease, mouse models suggest that PE and FGR can be acheived by various mechanisms. The murine models have been instrumental in defining the maternal and fetoplacental contributions to PE and FGR and in investigating the progression of these pathologies.

The etiology and pathogenesis of PE is complex and poorly understood. One of the reasons for its complexity may be due to its multifactorial nature. There

are number of factors, which are dysregulated in PE; these include angiogenic factors, immunological factors and endothelial function. Therefore it may be impossible to have a single animal model, targeting all aspects of this disease. Nevertheless, these models have allowed us to gain a better understanding of etiology and pathogenesis of the disease.

1.6 Potential treatments for PE and FGR

1.6.1 Clinical trials involving PE

A number of clinical trials have investigated the role of various therapies to reduce the rate or severity of PE. In summary, these therapies have included protein or salt restriction, zinc, magnesium, (Sibai, 1998) calcium, (Coomarasamy et al., 2003) l-arginine, (Vadillo-Ortega et al., 2011) fish oil (Makrides et al., 2007) or vitamin C and E supplementation, (Chappell et al., 1999) aspirin (Knight et al., 2001) heparin. (North et al., 1995, Kupferminc et al., 2001) These trials, however, showed a minimal to no benefit and did not reduce the rate of adverse maternal and perinatal outcomes related to PE. Hence, there is insufficient evidence to recommend any of these therapies for treatment of PE. New approaches for prevention or treatment of PE are therefore needed.

1.6.2 Clinical trials involving FGR

Several randomized trials have reported numerous approaches in order to increase the birth weight or to extend the gestational age at delivery. These include nutritional supplementation, (Hofmeyr and Gulmezoglu, 2000) low-dose aspirin, (Bujold et al., 2009b) calcium channel blockers (Hofmeyr and

Gulmezoglu, 2000) maternal oxygen administration, (Hofmeyr and Gulmezoglu, 2000) hormones, (Say et al., 2003) betamimetics (Say et al., 2001) and heparin. (Dodd et al., 2010) Unfortunately, none of the treatments have been of value. There are currently no treatment options available for FGR. Hence, an effective therapeutic strategy would have a major clinical significance.

1.6.3 Rationale behind interventions in the current study

Therapeutic advances for PE and FGR have been severely limited as none of the therapeutic agents tested for these conditions have been of value. As a result, new approaches for prevention/treatment of these conditions are needed. For the purpose of this thesis we have chosen to study two potential therapies based on their vascular targets and clinical potential (discussed below).

1.6.3.1 2-Methoxyestradiol

As detailed above, the concentration of 2-ME is elevated during the third trimester of normal pregnancy but is reduced in women with severe PE. (Kanasaki et al., 2008, Barnea et al., 1988) Preliminary studies in our laboratory have shown that 2-ME is able to induce significant vasodilation of uterine arteries (*ex vivo)* from normal pregnant mice and myometrial arteries from pregnant women. (Stanley et al., 2010) In addition, uterine artery endothelial cells are known to express COMT enzyme, required for 2-ME synthesis. (Jobe et al., 2010) Furthermore, recent evidence suggests that 2-ME

might be necessary for cytotrophoblast invasion of the maternal decidua, and therefore contribute to the prevention of PE and FGR by promoting normal placental vascular formation. (Lee et al., 2010) Taken together there is evidence to suggest that 2-ME may be able to improve uterine artery vasodilation and therefore uterine artery resistance in women with PE. It also remains to be investigated if 2-ME improves uteroplacental blood flow and therefore rescues the fetal growth in the $COMT^{-/-}$ mice. Hence this will be one of the major focus of this thesis.

1.6.3.2 Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring polyphenol found in many plant species (Juan et al., 2012) that may have a beneficial effect by targeting some of the common pathophysiological mechanisms described in PE and FGR. It is widely known for its cardioprotective effects in humans and various animal models. The beneficial effects of resveratrol appear to be mediated via a plethora of biochemical pathways including stimulation of NO production and eNOS, iNOS and nNOS expression, as well as a reduction in oxidative stress and a decrease in ischemia reperfusion injury. (Leifert and Abeywardena, 2008)

Previous studies have evaluated safety, pharmacokinetics and metabolism of resveratrol in humans following oral ingestion of either the synthetic agent or polyphenol rich diets. (Brown et al., 2010, Chow et al., 2010) The majority of studies to date have reported resveratrol to be safe, suggesting that it is well tolerated in humans even at very high doses. (Patel et al., 2011) In addition,

rodent models have shown no evidence of teratogenesis associated with this compound. (Edwards et al., 2011, Williams et al., 2009) Although previous use of resveratrol in animal models of PE is very limited (Moraloglu et al., 2011), it has been shown to ameliorate high blood pressure, (Rivera et al., 2009) proteinuria (Nihei et al., 2001) and improve fetal weight. (Singh et al., 2011) In addition, it has been demonstrated that resveratrol induces vasorelaxation of uterine arteries in non-pregnant guinea pigs *ex vivo* through both endothelium dependent and independent mechanisms. (Naderali et al., 2000)

In study-2 we tested the hypothesis that supplementing the diet of $eNOS^{-/-}$ and $COMT^{-/-}$ mice with resveratrol during pregnancy will improve uterine artery blood flow velocity and therefore ameliorate PE like phenotype and FGR in these murine models.

1.6 Hypothesis

My overall hypothesis is that restoration of uteroplacental blood flow will ameliorate signs of PE and rescue FGR in relevant mouse models.

1.7 Specific aims

1.7.1 Study 1: Effect of 2-ME administration in COMT$^{-/-}$ mice during pregnancy

1. To determine the ability of 2-ME to increase uterine artery flow *in vivo* in COMT$^{-/-}$ mice.
2. To determine the effect of 2-ME on maternal blood pressure and proteinuria in COMT$^{-/-}$ mice.
3. To determine the effect of 2-ME on fetal growth in COMT$^{-/-}$ mice.
4. To characterize the *ex vivo* uterine artery function in response to vasoconstrictor (phenylephrine) and vaso-relaxant (Methacholine) in 2-ME treated vs. untreated COMT$^{-/-}$ mice.
5. To determine whether there are any major teratogenic effects (external malformations) associated with 2-ME therapy.

1.7.2 Study 2: Effect of resveratrol treatment in eNOS$^{-/-}$ and COMT$^{-/-}$ during pregnancy

1. To evaluate if resveratrol can increase uterine artery blood flow *in vivo* in eNOS$^{-/-}$ and COMT$^{-/-}$ during pregnancy.

2. To determine the effect of resveratrol on maternal blood pressure and proteinuria and in eNOS$^{-/-}$ and COMT$^{-/-}$ during pregnancy.
3. To determine the ability of resveratrol to ameliorate FGR in the eNOS$^{-/-}$ and COMT$^{-/-}$ during pregnancy.
4. To characterize the *ex vivo* uterine artery function in response to vasoconstrictor (phenylephrine) and vaso-relaxant (Methacholine) in resveratrol treated vs. untreated eNOS$^{-/-}$ and COMT$^{-/-}$ mice.
5. To investigate whether there are any major teratogenic effects external malformations associated with the administration of resveratrol during pregnancy in these murine models.

CHAPTER 2: MATERIALS AND METHODS

2.1 Ethics

The University of Alberta Health Sciences Animal Care and Use Committee approved all the experimental procedures in this study in accordance with the guidelines of Canadian Council on Animal Care and the *Guide for the Care and Use of Laboratory Animals* (copyright 1996, National Academy of Science).

2.2 Animal care

All animals used in this study were housed in polypropylene cages in a temperature (20°C) and humidity controlled environment with 12h-12h light-dark cycle. Breeding pairs of COMT mice were obtained initially from Professor J Gogos, Columbia University and used to establish our own colony. Female $COMT^{-/-}$, $eNOS^{-/-}$ mice (Jackson Laboratories; Bar Harbor, ME) and control, C57BL/6J mice (Jackson Laboratories; Bar Harbor, ME) of 2-3 months of age were mated with strain-matched males nightly. Females were checked the next morning for the presence of vaginal plug by gentle probing of the vaginal orifice with a blunt pipette tip. The day of vaginal plug detection was designated as 0.5 day of pregnancy.

2.3 Treatments

2.3.1 2-ME

Pregnant $COMT^{-/-}$ and C57BL/6J mice were provided with regular chow and water *ad libitum*. From gestational day (GD) 12.5 to GD 18.5 pregnant dams were injected subcutaneously every day with 10 ng (100 μL**)** of 2-ME or

vehicle (olive oil-100 μL) (Figure 2-1). The timing of the dose was chosen because this is when placental development is equivalent to the end of the first trimester in the human. (Stanley et al., 2012a) In addition, we have administered Sildenafil Citrate in $COMT^{-/-}$ mice and Tempol in $eNOS^{-/-}$ mice in the identical time frame and were able to rescue fetal growth. (Stanley et al., 2012a, Stanley et al., 2012b) The dose of 2-ME was selected based on previous studies. (Kanasaki et al., 2008, Lee et al., 2010) In addition, the dose administered in the current study increased the concentration of 2-ME in $COMT^{-/-}$ mice to levels similar to those observed in the control wild-type pregnant mice. (Kanasaki et al., 2008)

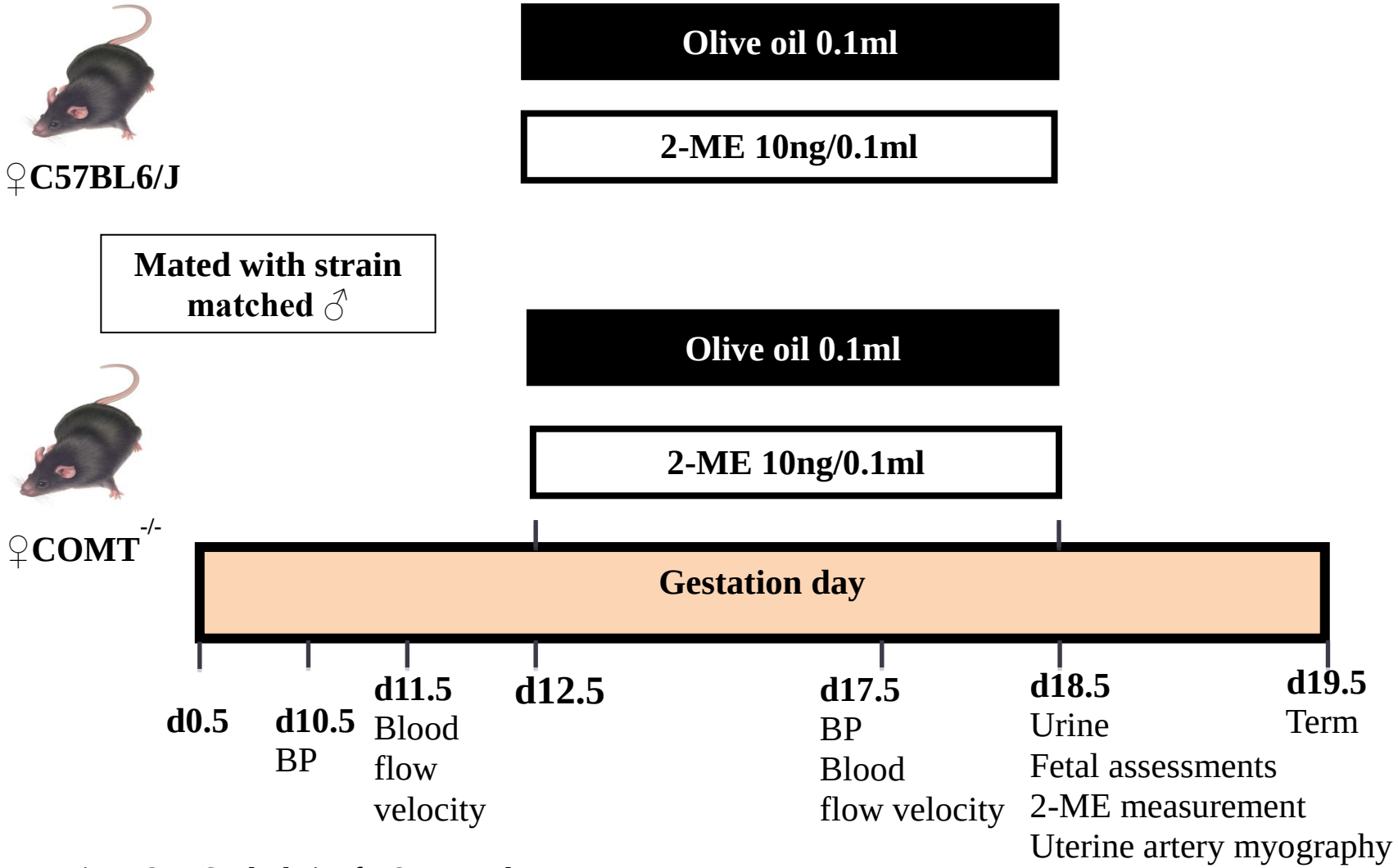


Figure 2- 1: Study design for 2-ME study

2.3.2 Resveratrol

Pregnant mice were housed in a single occupancy cages from GD 0.5 until the end of the experiments in order to monitor their food intake. Pregnant dams were randomly allocated to receive purified control diet (AIN-93G diet, Dyets Inc., Bethlehem, PA) or resveratrol diet (AIN-93G diet supplemented with 4-gram resveratrol/kg diet) from GD 0.5 to 18.5 (Figure 2-2). The duration of administration and dose of resveratrol in the diet was based on a previous study (Lagouge et al., 2006) as well as results from our own laboratory following administration of the resveratrol supplemented diet in pregnant rats. (Bourque et al., 2012) Resveratrol bioavailability is low when it is administered through diet as a result of poor intestinal absorption and rapid clearance by the liver. (Wenzel and Somoza, 2005) However, a therapeutic concentration range of resveratrol in the plasma was achieved by Dolinsky et al., (2011) following the use of dose administered in this study.

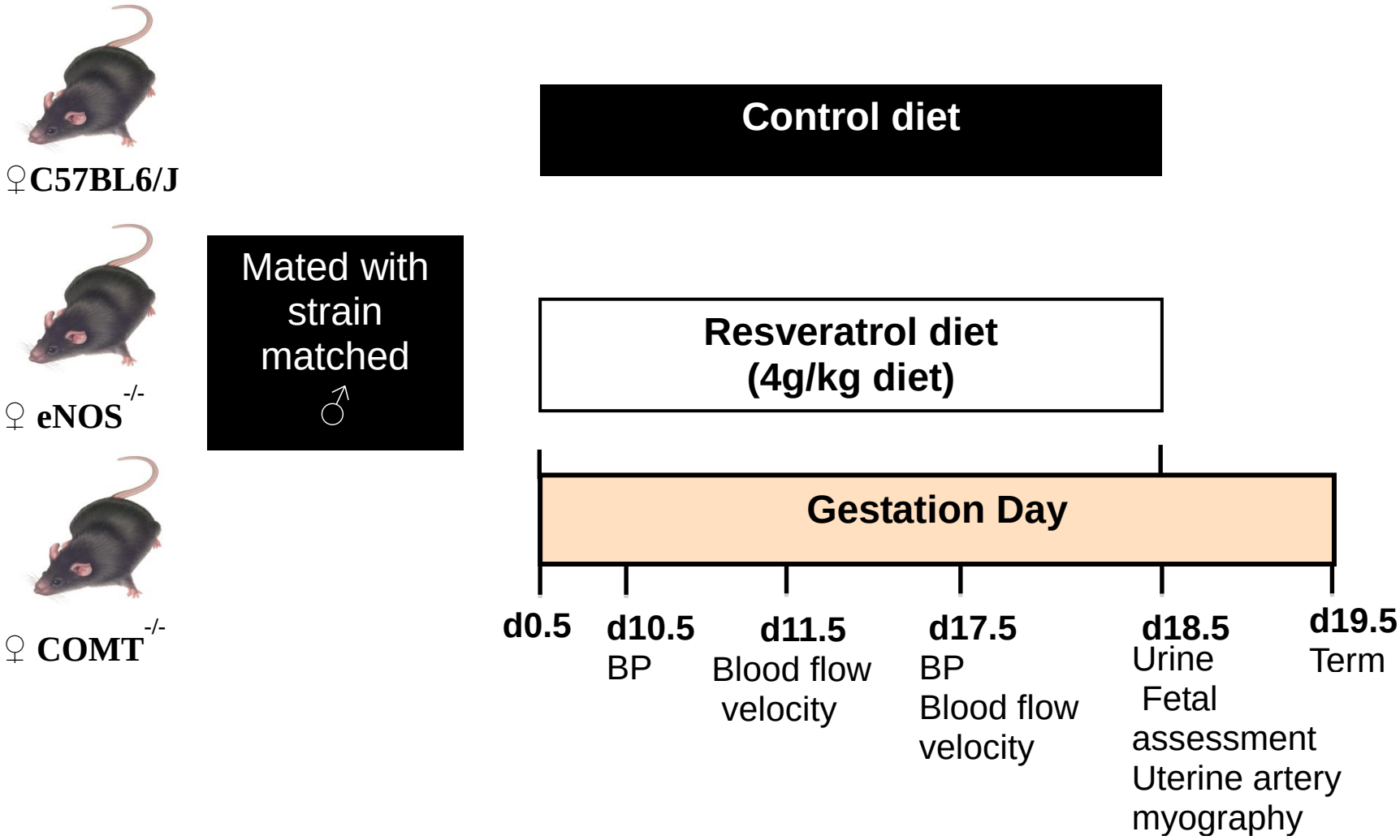


Figure 2-2: Study design for resveratrol study

2.4 Measurement of Blood Pressure and Heart Rate

Mice were trained in restraint tubes for 5 minutes each on three successive days prior to mating and measurement of blood pressure in order to accustom them to the procedure. Blood pressure and heart rate were measured by the staffs at Cardiovascular Research Centre, University of Alberta, facility using tail-cuff plethysmography (IITC Life Science, Woodland Hills, CA, USA) as previously validated. (Whitesall et al., 2004) Both systolic and diastolic blood pressure and heart rate measurements were conducted on GD 10.5 and GD 17.5.

2.5 Assessment of Proteinuria

Urine samples were collected on GD 18.5 of pregnancy. Samples were then stored at -80°C until assayed for urinary albumin using an Enzyme-linked immunosorbent assay (ELISA) (AssayPro, St Charles, MO, USA) and urinary creatinine by colorimetric kit assay (Cayman Chemical Company, Ann Arbor, MI, USA). From these measurements the albumin to creatinine ratio was determined.

2.6 Uterine and umbilical artery blood flow velocity

Uterine and umbilical artery and vein blood flow velocity was assessed *in vivo* on GD 11.5 and GD 17.5. Blood flow velocities were measured using previously established protocols. (Stanley et al., 2012b, Mu and Adamson, 2006) Briefly, mice were anesthetized with isofluorane (3%) in air and placed in a supine position on a temperature controlled heating pad. Heating was

adjusted to maintain rectal temperature between 36° and 38°C. Then the anesthetic concentration was reduced to (~0.5 to 1.5%) in order to maintain the constant maternal heart rate of 550 ± 50 bpm and a respiratory rate of 150 ± 20 cpm. Abdominal hair was removed by the application of chemical hair remover. Pre-warmed gel was applied to the shaved abdomen, as an ultrasound-coupling medium before mice were imaged transcutaneously using a high resolution ultrasound biomicroscope ((model Vevo 770 for 2-ME study (study-1) & model Vevo 2100 for resveratrol study (study-2), VisualSonics®, Toronto, ON, Canada)). The transducer used for Vevo 770 was 30 MHz operating at 100 frames/s. Vevo 2100 system was also equipped with a 30 MHz transducer but was operating at 300 frames/s. The angle between the Doppler beam and the vessel was <30 degrees. Doppler waveforms were then obtained from both left and right uterine artery near the uterocervical junction close to the iliac artery (Figure 2-3). Umbilical artery and vein (Figure 2-4) from at least two fetuses near the placental surface. Peak systolic velocity (PSV) and end diastolic velocity (EDV) were measured from at least three consecutive cardiac cycles that were not affected by motion caused by maternal breathing and the results were averaged. The resistance index (RI) = ((PSV-EDV)/PSV) was calculated.

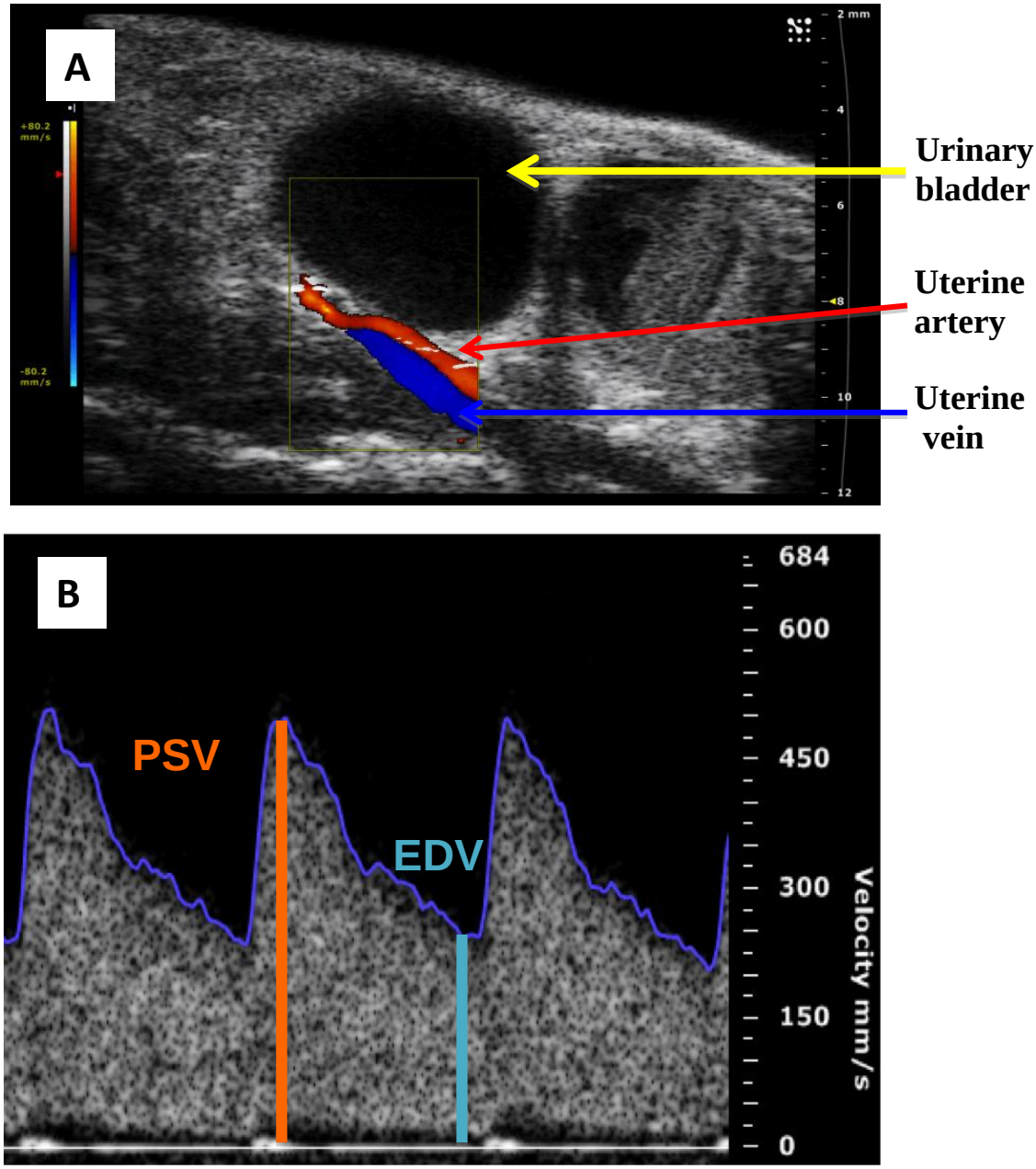


Figure 2-3: Representative images of uterine artery and vein obtained from Vevo 2100 biomicroscope.

(A)Uterine artery and vein and their respective location and (B) uterine artery Doppler waveforms. Orange line indicates peak systolic blood flow velocity (PSV) and the blue line indicates end diastolic blood flow velocity (EDV).

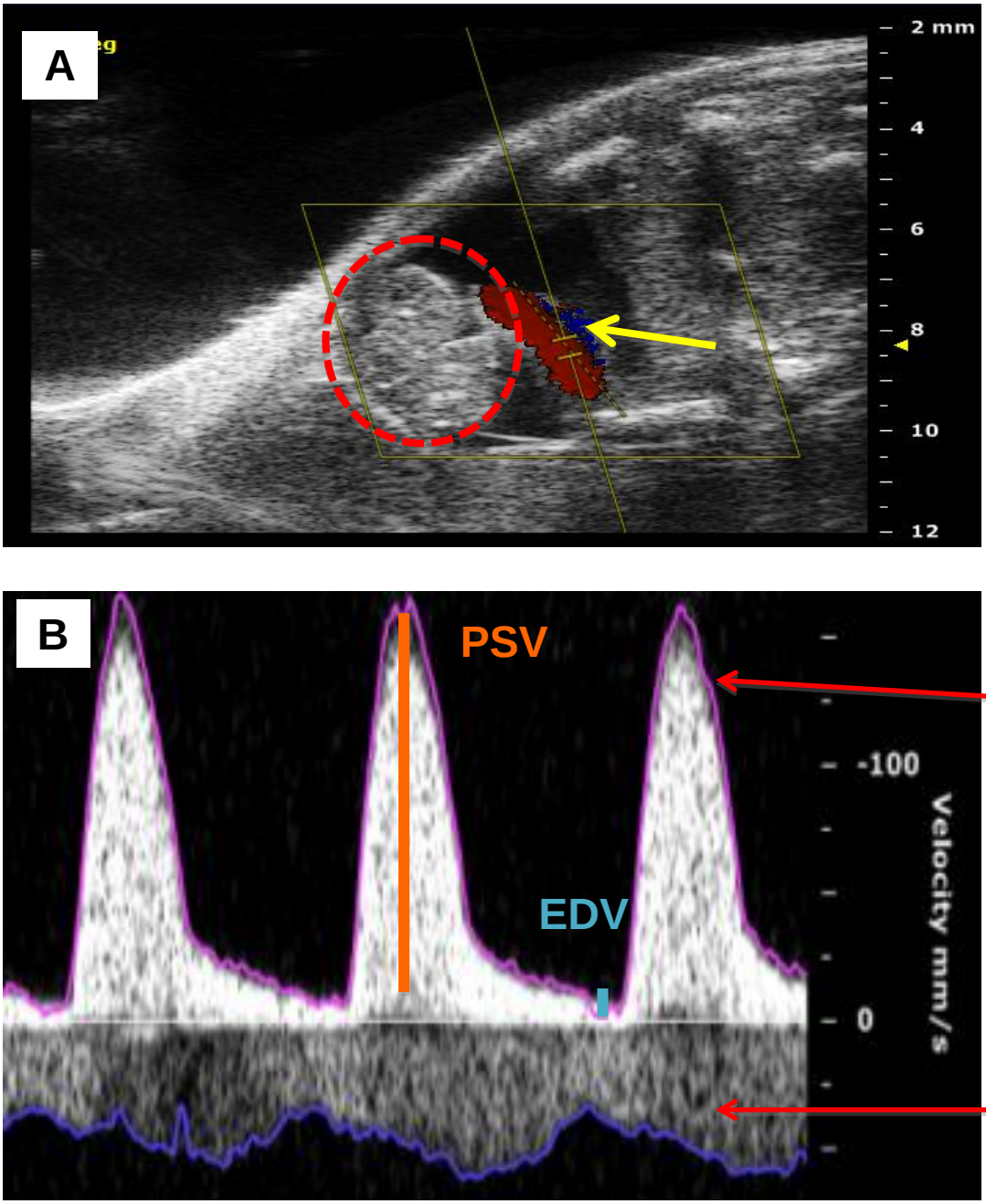


Figure 2-4: Representative images of an umbilical cord and the respective Doppler waveforms obtained from Vevo 2100 biomicroscope.

(A) Red circle represents a fetus and the yellow arrow indicates the umbilical cord. (B) Umbilical artery and vein Doppler waveforms. Orange line indicates peak systolic blood flow velocity (PSV) and the blue line indicates end diastolic blood flow velocity (EDV).

2.7 Measurement of serum 2-ME concentration

On day 18.5 of pregnancy mice were subjected to terminal anesthesia using isofluorane and blood was withdrawn by heart puncture. Blood was then allowed to clot for ~1hr in ice. Serum was then extracted following centrifugation for 10 minutes at 5000 rpm and stored in -80°C. 2-ME measurements were performed by Dr. Timothy D. Veenstra at Frederick National Laboratory for Cancer Research in Frederick, USA. The protocol supplied by the facility to measure serum 2-ME concentration is described below.

Serum 2-ME concentration at GD 18.5 in C57BL6/J, $COMT^{-/-,}$ and $COMT^{-/-}$ supplemented with 2-ME was measured by liquid chromatography-mass spectroscopy. Briefly, 0.05 ml of freshly prepared 0.15 M sodium acetate buffer (pH 4.6) containing 16 pg of d_5-2-$MeOE_2$ and 2 mg of L-ascorbic acid were added to 0.05 ml of the serum sample. After extraction, the organic solvent portion was transferred into a clean glass tube and evaporated to dryness at 60 °C under nitrogen gas (Reacti-Vap III™, Pierce, Rockford, IL). To each dried sample, 32 µL of 0.1 M sodium acetate buffer (pH at 9.0) and 32 µL of dansyl chloride solution (1 mg/mL in acetone) were added. After vortexing, the sample was heated at 60 °C (Reacti-Therm III™ Heating Module, Pierce, Rockford, IL) for 10 min to form 2-$MeOE_2$-Dansyl and d_5-2-$MeOE_2$-Dansyl. Calibration standards and quality control samples are hydrolyzed, extracted, and derivatized following the same procedure as that used for unknown serum samples. After derivatization, all samples are analyzed by capillary LC-ESI-MS.

LC-MS^2 analysis was performed using an Agilent 1200 series nanoflow LC system (Agilent Technologies, Palo Alto, CA) coupled to a TSQ™ Quantum Ultra triple quadrupole mass spectrometer (Thermo Electron, San Jose, CA). The LC separation was carried out on a 150 mm long x 300 μm i.d. column packed with 4 μm Synergi Hydro-RP particles (Phenomenex, Torrance, CA) and maintained at 40 °C. A total of 8 μL of each sample was injected onto the column. The mobile phase, operating at a flow rate of 4 μL/min, consists of methanol as solvent A and 0.1% (v/v) formic acid in water as solvent B. A linear gradient from 72-85% solvent B in 75 min. was employed for separation of 2-$MeOE_2$ and 2-$MeOE_2$. The mass spectrometer setting were as follows: source: ESI; ion polarity: positive; spray voltage: 3200 V; sheath and auxiliary gas: nitrogen; sheath gas pressure: 10 arbitrary units; ion transfer capillary temperature, 270 °C; scan type: selected reaction monitoring (SRM); collision gas: argon; collision gas pressure: 1.5 mTorr; scan width: 0.7 u; scan time: 0.30 s; Q1 peak width: 0.70 u full-width half-maximum (FWHM); Q3 peak width: 0.70 u FWHM. Quantitation of d_5-2-$MeOE_2$ was carried out using Xcalibur™ Quan Browser (Thermo Electron). Calibration curves were constructed by plotting 2-$MeOE_2$-Dansyl/d_5-2-$MeOE_2$-Dansyl peak area ratios obtained from calibration standards versus amounts injected on column and fitting these data using linear regression with 1/*X* weighting. The amounts of 2-$MeOE_2$ were interpolated using this linear function.

2.8 Fetal and placental measurements

Dams were euthanized on GD 18.5 and the entire uterus was removed and immediately placed in freshly prepared cold physiological salt solution (PSS;

mmol/L: 10 HEPES, 142 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.6 $CaCl_2$, 1.18 KH_2PO_4, 5.5 glucose, 0.034 EDTA; pH 7.4). Pups and placentas were then dissected out and number of live fetuses and resorptions were recorded. In addition, all fetuses were examined for external malformations. The fetuses and placentas were blotted dry and weighed; fetal crown to rump length (length from crown of the head to the apices of the tail) and abdominal circumference (length around the outer edge of navel center) were also recorded. Finally, placentas were dried at 50°C overnight and dry placental weight was recorded.

2.9 Assessment of uterine artery function

Wire myography is a well established *ex vivo* technique that enables the examination of the functional responses and vascular reactivity of small blood vessels.(Mulvany and Halpern, 1977) The vascular experiments in this study were conducted using DMT wire myograph (610m, Danish Myo Technology, Aarhus, Denmark) and myography software (Myodata, Danish Myotech, Aarhus, Denmark). The equipment consists of four sets of baths and therefore allows the study of four vessels simultaneously (Figure 2-5).

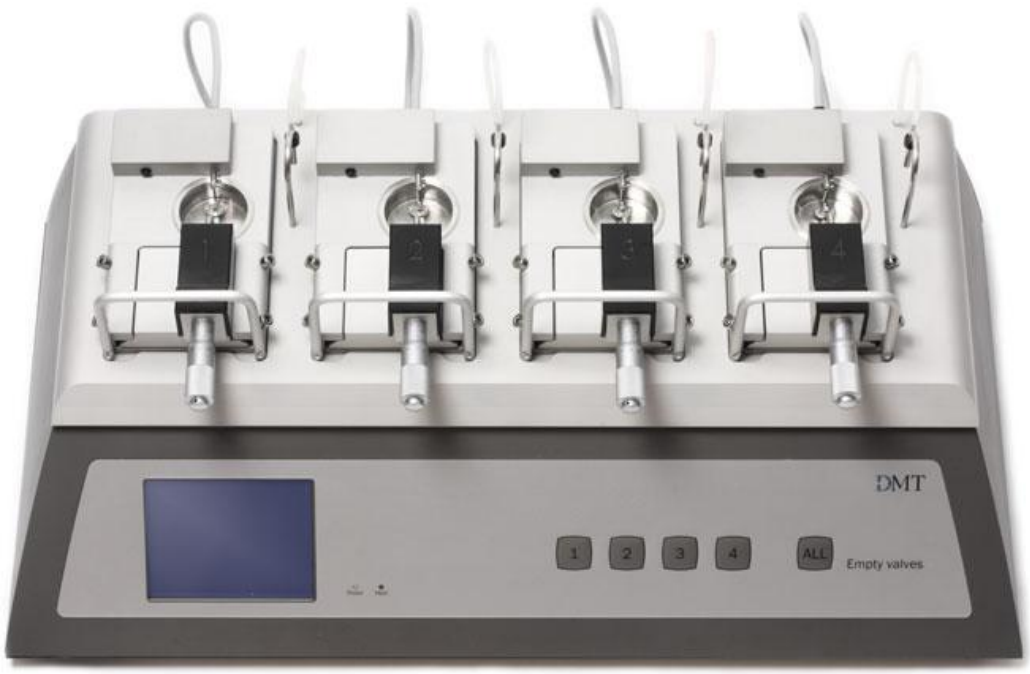


Figure 2-5: Wire myograph system equipped with four baths; allows the study of four vessels simultaneously.

Uterine arteries from the right horn were carefully dissected under a dissecting microscope in cold PSS and surrounding adipose and connective tissue was removed. They were then carefully cut into four small segments (~2mm each) and mounted between the jaws of each bath using 25μm tungsten wire (ADinstruments, Colorado Springs, USA) (Figure 2-6). The preparations were bathed in 6 ml of PSS maintained at a temperature of 37°C and pH of 7.4 and constantly oxygenated with dry air. In the next step, using a normalization software (Myodata, Danish Myotech, Aarhus, Denmark) the vessels were normalized to set vessels to standard initial conditions by incremental stretching (with a micrometer) to luminal pressure of 13.3kPa (resting tension), where the internal circumference of the vessel would have had *in vivo* under a transmural pressure of ~90 mmHg. (Mulvany and Halpern, 1977)

The fixed head of the myograph connected to a force transducer recorded the tension across the vessel wall under resting conditions and when challenged with various agonists.

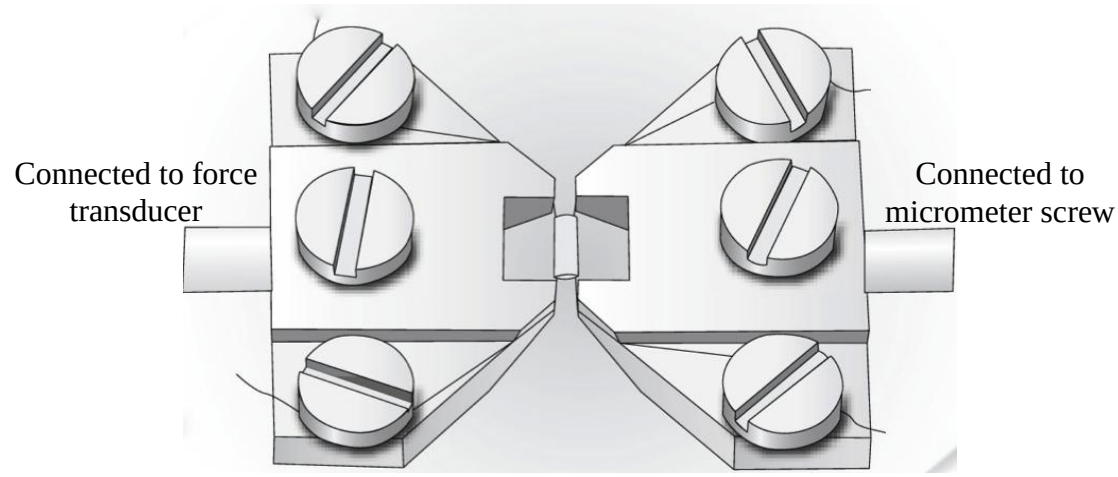


Figure 2-6: Diagram of segment of uterine artery mounted in wire myograph.

Following normalization, all vessels were washed with 2 x PSS and allowed to equilibrate under resting tension for 30 minutes. To obtain optimal data from the vessels, it is important to ensure that the technique of isolating and mounting vessel has no negative effect on functional response. Thus, vascular integrity was assessed using a single dose of phenylephrine (Phe), providing a final bath concentration of 10^{-5}M. The resulting constriction was allowed to plateau before being washed with 2 x PSS back to baseline. The vessels were then left to recover at baseline for 15 minutes and second dose of Phe was added to the baths. Once a plateau of constriction was reached, integrity of the endothelium dependent vasodilation was assessed by adding methacholine (MCh, 10^{-5} M in the bath) to the baths. Vessels that did not relax following the addition of MCh, were excluded from the study.

Next, vessels were washed with 2 x PSS and allowed to recover for 30 minutes. A Phe dose-response curve (10^{-10} to 10^{-5} M in nine steps) was then performed, followed by a rinse with 2 x PSS. To investigate the contribution of NO to endothelium-dependent vasodilation, we added NO synthase inhibitor $_{L}$-NAME (10^{-4} M) to one of the baths. Remaining sections were left in PSS only and these served as controls. Vessels were then allowed to rest for 30 minutes, after which all sections were preconstricted with Phe (EC_{80}) and the constriction was allowed to plateau. The endothelium-dependent relaxation response was then tested by constructing a vasodilation curve to MCh (10^{-10} to 10^{-5} M in 9 steps). Each dose of MCh was added to the bath at 2-minute intervals.

After the MCh curve, vessels were rinsed two times with PSS and left to recover for 30 minutes. All four sections were then again preconstricted with Phe (EC_{80}) and the constriction was allowed to plateau. Finally, a dose curve was determined in response to sodium nitroprusside (SNP; 10^{-10} to 10^{-5} M in six steps) to validate endothelium-independent NO-mediated smooth muscle relaxation. SNP donates NO, which induces vasodilation by directly stimulating the smooth muscle. SNP was added to the baths at 2 min intervals.

2.10 Statistical analyses

All data are presented as mean ± standard error of the mean (SEM). Graphpad Prism (La Jolla, CA) 5.0 software was used for statistical analysis. Analyses with two experimental groups were made using Student's t-test. One-way ANOVA was used to compare the serum 2-ME concentration between C57BL6/J, $COMT^{-/-}$ and $COMT^{-/-}$ supplemented with 2-ME, followed by

Bonferroni post-hoc test to determine statistical significance. Two-way ANOVA was performed in order to make a comparison between genotype and treatment (independent variables). Significance was determined using Bonferroni post-hoc test. Statistical significance was defined as $p<0.05$. For myography experiments, sigmoidal dose-response curve fitting was performed and analysed using GraphPad Prism. The EC_{50} or EC_{80} concentration of the drug was calculated from the concentration-response curves by fitting data to a logistic sigmoid equation using the GraphPad Prism program.

CHAPTER 3: RESULTS

Study 1: Effect of 2-ME administration in COMT$^{-/-}$ mice during pregnancy

3.1.1 Blood pressure and heart rate

Systolic blood pressure (SBP) trended towards increase in the $COMT^{-/-}$ mice compared to C57BL/6J prior to treatment on GD 10.5 of pregnancy (126.7 ± 3.4 vs 136.1 ± 3.9 mmHg p=0.096). Diastolic blood pressure (DBP) (74.1 ± 4.9 vs 78.0 ± 4.0 mmHg) and heart rate (HR) (479.8 ± 17.0 vs 513.3 ± 23.8 bpm) on GD 10.5 were not different between the C57BL/6J and $COMT^{-/-}$ mice. In addition, SBP, DBP and HR were not different between placebo and 2-ME treated groups on GD 17.5 (Table 3-1).

3.1.2 Proteinuria

There was a significant effect of genotype and treatment on albumin:creatinine ratio. It was significantly elevated in the $COMT^{-/-}$ mice on GD 18.5 compared to C57BL/6J on placebo (Figure 3-1; $p<0.05$). This increase was normalized to control levels following 2-ME administration in the $COMT^{-/-}$ mice (Figure 3-1; $p<0.01$).

3.1.3 Uterine and umbilical artery and vein blood flow velocity

The hemodynamic and waveform parameters, as measured at GD 11.5 and 17.5 are listed in Tables 3-2 and 3-3 respectively. There were no differences in uterine artery and umbilical vein blood flow velocities in C57BL/6J and $COMT^{-/-}$ mice at either GD 11.5 or 17.5 (Tables 3-2 and 3-3). Umbilical artery maximum velocities were not different among the groups (Figure 3-2A). However, a significant effect of genotype and treatment on umbilical artery end diastolic velocity and resistance index was observed on GD 17.5 (Figure 3-2B and 3-2C). Minimum umbilical artery velocity was significantly reduced and (Figure 3-2B) resistance index was increased in the $COMT^{-/-}$ mice (Figure

3-2C). Importantly, both of these measures were normalized following treatment with 2-ME (Figure 3-2B and 3-2C). Reverse umbilical Doppler waveforms observed in $COMT^{-/-}$ mice were also normalized following 2-ME administration (Figure 3-2D).

3.1.4 Maternal weight and litter size

There was no difference in maternal weight gain at term among the groups and there was no effect of 2-ME treatment on this measure (C57BL6/J; 34.4 ± 0.6 vs 30.3 ± 2.1, $COMT^{-/-}$ 33.2 ± 0.9 vs 34.8 ± 1.4g). Additionally, there was no effect of genotype or 2-ME treatment on litter size (C57BL6/J; 8.6 ± 0.6 vs 5.8 ± 1.6, $COMT^{-/-}$ 6.8 ± 0.7 vs 7.2 ± 0.3) in either of the groups.

3.1.5 Fetal and placental measurements

There was no difference in pup weight (Figure 3-3A), crown-rump length (Figure 3-3B), or abdominal circumference (Figure 3-3C) among the groups.

Placental wet (Figure 3-4A) and dry weights (Figure 3-4B) were not different in either of the groups. Similarly, pup weight: placental weight ratio was not different (Figure 3-4C); again 2-ME did not have any effect on this measure. There was no evidence of gross abnormalities in fetuses from 2-ME treated dams.

3.1.6 Uterine artery *ex vivo* vascular function

There was no effect of genotype or 2-ME treatment on Phe-induced constriction in any of the groups (Figure 3-5A). Similarly, there was no effect of genotype or 2-ME treatment on sensitivity to Phe (Figure 3-5B).

Additionally, MCh-induced relaxation or MCh EC_{50} concentrations were not significantly different between C57BL/6J and $COMT^{-/-}$ mice (Figure 3-5C and D). Supplementation with 2-ME did not have any effect on these measures (Figure 3-5C and D). NO contribution to endothelium-dependent vasodilation could not be statistically analyzed for this study as sample size was very low. No difference in uterine artery relaxation was observed among the groups in response to the NO donor SNP (Figure 3-6A and B).

3.1.7 2-ME measurement in the serum

Subcutaneous administration of 2-ME increased circulating levels of this molecule in $COMT^{-/-}$ mice to levels similar to those observed in the C57BL6/J mice on GD 18.5 (Figure 3-7).

Study 2: Effect of resveratrol treatment in eNOS$^{-/-}$ and COMT$^{-/-}$ during pregnancy

3.2.8 Blood pressure and heart rate

Systolic and diastolic blood pressures and heart rate were not different between the C57BL/6J, eNOS$^{-/-}$ and COMT$^{-/-}$ mice on control diet on GD 10.5 or 17.5 (Table 3-4). In addition, resveratrol treatment did not have any effect on these measurements (Table 3-4).

3.2.9 Proteinuria

There was a significant effect of genotype on maternal proteinuria at GD 18.5 (Figure 3-8; $p<0.001$). A significantly higher albumin:creatinine ratio was observed in eNOS$^{-/-}$ compared to C57BL/6J controls. Supplementation with resveratrol, however, had no effect on proteinuria in any of the groups.

3.2.10 Uterine and umbilical artery and vein blood flow velocity

The hemodynamic and waveform parameters, as measured at GD 11.5 and 17.5, are listed in Tables 3-5 and 3-6 respectively. There were no differences in hemodynamic and waveform parameters on GD 11.5. A significant effect of genotype on both maximum (Figure 3-9A) and minimum (Figure 3-9B) uterine artery blood flow velocity was observed on GD 17.5 ($p<0.05$). In addition, there was a significant interaction of genotype and treatment on maximum uterine artery blood flow velocity ($p<0.05$). Interestingly, resveratrol treatment increased both of these measures in COMT$^{-/-}$ mice but not in C57BL/6J; in eNOS$^{-/-}$ mice trends to increased blood flow velocity were not significant (Figure 3-9). There were no effects of genotype or resveratrol treatment on umbilical artery or vein velocity in either of the groups. (Table 3-5).

3.2.11 Maternal weight gain, food consumption and litter size

There was no difference in maternal weight among the groups at term and there was no effect of resveratrol treatment on this measure (C57BL6/J; 30.1 ± 1.1 vs 32.5 ± 1.2, $eNOS^{-/-}$; 30.4 ± 0.8 vs 28.7 ± 0.8, $COMT^{-/-}$ 33.7 ± 0.4 vs 33.2 ± 1.3g). In addition, food consumption was not affected by genotype or resveratrol treatment in C57BL/6J (56.1 ± 1.0 vs 58.6 ± 2.2g), $eNOS^{-/-}$ (58.1 ± 1.9 vs 55.5 ± 1.8g) and $COMT^{-/-}$ (61.6 ± 1.9 vs 60.4 ± 2.5g) mice.

Additionally, there was no effect of genotype or resveratrol treatment on litter size (C57BL6/J; 6.5 ± 0.3 vs 7.2 ± 0.6, $eNOS^{-/-}$; 7.1 ± 0.8 vs 6.6 ± 0.5, $COMT^{-/-}$ 7.8 ± 0.3 vs 7.8 ± 0.9) in any of the groups.

3.2.12 Fetal and placental measurements

There was a significant effect of both genotype and treatment on pup weight ($p<0.001$, Figure 3-10A). Pup weight in untreated $eNOS^{-/-}$ mice was significantly reduced compared to C57BL/6J mice on control diet. There was no difference in pup weight between untreated and resveratrol treated C57BL/6J (1.052 ± 0.02 vs 1.088 ± 0.01g) mice. There was a trend towards increase in pup weight in resveratrol treated $eNOS^{-/-}$ mice (0.910 ± 0.02 vs 0.975 ± 0.02g), although the difference did not reach statistical significance at the 5% level. A significant increase in pup weight was observed in $COMT^{-/-}$ (1.031 ± 0.02 vs 1.119 ± 0.01g, $p<0.05$; Figure 3-10A) mice following resveratrol administration. Crown to rump length was significantly greater in the resveratrol treated $COMT^{-/-}$ (28.4 ± 0.2 vs 30.14 ± 0.2mm; $p<0.05$; Figure 3-10B) but not in $eNOS^{-/-}$ (28.3 ± 0.3 vs 28.9 ± 0.3mm) or C57BL/6J (28.5 ± 0.2 vs 29.7 ± 0.3mm) mice. Abdominal circumference was significantly

elevated in resveratrol treated eNOS$^{-/-}$ compared with untreated genotype controls (23.6 ± 0.3 vs 25.0 ± 0.4mm, $p<0.05$; Figure 3-10C) but not in COMT$^{-/-}$ or C57BL/6J (24.1 ± 0.4 vs 24.8 ± 0.4mm) mice. Placental wet (Figure 3-11A) and dry weight (Figure 3-11B) in C57BL/6J, eNOS$^{-/-}$ or COMT$^{-/-}$ were not affected by either genotype or resveratrol treatment. Similarly, pup weight: placental ratio was comparable across genotypes and did not change following resveratrol treatment (3-11C). No evidence of gross abnormalities in fetuses from resveratrol treated dams was found.

3.2.13 Uterine artery *ex vivo* vascular function

There was no effect of genotype or resveratrol treatment on Phe-induced constriction in any of the groups (Figure 3-12A). Similarly, there was no effect of genotype or resveratrol treatment on sensitivity to phenylephrine (Figure 3-12B). MCh-induced relaxation, on the other hand, was significantly impaired in eNOS$^{-/-}$ compared with C57BL/6J mice. Supplementation with resveratrol did not improve this impairment (Figure 3-13A). A significant effect of genotype was observed on MCh EC_{50} concentration, such that it was significantly elevated in the eNOS$^{-/-}$ mice; again resveratrol treatment did not have any effect on this measure (Figure 3-13B). L-NAME was used to study NO contribution to total relaxation. In the uterine arteries from C57BL/6J and COMT$^{-/-}$ mice on control diet NO had a similar contribution to total relaxation. In eNOS$^{-/-}$ mice, NO contribution to total relaxation was significantly reduced. Resveratrol did not have any effect on NO contribution to total relaxation in any of the groups (Figure 3-13C). No difference in uterine artery relaxation

was observed among the groups in response to the NO donor SNP (3-14A and B).

CHAPTER 4: DISCUSSION

4.1 Effects of 2-ME administration in COMT$^{-/-}$ mouse model of PE and FGR.

PE and FGR are the leading causes of fetal and maternal morbidity and mortality during pregnancy. Despite intensive research and clinical trials, there are currently no therapeutic approaches available for either prevention or treatment of PE and FGR. Although maternal manifestations differ, both of these conditions are associated with impaired uterine artery blood flow. Thus, treatments directed at improving uterine artery blood flow may ameliorate signs of PE and rescue fetal growth.

In the first study, the effect of 2-ME administration in the COMT$^{-/-}$ mouse was studied. COMT and 2-ME are significantly reduced in pregnancies presenting with PE compared to normal pregnancies. (Fitzgerald and Drumm, 1977, Kanasaki et al., 2008) 2-ME has been shown to induce relaxation of uterine arteries from pregnant mice *ex vivo* (Stanley et al., 2010) and to improve many pathological features that are associated with PE, including increased blood pressure and proteinuria. (Kanasaki et al., 2008) In the present study, using the COMT$^{-/-}$ mouse model, we investigated whether 2-ME therapy could increase uterine artery blood flow velocity and therefore ameliorate PE-like symptoms and rescue fetal growth. In contrast to our hypothesis, however, 2-ME therapy did not increase uterine artery blood flow velocity in the COMT$^{-/-}$ mice despite wild-type levels of serum 2-ME concentrations in treated mice. Interestingly, 2-ME treatment led to normalization of proteinuria and normalization of umbilical artery Doppler waveforms (which were characterized by decreased minimum umbilical artery blood flow velocity and increased resistance) in the COMT$^{-/-}$ mice. No toxic effects were observed in the pups from COMT$^{-/-}$ mice

based on our assessment of external malformations, litter size, and fetal resorption.

2-ME is known to induce significant vasodilation of uterine arteries from normal pregnant mice (Stanley et al., 2010) (C57BL/6J) and aortic segments from male rats. (Gui et al., 2008) In both studies, 2-ME abolished vascular contraction in the presence, but not in the absence, of endothelium and a NOS inhibitor blocked this effect, suggesting that 2-ME abrogates vascular contraction via endothelium-dependent NO production. (Wenzel and Somoza, 2005, Gui et al., 2008) Given the ability of 2-ME to induce vascular relaxation, we investigated its ability to increase uterine artery blood flow velocity in the $COMT^{-/-}$ mice. In agreement with our previous study (Stanley et al., 2012b), there was no difference in uterine artery blood flow velocity in the $COMT^{-/-}$ compared to C57BL/6J mice and 2-ME did not have an effect on this measure. In line with this finding, we did not observe any differences between the normal control mice C57BL6/ and $COMT^{-/-}$ mice in *ex vivo* uterine artery endothelial function studies.

$COMT^{-/-}$ mice have previously (Stanley et al., 2012b) demonstrated abnormal umbilical Doppler waveforms, including decreased minimum umbilical artery blood flow velocity and increased umbilical artery resistance. Interestingly, 2-ME administration led to normalization of these measures in the $COMT^{-/-}$ mice. Although the abnormal umbilical Doppler waveforms were associated with FGR in the $COMT^{-/-}$ mice in our previous study (Stanley et al., 2012b), FGR was not observed in the current study. Kanasaki et al., (2008) also did not report FGR in the pups from $COMT^{-/-}$ mice. It is important to note that the growth restriction observed in the pups from $COMT^{-/-}$ mice in our previous

study was subtle. (Stanley et al., 2012b) In the current study, it is possible that the abnormal umbilical Doppler waveforms were present only in a subset of fetuses and therefore growth restriction did not appear in all fetuses. In a study of 1126 human cases with absent or reversed umbilical artery diastolic flow not all fetuses were associated with FGR, 68% of them were small for gestational age. (Maulik et al., 2011) This suggests that abnormal umbilical Doppler waveforms may not always indicate FGR, although it is definitely associated with a greater risk of FGR. (Alfirevic et al., 2010) Given that 2-ME administration led to normalization of abnormal umbilical Doppler waveforms in the $COMT^{-/-}$ mice; its therapeutic potential in cases of FGR with abnormal umbilical Doppler waveforms is apparent. However, the mechanisms by which 2-ME normalizes umbilical Doppler waveforms in the $COMT^{-/-}$ mice remain to be investigated.

Placentas from $COMT^{-/-}$ mice exhibit an increased in the anti-angiogenic factor sFlt-1. (Kanasaki et al., 2008) This can lead to a reduction in placental angiogenesis in these mice, leading to increased placental resistance and therefore abnormal umbilical Doppler waveforms. One of the mechanisms by which 2-ME can lead to normalization of abnormal umbilical Doppler waveforms might be via its effect on hypoxia-inducible factor HIF-1α, a transcription factor that senses tissue oxygen tension and regulates the expression of hypoxia-induced genes. 2-ME might reduce placental resistance through inhibition of HIF-1α, which is known to upregulate sFlt1. (Mabjeesh et al., 2003, Gerber et al., 1997) Another possible mechanism by which 2-ME could normalize the abnormal umbilical Doppler waveforms in the $COMT^{-/-}$ mice may be through restoration of the placental eNOS enzyme expression as

reported by (Kanasaki et al., 2008) This might have led to an increase in NO production in the placental vascular bed leading to a reduction in placental resistance. Recently, Barnes et al., (2010) measured 2-ME in 157 cord blood samples spanning a wide range of gestation (24 – 42 weeks). It was discovered that infants born between 37 and 42 weeks had significantly increased levels of 2-ME compared to infants born at earlier gestation. Although it remains to be verified, it is plausible that 2-ME supplementation might have led to an increase in umbilical 2-ME concentrations in the $COMT^{-/-}$ mice and therefore might elicit its vasorelaxing effects in the umbilical circulation. This could in part explain the normalization of impaired umbilical Doppler waveforms in these mice.

Kanasaki et al., (2008) first demonstrated that $COMT^{-/-}$ mice displayed a PE-like phenotype, including significantly elevated blood pressure and proteinuria at GD 17.5. 2-ME therapy led to amelioration of these signs. In agreement with (Kanasaki et al., 2008), $COMT^{-/-}$ in our laboratory exhibited increased proteinuria and this was normalized to wild-type levels following 2-ME administration. In line with our previous study, we observed a trend towards an increase in SBP in $COMT^{-/-}$ mice at GD 10.5. (Stanley et al., 2012b) In contrast to Kanasaki et al., (2008) but consistent with our previous study (Stanley et al., 2012b), $COMT^{-/-}$ mice did not display hypertension on GD 17.5. The use of different control mice in the two studies might account for the observed difference in blood pressure. Kanasaki et al., (2008) used $COMT^{+/+}$ (littermates of $COMT^{-/-}$) as controls in their study whereas we used C57BL6/J mice, which are genetic background for the $COMT^{-/-}$ mice. It is important to note that $COMT^{+/+}$ mice in the study by Kanasaki et al., (2008) did not display

the pregnancy-induced blood pressure adaptation observed in C57BL/6J mice (Burke et al., 2010) and in human pregnancy. In normal pregnancy in both humans and mice (Burke et al., 2010), there is a steady decrease in blood pressure up to mid-pregnancy and blood pressure returns to normal in the last weeks of pregnancy. In the $COMT^{+/+}$ mice used by Kanasaki et al., (2008), blood pressure continued to decrease until the end of pregnancy, which might explain the blood pressure difference found between the two studies.

In summary, the current study demonstrated that 2-ME treatment in $COMT^{-/-}$ mice led to normalization of proteinuria and umbilical artery Doppler waveforms, suggesting that 2-ME might have therapeutic potential in cases of FGR associated with abnormal Doppler waveforms. The utility of 2-ME in cases of PE needs to be further explored in PE models that exhibit more severe disease phenotypes.

4.2 Effects of resveratrol administration in transgenic mouse models of PE and FGR.

Resveratrol has been shown to induce vasorelaxation of uterine arteries from nonpregnant guinea pigs *ex vivo*. (Naderali et al., 2000) In addition, it is known to ameliorate high blood pressure, (Rivera et al., 2009) proteinuria, (Nihei et al., 2001) and improve fetal weight (Singh et al., 2011) in other animal models of human disease. We hypothesized that treatment of $COMT^{-/-}$ and $eNOS^{-/-}$ mice with resveratrol during pregnancy would improve uterine artery blood flow velocity and therefore ameliorate signs of PE and rescue fetal growth. In the current study we observed that resveratrol administration during pregnancy led to a significant increase in uterine artery blood flow velocity and a concomitant increases in fetal weight in $COMT^{-/-}$ mice. In the $eNOS^{-/-}$ mice, increases in uterine artery blood flow velocity and fetal weight following resveratrol administration did not reach significance.

Many clinical studies have shown a decrease in uteroplacental blood flow in cases of PE and FGR. (Cnossen et al., 2008a, Campbell et al., 1983) In this study, we observed a decrease in uterine artery blood flow velocity in $eNOS^{-/-}$ mice, in agreement with a study by Kulandavelu et al., (2012). $COMT^{-/-}$ mice also exhibited a decrease in uterine artery blood flow velocity. Following resveratrol administration, uterine artery blood flow velocity was normalized only in the $COMT^{-/-}$ mice but not in $eNOS^{-/-}$ mice. These results are dissimilar to those of a rat model of PE studied by Moraloglu et al., (2011), which showed no difference in blood flow velocity to placenta following resveratrol administration. Although oral absorption of resveratrol is about 75%, extensive metabolism in the intestine and liver results in an oral bioavailability

of less than 1%.(Walle, 2011) Differences in the study by Moraloglu et al., (2011) and the current study might be due to a difference in models (rat vs. mouse) or method of resveratrol administration. For instance, Moraloglu and colleagues (2011) gavaged rats with 20 mg/kg of resveratrol per day and this method of administration may not be optimal in terms of its bioavailability. Because the plasma half-life of resveratrol is ~9 hours (Walle et al., 2004), the rats might not have had resveratrol in their circulation at all times. Animals in our study had constant access to food containing resveratrol; therefore, resveratrol might have had better bioavailability. In addition, following the exact dose and diet formulation used in our study, a therapeutic concentration range of resveratrol in the plasma has been reported. (Dolinsky et al., 2011) Moraloglu et al., (2011), however, did not measure the blood resveratrol levels. It is not possible to conclude from the Moraloglu et al., (2011) study that the minimum effective blood concentration of resveratrol was achieved, therefore, the described absence of effects on uteroplacental blood flow cannot be linked directly with the plasma concentration of resveratrol. A third factor that could be responsible for the difference in results between studies was the different brands of resveratrol used in each study.

Indirect evidence supports the uterine artery blood flow velocity increase observed in $COMT^{-/-}$ mice receiving resveratrol. Resveratrol has been shown to increase cerebral blood flow in humans (Kennedy et al., 2010) and animals. In a mouse model of sepsis-induced acute kidney injury, blood flow to the kidney was increased by resveratrol therapy, (Holthoff et al., 2011) as it was in coronary arteries in a swine model of experimental acute coronary occlusion. (Wang et al., 2011) The mechanisms by which resveratrol increases uterine

artery blood flow velocity in $COMT^{-/-}$ mice might be through vasorelaxation of uterine arteries. Naderali et al., (2000) demonstrated significant resveratrol-induced vasorelaxation in uterine arteries from non pregnant guinea pigs *ex vivo*. In the same study, pretreatment of the uterine arteries with L-NAME (a NOS inhibitor) had no effect against resveratrol-induced vasorelaxation, (Naderali et al., 2000) suggesting that resveratrol induces vasorelaxation through NO independent mechanisms. When assessed *in vivo*, resveratrol has been shown to increase both endothelial and inducible nitric oxide synthase. (Das et al., 2005) Therefore, resveratrol could induce vasorelaxation of uterine arteries in $COMT^{-/-}$ mice through multiple mechanisms. Interestingly, resveratrol treatment increased uterine artery blood flow velocity in the $eNOS^{-/-}$ mice but the increase was not significant. It is plausible that in part, resveratrol increased uterine artery blood flow velocity in the $COMT^{-/-}$ mice via upregulation of eNOS. However, this might not be the case here as our *ex vivo* studies showed no difference in endothelium dependent relaxation of uterine arteries in C57BL/6J and $COMT^{-/-}$ mice after resveratrol treatment. In addition, NO contribution to total endothelium dependent relaxation was not affected by resveratrol treatment. In agreement with our previous study (Stanley et al., 2012a) and our uterine artery blood flow velocity data, $eNOS^{-/-}$ mice exhibited significantly impaired endothelium dependent relaxation of uterine arteries and this impairment was not improved following resveratrol administration. Taken together, these results indicate that resveratrol might increase uterine artery blood flow velocity in the $COMT^{-/-}$ mice through an NO independent pathway but have minimal effects on $eNOS^{-/-}$ mice.

Following resveratrol treatment, pups from COMT$^{-/-}$ mice showed a significant increase in weight. This might be attributed to an increase in uterine artery blood flow velocity in these mice. Our results are consistent with those of Singh et al., (2011) who reported an increase in fetal weight and crown-rump length following resveratrol administration in a rat model of diabetic embryopathy, which, similar to PE and FGR, is associated with a decrease in fetal weight, an increase in oxidative stress, and endothelial dysfunction.

Previous studies have evaluated safety, pharmacokinetics, and metabolism of resveratrol and have reported resveratrol to be well tolerated in humans even at very high doses.(Patel et al., 2011) No evidence of teratogenesis associated with this compound was found in rodents. (Williams et al., 2009) In agreement with previous studies we did not observe external malformations or treatment related effects on litter size and fetal resorption in any of the mouse models tested. Thus, it is likely that resveratrol would be a safe and beneficial drug to administer during human pregnancy, but rigorous safety/toxicity studies in animal models are needed before therapeutic use in human is initiated.

The hypertension and proteinuria associated with PE pose serious risks to maternal health and are linked to adverse neonatal outcomes. In this study there was no significant difference in systolic blood pressure in C57BL/6J, eNOS$^{-/-}$ or COMT$^{-/-}$ mice. The blood pressure results in COMT$^{-/-}$ mice in the current study are consistent with our previous findings. (Stanley et al., 2012b) However, these results differ from those of Kanasaki and colleagues (2008), who observed a significant increase in systolic blood pressure in COMT$^{-/-}$ mice at GD 17.5. Differences in blood pressure results in COMT$^{-/-}$ mice

between our study and the study by Kanasaki et al., (2008) are addressed in section (4.1). There were no differences observed in systolic and diastolic blood pressure between C57BL/6J and eNOS$^{-/-}$ mice. (Hefler et al., 2001b) reported a significant increase in blood pressure in eNOS$^{-/-}$ mice during pregnancy, while another study (Shesely et al., 2001) reported a decrease in blood pressure in eNOS$^{-/-}$ mice during pregnancy. Result discrepancies among the groups can be explained by differences in experimental methods (i) Hefler et al., (2001b) conditioned the mice to tail-cuff blood pressure apparatus for 15 min daily for 5 days, (ii) Shesely et al., (2001) trained mice to acclimatize to the tail-cuff system for a period of two weeks before recording experimental data, and (iii) our mice were trained in restraint tubes for 5 minute on three successive days prior to mating.

Although resveratrol has been shown to reduce blood pressure in several animal models of human diseases, (Rivera et al., 2009, Chan et al., 2011) it did not have any effect on blood pressure in our study. It is noteworthy that there were no adverse effects of resveratrol on blood pressure in the C57BL/6J, control mice. The lack of efficacy in COMT$^{-/-}$ and eNOS$^{-/-}$ mice might be due to the fact that they were not initially hypertensive, unlike the animal models tested in other preclinical studies. (Rivera et al., 2009, Chan et al., 2011) Antihypertensive treatments of women with PE pose a dilemma among clinicians because of their risk of decreasing blood flow by reducing perfusion pressure in an already compromised uteroplacental unit. (Hanretty et al., 1989) A drug that can simultaneously reduce maternal blood pressure and improve uteroplacental blood flow is currently unavailable. It is therefore imperative to further investigate the use of resveratrol in an animal model that exhibits

hypertension during pregnancy, particularly since it increases uterine artery blood flow velocity in the $COMT^{-/-}$ mice.

The proteinuria observed in $eNOS^{-/-}$ mice was in agreement with a study by Kusinski et al., (2012) however, the proteinuria results in $COMT^{-/-}$ mice in this study were dissimilar to two previous studies. (Kanasaki et al., 2008, Stanley et al., 2012b) The reason for absence of proteinuria in the current study might be explained by the lack of hypertension observed in the $COMT^{-/-}$ mice. High blood pressure is often linked to a decline in kidney function and proteinuria, as it induces damage to the glomeruli within the kidney. (Yan et al., 2012) The fact that high blood pressure and proteinuria was not present in the $COMT^{-/-}$ mice in this study might mean that kidney function was not impaired.

In summary, resveratrol treatment leads to an increase in fetal growth in $COMT^{-/-}$ mice, which may be mediated by an increase in uterine artery blood flow velocity. No evidence of treatment-related external malformations or effects on litter size and fetal resorption in any of the mouse models was observed. Although our study indicates that resveratrol may offer therapeutic potential in FGR and PE, further studies are needed to verify its potential in phenotypically stronger animal models of these conditions.

4.3 Study limitations

4.3.1 Animal models

As discussed in chapter 1, PE is a condition unique to human pregnancy, therefore mouse models of PE pose some limitations. Analogous to humans, the mouse has a hemochorial placentation, however, compared to humans, the

mouse trophoblast invasion of the uterine spiral artery is shallow and restricted to decidua. In addition, blood vessels of the decidua are lined by endothelial cells rather than trophoblasts. (Pijnenborg et al., 2006) Therefore, mouse models of PE and FGR might not reflect the impaired trophoblast invasion characteristic of the human condition. Consequently, uterine artery blood flow changes during pregnancy might not be as pronounced as in the human.

While eNOS$^{-/-}$ and COMT$^{-/-}$ mice have enabled us to understand PE and FGR pathogenesis and allowed us to test potential therapies for these conditions, these mouse models, however, have a deficiency of eNOS or COMT enzymes since birth. In human PE and FGR cases, the pathological mechanisms almost always arise during pregnancy and resolve after giving birth. In addition, pathogenesis of PE and FGR in humans is much more complex and knockout mice do not recapitulate all of the pathological features observed in these conditions.

4.3.2 Loss of the COMT$^{-/-}$ mice phenotype

The COMT$^{-/-}$ mice displayed some varied phenotypes between study 1 and study 2. For instance, consistent with two previous studies (Kanasaki et al., 2008, Stanley et al., 2012b) the COMT$^{-/-}$ mice in study 1 exhibited proteinuria, however, this phenotype was no longer present in study 2. In addition, the COMT$^{-/-}$ mice in the current study did not exhibit FGR demonstrated in our earlier study. (Stanley et al., 2012b) Furthermore, abnormal umbilical Doppler waveforms were absent in mice in study 2 of this thesis. Although we saw a much stronger disease phenotype in COMT$^{-/-}$ mice initially; we observed a gradual loss of phenotype in the COMT$^{-/-}$ mice across studies. This

inconsistency in phenotype made it difficult to measure the degree of treatment effect in these mice. We speculate that this might be due to a phenomenon called "genetic drift.'' Genetic drift is a result of tendency of genes to evolve over generations, which can lead to a loss of phenotypic characteristics in a species. Studies have reported this phenomenon in transgenic knock out mouse models of other human diseases. (Watkins-Chow and Pavan, 2008) Genetic drifting can be avoided by minimizing the generations (10 generations recommended) produced by breeder pairs and/or by replacing the breeding stock (every 10 generations). (Powell, 2008) It is difficult to determine the generation of breeding pairs supplied to us to initiate the $COMT^{-/-}$ colony in our facility, therefore we do not know at which generation the mice were when we received them. Consequently, it is important to re-establish our $COMT^{-/-}$ colony and investigate whether the phenotype lost due to genetic drift can be restored.

4.3.3 Blood pressure

In the current study we used a tail-cuff method to measure blood pressure because of some obvious advantages: (1) no surgery is required to monitor blood pressure, (2) the tail-cuff method is less expensive than other blood pressure measurement methods (e.g., telemetry), and (3) the tail-cuff method has the capacity to screen large number of animals quickly. (Whitesall et al., 2004) While, the tail cuff method is widely used in reported studies and is a well-validated technique for measuring blood pressure, it has inherent limitations. It utilizes a tail-cuff placed on the tail to occlude the blood flow. Upon deflation, blood pressure sensors, placed distal to the occlusion cuff record the amplitude of a single pulse from which systolic blood pressure and

the heart rate can be calculated. Diastolic blood pressure cannot be measured by this method because the technology records only the first appearance of the pulse. A software algorithm calculates diastolic blood pressure, therefore, it is only an estimation rather than a true measurement. In addition, the system is sensitive to animal movement leading to motion artifacts, which can result in variability in blood pressure readings. Continuous blood pressure measurements cannot be taken using the tail-cuff method, as keeping mice in the restraint tubes for prolonged a period of time can induce additional stress. (Kubota et al., 2006)

In the current study we did not observe differences in blood pressure or treatments effects on blood pressure in the mouse models utilized. It is difficult to distinguish whether this lack of treatment efficacy was because the treatment did not have any effect or because limitations associated with tail-cuff method confounded the treatment effects. It is therefore important that the future studies utilize telemetry, i.e., implantation of radio transmitters in the mice. Telemetry is well validated and is known to produce excellent correlation with direct blood pressure measurements. Additionally, telemetry allows the continuous measurement of both systolic and diastolic blood pressure in freely motile mice. (Anderson et al., 1999)

4.4 Future directions

4.4.1 Mechanisms of increased pup weight by resveratrol

The ability of resveratrol to increase uterine artery blood flow velocity and increase pup weight in $COMT^{-/-}$ mice and to some extent in $eNOS^{-/-}$ mice is an exciting finding that could have a significant impact on human pregnancies

complicated by PE and FGR. However, the mechanisms behind these findings, however, need to be addressed in future studies. The extent of trophoblast invasion of spiral arteries can be assessed, as this invasion could account for the increase uterine artery blood flow velocity and therefore increased pup weight observed in these mice. Resveratrol did not have an effect on endothelium dependent uterine artery relaxation in $eNOS^{-/-}$ or $COMT^{-/-}$ mice, however, it is known to induce uterine artery relaxation through endothelium independent mechanisms, (Naderali et al., 2000) which could be explored in future studies. Several studies have reported that polyphenols inhibit cyclic nucleotide phosphodiesterases, which break down vasorelaxant cAMP and cGMP. It is known that other inhibitors of cyclic nucleotide phosphodiesterases lead to relaxation of endothelium denuded vessels. (Dell'Agli et al., 2005) Such mechanisms might be involved in the endothelium-independent relaxation elicited by resveratrol. Ke Chen and Pace-Asciak, (1996) showed that resveratrol led to relaxation of endothelium-denuded aortic rings, indicating that resveratrol might act directly on the vascular smooth cells. A potential mechanism for this observation is ability of resveratrol to induce vascular relaxation through modulation of potassium channels located in smooth muscle cells. (Novakovic et al., 2006) Uterine artery vessel function could be assessed in the presence or absence of potassium channel inhibitors to determine whether they play a role in improved uterine artery function and therefore increased uterine artery blood flow velocity.

In addition to its vascular benefits, resveratrol is known to increase glucose and protein metabolism through an increase of Akt phosphorylation. Akt, or

protein kinase B, is a serine/threonine protein kinase that plays a significant role in many cellular processes including glucose and protein metabolism. Akt phosphorylation has been reported to be reduced in placentas from women exhibiting FGR (Yung et al., 2007) and PE. (Cudmore et al., 2012) In addition, genetically modified mice that lack the gene that encodes Akt are known to exhibit placental and fetal growth restriction and a significant correlation between Akt levels and placental growth has been documented. (Yang et al., 2003)

Dudley et al., (2008) showed that resveratrol treatment of rats subjected to ischaemia re-perfusion injury lead to a decrease in infarct size concomitant with increased Akt expression. Likewise, Das and colleagues reported decreased numbers of apoptotic cardiomyocytes associated with increased Akt phosphorylation in a similar study. (Das et al., 2006) The evidence presented above suggests that resveratrol has the potential to restore Akt phosphorylation in placentas from pregnancies complicated by PE and FGR. Resveratrol induced increases in glucose and protein metabolism, and therefore in fetal weight, through an increase in Akt phosphorylation in placentas from eNOS $^{-/-}$ and COMT$^{-/-}$ mice remains to be investigated.

4.4.2 Assessment of placental oxidative stress

Reactive oxygen species (ROS) production such as superoxide anions can react with nitric oxide, to form peroxynitrite and thus reduce vasodilation. Reduced nitric oxide-mediated vasodilation, via scavenging by increased ROS or aberrant nitric oxide signaling can therefore reduce uteroplacental perfusion. In human umbilical vein endothelial cells pretreated with

resveratrol, Arunachalam et al., (2010) showed a significant increase in NO production. In addition, resveratrol induced an increase in ROS-inactivating enzymes superoxide dismutase and gluthathione peroxidase in human endothelial cells. Our group has previously observed an increase in superoxide production in placentas from eNOS$^{-/-}$ mice; this may lead to increase in placental resistance due to reduced NO availability. Given that resveratrol decreases ROS, future studies could assess whether it reduces the increased superoxide production observed in the eNOS$^{-/-}$ mice and therefore contributes towards an increase in uterine artery blood flow velocity and fetal weight.

4.4.3 New animal models

Ideally an animal model of PE/FGR would exhibit all the signs seen in women with PE/FGR, including hypertension, proteinuria and FGR. However, none of the models used in the current study developed a severe PE/FGR-like phenotype. The subtle nature of the disease phenotypes observed in eNOS$^{-/-}$ and COMT$^{-/-}$ mice did not allow us to test the full potential of the therapies. For example, the ability of 2-ME and resveratrol to reduce the blood pressure could not be investigated, as the models did not exhibit hypertension.

Recently, we have been able to exacerbate the PE/FGR phenotype in COMT$^{-/-}$ mice by feeding them a high fat diet prior to pregnancy. Our preliminary results demonstrate that during pregnancy these mice develop a more severe phenotype, including marked hypertension, impaired uterine artery blood flow velocity, and fetal growth restriction. These results indicate that clinically relevant animal models of PE/FGR might be developed by integrating genetic

and environmental factors. The high fat diet mouse model provides a useful tool to investigate the full potential of the therapies tested in the current study.

4.5 Conclusions

In this study the abilities of two potential therapies were investigated, based on their vascular targets and clinical potential to ameliorate PE and FGR in relevant mouse models. In the first study we demonstrated that administration of 2-ME in pregnant $COMT^{-/-}$ mice led to normalization of proteinuria and umbilical artery Doppler waveforms. This study suggests that 2-ME might have therapeutic potential in cases of FGR associated with abnormal Doppler waveforms, although its utility in cases of PE needs further investigation. In the second study we showed that supplementation of resveratrol in $eNOS^{-/-}$ and $COMT^{-/-}$ mice resulted in increased uterine artery blood flow velocity and pup weight in both groups, although statistical significance was only reached in the $COMT^{-/-}$ mice. Our results in this study indicate that resveratrol might have the therapeutic potential to alleviate PE and improve FGR. Although our studies are still in early stages, our results provide a promising perspective for an intervention in cases of PE and FGR.

Table 3-1 Effect of 2-ME on maternal blood pressure and heart rate on GD 17.5 in C57BL/6J and COMT$^{-/-}$ mice.

	C57BL6J + Olive oil	**C57BL6J + 2-ME**	**COMT$^{-/-}$ + Olive oil**	**COMT$^{-/-}$ + 2-ME**
SBP, mmHg	132 ± 9	135 ± 9	113 ± 5	126 ± 6
DBP, mmHg	86 ± 11	85 ± 9	77 ± 2	90 ± 5
HR, bpm	467 ± 29	485 ± 34	477 ± 45	480 ± 31

GD: Gestational day, 2-ME: 2-Methoxyestradiol, SBP: Systolic blood pressure, DBP: Diastolic blood pressure. Mean ± SEM, n=5-6, two-way ANOVA.

Table 3-2: Hemodynamic parameters of uterine and umbilical vasculature evaluated by ultrasound biomicroscopy pre-treatment on GD 11.5.

	C57BL/6J	**COMT$^{-/-}$**
Maternal heart rate (bpm)	580.7 ± 7.2	585.1 ± 7.2
Fetal heart rate (bpm)	158.7 ± 4.9	160 ± 6.4
Uterine Artery		
PSV (mm/s)	342.07 ± 17.43	301.49 ± 12.99
EDV (mm/s)	143.26 ± 8.23	131.42 ± 5.55
RI	0.57 ± 0.01	0.55 ± 0.02
Umbilical Artery		
PSV (mm/s)	73.16 ± 1.83	74.85 ± 1.02
EDV (mm/s)	12.49 ± 1.10	16.01 ± 2.23
RI	0.82 ± 0.01	0.78 ± 0.02
Umbilical Vein		
PSV (mm/s)	52.76 ± 3.81	51.07 ± 4.5
EDV (mm/s)	16.24 ± 2.55	18.65 ± 3.35
RI	0.70 ± 0.02	0.65 ± 0.03

PSV: Peak systolic velocity, EDV: End diastolic velocity, RI: Resistance index. Mean ± SEM, n=6-9, two-way ANOVA.

Table 3-3: Hemodynamic parameters of uterine and umbilical vasculature evaluated by ultrasound biomicroscopy on GD 17.5.

	C57BL6/J		COMT-/-		ANOVA		
	Olive oil	2-ME	Olive oil	2-ME	Int	Gen	2ME
Maternal Heart rate (bpm)	550.7 ± 17.6	569.3 ± 17.0	578.1 ± 17.1	567.3 ± 6.4			
Fetal heart rate (bpm)	215.0 ± 16.3	209.3 ± 11.5	239.8 ± 15.9	203.0 ± 19.5			
Uterine Artery							
PSV (mm/s)	573.34 ± 55.36	569.33 ± 17.03	513.1 ± 42.46	598.6 ± 66.2			
EDV (mm/s)	296.82 ± 42.2	284.82 ± 16.16	228.75 ± 4.71	254.76 ± 31.69			
RI	0.49 ± 0.02	0.52 ± 0.02	0.54 ± 0.03	0.57 ± 0.01			
Umbilical Artery							
PSV (mm/s)	136.20 ± 11.47	166.05 ± 17.40	175.55 ± 15.94	190.83 ± 39.39			
EDV (mm/s)	14.09 ± 0.54	15.31 ± 1.84	2.41 ± 0.42 #	16.91 ± 3.53		*	*
RI	0.91 ± 0.02	0.90 ± 0.003	0.98 ± 0.003#	0.90 ± 0.01		*	*
Umbilical Vein							
PSV (mm/s)	84.31 ± 18.73	68.86 ± 10.00	86.22 ± 7.53	81.72 ± 4.73			
EDV (mm/s)	46.24 ± 8.10	36.92 ± 3.70	42.01 ± 6.04	46.35 ± 5.34			
RI	0.43 ± 0.02	0.44 ± 0.05	0.51 ± 0.04	0.44 ± 0.04			

PSV: Peak systolic velocity, EDV: End diastolic velocity, RI: Resistance index. Mean ± SEM, n=3-6 *p<0.05, when effect of genotype (Gen), administration of 2-Methoxyestradiol (2-ME) or their interaction (Int) was evaluated by two-way ANOVA. #p<0.05, Bonferroni post-hoc test indicating differences within genotypes in placebo groups.

Table 3-4: Effect of resveratrol on maternal blood pressure and heart rate on GD 10.5 and 17.5 in C57BL/6J, eNOS$^{-/-}$ and COMT$^{-/-}$ mice.

	GD	C57BL6J + CD	C57BL6J + RD	eNOS$^{-/-}$ + CD	eNOS$^{-/-}$ + RD	COMT$^{-/-}$ + CD	COMT$^{-/-}$ + RD
SBP, mmHg	10.5	127 ± 6	132 ± 7	142 ± 8	134 ± 4	141 ± 3	128 ± 4
DBP, mmHg	10.5	83 ± 6	92 ± 6	95 ± 5	89 ± 4	94 ± 5	87 ± 5
HR, bpm	10.5	480 ± 16	544 ± 36	549 ± 31	502 ± 18	484 ± 30	490 ± 28
SBP, mmHg	17.5	133 ± 2	134 ± 4	131 ± 4	118 ± 3	131 ± 7	138 ± 5
DBP, mmHg	17.5	98 ± 6	85 ± 8	87 ± 3	75 ± 4	89 ± 8	96 ± 5
HR, bpm	17.5	470 ± 14	469 ± 24	473 ± 23	477 ± 14	488 ± 25	503 ± 26

GD: Gestational day, CD: Control diet, RD: Resveratrol diet, SBP: Systolic blood pressure, DBP: Diastolic blood pressure. Mean ± SEM, n=6-11, two-way ANOVA.

Table 3-5: Hemodynamic parameters of uterine and umbilical vasculature evaluated by ultrasound biomicroscopy at GD 11.5

	C57BL6/J		$eNOS^{-/-}$		$COMT^{-/-}$	
	Control diet	Res diet	Control diet	Res diet	Control diet	Res diet
Maternal Heart rate (bpm)	595.6 ± 42.3	591.9 ± 17.1	618.6 ± 4.8	607.4 ± 25.0	601.1 ± 2.2	593.9 ± 18.8
Fetal heart rate (bpm)	189.5 ± 4.6	210.4 ± 15.08	182.04 ± 15.9	177.5 ± 10.6	211.8 ± 15.1	196.6 ± 22.3
Uterine Artery						
PSV (mm/s)	324.86 ± 5.87	319.10 ± 32.19	277.78 ± 14.23	285.76 ± 36.33	297.95 ± 5.07	325.27 ± 9.61
EDV (mm/s)	197.34 ± 7.76	180.97 ± 21.05	121.74 ± 16.90	147.85 ± 25.16	135.93 ± 3.07	151.94 ± 6.12
RI	0.39 ± 0.01	0.43 ± 0.01	0.56 ± 0.05	0.48 ± 0.05	0.54 ± 0.01	0.52 ± 0.03
Umbilical Artery						
PSV (mm/s)	45.20 ±2.99	54.14 ± 7.99	43.28 ± 4.31	56.59 ± 3.47	46.66 ± 12.79	51.29 ± 4.83
EDV (mm/s)	3.73 ± 0.97	4.54 ± 1.58	2.29 ± 0.19	3.56 ± 0.18	4.32. ± 1.06	4.58 ± 0.87
RI	0.91 ± 0.01	0.92 ± 0.02	0.94 ± 0.09	0.93 ± 0.06	0.88 ± 0.03	0.91 ± 0.01
Umbilical Vein						
PSV (mm/s)	28.35 ± 4.42	30.94 ± 1.09	26.72 ± 3.41	31.36 ± 2.18	32.33 ± 2.18	29.18 ± 3.82
EDV (mm/s)	16.54 ± 2.39	13.72 ± 1.30	13.08 ± 2.38	16.42 ± 2.24	15.14 ± 1.47	13.99 ± 3.65
RI	0.40 ± 0.04	0.42 ± 0.01	0.38 ± 0.01	0.44 ± 0.02	0.36 ± 0.01	0.40 ± 0.01

PSV: Peak systolic velocity, EDV: End diastolic velocity, RI: Resistance index, RES: Resveratrol, Mean ± SEM, n=6-11, two-way ANOVA.

Table 3-6: Hemodynamic parameters of uterine and umbilical vasculature evaluated by ultrasound biomicroscopy on GD 17.5

	C57BL6/J		eNOS$^{-/-}$		COMT$^{-/-}$		ANOVA		
	Control diet	**Res diet**	**Control diet**	**Res diet**	**Control diet**	**Res diet**	**Int**	**Gen**	**Res**
Maternal Heart rate (bpm)	585.5 ± 15.9	565.6 ± 14.7	608.6 ± 10.71	591.4 ± 14.2	588.3 ± 10.1	577.8 ± 11.9			
Fetal heart rate (bpm)	227.1 ± 7.2	229.6 ± 12.9	253.8 ± 7.3	229.7 ± 3.6	243 ± 10.7	249 ± 14.0			
Uterine Artery									
PSV (mm/s)	569.13 ± 32.39	554.61 ± 36.76	396.89 ± 41.87#	500.96 ± 42.23	430.69 ± 35.60#	604.57 ± 17.72	*	**	**
EDV (mm/s)	275.52 ± 14.06	278.32 ± 21.65	175.19 ± 21.61#	231.91 ± 31.31	195.00 ± 21.98#	276.76 ± 22.55		*	*
RI	0.51 ± 0.02	0.49 ± 0.01	0.56 ± 0.02	0.52 ± 0.06	0.55± 0.02	0.53 ± 0.03			
Umbilical Artery									
PSV (mm/s)	145.01 ± 11.81	119.59 ± 4.83	103.47 ± 11.22	129.22 ± 19.55	119.77 ± 7.37	150.96 ± 22.00			
EDV (mm/s)	9.22 ± 0.94	10.27 ± 1.58	12.86 ± 1.95	9.58 ± 1.12	8.47 ± 1.26	13.82 ± 4.63			
RI	0.93 ± 0.007	0.92 ± 0.008	0.86 ± 0.03	0.92 ± 0.009	0.92 ± 0.01	0.91 ± 0.01			
Umbilical Vein									
PSV (mm/s)	70.08 ± 5.09	63.70 ± 5.80	57.33 ± 7.45	50.64 ± 1.91	63.43 ± 3.86	95.62 ± 23.81			
EDV (mm/s)	41.62 ± 2.70	37.81 ± 2.36	35.44 ± 4.76	28.16 ± 0.52	40.05 ± 1.82	45.84 ± 5.58			
RI	0.40 ± 0.01	0.40 ± 0.02	0.38 ± 0.01	0.44 ± 0.02	0.36 ± 0.01	0.40 ± 0.01			

PSV: Peak systolic velocity, EDV: End diastolic velocity, RI: Resistance index. *p<0.05, **p<0.01, ***p<0.001 when effect of genotype (Gen), administration of resveratrol (Res) or their interaction (Int) was evaluated by two-way ANOVA. #p<0.05, Bonferroni post-hoc test indicating differences in control diet.

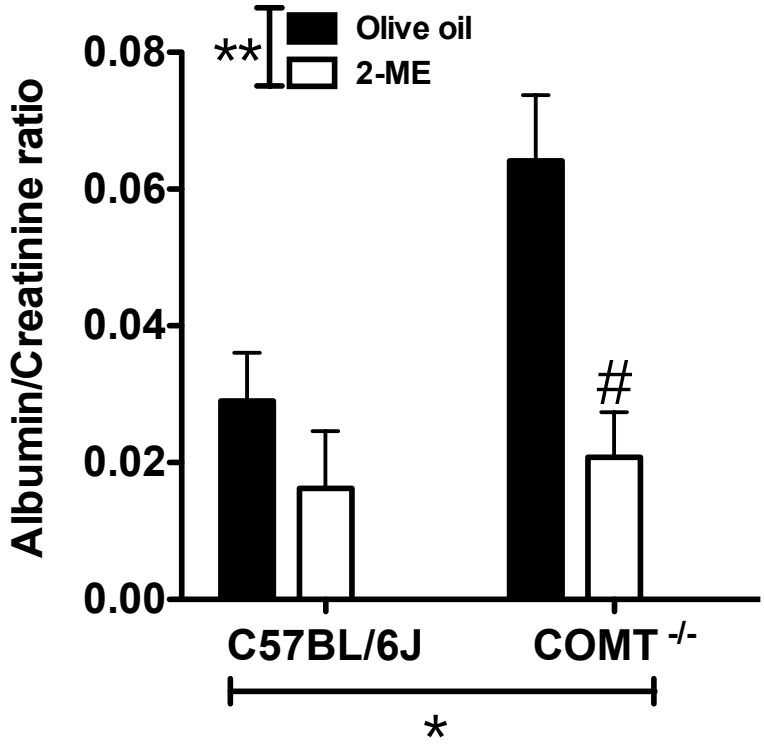


Figure 3-1: Proteinuria in C57BL/6J and COMT$^{-/-}$ mice on day 18.5 of gestation.

The albumin: creatinine ratio was significantly elevated in the COMT$^{-/-}$ mice; this was normalized following treatment with 2-ME. Mean ± SEM, n=4-6, **p<0.01, *p<0.05, two-way ANOVA, #p<0.05, Bonferroni post-hoc test indicating differences compared with COMT$^{-/-}$ mice on olive oil.

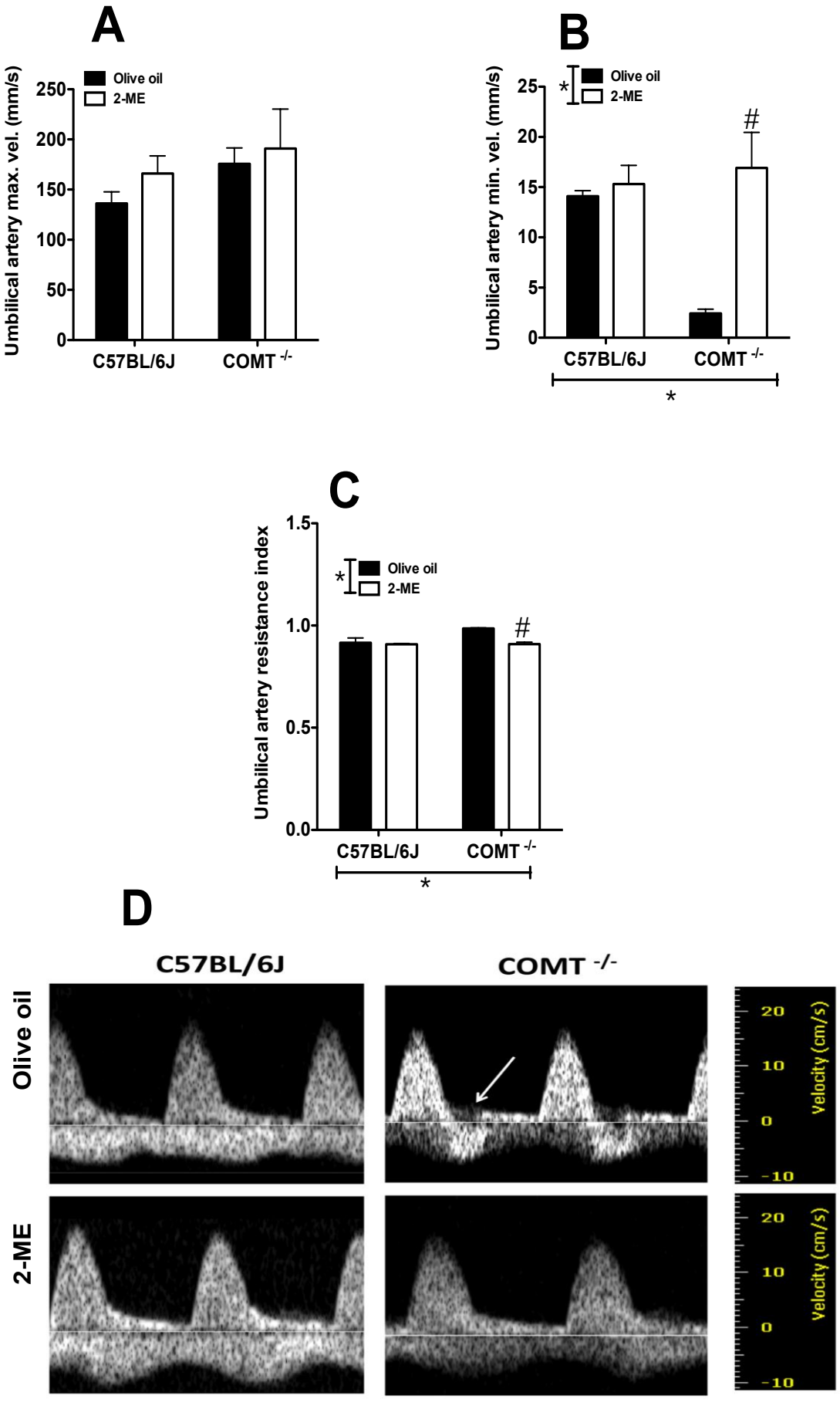

A
Umbilical artery max. vel. (mm/s)
250
200
150
100
50
0
Olive oil
2-ME
C57BL/6J
COMT -/-
B
Umbilical artery min. vel. (mm/s)
25
20
15
10
5
0
*
Olive oil
2-ME
#
C57BL/6J
COMT -/-
*
C
Umbilical artery resistance index
1.5
1.0
0.5
0.0
*
Olive oil
2-ME
#
C57BL/6J
COMT -/-
*
D
C57BL/6J
COMT -/-
Olive oil
2-ME
20
10
0
-10
Velocity (cm/s)
20
10
0
-10
Velocity (cm/s)

Figure 3-2: Abnormal umbilical artery Doppler waveform was normalized following 2-ME administration in the COMT$^{-/-}$ mice on day 17.5 of gestation.

A) Umbilical artery maximum velocity was not different between among the groups. B) Umbilical artery minimum velocity was significantly decreased and resistance index (C) was increased in COMT$^{-/-}$ mice; this was normalized following 2-ME administration. D) Example of umbilical artery Doppler waveform from C57BL6/J and COMT$^{-/-}$ mice ± 2-ME. Reverse end diastolic blood flow velocity (arrow) observed in COMT$^{-/-}$ mice was normalized by 2-ME. Mean ± SEM, n=3-6, *$p<0.05$, two-way ANOVA, #$p<0.05$, Bonferroni post-hoc test indicating differences compared with COMT$^{-/-}$ mice on olive oil.

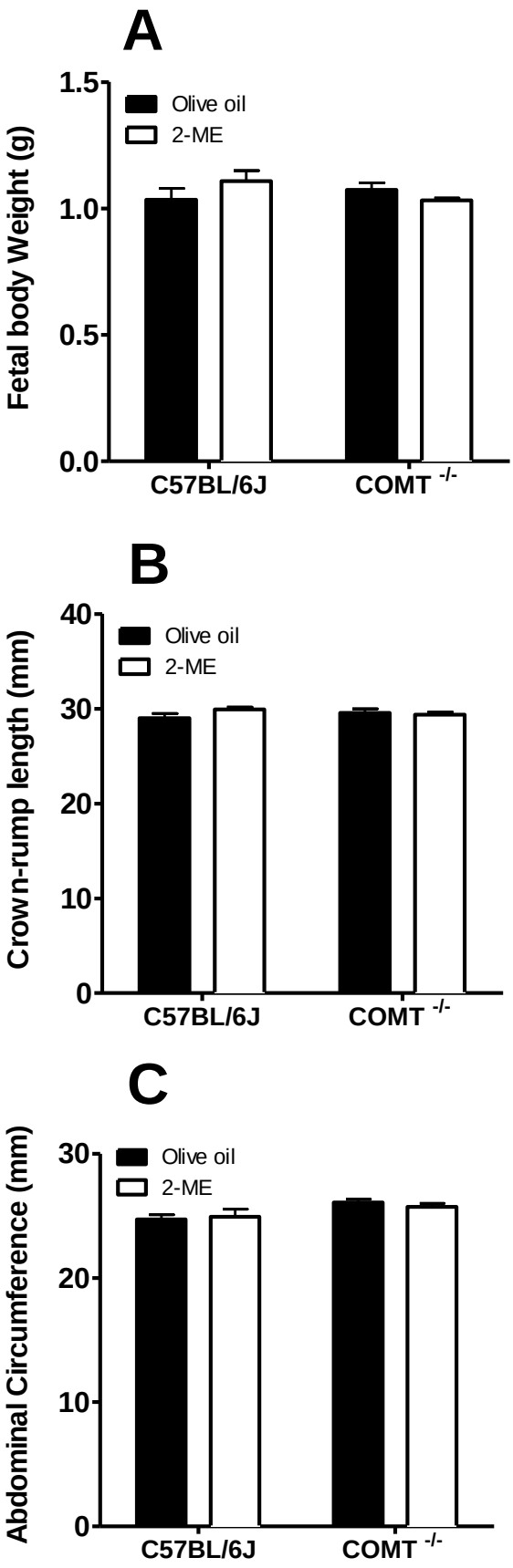

A
Fetal body Weight (g)
1.5
1.0
0.5
0.0
Olive oil
2-ME
C57BL/6J
COMT -/-
B
Crown-rump length (mm)
40
30
20
10
0
Olive oil
2-ME
C57BL/6J
COMT -/-
C
Abdominal Circumference (mm)
30
20
10
0
Olive oil
2-ME
C57BL/6J
COMT -/-

Figure 3-3: Fetal outcome between C57BL/6J and COMT$^{-/-}$ mice on day 18.5 of gestation.

There were no significant differences between fetal body weight (A), crown-rump length (B) and abdominal circumference (C) among the groups. In addition, 2-ME did not have any effect on these parameters. Mean ± SEM, n=5 each, two-way ANOVA.

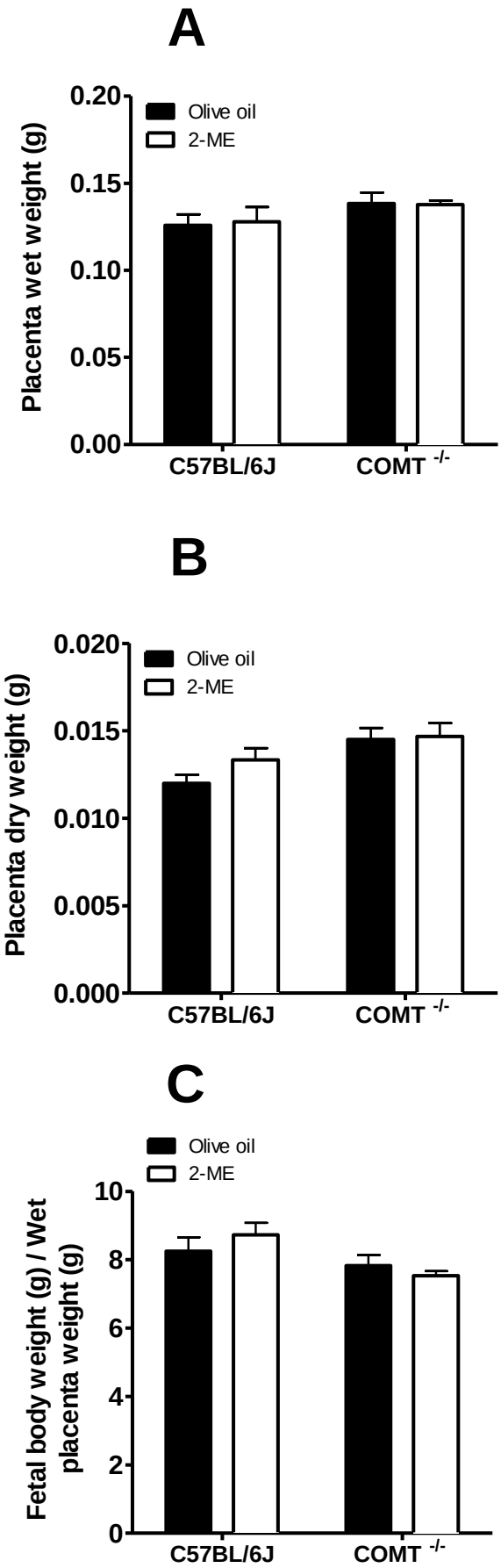

A
Placenta wet weight (g)
0.20
0.15
0.10
0.05
0.00
Olive oil
2-ME
C57BL/6J
COMT -/-
B
Placenta dry weight (g)
0.020
0.015
0.010
0.005
0.000
Olive oil
2-ME
C57BL/6J
COMT -/-
C
Fetal body weight (g) / Wet placenta weight (g)
10
8
6
4
2
0
Olive oil
2-ME
C57BL/6J
COMT -/-

Figure 3-4: Placental outcome and pup weight: placental weight ratio between C57BL/6J and COMT$^{-/-}$ mice on day 18.5 of gestation.

There were no significant differences between wet placental weight (A), dry placental weight (B) or body weight: placental weight ratio (C) among the groups.

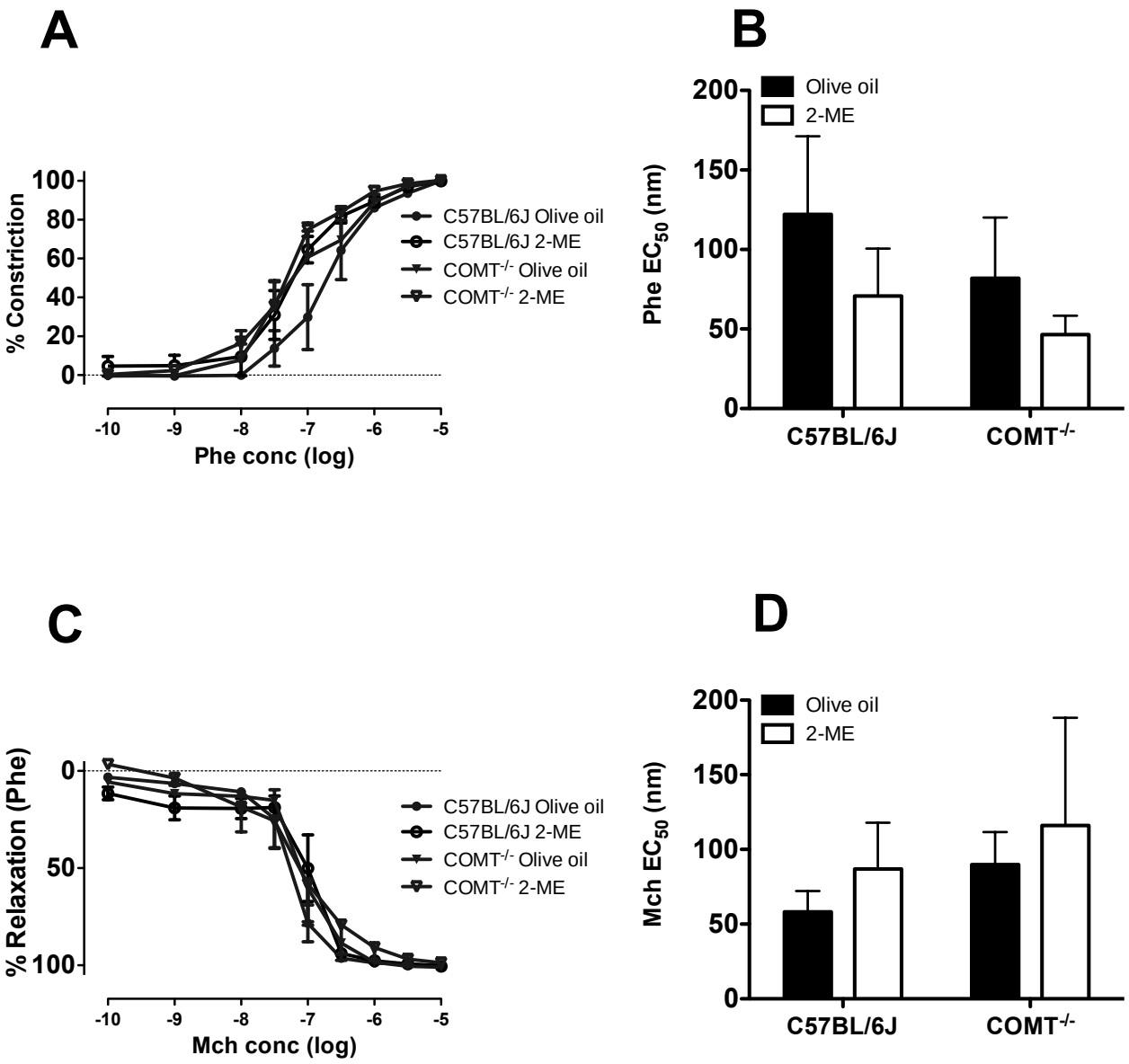


Figure 3-5: Response curves and EC50 concentrations of uterine arteries from C57BL/6J and COMT$^{-/-}$ mice to cumulative concentrations of phenylephrine and methacholine.

A and B: There was no effect of genotype or treatment on phenylephrine induced vasoconstriction of the uterine arteries in either of the groups. C and D: There were no significant differences between relaxation responses to methacholine in COMT$^{-/-}$ compared to C57BL/6J mice. Mean ± SEM, n=3-4, two-way ANOVA.

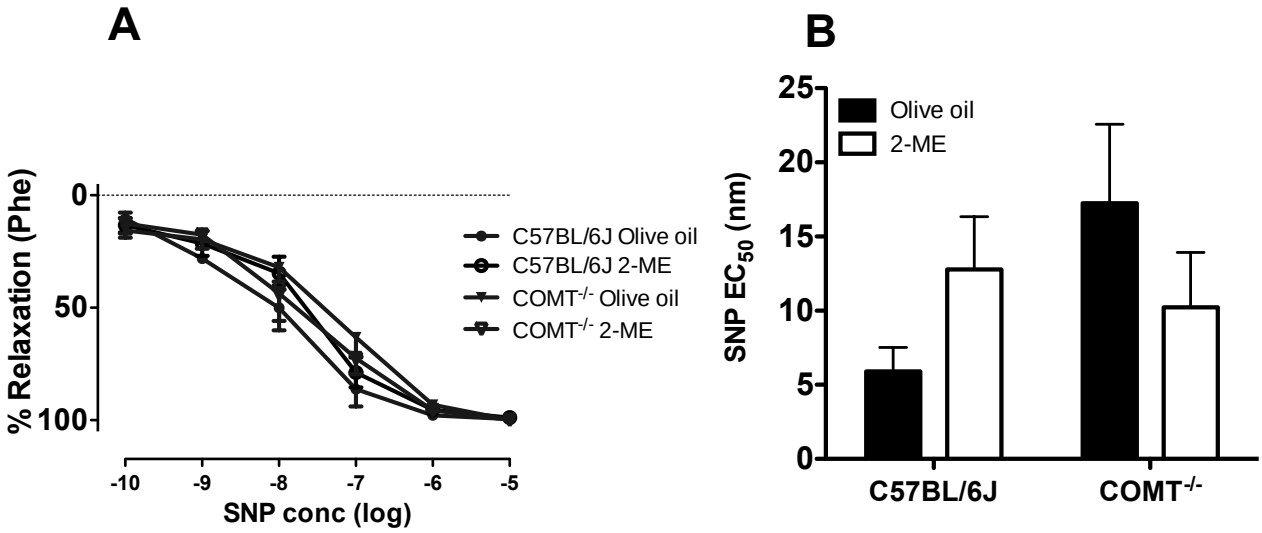


Figure 3-6: Response curves of uterine arteries from C57BL/6J and COMT$^{-/-}$ mice to cumulative concentrations of SNP.

A) There was no significant difference between relaxation responses to SNP in C57BL/6J and COMT$^{-/-}$ mice. B) EC_{50} concentrations were not different among the groups. Mean ± SEM, n=3-4, two-way ANOVA.

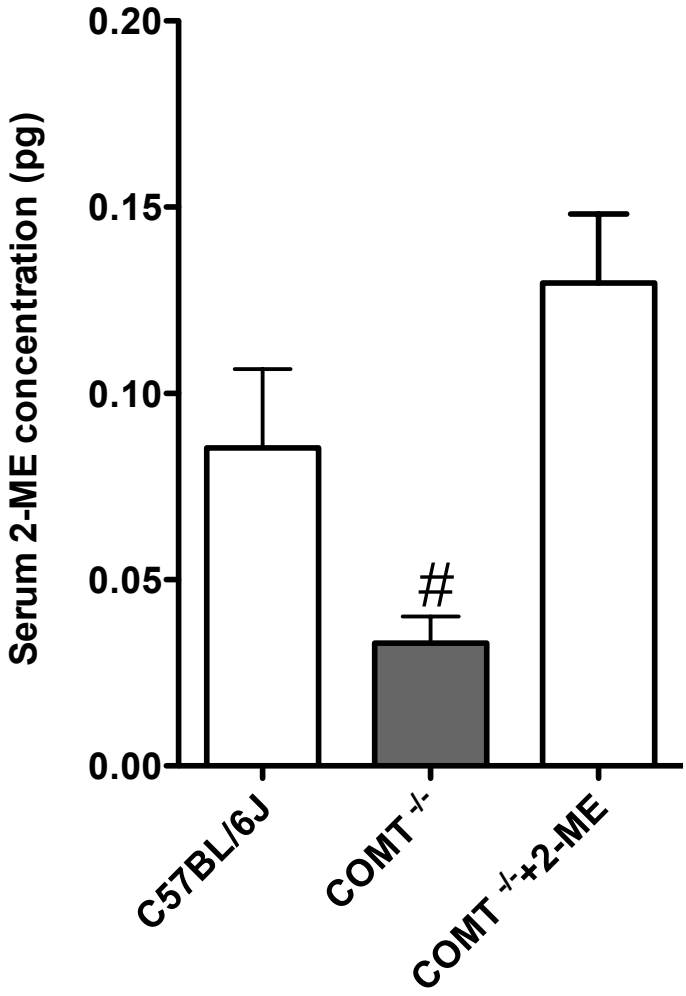


Figure 3-7: Serum 2-ME concentrations reached control levels in COMT$^{-/-}$ mice treated with 2-ME on day 18.5 of gestation.

Administration of 2-ME increased circulating levels of this molecule in COMT$^{-/-}$ mice such that they were comparable to C57BL/6J mice. Mean ± SEM, n=6 each, one-way ANOVA, #$p<0.05$, Bonferroni post-hoc test indicating significant difference.

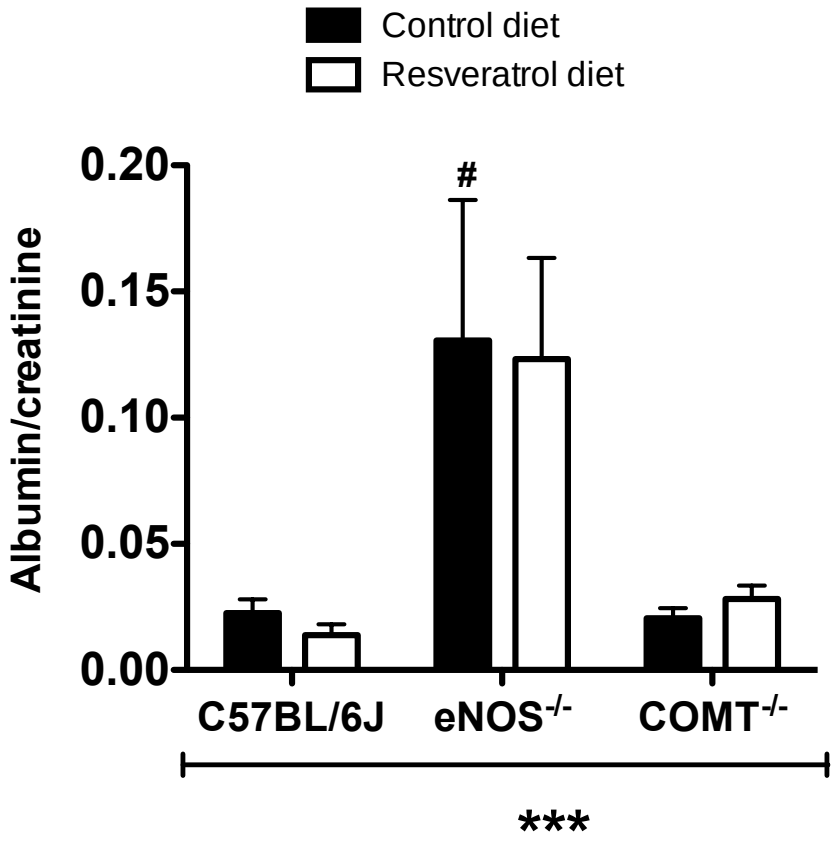


Figure 3-8: Urinary albumin and creatinine ratio on C57BL/6J, eNOS$^{-/-}$ and COMT$^{-/-}$ mice on day 18.5 of gestation.

There was a significant effect of genotype on albumin: creatinine ratio. This was significantly elevated in the eNOS$^{-/-}$ mice. There was however no effect of resveratrol treatment in this measure. Mean ± SEM, n=5-7, ***p<0.001 two-way ANOVA, #p<0.05, Bonferroni post-hoc test indicating differences within genotypes in control diet.

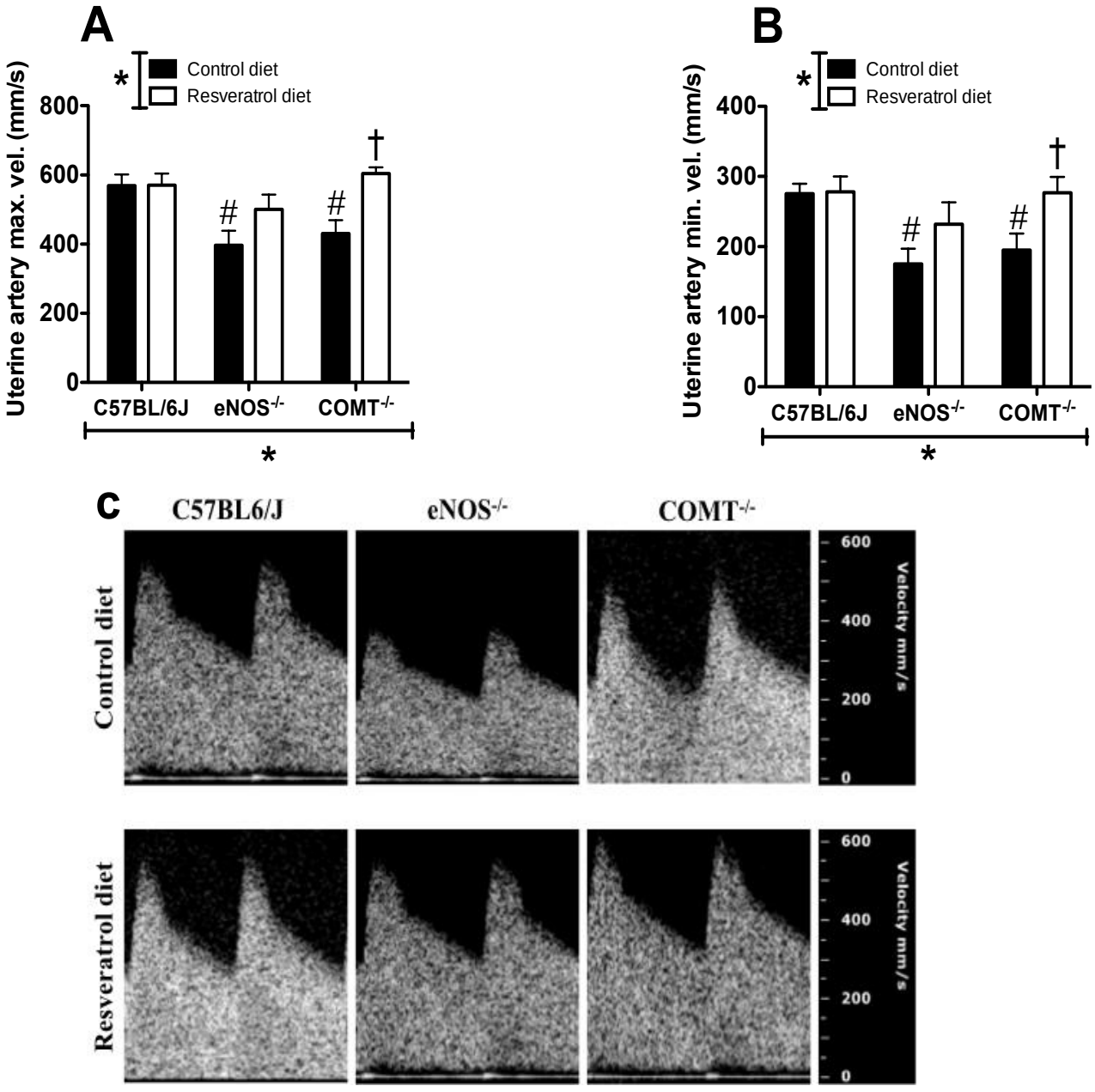

A
Uterine artery max. vel. (mm/s)
800
600
400
200
0
*
Control diet
Resveratrol diet
#
#
†
C57BL/6J
eNOS-/-
COMT-/-
*
B
Uterine artery min. vel. (mm/s)
400
300
200
100
0
*
Control diet
Resveratrol diet
#
#
†
C57BL/6J
eNOS-/-
COMT-/-
*
C
C57BL6/J
eNOS-/-
COMT-/-
Control diet
Resveratrol diet
600
400
200
0
Velocity mm/s

Figure 3-9: Effect of genotype and resveratrol treatment in uterine artery Doppler indices on day 17.5 of gestation.

There was a significant effect of genotype on both maximum (A) and minimum (B) uterine artery blood flow velocity. Resveratrol treatment significantly increased both maximum and minimum uterine artery blood flow velocity in $COMT^{-/-}$ mice. A significant interaction of genotype and treatment on maximum uterine artery blood flow velocity ($p<0.05$, two-way ANOVA) was observed. (C) Example uterine artery Doppler waveforms from C57BL6/J, $eNOS^{-/-}$ and $COMT^{-/-}$ mice ± Resveratrol. Mean ± SEM, n=6-10, *$p<0.05$, two-way ANOVA, #$p<0.05$, Bonferroni post-hoc test indicating differences within genotypes in control diet. †$p<0.05$ Bonferroni post-hoc test indicating differences between control and resveratrol diet within the same genotype.

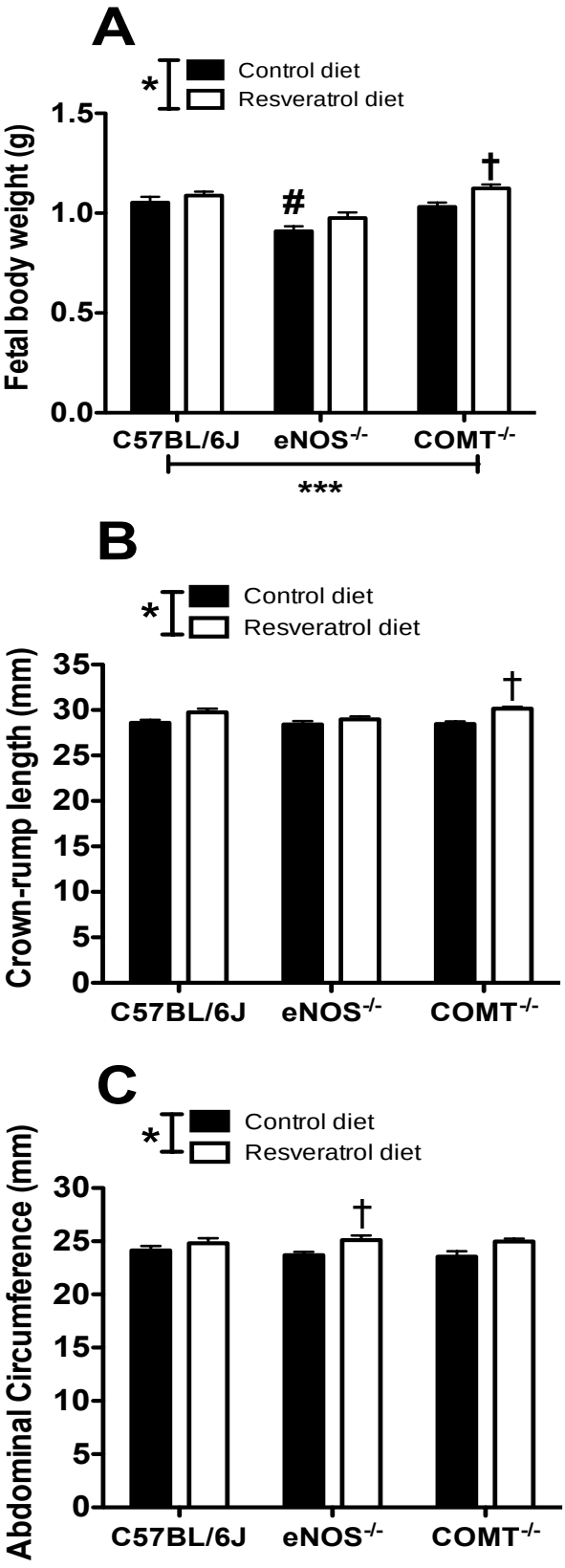
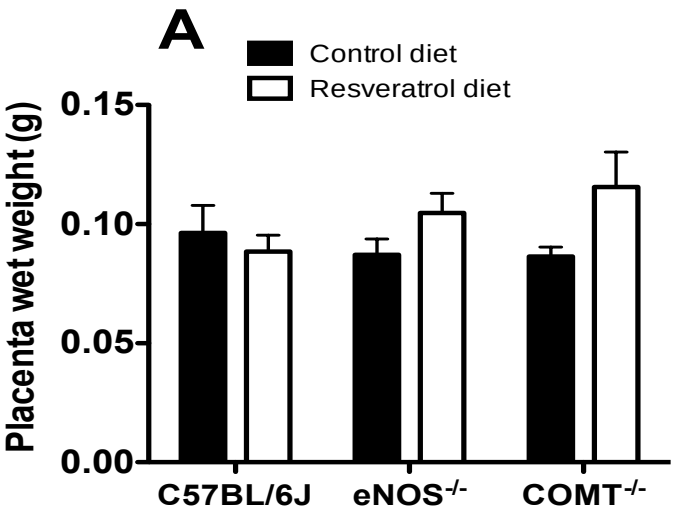
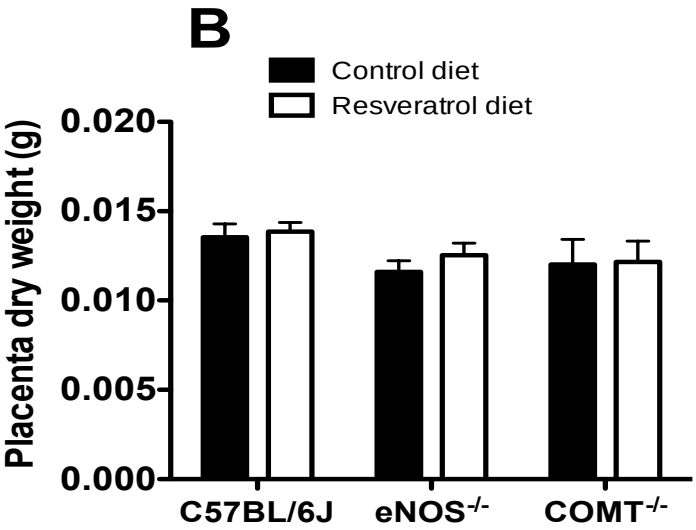

A
Control diet
Resveratrol diet
*
Fetal body weight (g)
1.5
1.0
0.5
0.0
#
†
C57BL/6J
eNOS-/-
COMT-/-
***
B
Control diet
Resveratrol diet
*
Crown-rump length (mm)
35
30
25
20
15
10
5
0
†
C57BL/6J
eNOS-/-
COMT-/-
C
Control diet
Resveratrol diet
*
Abdominal Circumference (mm)
30
25
20
15
10
5
0
†
C57BL/6J
eNOS-/-
COMT-/-

Figure 3-10: Effect of genotype and resveratrol treatment on fetal growth parameters.

A) Fetal body weight was significantly affected by both genotype and treatment. A significant reduction in fetal weight in $eNOS^{-/-}$ mice was observed. There was a significant increase in fetal body weight in $COMT^{-/-}$ mice following resveratrol administration. B) Resveratrol treatment leads to significant increase in crown-rump length only in the $COMT^{-/-}$ group. C) Resveratrol treatment leads to significant increase in abdominal circumference in $eNOS^{-/-}$ mice. Mean ± SEM, n=7-11, ***p<0.001, *p<0.05, two-way ANOVA, #p<0.05, Bonferroni post-hoc test indicating differences within genotypes in control diet. †p<0.05 Bonferroni post-hoc test indicating differences between control and resveratrol diet within same genotype.


A
Control diet
Resveratrol diet
Placenta wet weight (g)
0.15
0.10
0.05
0.00
C57BL/6J
eNOS-/-
COMT-/-


B
Control diet
Resveratrol diet
Placenta dry weight (g)
0.020
0.015
0.010
0.005
0.000
C57BL/6J
eNOS-/-
COMT-/-

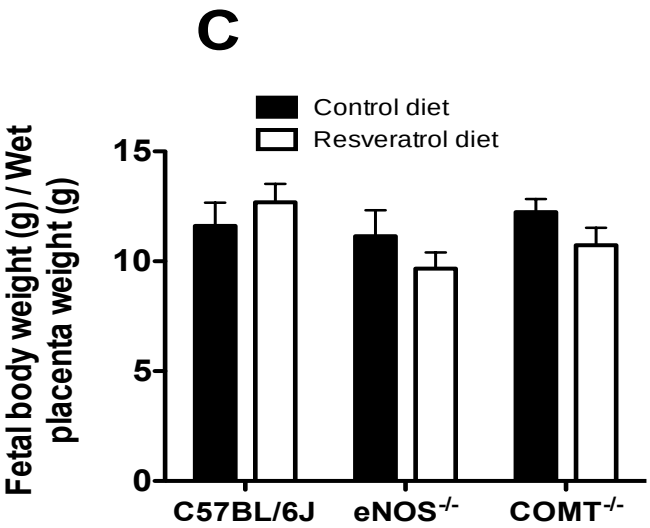

C
Control diet
Resveratrol diet
Fetal body weight (g) / Wet placenta weight (g)
15
10
5
0
C57BL/6J
eNOS-/-
COMT-/-

Figure 3-11: Placental outcome and fetal body weight: placental weight ratio between C57BL/6J, eNOS$^{-/-}$, COMT$^{-/-}$ mice on day 18.5 of gestation.

There were no significant differences between wet placental weight (A), dry placental weight (B) or fetal body weight: placental weight ratio (C) among the groups. Mean ± SEM, n=7-11, two-way ANOVA.

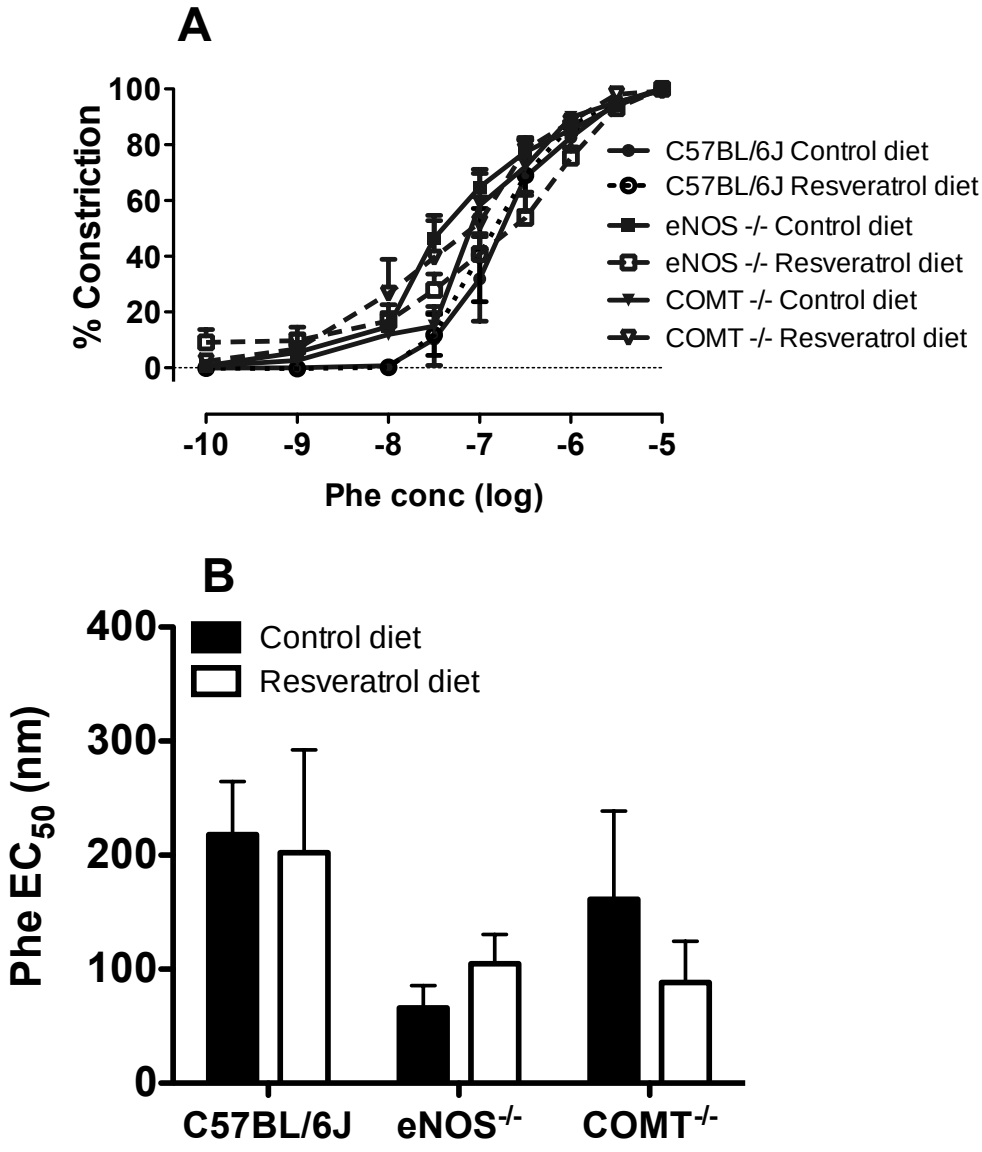


Figure 3-12: Response curves and EC50 concentrations of uterine arteries from C57BL/6J, eNOS$^{-/-}$, COMT$^{-/-}$ mice to cumulative concentrations of phenylephrine.

A and B: There was no effect of genotype or treatment on phenylephrine induced vasoconstriction of the uterine arteries in either of the groups. Mean ± SEM, n=5-6, two-way ANOVA.

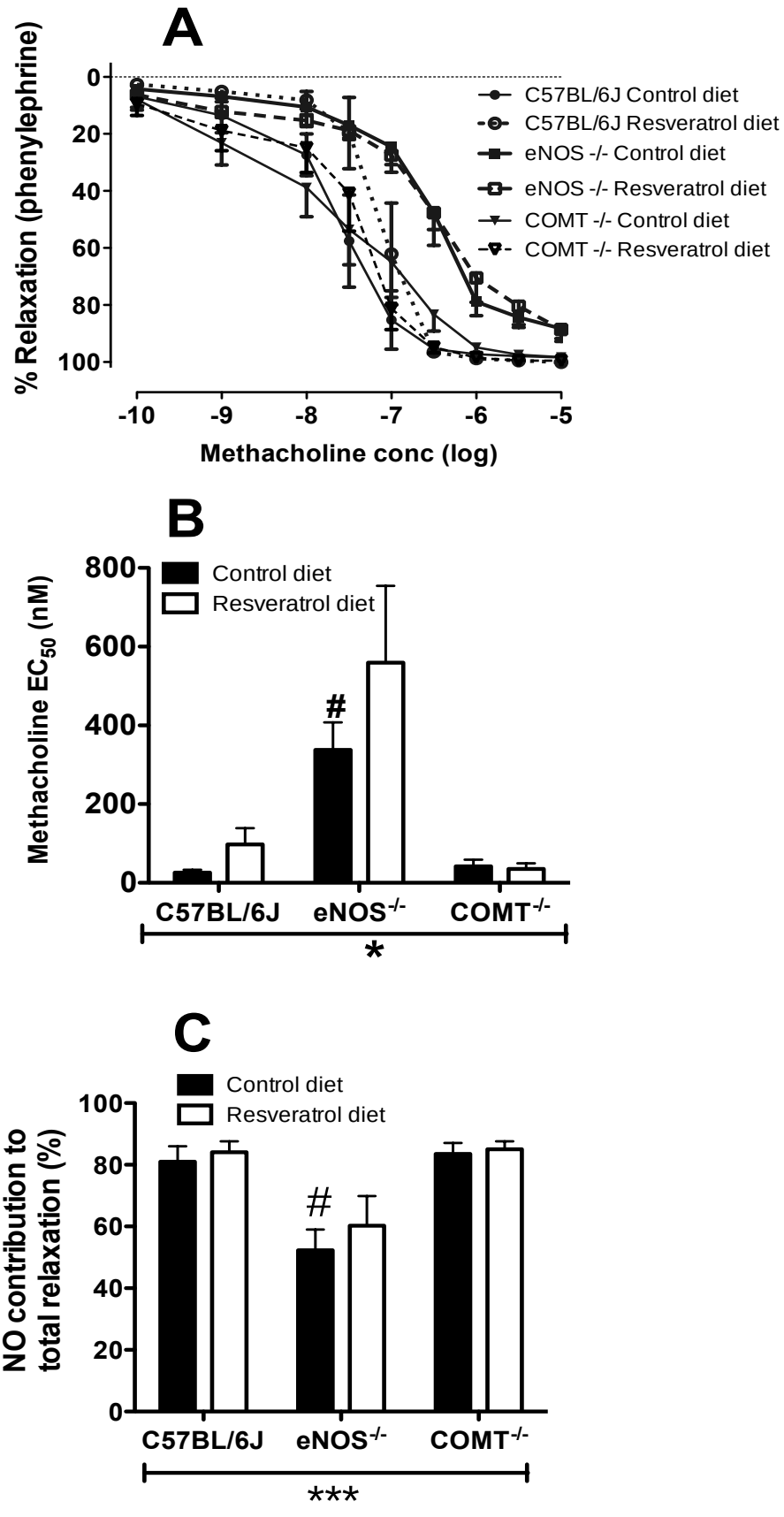

A
% Relaxation (phenylephrine)
0
20
40
60
80
100
-10
-9
-8
-7
-6
-5
Methacholine conc (log)
C57BL/6J Control diet
C57BL/6J Resveratrol diet
eNOS -/- Control diet
eNOS -/- Resveratrol diet
COMT -/- Control diet
COMT -/- Resveratrol diet
B
Methacholine EC50 (nM)
800
600
400
200
0
Control diet
Resveratrol diet
#
C57BL/6J
eNOS-/-
COMT-/-
*
C
NO contribution to total relaxation (%)
100
80
60
40
20
0
Control diet
Resveratrol diet
#
C57BL/6J
eNOS-/-
COMT-/-
***

Figure 3-13: Response curves, EC50 concentrations and contribution of NO to total relaxation in uterine arteries from C57BL/6J, eNOS$^{-/-}$, COMT$^{-/-}$ mice to cumulative concentrations of methacholine.

A and B) There was a significant decrease in percent relaxation induced by methacholine in eNOS$^{-/-}$ compared to C57BL6/J mice. Methacholine EC_{50} concentration was significantly elevated in eNOS$^{-/-}$ mice. (C) Summary graph showing NO contribution to total relaxation in C57BL/6J, eNOS$^{-/-}$ and COMT$^{-/-}$ mice. NO contribution was calculated as the difference between maximal MCh-induced relaxation and the maximal MCh-induced relaxation in the presence of L-NAME and expressed as percent of maximal MCh-induced relaxation. Mean ± SEM, n=5-6, two-way ANOVA. #$p<0.05$, Bonferroni post-hoc test indicating differences within genotypes in control diet.

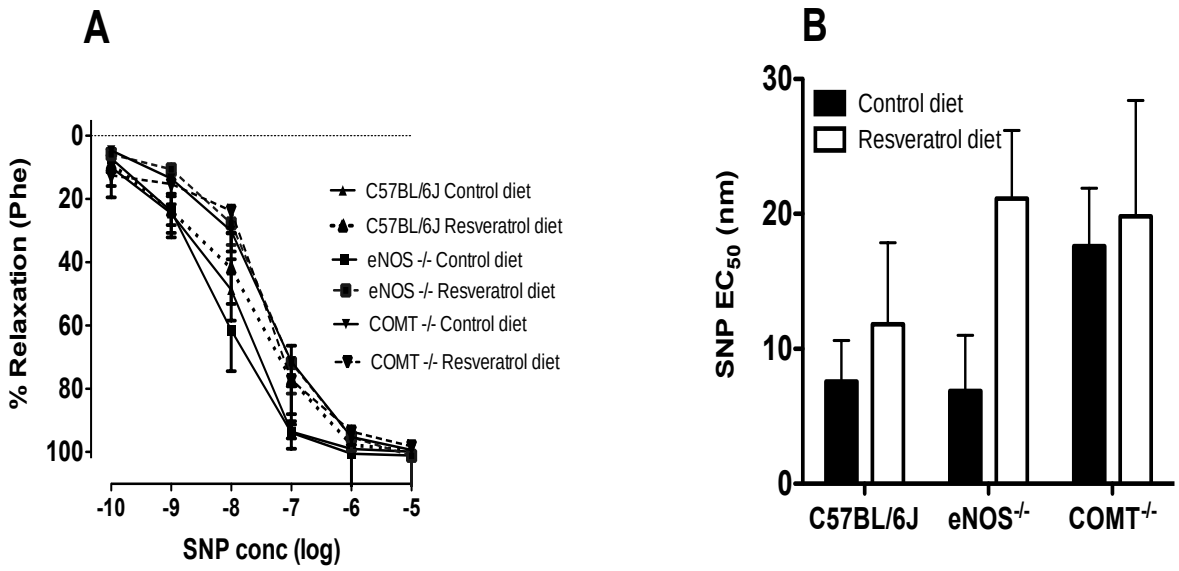


Figure 3-14: Response curves of uterine arteries from C57BL/6J, eNOS$^{-/-}$ and COMT$^{-/-}$ mice to cumulative concentrations of SNP.

A) There was no significant difference between relaxation responses to SNP in C57BL/6J, eNOS$^{-/-}$ and COMT$^{-/-}$ mice. B) EC_{50} concentrations were not different among the groups. Mean ± SEM, n=3-4, two-way ANOVA.

BIBLIOGRAPHY

Aardema, M., Oosterhof, H., Timmer, A., Van Rooy, I. & Aarnoudse, J. 2001. Uterine artery Doppler flow and uteroplacental vascular pathology in normal pregnancies and pregnancies complicated by pre-eclampsia and small for gestational age fetuses. *Placenta,* 22**,** 405-411.

Abbas, A. E., Lester, S. J. & Connolly, H. 2005. Pregnancy and the cardiovascular system. *International journal of cardiology,* 98**,** 179-189.

Abouzahr, C. 2003. Global burden of maternal death and disability. *British medical bulletin,* 67**,** 1-11.

Adamson, S. L., Lu, Y., Whiteley, K. J., Holmyard, D., Hemberger, M., Pfarrer, C. & Cross, J. C. 2002. Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. *Developmental biology,* 250**,** 358-373.

Aird, W. C. 2007. Phenotypic heterogeneity of the endothelium II. Representative vascular beds. *Circulation research,* 100**,** 174-190.

Alfirevic, Z. & Neilson, J. P. 2010. Doppler ultrasound for fetal assessment in high risk pregnancies. *The Cochrane Library*.

Alfirevic, Z., Stampalija, T. & Gyte, G. M. L. 2010. Fetal and umbilical Doppler ultrasound in high‐risk pregnancies. *The Cochrane Library*.

Amit, A., Thaler, I., Paz, Y. & Itskovitz‐Eldor, J. 1998. The effect of a nitric oxide donor on Doppler flow velocity waveforms in the uterine artery during the first trimester of pregnancy. *Ultrasound in obstetrics & gynecology,* 11**,** 94-98.

Ananth, C. V. & Vintzileos, A. M. 2009. Distinguishing pathological from constitutional small for gestational age births in population-based studies. *Early human development,* 85**,** 653-658.

Anderson, N. H., Devlin, A. M., Graham, D., Morton, J. J., Hamilton, C. A., Reid, J. L., Schork, N. J. & Dominiczak, A. F. 1999. Telemetry for cardiovascular monitoring in a pharmacological study: new approaches to data analysis. *Hypertension,* 33**,** 248-255.

Antsaklis, A., Daskalakis, G., Tzortzis, E. & Michalas, S. 2000. The effect of gestational age and placental location on the prediction of pre‐eclampsia by uterine artery Doppler velocimetry in low‐risk nulliparous women. *Ultrasound in obstetrics & gynecology,* 16**,** 635-639.

Arias, F., Rodriquez, L., Rayne, S. & Kraus, F. 1993. Maternal placental vasculopathy and infection: two distinct subgroups among patients with preterm labor and preterm ruptured membranes. *American journal of obstetrics and gynecology,* 168**,** 585.

Arngrìmsson, R., BjˆRnsson, H. & Geirsson, R. T. 1995. Analysis of different inheritance patterns in preeclampsia/eclampsia syndrome. *Hypertension in pregnancy,* 14**,** 27-38.

Arunachalam, G., Yao, H., Sundar, I. K., Caito, S. & Rahman, I. 2010. SIRT1 regulates oxidant-and cigarette smoke-induced eNOS acetylation in endothelial cells: Role of resveratrol. *Biochemical and biophysical research communications,* 393**,** 66-72.

Ashworth, J. R., Warren, A. Y., Baker, P. N. & Johnson, I. R. 1997. Loss of endothelium‐dependent relaxation in myometrial resistance arteries in

pre‐eclampsia. *BJOG: An International Journal of Obstetrics & Gynaecology,* 104**,** 1152-1158.
Assali, N., Douglass Jr, R., Baird, W., Nicholson, D. & Suyemoto, R. 1953. Measurement of uterine blood flow and uterine metabolism. IV. Results in normal pregnancy. *American journal of obstetrics and gynecology,* 66**,** 248.
Assali, N., Rauramo, L. & Peltonen, T. 1960. Measurement of uterine blood flow and uterine metabolism. 8. Uterine and fetal blood flow and oxygen consumption in early human pregnancy. *American journal of obstetrics and gynecology,* 79**,** 86-98.
Barnea, E., Maclusky, N., Decherney, A. & Naftolin, F. 1988. Catechol-O-methyl transferase activity in the human term placenta. *Am J Perinatol,* 5**,** 121-7.
Barnes, C. M., Mcelrath, T. F., Folkman, J. & Hansen, A. R. 2010. Correlation of 2-methoxyestradiol levels in cord blood and complications of prematurity. *Pediatric research,* 67**,** 545-550.
Battistini, B., Berthiaume, N., Kelland, N. F., Webb, D. J. & Kohan, D. E. 2006. Profile of past and current clinical trials involving endothelin receptor antagonists: the novel"-sentan" class of drug. *Experimental Biology and Medicine,* 231**,** 653.
Bellamy, L., Casas, J. P., Hingorani, A. D. & Williams, D. J. 2007. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ,* 335**,** 974.
Bird, I. M., Sullivan, J. A., Di, T., Cale, J. M., Zhang, L., Zheng, J. & Magness, R. R. 2000. Pregnancy-dependent changes in cell signaling underlie changes in differential control of vasodilator production in uterine artery endothelial cells. *Endocrinology,* 141**,** 1107-1117.
Boffa, M. B., Wang, W., Bajzar, L. & Nesheim, M. E. 1998. Plasma and recombinant thrombin-activable fibrinolysis inhibitor (TAFI) and activated TAFI compared with respect to glycosylation, thrombin/thrombomodulin-dependent activation, thermal stability, and enzymatic properties. *Journal of Biological Chemistry,* 273**,** 2127-2135.
Bourque, S., Dolinsky, V., Dyck, J. & Davidge, S. 2012. Maternal resveratrol treatment during pregnancy improves adverse fetal outcomes in a rat model of severe hypoxia. *Placenta*.
Brandão, A. H. F., Pereira, L. M. G., Gonçalves, A. C. O., Reis, Z. S. N., Leite, H. V. & Cabral, A. C. V. 2012. Comparative Study of Endothelial Function and Uterine Artery Doppler Velocimetry between Pregnant Women with or without Preeclampsia Development. *Journal of Pregnancy,* 2012.
Brooks, V. L., Clow, K. A., Welch, L. S. & Giraud, G. D. 2001. Does nitric oxide contribute to the basal vasodilation of pregnancy in conscious rabbits? *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 281**,** R1624-R1632.
Brosens, I., Robertson, W. & Dixon, H. 1967. The physiological response of the vessels of the placental bed to normal pregnancy. *The Journal of pathology and bacteriology,* 93**,** 569-579.

Brosens, I., Robertson, W. & Dixon, H. 1972. The role of the spiral arteries in the pathogenesis of preeclampsia. *Obstetrics and gynecology annual,* 1**,** 177.

Brown, M. A. 2007. The physiology of preeclampsia. *Clinical and experimental pharmacology and physiology,* 22**,** 781-791.

Brown, M. A., Tibben, E., Zammit, V. C., Cario, G. M. & Carlton, M. A. 1995. Nitric oxide excretion in normal and hypertensive pregnancies. *Hypertension in pregnancy,* 14**,** 319-326.

Brown, V. A., Patel, K. R., Viskaduraki, M., Crowell, J. A., Perloff, M., Booth, T. D., Vasilinin, G., Sen, A., Schinas, A. M. & Piccirilli, G. 2010. Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer research,* 70**,** 9003-9011.

Bujold, E., Effendi, M., Girard, M., Gouin, K., Forest, J. C., Couturier, B. & Giguère, Y. 2009a. Reproducibility of first trimester three-dimensional placental measurements in the evaluation of early placental insufficiency. *J Obstet Gynaecol Can,* 31**,** 1144-1148.

Bujold, E., Morency, A. M., Roberge, S., Lacasse, Y., Forest, J. C. & Giguère, Y. 2009b. Acetylsalicylic acid for the prevention of preeclampsia and intra-uterine growth restriction in women with abnormal uterine artery Doppler: a systematic review and meta-analysis. *J Obstet Gynaecol Can,* 31**,** 818-826.

Burke, S. D., Barrette, V. F., Gravel, J., Carter, A. L. I., Hatta, K., Zhang, J., Chen, Z., Leno‐Durán, E., Bianco, J. & Leonard, S. 2010. Uterine NK Cells, Spiral Artery Modification and the Regulation of Blood Pressure During Mouse Pregnancy. *American Journal of Reproductive Immunology,* 63**,** 472-481.

Calvin, S., Corrigan, J., Weinstein, L. & Jeter, M. 1988. Factor VIII: von Willebrand factor patterns in the plasma of patients with pre-eclampsia. *American journal of perinatology,* 5**,** 29.

Campbell, S., Griffin, D., Pearce, J., Diaz-Recasens, J., Cohen-Overbeek, T., Willson, K. & Teague, M. 1983. New Doppler technique for assessing uteroplacental blood flow. *The Lancet,* 321**,** 675-677.

Campbell, S., Warsof, S. L., Little, D. & Cooper, D. J. 1985. Routine ultrasound screening for the prediction of gestational age. *Obstetrics and gynecology,* 65**,** 613.

Cartwright, J. E., Fraser, R., Leslie, K., Wallace, A. E. & James, J. L. 2010. Remodelling at the maternal–fetal interface: relevance to human pregnancy disorders. *Reproduction,* 140**,** 803-813.

Casanello, P. & Sobrevia, L. 2002. Intrauterine growth retardation is associated with reduced activity and expression of the cationic amino acid transport systems y+/hCAT-1 and y+/hCAT-2B and lower activity of nitric oxide synthase in human umbilical vein endothelial cells. *Circulation research,* 91**,** 127-134.

Casper, F. & Seufert, R. 1995. Atrial natriuretic peptide (ANP) in preeclampsia-like syndrome in a rat model. *Experimental and Clinical Endocrinology and Diabetes,* 103**,** 292-292.

Chan, V., Fenning, A., Iyer, A., Hoey, A. & Brown, L. 2011. Resveratrol improves cardiovascular function in DOCA-salt hypertensive rats. *Current Pharmaceutical Biotechnology,* 12**,** 429-436.

Chang, J., Roman, C. & Heymann, M. 1992. Effect of endothelium-derived relaxing factor inhibition on the umbilical-placental circulation in fetal lambs in utero. *American journal of obstetrics and gynecology,* 166**,** 727.

Chappell, L. C., Enye, S., Seed, P., Briley, A. L., Poston, L. & Shennan, A. H. 2008. Adverse perinatal outcomes and risk factors for preeclampsia in women with chronic hypertension a prospective study. *Hypertension,* 51**,** 1002-1009.

Chappell, L. C., Seed, P. T., Briley, A. L., Kelly, F. J., Lee, R., Hunt, B. J., Parmar, K., Bewley, S. J., Shennan, A. H. & Steer, P. J. 1999. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. *The Lancet,* 354**,** 810-816.

Chien, P. F. W., Arnott, N., Gordon, A., Owen, P. & Khan, K. S. 2005. How useful is uterine artery Doppler flow velocimetry in the prediction of pre‐eclampsia, intrauterine growth retardation and perinatal death? An overview. *BJOG: An International Journal of Obstetrics & Gynaecology,* 107**,** 196-208.

Chow, H. H. S., Garland, L. L., Hsu, C. H., Vining, D. R., Chew, W. M., Miller, J. A., Perloff, M., Crowell, J. A. & Alberts, D. S. 2010. Resveratrol modulates drug-and carcinogen-metabolizing enzymes in a healthy volunteer study. *Cancer Prevention Research,* 3**,** 1168-1175.

Cines, D. B., Pollak, E. S., Buck, C. A., Loscalzo, J., Zimmerman, G. A., Mcever, R. P., Pober, J. S., Wick, T. M., Konkle, B. A. & Schwartz, B. S. 1998. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood,* 91**,** 3527-3561.

Clark, D., Smith, S., Sharkey, A. & Charnock-Jones, D. 1996. Localization of VEGF and expression of its receptors flit and KDR in human placenta throughout pregnancy. *Human Reproduction,* 11**,** 1090-1098.

Clark, S., Cotton, D., Lee, W., Bishop, C., Hill, T., Southwick, J., Pivarnik, J., Spillman, T., Devore, G. & Phelan, J. 1989. Central hemodynamic assessment of normal term pregnancy. *American journal of obstetrics and gynecology,* 161**,** 1439.

Clark, S. L., Belfort, M. A., Dildy, G. A., Herbst, M. A., Meyers, J. A. & Hankins, G. D. 2008. Maternal death in the 21st century: causes, prevention, and relationship to cesarean delivery. *American journal of obstetrics and gynecology,* 199**,** 36. e1-36. e5.

Cnossen, J. S., Morris, R. K., Gerben Ter Riet, M., Mol, B. W. J., Van Der Post, J. a. M., Coomarasamy, A., Zwinderman, A. H., Robson, S. C., Bindels, P. J. E. & Kleijnen, J. 2008a. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. *Canadian Medical Association Journal,* 178**,** 701-711.

Cnossen, J. S., Morris, R. K., Ter Riet, G., Mol, B. W. J., Van Der Post, J. a. M., Coomarasamy, A., Zwinderman, A. H., Robson, S. C., Bindels, P. J. E. &

Kleijnen, J. 2008b. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. *Canadian Medical Association Journal,* 178**,** 701-711.

Cogswell, M. E. & Yip, R. Year. The influence of fetal and maternal factors on the distribution of birthweight. *In*, 1995. 222.

Collins, S., Stevenson, G., Noble, J. & Impey, L. 2012. Developmental changes in spiral artery blood flow in the human placenta observed with colour Doppler ultrasonography. *Placenta*.

Conde-Agudelo, A., Villar, J. & Lindheimer, M. 2004. World Health Organization systematic review of screening tests for preeclampsia. *Obstetrics & Gynecology,* 104**,** 1367-1391.

Conrad, K. P., Joffe, G. M., Kruszyna, H., Kruszyna, R., Rochelle, L., Smith, R., Chavez, J. & Mosher, M. 1993. Identification of increased nitric oxide biosynthesis during pregnancy in rats. *The FASEB journal,* 7**,** 566-571.

Const,Ncia, M., Hemberger, M., Hughes, J., Dean, W., Ferguson-Smith, A., Fundele, R., Stewart, F., Kelsey, G., Fowden, A. & Sibley, C. 2002. Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature,* 417**,** 945-948.

Coomarasamy, A., Honest, H., Papaioannou, S., Gee, H. & Khan, K. S. 2003. Aspirin for prevention of preeclampsia in women with historical risk factors: a systematic review. *Obstetrics & Gynecology,* 101**,** 1319-1332.

Cooper, A., Robinson, G., Vinson, G., Cheung, W. & Broughton Pipkin, F. 1999. The localization and expression of the renin-angiotensin system in the human placenta throughout pregnancy. *Placenta,* 20**,** 467-474.

Cross, J., Hemberger, M., Lu, Y., Nozaki, T., Whiteley, K., Masutani, M. & Adamson, S. 2002. Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta. *Molecular and cellular endocrinology,* 187**,** 207-212.

Cross, J. C. 1996. Trophoblast function in normal and preeclamptic pregnancy. *Fetal and Maternal Medicine Review,* 8**,** 57-66.

Cudmore, M. J., Ahmad, S., Sissaoui, S., Ramma, W., Ma, B., Fujisawa, T., Al-Ani, B., Wang, K., Cai, M. & Crispi, F. 2012. Loss of Akt activity increases circulating soluble endoglin release in preeclampsia: identification of inter-dependency between Akt-1 and heme oxygenase-1. *European heart journal,* 33**,** 1150-1158.

Curtis, N. E., Gude, N. M., King, R. G., Marriott, P. J., Rook, T. J. & Brennecke, S. P. 1995. Nitric oxide metabolites in normal human pregnancy and preeclampsia. *Hypertension in pregnancy,* 14**,** 339-349.

Das, S., Alagappan, V. K. T., Bagchi, D., Sharma, H. S., Maulik, N. & Das, D. K. 2005. Coordinated induction of iNOS–VEGF–KDR–eNOS after resveratrol consumption: A potential mechanism for resveratrol preconditioning of the heart. *Vascular pharmacology,* 42**,** 281-289.

Das, S., Tosaki, A., Bagchi, D., Maulik, N. & Das, D. K. 2006. Potentiation of a survival signal in the ischemic heart by resveratrol through p38 mitogen-activated protein kinase/mitogen-and stress-activated protein kinase

1/cAMP response element-binding protein signaling. *Journal of Pharmacology and Experimental Therapeutics,* 317**,** 980-988.

Davisson, R. L., Hoffmann, D. S., Butz, G. M., Aldape, G., Schlager, G., Merrill, D. C., Sethi, S., Weiss, R. M. & Bates, J. N. 2002. Discovery of a spontaneous genetic mouse model of preeclampsia. *Hypertension,* 39**,** 337-342.

Dechanet, C., Fort, A., Barbero-Camps, E., Dechaud, H., Richard, S. & Virsolvy, A. 2011. Endothelin-dependent vasoconstriction in human uterine artery: application to preeclampsia. *PloS one,* 6**,** e16540.

Dekker, G. & Sibai, B. 2001. Primary, secondary, and tertiary prevention of pre-eclampsia. *The Lancet,* 357**,** 209-215.

Dell'agli, M., Galli, G. V., Vrhovsek, U., Mattivi, F. & Bosisio, E. 2005. In vitro inhibition of human cGMP-specific phosphodiesterase-5 by polyphenols from red grapes. *Journal of agricultural and food chemistry,* 53**,** 1960-1965.

Dieckmann, W. J. & Wegner, C. R. 1934. The blood in normal pregnancy: I. Blood and plasma volumes. *Archives of Internal Medicine,* 53**,** 71.

Dodd, J., Mcleod, A. & Windrim, R. 2010. Kingdom J. Antithrombotic therapy for improving maternal or infant health outcomes in women considered at risk of placental dysfunction. *Cochrane Database Syst Rev,* 6.

Dolinsky, V. W., Rueda-Clausen, C. F., Morton, J. S., Davidge, S. T. & Dyck, J. R. B. 2011. Continued postnatal administration of resveratrol prevents diet-induced metabolic syndrome in rat offspring born growth restricted. *Diabetes,* 60**,** 2274-2284.

Dowell, R. T. & Kauer, C. D. 1997. Maternal hemodynamics and uteroplacental blood flow throughout gestation in conscious rats. *Methods and findings in experimental and clinical pharmacology,* 19**,** 613-626.

Dudley, J. I., Lekli, I., Mukherjee, S., Das, M., Bertelli, A. a. A. & Das, D. K. 2008. Does white wine qualify for French paradox? Comparison of the cardioprotective effects of red and white wines and their constituents: resveratrol, tyrosol, and hydroxytyrosol. *Journal of agricultural and food chemistry,* 56**,** 9362-9373.

Edwards, J., Beck, M., Riegger, C. & Bausch, J. 2011. Safety of resveratrol with examples for high purity, trans‐resveratrol, resVida≫. *Annals of the New York Academy of Sciences,* 1215**,** 131-137.

El-Hamedi, A., Shillito, J., Simpson, N. a. B. & Walker, J. J. 2005. A prospective analysis of the role of uterine artery Doppler waveform notching in the assessment of at-risk pregnancies. *Hypertension in pregnancy,* 24**,** 137-145.

Ellis, G., Adatia, I., Yazdanpanah, M. & Makela, S. K. 1998. Nitrite and nitrate analyses: a clinical biochemistry perspective. *Clinical biochemistry,* 31**,** 195-220.

Eskenazi, B., Fenster, L. & Sidney, S. 1991. A multivariate analysis of risk factors for preeclampsia. *JAMA: the journal of the American Medical Association,* 266**,** 237.

Fitzgerald, D. & Drumm, J. 1977. Non-invasive measurement of human fetal circulation using ultrasound: a new method. *British medical journal,* 2**,** 1450.

Friedman, S. A., Schiff, E., Emeis, J. J., Dekker, G. A. & Sibai, B. M. 1995. Biochemical corroboration of endothelial involvement in severe preeclampsia. *American journal of obstetrics and gynecology,* 172**,** 202-203.

Furchgott, R. F. 1983. Role of endothelium in responses of vascular smooth muscle. *Circulation research,* 53**,** 557-573.

Furchgott, R. F. & Zawadzki, J. V. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine.

Gagnon, R. 2003. Placental insufficiency and its consequences. *European Journal of Obstetrics & Gynecology and Reproductive Biology,* 110**,** S99-S107.

Gerber, H. P., Condorelli, F., Park, J. & Ferrara, N. 1997. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. *Journal of Biological Chemistry,* 272**,** 23659-23667.

Goodlin, R., Dobry, C., Anderson, J., Woods, R. & Quaife, M. 1983. Clinical signs of normal plasma volume expansion during pregnancy. *American journal of obstetrics and gynecology,* 145**,** 1001.

Grollman, A. 1932. *The cardiac output of man in health and disease,* CC Thomas.

Groom, K. M., North, R. A., Stone, P. R., Chan, E. H. Y., Taylor, R. S., Dekker, G. A. & Mccowan, L. M. E. 2009. Patterns of change in uterine artery Doppler studies between 20 and 24 weeks of gestation and pregnancy outcomes. *Obstetrics & Gynecology,* 113**,** 332-338.

Gui, Y., Zheng, X. L., Zheng, J. & Walsh, M. P. 2008. Inhibition of rat aortic smooth muscle contraction by 2-methoxyestradiol. *American Journal of Physiology-Heart and Circulatory Physiology,* 295**,** H1935-H1942.

Hanretty, K. P., Whittle, M. J., Howie, C. A. & Rubin, P. C. 1989. Effect of nifedipine on Doppler flow velocity waveforms in severe pre-eclampsia. *British medical journal,* 299**,** 1205-1206.

Harrington, K., Cooper, D., Lees, C., Hecher, K. & Campbell, S. 1996. Doppler ultralsound of the uterine arteries: the importance of bilateral notching in the prediction of pre‐eclampsia, placental abruption or delivery of a small‐for‐gestational‐age baby. *Ultrasound in obstetrics & gynecology,* 7**,** 182-188.

Hefler, L. A., Reyes, C. A., O'brien, W. E. & Gregg, A. R. 2001a. Perinatal development of endothelial nitric oxide synthase-deficient mice. *Biology of reproduction,* 64**,** 666-673.

Hefler, L. A., Tempfer, C. B., Moreno, R. M., O'brien, W. E. & Gregg, A. R. 2001b. Endothelial-derived nitric oxide and angiotensinogen: blood pressure and metabolism during mouse pregnancy. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 280**,** R174-R182.

Higgins, J. R., Papayianni, A., Brady, H. R., Darling, M. & Walshe, J. J. 1998. Circulating vascular cell adhesion molecule–1 in pre-eclampsia, gestational hypertension, and normal pregnancy: Evidence of selective

dysregulation of vascular cell adhesion molecule–1 homeostasis in pre-eclampsia. *American journal of obstetrics and gynecology,* 179**,** 464-469.
Hofmeyr, G. & Gulmezoglu, A. 2000. Vaginal misoprostol for cervical ripening and labour induction in late pregnancy (Cochrane Review). *The Cochrane Library,* 2.
Holthoff, J. H., Wang, Z., Seely, K. A., Gokden, N. & Mayeux, P. R. 2011. Resveratrol improves renal microcirculation, protects the tubular epithelium, and prolongs survival in a mouse model of sepsis-induced acute kidney injury. *Kidney international*.
Huang, P. L., Huang, Z., Mashimo, H., Bloch, K. D., Moskowitz, M. A., Bevan, J. A. & Fishman, M. C. 1995. Hypertension in mice lacking the gene for endothelial nitric oxide synthase.
Hurairah, H. & Ferro, A. 2004. The role of the endothelium in the control of vascular function. *International journal of clinical practice,* 58**,** 173-183.
Hytten, F. & Paintin, D. 1963. Increase in plasma volume during normal pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology,* 70**,** 402-407.
Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E. & Chaudhuri, G. 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences,* 84**,** 9265-9269.
James, J., Stone, P. & Chamley, L. 2006. The regulation of trophoblast differentiation by oxygen in the first trimester of pregnancy. *Human reproduction update,* 12**,** 137-144.
Jobe, S. O., Ramadoss, J., Koch, J. M., Jiang, Y., Zheng, J. & Magness, R. R. 2010. Estradiol-17β and Its Cytochrome P450-and Catechol-O-Methyltransferase–Derived Metabolites Stimulate Proliferation in Uterine Artery Endothelial Cells Role of Estrogen Receptor-α Versus Estrogen Receptor-β. *Hypertension,* 55**,** 1005-1011.
Jouppila, R., Jouppila, P., Kuikka, J. & Hollmen, A. 1978. Placental blood flow during caesarean section under lumbar extradural analgesia. *British journal of anaesthesia,* 50**,** 275-279.
Juan, M. E., Alfaras, I. & Planas, J. M. 2012. Colorectal cancer chemoprevention by trans-resveratrol. *Pharmacological Research*.
Kanasaki, K., Palmsten, K., Sugimoto, H., Ahmad, S., Hamano, Y., Xie, L., Parry, S., Augustin, H. G., Gattone, V. H. & Folkman, J. 2008. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. *Nature,* 453**,** 1117-1121.
Kanayama, N., Takahashi, K., Matsuura, T., Sugimura, M., Kobayashi, T., Moniwa, N., Tomita, M. & Nakayama, K. 2002. Deficiency in p57Kip2 expression induces preeclampsia-like symptoms in mice. *Molecular human reproduction,* 8**,** 1129.
Kaya, A., Boztosun, A., Seckin, H., Guven, A. S., Kucukdurmaz, Z., Gulturk, S. & Cevit, O. 2011. The Evaluation of Hypoxia-Inducible Factor 1 in N-Nitro-L-Arginine Methyl Ester Preeclampsia Model of Pregnant Rats. *Journal of Investigative Medicine,* 59**,** 1268.

Ke Chen, C. & Pace-Asciak, C. R. 1996. Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta. *General Pharmacology: The Vascular System,* 27**,** 363-366.

Kennedy, D. O., Wightman, E. L., Reay, J. L., Lietz, G., Okello, E. J., Wilde, A. & Haskell, C. F. 2010. Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. *The American journal of clinical nutrition,* 91**,** 1590-1597.

Khan, K. S., Wojdyla, D., Say, L., Gulmezoglu, A. M. & Van Look, P. F. 2006. WHO analysis of causes of maternal death: a systematic review. *Lancet,* 367**,** 1066-74.

Khong, T., Wolf, F., Robertson, W. & Brosens, I. 1986. Inadequate maternal vascular response to placentation in pregnancies complicated by pre‐eclampsia and by small‐for‐gestational age infants. *BJOG: An International Journal of Obstetrics & Gynaecology,* 93**,** 1049-1059.

Knight, M., Duley, L., Henderson‐Smart, D. J. & King, J. F. 2001. Antiplatelet agents for preventing and treating pre‐eclampsia. *The Cochrane Library*.

Knock, G. A. & Poston, L. 1996. Bradykinin-mediated relaxation of isolated maternal resistance arteries in normal pregnancy and preeclampsia. *American journal of obstetrics and gynecology,* 175**,** 1668-1674.

Kopp, L., Paradiz, G. & Tucci, J. R. 1977. Urinary excretion of cyclic 3′, 5′-adenosine monophosphate and cyclic 3′, 5′-guanosine monophosphate during and after pregnancy. *Journal of Clinical Endocrinology & Metabolism,* 44**,** 590-594.

Kublickiene, K. R., Grunewald, C., Lindblom, B. & Nisell, H. 1998. Myogenic and endothelial properties of myometrial resistance arteries from women with preeclampsia. *Hypertension in pregnancy,* 17**,** 271-281.

Kuboki, K., Jiang, Z. Y., Takahara, N., Ha, S. W., Igarashi, M., Yamauchi, T., Feener, E. P., Herbert, T. P., Rhodes, C. J. & King, G. L. 2000. Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. *Circulation,* 101**,** 676-681.

Kubota, Y., Umegaki, K., Kagota, S., Tanaka, N., Nakamura, K., Kunitomo, M. & Shinozuka, K. 2006. Evaluation of blood pressure measured by tail-cuff methods (without heating) in spontaneously hypertensive rats. *Biological and Pharmaceutical Bulletin,* 29**,** 1756-1758.

Kulandavelu, S., Whiteley, K. J., Qu, D., Mu, J., Bainbridge, S. A. & Adamson, S. L. 2012. Endothelial Nitric Oxide Synthase Deficiency Reduces Uterine Blood Flow, Spiral Artery Elongation, and Placental Oxygenation in Pregnant MiceNovelty and Significance. *Hypertension,* 60**,** 231-238.

Kumasawa, K., Ikawa, M., Kidoya, H., Hasuwa, H., Saito-Fujita, T., Morioka, Y., Takakura, N., Kimura, T. & Okabe, M. 2011. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. *Proceedings of the National Academy of Sciences,* 108**,** 1451-1455.

Kupferminc, M. J., Fait, G., Many, A., Lessing, J. B., Yair, D., Bar-Am, A. & Eldor, A. 2001. Low-molecular-weight heparin for the prevention of

obstetric complications in women with thrombophilias. *Hypertension in pregnancy,* 20**,** 35-44.

Kusinski, L. C., Stanley, J. L., Dilworth, M. R., Hirt, C. J., Andersson, I. J., Renshall, L. J., Baker, B. C., Baker, P. N., Sibley, C. P. & Wareing, M. 2012. eNOS knockout mouse as a model of fetal growth restriction with an impaired uterine artery function and placental transport phenotype. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 303**,** R86-R93.

Lachmeijer, A., Dekker, G. A., Pals, G., Aarnoudse, J. G., Ten Kate, L. P. & Arngrimsson, R. 2002. Searching for preeclampsia genes: the current position. *European Journal of Obstetrics & Gynecology and Reproductive Biology,* 105**,** 94-113.

Lackman, F., Capewell, V., Richardson, B., Dasilva, O. & Gagnon, R. 2001. The risks of spontaneous preterm delivery and perinatal mortality in relation to size at birth according to fetal versus neonatal growth standards. *American journal of obstetrics and gynecology,* 184**,** 946.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P. & Elliott, P. 2006. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 [alpha]. *Cell,* 127**,** 1109-1122.

Lain, K. Y. & Roberts, J. M. 2002. Contemporary concepts of the pathogenesis and management of preeclampsia. *JAMA: the journal of the American Medical Association,* 287**,** 3183-3186.

Langille, B. & O'donnell, F. 1986. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science (New York, NY),* 231**,** 405.

Lee, S. B., Wong, A. P., Kanasaki, K., Xu, Y., Shenoy, V. K., Mcelrath, T. F., Whitesides, G. M. & Kalluri, R. 2010. Preeclampsia: 2-methoxyestradiol induces cytotrophoblast invasion and vascular development specifically under hypoxic conditions. *The American journal of pathology,* 176**,** 710-720.

Leifert, W. R. & Abeywardena, M. Y. 2008. Cardioprotective actions of grape polyphenols. *Nutrition Research,* 28**,** 729-737.

Li, Y. & Behringer, R. R. 1998. Esx1 is an X-chromosome-imprinted regulator of placental development and fetal growth. *Nature Genetics,* 20**,** 309-311.

Lockwood, C. J. 2002. Inherited thrombophilias in pregnant patients: detection and treatment paradigm. *Obstetrics & Gynecology,* 99**,** 333-341.

Lückhoff, A. & Busse, R. 1986. Increased free calcium in endothelial cells under stimulation with adenine nucleotides. *Journal of cellular physiology,* 126**,** 414-420.

Lunell, N., Sarby, B., Lewander, R. & Nylund, L. 1979. Comparison of uteroplacental blood flow in normal and in intrauterine growth-retarded pregnancy. *Gynecologic and obstetric investigation,* 10**,** 106-118.

Lyall, F. 2005. Priming and remodelling of human placental bed spiral arteries during pregnancy–a review. *Placenta,* 26**,** S31-S36.

Lyall, F. & Greer, I. A. 1996. The vascular endothelium in normal pregnancy and pre-eclampsia. *Reviews of reproduction,* 1**,** 107-116.
Mabie, W. C., Disessa, T. G., Crocker, L. G., Sibai, B. M. & Arheart, K. L. 1994. A longitudinal study of cardiac output in normal human pregnancy. *American journal of obstetrics and gynecology,* 170**,** 849.
Mabjeesh, N. J., Escuin, D., Lavallee, T. M., Pribluda, V. S., Swartz, G. M., Johnson, M. S., Willard, M. T., Zhong, H., Simons, J. W. & Giannakakou, P. 2003. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer cell,* 3**,** 363-375.
Magee, L. A., Cham, C., Waterman, E. J., Ohlsson, A. & Von Dadelszen, P. 2003. Hydralazine for treatment of severe hypertension in pregnancy: meta-analysis. *Bmj,* 327**,** 955.
Magness, R. R., Shaw, C., Phernetton, T., Zheng, J. & Bird, I. 1997. Endothelial vasodilator production by uterine and systemic arteries. II. Pregnancy effects on NO synthase expression. *American Journal of Physiology-Heart and Circulatory Physiology,* 272**,** H1730-H1740.
Magnussen, E. B., Vatten, L. J., Smith, G. D. & Romundstad, P. R. 2009. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. *Obstetrics & Gynecology,* 114**,** 961.
Makrides, M., Duley, L. & Olsen, S. F. 2007. Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre‐eclampsia or intrauterine growth restriction. *The Cochrane Library*.
Makris, A., Thornton, C., Thompson, J., Thomson, S., Martin, R., Ogle, R., Waugh, R., Mckenzie, P., Kirwan, P. & Hennessy, A. 2007. Uteroplacental ischemia results in proteinuric hypertension and elevated sFLT-1. *Kidney international,* 71**,** 977-984.
Malassine, A., Frendo, J. L. & Evain Brion, D. 2003. A comparison of placental development and endocrine functions between the human and mouse model. *Human reproduction update,* 9**,** 531.
Maulik, D., Mundy, D. & Heitmann, E. 2011. Umbilical artery Doppler in the assessment of fetal growth restriction. *Clinics in perinatology,* 38**,** 65.
Maynard, S. E., Min, J. Y., Merchan, J., Lim, K. H., Li, J., Mondal, S., Libermann, T. A., Morgan, J. P., Sellke, F. W. & Stillman, I. E. 2003. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *Journal of clinical investigation,* 111**,** 649-658.
Mazzuca, M. Q., Tare, M., Parkington, H. C., Dragomir, N. M., Parry, L. J. & Wlodek, M. E. 2012. Uteroplacental insufficiency programmes vascular dysfunction in non‐pregnant rats: compensatory adaptations in pregnancy. *The Journal of Physiology,* 590**,** 3375-3388.
Mcintire, D. D., Bloom, S. L., Casey, B. M. & Leveno, K. J. 1999. Birth weight in relation to morbidity and mortality among newborn infants. *New England journal of medicine,* 340**,** 1234-1238.
Mcmillen, I. C., Adams, M. B., Ross, J. T., Coulter, C. L., Simonetta, G., Owens, J. A., Robinson, J. S. & Edwards, L. J. 2001. Fetal growth restriction: adaptations and consequences. *Reproduction,* 122**,** 195-204.

Mishra, N., Nugent, W. H., Mahavadi, S. & Walsh, S. W. 2011. Mechanisms of enhanced vascular reactivity in preeclampsia. *Hypertension,* 58**,** 867-873.
Moll, W., Nienartowicz, A., Hees, H., Wrobel, K. & Lenz, A. 1988. Blood flow regulation in the uteroplacental arteries. *Trophoblast Res,* 3**,** 83-96.
Moncada, S. & Vane, J. 1979. Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls. *New England journal of medicine,* 300**,** 1142-1147.
Moore, R., Strachan, B., Tyler, D., Duncan, K., Baker, P., Worthington, B., Johnson, I. & Gowland, P. 2000. In utero perfusing fraction maps in normal and growth restricted pregnancy measured using IVIM echo-planar MRI. *Placenta,* 21**,** 726-732.
Moore, R. J., Ong, S. S., Tyler, D. J., Duckett, R., Baker, P. N., Dunn, W. R., Johnson, I. R. & Gowland, P. A. 2007. Spiral artery blood volume in normal pregnancies and those compromised by pre‐eclampsia. *NMR in Biomedicine,* 21**,** 376-380.
Moraloglu, O., Engin-Ustun, Y., Tonguç, E., Var, T., Tapisiz, Ö. L., Ergün, H., Guvenc, T. & Gacar, A. 2011. The effect of resveratrol on blood pressure in a rat model of preeclampsia. *Journal of Maternal-Fetal and Neonatal Medicine***,** 1-4.
Morris, N., Carroll, S., Nicolaides, K., Steer, P. & Warren, J. 2008. Exhaled nitric oxide concentration and amniotic fluid nitrite concentration during pregnancy. *European journal of clinical investigation,* 25**,** 138-141.
Mu, J. & Adamson, S. L. 2006. Developmental changes in hemodynamics of uterine artery, utero-and umbilicoplacental, and vitelline circulations in mouse throughout gestation. *American Journal of Physiology-Heart and Circulatory Physiology,* 291**,** H1421-H1428.
Mulvany, M. J. & Halpern, W. 1977. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circulation research,* 41**,** 19-26.
Naderali, E. K., Doyle, P. J. & Williams, G. 2000. Resveratrol induces vasorelaxation of mesenteric and uterine arteries from female guinea-pigs. *Clinical Science,* 98**,** 537-543.
Nelson, S. H., Steinsland, O. S., Wang, Y., Yallampalli, C., Dong, Y. L. & Sanchez, J. M. 2000. Increased nitric oxide synthase activity and expression in the human uterine artery during pregnancy. *Circulation research,* 87**,** 406-411.
Neri, I., Mazza, V., Galassi, M. C., Volpe, A. & Facchinetti, F. 1996. Effects of L-arginine on utero-placental circulation in growth-retarded fetuses. *Acta obstetricia et gynecologica Scandinavica,* 75**,** 208-212.
Ness, R. B. & Sibai, B. M. 2006. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *American journal of obstetrics and gynecology,* 195**,** 40.
Ni, Y., Meyer, M. & Osol, G. 1997. Gestation increases nitric oxide–mediated vasodilation in rat uterine arteries. *American journal of obstetrics and gynecology,* 176**,** 856-864.

Nihei, T., Miura, Y. & Yagasaki, K. 2001. Inhibitory effect of resveratrol on proteinuria, hypoalbuminemia and hyperlipidemia in nephritic rats. *Life sciences,* 68**,** 2845-2852.

North, R., Ferrier, C., Gamble, G., Fairley, K. & Kincaid‐Smith, P. 1995. Prevention of preeclampsia with heparin and antiplatelet drugs in women with renal disease. *Australian and New Zealand journal of obstetrics and gynaecology,* 35**,** 357-362.

Novakovic, A., Bukarica, L. G., Kanjuh, V. & Heinle, H. 2006. Potassium Channels‐Mediated Vasorelaxation of Rat Aorta Induced by Resveratrol. *Basic & clinical pharmacology & toxicology,* 99**,** 360-364.

Nylund, L., Lunell, N. O., Lewander, R. & Sarby, B. 1983. Uteroplacental blood flow index in intrauterine growth retardation of fetal or maternal origin. *BJOG: An International Journal of Obstetrics & Gynaecology,* 90**,** 16-20.

O'day, M. P. Year. Cardio-respiratory physiological adaptation of pregnancy. *In,* 1997. *Elsevier,* 268-275.

Ott, W. J. 2000. Intrauterine growth restriction and Doppler ultrasonography. *Journal of ultrasound in medicine,* 19**,** 661-665.

Owens, J., Owens, P. & Robinson, J. 1989. Experimental fetal growth retardation: metabolic and endocrine aspects. *Advances in Fetal physiology***,** 263-286.

Ozkor, M. A., Murrow, J. R. & Quyyumi, A. A. 2010. Endothelium: Dysfunction and Repair. *Advances in Vascular Medicine***,** 187-210.

Palmer, R., Rees, D. D., Ashton, D. S. & Moncada, S. 1988. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochemical and biophysical research communications,* 153**,** 1251.

Papageorghiou, A., Yu, C., Cicero, S., Bower, S. & Nicolaides, K. 2002. Second-trimester uterine artery Doppler screening in unselected populations: a review. *Journal of Maternal-Fetal and Neonatal Medicine,* 12**,** 78-88.

Patel, K. R., Scott, E., Brown, V. A., Gescher, A. J., Steward, W. P. & Brown, K. 2011. Clinical trials of resveratrol. *Annals of the New York Academy of Sciences,* 1215**,** 161-169.

Pijnenborg, R., Anthony, J., Davey, D. A., Rees, A., Tiltman, A., Vercruyssel, L. & Assche, A. 1991. Placental bed spiral arteries in the hypertensive disorders of pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology,* 98**,** 648-655.

Pijnenborg, R., Dixon, G., Robertson, W. & Brosens, I. 1980. Trophoblastic invasion of human decidua from 8 to 18 weeks of pregnancy. *Placenta,* 1**,** 3-19.

Pijnenborg, R., Vercruysse, L. & Hanssens, M. 2006. The uterine spiral arteries in human pregnancy: facts and controversies. *Placenta,* 27**,** 939-958.

Pirani, B., Campbell, D. & Macgillivray, I. 1973. Plasma volume in normal first pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology,* 80**,** 884-887.

Poston, L., Mccarthy, A. & Ritter, J. 1995. Control of vascular resistance in the maternal and feto-placental arterial beds. *Pharmacology & therapeutics,* 65**,** 215-239.

Poston, L. & Williams, D. 2002. Vascular function in normal pregnancy and pre-eclampsia. *An Introduction to Vascular Biology: From Basic Science to Clinical Practice***,** 398.

Powell, C. M. 2008. What's Wrong With My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice. *Archives of Neurology,* 65**,** 153.

Ralevic, V. & Burnstock, G. 2012. Mesenteric arterial function in the rat in pregnancy: role of sympathetic and sensory‐motor perivascular nerves, endothelium, smooth muscle, nitric oxide and prostaglandins. *British journal of pharmacology,* 117**,** 1463-1470.

Rasmussen, S. & Irgens, L. M. 2006. The effects of smoking and hypertensive disorders on fetal growth. *BMC pregnancy and childbirth,* 6**,** 16.

Redman, C. W. & Sargent, I. L. 2005. Latest advances in understanding preeclampsia. *Science Signalling,* 308**,** 1592.

Resnik, R. 2002. Intrauterine growth restriction. *Obstetrics & Gynecology,* 99**,** 490-496.

Rivera, L., Morón, R., Zarzuelo, A. & Galisteo, M. 2009. Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochemical pharmacology,* 77**,** 1053-1063.

Roberts, J. & Cooper, D. 2001. Pathogenesis and genetics of pre-eclampsia. *The Lancet,* 357**,** 53-56.

Roberts, J. M. & Hubel, C. A. 2009. The two stage model of preeclampsia: variations on the theme. *Placenta,* 30**,** 32-37.

Roberts, J. M., Pearson, G., Cutler, J. & Lindheimer, M. 2003. Summary of the NHLBI working group on research on hypertension during pregnancy. *Hypertension,* 41**,** 437-445.

Roccella, E. 2000. Report of the national high blood pressure education program working group on high blood pressure in pregnancy. *American journal of obstetrics and gynecology,* 183**,** S1-S22.

Rosenberg, A. Year. The IUGR newborn. *In*, 2008. Elsevier, 219-224.

Rosenfeld, C., Morriss Jr, F., Makowski, E., Meschia, G. & Battaglia, F. 1974. Circulatory changes in the reproductive tissues of ewes during pregnancy. *Gynecologic and obstetric investigation,* 5**,** 252-268.

Roten, L., Fenstad, M., Forsmo, S., Johnson, M., Moses, E., Austgulen, R. & Skorpen, F. 2011. A low COMT activity haplotype is associated with recurrent preeclampsia in a Norwegian population cohort (HUNT2). *Molecular human reproduction,* 17**,** 439-446.

Rubanyi, G. & Vanhoutte, P. 1987. Nature of endothelium-derived relaxing factor: are there two relaxing mediators? *Circulation research,* 61**,** II61.

Sand, A., Andersson, E. & Fried, G. 2002. Effects of nitric oxide donors and inhibitors of nitric oxide signalling on endothelin‐and serotonin‐induced contractions in human placental arteries. *Acta physiologica scandinavica,* 174**,** 217-223.

Sata, F., Yamada, H., Suzuki, K., Saijo, Y., Yamada, T., Minakami, H. & Kishi, R. 2006. Functional maternal catechol-O-methyltransferase polymorphism and fetal growth restriction. *Pharmacogenetics and genomics,* 16**,** 775-781.

Say, L., Gülmezoglu, A. & Hofmeyr, G. 2001. Betamimetics for suspected impaired fetal growth. *Cochrane Database of Systematic Reviews*.
Say, L., Gülmezoglu, A. & Hofmeyr, G. 2003. Hormones for suspected impaired fetal growth. *Cochrane Database Syst Rev*, 1.
Schiff, E., Ben‐Baruch, G., Galron, R., Mashiach, S. & Sokolovsky, M. 1993. Endothelin‐1 receptors in the human myometrium: evidence for different binding properties in post‐menopausal as compared to premenopausal and pregnant women. *Clinical endocrinology,* 38**,** 321-324.
Schutte, J. M., Steegers, E. A., Schuitemaker, N. W., Santema, J. G., De Boer, K., Pel, M., Vermeulen, G., Visser, W. & Van Roosmalen, J. 2010. Rise in maternal mortality in the Netherlands. *BJOG,* 117**,** 399-406.
Seligman, S. P., Buyon, J. P., Clancy, R. M., Young, B. K. & Abramson, S. B. 1994. The role of nitric oxide in the pathogenesis of preeclampsia. *American journal of obstetrics and gynecology,* 171**,** 944.
Shaamash, A., Elsnosy, E., Makhlouf, A., Zakhari, M., Ibrahim, O. & El-Dien, H. 2000. Maternal and fetal serum nitric oxide (NO) concentrations in normal pregnancy, pre-eclampsia and eclampsia. *International Journal of Gynecology & Obstetrics,* 68**,** 207-214.
Shesely, E. G., Gilbert, C., Granderson, G., Carretero, C. D., Carretero, O. A. & Beierwaltes, W. H. 2001. Nitric oxide synthase gene knockout mice do not become hypertensive during pregnancy. *American journal of obstetrics and gynecology,* 185**,** 1198-1203.
Sibai, B. M. 1998. Prevention of preeclampsia: a big disappointment. *American journal of obstetrics and gynecology,* 179**,** 1275-1278.
Sibai, B. M. 2004. Magnesium sulfate prophylaxis in preeclampsia: lessons learned from recent trials. *American journal of obstetrics and gynecology,* 190**,** 1520-1526.
Sibai, B. M. Year. Preeclampsia as a cause of preterm and late preterm (near-term) births. *In*, 2006. *Elsevier*, 16-19.
Sibai, B. M., Gordon, T., Thom, E., Caritis, S. N., Klebanoff, M., Mcnellis, D. & Paul, R. H. 1995. Risk factors for preeclampsia in healthy nulliparous women: A prospective multicenter study* 1. *American journal of obstetrics and gynecology,* 172**,** 642-648.
Singer, H. A. & Peach, M. J. 1982. Calcium-and endothelial-mediated vascular smooth muscle relaxation in rabbit aorta. *Hypertension,* 4**,** 19-25.
Singh, C. K., Kumar, A., Hitchcock, D. B., Fan, D., Goodwin, R., Lavoie, H. A., Nagarkatti, P., Dipette, D. J. & Singh, U. S. 2011. Resveratrol prevents embryonic oxidative stress and apoptosis associated with diabetic embryopathy and improves glucose and lipid profile of diabetic dam. *Molecular nutrition & food research,* 55**,** 1186-1196.
Stanley, J. L., Andersson, I. J., Hirt, C. J., Moore, L., Dilworth, M. R., Chade, A. R., Sibley, C. P., Davidge, S. T. & Baker, P. N. 2012a. Effect of the Anti-Oxidant Tempol on Fetal Growth in a Mouse Model of Fetal Growth Restriction. *Biology of reproduction,* 87**,** 25.
Stanley, J. L., Andersson, I. J., Poudel, R., Rueda-Clausen, C. F., Sibley, C. P., Davidge, S. T. & Baker, P. N. 2012b. Sildenafil Citrate Rescues Fetal

Growth in the Catechol-O-Methyl Transferase Knockout Mouse Model. *Hypertension,* 59**,** 1021-1028.
Stanley, J. L., Zhang, Y., Crocker, I. & Baker, P. N. 2010. The effect of 2-methoxyestradiol on uterine artery function. *Reproductive Sciences,* 17**,** 312A.
Surks, H. K., Mochizuki, N., Kasai, Y., Georgescu, S. P., Tang, K. M., Ito, M., Lincoln, T. M. & Mendelsohn, M. E. 1999. Regulation of myosin phosphatase by a specific interaction with cGMP-dependent protein kinase Iα. *Science,* 286**,** 1583-1587.
Takahashi, K., Kobayashi, T. & Kanayama, N. 2000. p57Kip2 regulates the proper development of labyrinthine and spongiotrophoblasts. *Molecular human reproduction,* 6**,** 1019.
Takata, M., Nakatsuka, M. & Kudo, T. 2002. Differential blood flow in uterine, ophthalmic, and brachial arteries of preeclamptic women. *Obstetrics & Gynecology,* 100**,** 931-939.
Takimoto, E., Ishida, J., Sugiyama, F., Horiguchi, H., Murakami, K. & Fukamizu, A. 1996. Hypertension induced in pregnant mice by placental renin and maternal angiotensinogen. *Science,* 274**,** 995.
Thornton, J., Hornbuckle, J., Vail, A., Spiegelhalter, D. & Levene, M. 2004. Infant wellbeing at 2 years of age in the Growth Restriction Intervention Trial (GRIT): multicentred randomised controlled trial. *Lancet,* 364**,** 513.
Thornton, J. & Onwude, J. 1992. Convulsions in pregnancy in related gorillas. *American journal of obstetrics and gynecology,* 167**,** 240.
Trudinger, B. J., Giles, W. B. & Cook, C. M. 1985. Uteroplacental blood flow velocity‐time waveforms in normal and complicated pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology,* 92**,** 39-45.
Vadillo-Ortega, F., Perichart-Perera, O., Espino, S., Avila-Vergara, M. A., Ibarra, I., Ahued, R., Godines, M., Parry, S., Macones, G. & Strauss, J. F. 2011. Effect of supplementation during pregnancy with L-arginine and antioxidant vitamins in medical food on pre-eclampsia in high risk population: randomised controlled trial. *BMJ: British Medical Journal,* 342.
Van Asselt, K., Gudmundsson, S., Lindqvist, P. & Marsal, K. 1998. Uterine and umbilical artery velocimetry in pre-eclampsia. *Acta obstetricia et gynecologica Scandinavica,* 77**,** 614-614.
Van Der Heijden, O. W., Essers, Y. P., Fazzi, G., Peeters, L. L., De Mey, J. G. & Van Eys, G. J. 2005a. Uterine artery remodeling and reproductive performance are impaired in endothelial nitric oxide synthase-deficient mice. *Biol Reprod,* 72**,** 1161-8.
Van Der Heijden, O. W. H., Essers, Y. P. G., Fazzi, G., Peeters, L. L. H., De Mey, J. G. R. & Van Eys, G. J. J. M. 2005b. Uterine artery remodeling and reproductive performance are impaired in endothelial nitric oxide synthase-deficient mice. *Biology of reproduction,* 72**,** 1161-1168.
Venuto, R. C. & Lindheimer, M. D. 2009. Animal models. *Chesley's Hypertensive Disorders in Pregnancy***,** 171-190.

Verkeste, C., Slangen, B., Daemen, M., Van Straaten, H., Kohnen, G., Kaufmann, P. & Peeters, L. 1998. The extent of trophoblast invasion in the preplacental vasculature of the guinea-pig. *Placenta,* 19**,** 49-54.

Wagner, L. K. 2004. Diagnosis and management of preeclampsia. *Am Fam Physician,* 70**,** 2317-24.

Walle, T. 2011. Bioavailability of resveratrol. *Annals of the New York Academy of Sciences,* 1215**,** 9-15.

Walle, T., Hsieh, F., Delegge, M. H., Oatis, J. E. & Walle, U. K. 2004. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metabolism and Disposition,* 32**,** 1377-1382.

Wang, Z., Xu, W., Zhang, C., Xie, W., Zhou, Z., Zhou, X. & Zhang, Z. 2011. Resveratrol improves coronary collateral circulation in pigs with experimental acute coronary occlusion]. *Zhonghua xin xue guan bing za zhi,* 39**,** 946.

Ward, K., Hata, A., Jeunemaitre, X., Helin, C., Nelson, L., Namikawa, C., Farrington, P., Ogasawara, M., Suzumori, K. & Tomoda, S. 1993. A molecular variant of angiotensinogen associated with preeclampsia. *Nature Genetics,* 4**,** 59-61.

Wareing, M., Myers, J. E., O'hara, M. & Baker, P. N. 2005. Sildenafil citrate (Viagra) enhances vasodilatation in fetal growth restriction. *Journal of Clinical Endocrinology & Metabolism,* 90**,** 2550-2555.

Watkins-Chow, D. E. & Pavan, W. J. 2008. Genomic copy number and expression variation within the C57BL/6J inbred mouse strain. *Genome research,* 18**,** 60-66.

Weiner, C., Liu, K., Thompson, L., Herrig, J. & Chestnut, D. 1991. Effect of pregnancy on endothelium and smooth muscle: their role in reduced adrenergic sensitivity. *American Journal of Physiology-Heart and Circulatory Physiology,* 261**,** H1275-H1283.

Weiner, C., Martinez, E., Chestnut, D. & Ghodsi, A. 1989. Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium. *American journal of obstetrics and gynecology,* 161**,** 1605.

Wenzel, E. & Somoza, V. 2005. Metabolism and bioavailability of trans‐resveratrol. *Molecular nutrition & food research,* 49**,** 472-481.

Whitesall, S. E., Hoff, J. B., Vollmer, A. P. & D'alecy, L. G. 2004. Comparison of simultaneous measurement of mouse systolic arterial blood pressure by radiotelemetry and tail-cuff methods. *American Journal of Physiology-Heart and Circulatory Physiology,* 286**,** H2408-H2415.

Williams, L. D., Burdock, G. A., Edwards, J. A., Beck, M. & Bausch, J. 2009. Safety studies conducted on high-purity trans-resveratrol in experimental animals. *Food and Chemical Toxicology,* 47**,** 2170-2182.

Winquist, R. J., Bunting, P. B. & Schofield, T. 1985. Blockade of endothelium-dependent relaxation by the amiloride analog dichlorobenzamil: possible role of Na+/Ca++ exchange in the release of endothelium-derived relaxant factor. *Journal of Pharmacology and Experimental Therapeutics,* 235**,** 644-650.

Wolin, M., Davidson, C., Kaminski, P., Fayngersh, R. & Mohazzab-H, K. 1998. Oxidant--nitric oxide signalling mechanisms in vascular tissue. *Biochemistry. Biokhimiia,* 63**,** 810.

Xiao, D., Pearce, W. J. & Zhang, L. 2001. Pregnancy enhances endothelium-dependent relaxation of ovine uterine artery: role of NO and intracellular Ca2+. *American Journal of Physiology-Heart and Circulatory Physiology,* 281**,** H183-H190.

Xiong, X., Mayes, D., Demianczuk, N., Olson, D. M., Davidge, S. T., Newburn-Cook, C. & Saunders, L. D. 1999. Impact of pregnancy-induced hypertension on fetal growth. *American journal of obstetrics and gynecology,* 180**,** 207-213.

Yallampalli, C. & Garfield, R. E. 1993. Inhibition of nitric oxide synthesis in rats during pregnancy produces signs similar to those of preeclampsia. *American journal of obstetrics and gynecology,* 169**,** 1316.

Yan, P., Zhu, X., Li, H., Shrubsole, M. J., Shi, H., Zhang, M., Harris, R. C., Hao, C. M. & Dai, Q. 2012. Association of High Blood Pressure with Renal Insufficiency: Role of Albuminuria, from NHANES, 1999–2006. *PloS one,* 7**,** e37837.

Yang, Z. Z., Tschopp, O., Hemmings-Mieszczak, M., Feng, J., Brodbeck, D., Perentes, E. & Hemmings, B. A. 2003. Protein kinase Bα/Akt1 regulates placental development and fetal growth. *Journal of Biological Chemistry,* 278**,** 32124-32131.

Yannopoulos, D. & Nadkarni, V. M. 2012. Regional Circulations. *Pediatric Critical Care Study Guide***,** 65-93.

Yung, H., Korolchuk, S., Tolkovsky, A. M., Charnock-Jones, D. S. & Burton, G. J. 2007. Endoplasmic reticulum stress exacerbates ischemia-reperfusion-induced apoptosis through attenuation of Akt protein synthesis in human choriocarcinoma cells. *The FASEB journal,* 21**,** 872-884.

Zhou, C. C., Irani, R. A., Dai, Y., Blackwell, S. C., Hicks, M. J., Ramin, S. M., Kellems, R. E. & Xia, Y. 2011. Autoantibody-Mediated IL-6ñDependent Endothelin-1 Elevation Underlies Pathogenesis in a Mouse Model of Preeclampsia. *The Journal of Immunology,* 186**,** 6024.