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PRE-ECLAMPSIA—STILL A DISEASE OF THEORIES

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Abstract: Pre-eclampsia is still one of the leading causes of maternal and fetal morbidity and mortality. Despite active research for many decades, the etiology of this disorder exclusive to human pregnancy is an enigma. Recent evidence suggests there may be several underlying causes or predispositions leading to endothelial dysfunction and causing the signs of hypertension, proteinuria, and edema—findings that allow us to make the diagnosis of the "syndrome" of pre-eclampsia. It is obvious that a single mechanism responsible for the syndrome pre-eclampsia does not exist. Instead, several mechanisms can act together and even multiply each other. The search for the underlying cause of this disorder and for a clinical marker to predict which women will develop pre-eclampsia is ongoing, with its prevention being the ultimate goal.

Key words: Pre-eclampsia, vascular factors, oxidative stress, genetics, angiogenesis

INTRODUCTION

Hypertensive disorders in pregnancy constitute a major risk factor for maternal mortality as well as fetal wastage and morbidity in the United States and in countries worldwide. They are the second leading cause of maternal mortality in the United States, representing almost 15% of pregnancy-related deaths and occurring in 3%-10% of pregnancies[1,2]. This is especially true in underdeveloped nations. About 20% of perinatal mortality and morbidity are related to hypertensive disorders in pregnancy[3].

Classifications of hypertensive disorders in pregnancy have varied in the past and led to some confusion in both the clinical management and research efforts

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toward the etiology of these disorders[4]. Currently, a classification established by the National Institutes of Health Working Group on High Blood Pressure in Pregnancy[2] is used in the United States and recommended by the International Society for the Study of Hypertension in Pregnancy:

1) *Chronic hypertension*:
   Hypertension present before pregnancy or first diagnosed before 20 weeks' gestation
2) *Preeclampsia-eclampsia*:
   Hypertension unique to pregnancy (blood pressure>140 mmHg systolic or 90 mmHg diastolic)
   Diagnosed after 20 weeks' gestation
   Associated by new onset proteinuria (≥0.3 g/24 h)
   Eclampsia, if seizures occur
3) *Pre-eclampsia superimposed upon chronic or preexisting hypertension*:
   New onset or acutely worse proteinuria, a sudden increase in blood pressure, thrombocytopenia, or elevated liver enzymes after 20 weeks' gestation in a woman with preexisting hypertension
4) *Gestational hypertension*:
   Hypertension first diagnosed after 20 weeks' gestation, not accompanied by proteinuria
   a) *Transient hypertension*
      The hypertension resolves by 12 weeks' postpartum
   b) *Chronic hypertension*
      The hypertension does not resolve by 12 weeks' postpartum.

HISTORY

Hippocrates first described the condition when he wrote in one of his aphorisms "*convulsions take place from either repletion or depletion*". Hippocrates had observed the sudden and unexpected appearance of maternal grand-mal seizures, which occur when preeclampsia progresses to eclampsia, the word being derived from the Greek word for *lightning*.

Presumably eclampsia was first confused with epilepsy and not described as a separate entity until 1739. The use of the term has been attributed to Gutsch in 1776, but this has not been well documented. Nonetheless, it was many years more before it was universally accepted as separate from epilepsy or hysteria.

Even so, it was not until about 150 years ago, when protein could be measured in the urine and by the introduction of blood pressure measurements at the turn of the 20th century, that the forerunner to eclampsia became apparent.

The triad of hypertension, proteinuria, and edema was termed preeclampsia. Because of the toxins that were believed to be in the pregnant woman's body, this disorder was also commonly called "toxemia of pregnancy," a term coined at least

150 years ago, but not currently used in today's nomenclature. Pre-eclampsia is now unanimously viewed as a multisystem disorder, as increases in blood pressure are rarely responsible for multi-organ dysfunction[5].

There is a vast diversity of additional symptoms and complications associated with pre-eclampsia. These can include cerebral edema[6], neurological manifestations (including headache, confusion, paralysis, coma, visual loss, and seizures)[7], liver capsule distension[8], renal failure[9,10], pulmonary edema[10,11], thrombocytopenia[12], coagulopathy[13], hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome[14], and nausea[15].

EPIDEMIOLOGY

Pre-eclampsia is a pregnancy-specific syndrome that is the principal cause of maternal and fetal morbidity and mortality[2,16], accounting for almost 15% of pregnancy-associated maternal deaths[2].

In the United States, from the years 1979 to 1986, pre-eclampsia was the second leading cause of maternal death[1] and ranks between the second and third leading cause of maternal death in more recent years[16].

Risk and predisposing factors :

Numerous maternal factors can predispose women to the disorder pre-eclampsia ; these may be genetic, behavioral, or environmental. The list of predisposing factors includes, besides the history of a previous pre-eclampsia, hypertension, diabetes, increased insulin resistance, increased testosterone, black race, and increased blood homocysteine concentration (Table 1).

In industrialized countries, pre-eclampsia complicates 3%-5% of pregnancies[17] and is more likely to occur at both extremes of reproductive age, but is greatest in women younger than 20 years of age[1].

Primigravid women are at higher risk for pre-eclampsia[18]. The incidence of pre-eclampsia in multiparous women is lower than in primiparous women, but higher if the multiparous woman has a different partner[19,20]. This finding supports the hypothesis that risk is reduced with repeated exposures to specific antigens from the same partner. However, the protective effect against pre-eclampsia of a previous pregnancy with the same partner was likely confounded by the time interval between births. Skjaerven *et al.*[21] showed that the risk of pre-eclampsia in subsequent pregnancies was related to the time that had elapsed since the index pregnancy, not to a change of partners. When the birth interval was greater than 10 years, the risk of the multiparous woman was identical to that of a primiparous woman.

Also, recent information suggests that a short interval of sexual cohabitation before conception is associated with an increased risk of pre-eclampsia[22]. Women with longer durations of sexual cohabitation before conception may be exposed to

Table 1. Risk factors for pre-eclampsia

Preconceptional and/or chronic risk factors	
Partner-related risk factors :	
Nulliparity	Limited sperm exposure
Primipaternity	Donor insemination
Teenage pregnancy	Partner who fathered a pre-eclamptic pregnancy in another woman
Maternal-specific risk factors :	
Previous pre-eclampsia	Interval between pregnancies
Family history	Patient requiring oocyte donation
Increasing maternal age	
Presence of specific underlying disorders :	
Chronic hypertension	Low maternal birth weight
Renal disease	APC resistance (factor V Leiden)
Obesity	Protein S deficiency
Insulin resistance	Antiphospholipid antibodies
Gestational diabetes	Hyperhomocysteinaemia
Diabetes mellitus Type-1	
Exogenous factors :	
Smoking (risk decrease)	Structural congenital anomalies
Stress	Hydrops fetalis (hydropic placenta)
Urinary tract infection	Hydatiform moles
Pregnancy-associated risk factors	Chromosomal anomalies (trisomy 13, triploidy)
Multiple pregnancy	

paternal antigens and presumably become more tolerant.

Pre-eclampsia is more likely to occur in women with underlying hypertension or other chronic illnesses such as autoimmune disease, renal disease, and diabetes. The risk of superimposed pre-eclampsia upon already existent hypertension is approximately 25%[23]. Women with a strong family history of hypertension are also more susceptible to this syndrome[24].

Additionally, women with thrombophilias, both inherited and acquired, may be more likely to develop pre-eclampsia[25-28]. An association with pre-eclampsia has been suggested for women who have antiphospholipid syndrome[25,29,30], factor V Leiden mutation[25-27,32] (whereby this mutation especially seems to be associated with hemolysis, elevated liver enzymes, low platelets [HELLP] syndrome[33-35]) activated protein C resistance[25,26,36], and hyperhomocysteinemia[26,37].

Women who are carriers of certain other inherited metabolic disorders, aside from those that predispose to thrombophilia, also appear to be more likely to develop pre-eclampsia. Specifically, women who are heterozygous carriers for beta-oxidation disorders appear to be at a higher risk for pre-eclampsia as well as other complications of pregnancy[38]. Mothers with long chain 3-hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency not only have a higher risk of pre-

eclampsia, and acute fatty liver of pregnancy, but also HELLP syndrome, as well as intrahepatic cholestasis and hyperemesis gravidarum[38,39].

Other lipid abnormalities may be associated with the development of pre-eclampsia. Wetzka *et al.*[40] found higher levels of triglycerides and lipoproteins in women with severe pre-eclampsia with or without HELLP syndrome.

Interestingly, these are also risk factors for other endothelial diseases, particularly atherosclerosis, and the late complications of diabetes mellitus[41]. Pre-eclampsia, atherosclerosis, and diabetes also share a common dyslipidemia. Increased triglycerides, decreased high density lipoproteins (HDL), and an increased concentration of small dense low density lipoproteins (LDL) are characteristic of these disorders. In addition, although Chesley *et al.*[42] showed that pre-eclampsia does not cause cardiovascular disease, the work of Fisher *et al.*[43] indicates that pre-eclamptic women have a higher risk of cardiovascular disease in later life. This finding supports common risk factors for pre-eclampsia and atherosclerosis, with normal pregnancy being a screening test indicating the absence of these factors.

Pre-eclampsia occurs only when placental tissue is present, and more often with an excess of placental tissue, even without the presence of a fetus. Women with multiple gestations (multiple placentas), i.e., twins and triplets, are more likely to develop pre-eclampsia[44], than women with a partial or complete molar pregnancy[45]. Interestingly, women with hydropic or extremely edematous fetuses (and hydropic, edematous placentas) may also show signs and symptoms of pre-eclampsia[46] that have actually been noted to resolve before delivery if the fetal hydrops resolves[47].

ETIOLOGY/PATHOPHYSIOLOGY

Pre-eclampsia, a life-threatening disease unique to pregnancy has been called a disease of theories. Even today, the etiology of pre-eclampsia is unknown, widely speculated about, and studied. A completely satisfactory, unifying hypothesis has not emerged. It is likely that there may be several etiologies or underlying predispositions with effects that result in the common group of signs and symptoms we can find with the syndrome pre-eclampsia.

Vasospasm or increased vascular reactivity and endothelial cell dysfunction may be the final common pathway of several different pathophysiologic mechanisms[48]. Nonetheless, the inciting organ in the syndrome of pre-eclampsia is the placenta[49]. The major underlying mechanism of pre-eclampsia is inadequate placentation with resultant placental ischemia[50]. According to this thesis, abnormal cytotrophoblast invasion of the spiral arteries of the uterus leads to failure of remodeling of these vascular channels into more spacious, lower resistance vessels. As a result, uteroplacental blood flow is compromised[51].

The characteristic pathologic finding in the pre-eclamptic placenta results from shallow or "inadequate" interstitial invasion by the cytotrophoblastic cells and limited endovascular invasion[52]. Thus, the trophoblast fails to develop into vascu-

lar cells, as they do in normal pregnancy. In consequence, the spiral arteries of the uterus remain small and narrow, with high resistance to flow, resulting in a failure of the uterine blood supply to adequately nourish the placenta[52,53]. These vessels, in pre-eclamptic patients, are estimated to be only about 40% of the diameter of those in normal pregnancy. This leads to placental hypoxia resulting in the villi of pre-eclamptic patients demonstrating abnormalities associated with growth in an environment of low oxygen tension.

In the past, several pathophysiologic mechanisms have been proposed suggesting that pre-eclampsia has multifactorial origins (Fig. 1):

1) An imbalance of vasodilative and vasoconstrictive substances[54-56] resulting in peripheral vasoconstriction, which causes the reduced organ perfusion[3,57]
2) Oxidative stress caused by an increased production of free radicals or by a deficiency of protective antioxidative substances[58-60]
3) Immunologic defects such as antiphospholipid syndrome[29,30] or angiotensin-1 receptor antibody[61,62]
4) An excessive maternal inflammatory reaction to pregnancy[59,63-65]
5) Coagulopathies and thrombophilias[25-28,31,32,36]
6) Genetic mutations may play an important role in the pathogenesis of pre-eclampsia[66,67]
7) Alterations in angiogenesis due to hypoxia and/or alterations in levels of angiogenic factors leading to inadequate placentation and immature vessel formation[49,68-80]

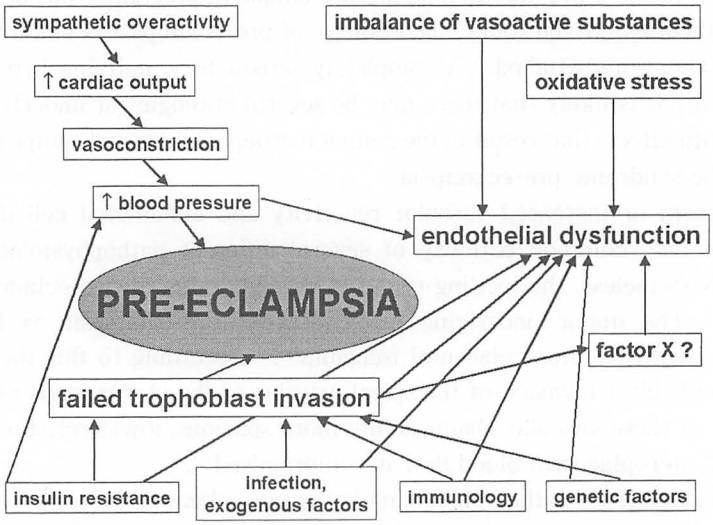

Fig. 1. Etiologic and pathophysiologic mechanisms in the development of pre-eclampsia.

VASCULAR FACTORS

During pregnancy, extensive haemostatic changes occur in the utero-placental circulation and, in pregnancies complicated by pre-eclampsia, a restricted physiological adaptation of the utero-placental blood vessels leads to increased vascular resistance and reduced blood flow. Nearly every vasoactive substance (which additionally may interact with each other) has been investigated with respect to its possible involvement in the pathogenesis of pre-eclampsia (Fig. 2). This categorization includes the prostaglandins, the renin-angiotensin-aldosterone axis, nitric oxide (NO), atrial natriuretic peptide (ANP), endothelin, adrenomedullin, and vasopressin (Table 2).

Nitric Oxide :

In the last years, extensive study of the nitric oxide (NO) system has been performed. Both, animal experiments and studies in pregnant women, have strongly suggested an important role for the nitric oxide synthase (NOS) system in pre-eclampsia. NO is a small, molecular weight mediator with diverse functions that include vasodilation, inhibition of platelet aggregation, and vascular remodeling.

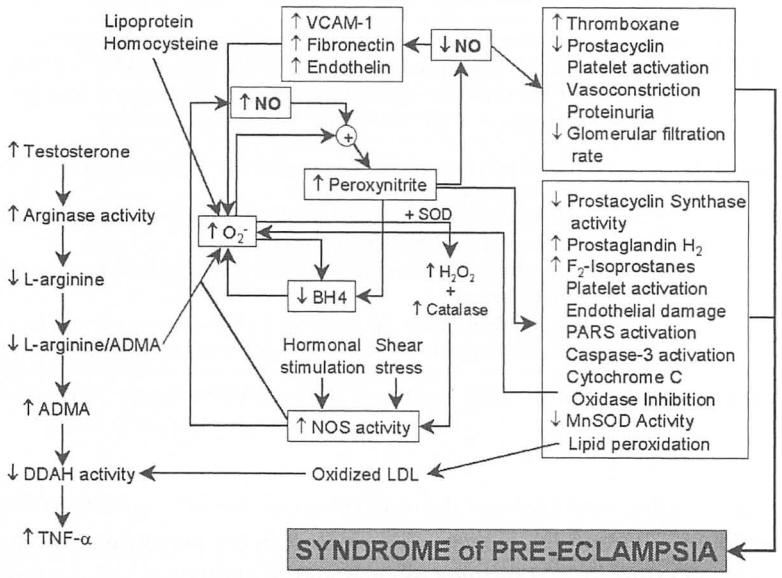

Fig. 2. Interactions involved in the pathophysiology and leading to the clinical syndrome of pre-eclampsia. Abbreviations: ADMA=asymmetrical dimethylarginine; BH4=tetrahydrobiopterin; DDAH=dimethylarginine dimethylaminohydrolase; H_2O_2=hydrogen peroxide; LDL=low-density lipoproteins; (Mn) SOD=(manganese) superoxide dismutase; NO=nitric oxide; NOS=nitric oxide synthase; O_2^-=superoxide anion; PARS=poly(ADP-ribose) synthetase; TNF-a=tumor necrosis factor alpha; VCAM-1=vascular cell adhesion molecule-1

Table 2. Circulating vasoactive substances in pregnancy and pre-eclampsia

Vasoactive Substance (s)	Alterations in Pre-eclampsia
Nitric oxide (NO)	Rat: Chronic NOS inhibition results in renal and peripheral vasoconstriction, proteinuria, increased fetal morbidity, and intrauterine growth restriction. Human: Studies report variable results (NO production and NOS inhibition), perhaps related to different vascular beds examined. NO metabolite levels increase through normal and particularly abnormal pregnancy, predominantly in the fetal compartments, suggesting that NO production is an additional instrument in the fetal control of the intrauterine environment
Endothelin (ET)	Human: Most studies have shown an increase in ET in plasma and placental tissue of pre-eclamptic women. ET-1 induces oxidative stress and alters secretion of vasoactive substances in human endothelial cells.
Prostaglandins	Postulate: An imbalance $TXA_2 > PGI_2$, is pathogenetic. However, use of prostaglandin inhibitors (low-dose aspirin) only effective in high risk group
Renin-Angiotensin-Aldosterone system (RAAS)	Rat: Chronic RAAS blockade throughout pregnancy does not play an important role in the hypertensive response to chronic reductions in uterine perfusion pressure. Human: Plasma renin activity and angiotensin II are lower than normal in pre-eclampsia. Role in causation of pre-eclampsia remains unclear.
Atrial natriuretic peptide (ANP)	Controversy results about increased maternal ANP levels. Placental ANP production and levels of pro-ANP mRNA do not differ between pre-eclamptics and normal pregnant females. → Changes in ANP levels may be a secondary effect.
Adrenomedullin	Levels of adrenomedullin do not differ from those measured in normal pregnant women. Levels in amniotic fluid and umbilical vein plasma have been reported to be several-fold higher in pre-eclampsia. Lower adrenomedullin mRNA expression in placental villi from pre-eclamptic women. Adrenomedullin may be involved in the adaptation of the vascular system to pregnancy and in the regulation of placental vascular tone.

* Abbreviations: NOS=nitric oxide synthase; TXA_2=thromboxane; PGI_2=prostacyclin

NO, originally called endothelium-derived relaxing factor[81] results from the enzymatic action of NOS, which converts L-arginine, in the presence of oxygen, to L-citrulline and NO (Fig. 3). Molecular oxygen and NADPH are cosubstrates in this reaction. Three NOS enzymes have been sequenced: 1) the constitutive enzyme present in the vascular endothelium (eNOS or NOS-3), 2) neuronal cells (nNOS or NOS-1), and 3) several other cell types[82]. The other is an inducible enzyme (iNOS or NOS-2) that has been found in macrophages and neutrophils and is activated by bacterial endotoxin or cytokines (e.g., IL-1, interferon-g)[82]. Human placental

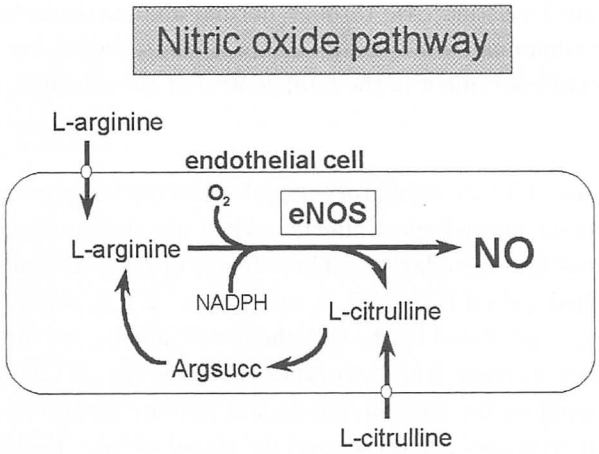

Fig. 3. Intracellular pathway of nitric oxide

syncytiotrophoblast is known to express eNOS but not iNOS. NO isoenzyme is also expressed on villous endothelial cells and NO produced from these cells is thought to be an important mediator for spiral artery transformation[83].

A series of studies in the rat have been performed[84,85] to examine the postulate that the pregnant animal responds to a chronic reduction in uteroplacental perfusion pressure with a reduction in renal NO synthesis. Evidence has accrued that indicates that NO synthesis is increased in normal pregnancy as are plasma and urine levels of cyclic guanosine 3′, 5′-cyclic monophosphate (cGMP), the second messenger of NO[86]. In the rat, it has been possible to show that chronic NOS inhibition results in renal and peripheral vasoconstriction, proteinuria, increased fetal morbidity, and intrauterine growth retardation[87,88]. Additionally, it has been reported that both eNOS and nNOS are upregulated in pregnant rats[89]. Furthermore, chronic inhibition of NOS reverses systemic vasodilation and glomerular hyperfiltration in the pregnant rat model[90]. Interestingly, chronic reduction in uterine perfusion pressure in the rat model is associated with no differences in whole body NO production and a decrease in the renal protein expression of neuronal NOS in pregnant animals compared with controls[91].

Studies performed in pregnant and pre-eclamptic women have unfortunately produced conflicting results. This may be explained in part by the difficulties in assessing the NOS system in clinical settings, but additionally by the different settings of these studies (different vessels, plasma/serum, amniotic fluid, fetal blood). NO production seems to be reduced using maternal serum[92,93], and unchanged or higher in maternal plasma and/or urine[94-96]. In amniotic fluid, NO levels are higher in pre-eclampsia compared with normal pregnancy[96]. In endothelial cell cultures and placental homogenates, NO production has been reported to be unchanged or higher in pre-eclampsia[97-99]. Recently a higher NO production has been reported in plasma from umbilical vein and artery[96].

NO metabolite levels increase through normal and particularly through abnormal pregnancy predominantly in the fetal compartments, suggesting that NO production is an additional instrument in the fetal control of the intrauterine environment.

Endothelins :

The endothelins (ET) are regulatory peptides, distributed in many organ systems and producing potent physiological effects. They are the most powerful vasoconstrictive substances known today[100]. Three forms of these 21-amino acid peptides have been described, called ET-1, ET-2, and ET-3. ET-1, the most important of the three peptides, is produced by the endothelium[101] and by smooth muscle cells[102].

These peptides interact with two types of receptors: ETA and ETB. ETA receptors are present on the smooth muscle and mediate contraction in response to ET-1. The ETB receptors are present on the endothelium. Both ET-1 and ET-3 are capable of inducing the release of NO and prostacyclin, thereby inducing vascular relaxation. Thus, ETB receptors are able to mediate both vasodilation and vasoconstriction[103]. ET-1 causes increased salt and water excretion, which represents a potentially hypotensive action, but in the vasculature it causes vasoconstriction[104]. The relevance of endothelin to the pathogenesis of pre-eclampsia remains unclear. Thus, most studies[56,104-108] have demonstrated an increase in endothelin in plasma or serum and in placental tissue[109] in pre-eclamptic women. Typically, ET-1 plasma levels are highest during the latter stage of the disease, suggesting that ET may not be involved in the initiation of pre-eclampsia, but rather in the progression of disease into a malignant phase[73]. Interestingly, immunoreactive endothelin levels have been reported to be higher in the plasma[110] and in the umbilical artery and vein blood of pregnant black women than in patients of European origin[111]. Therefore, this could contribute to the higher incidence of hypertension and pre-eclampsia noted in the former population compared with the latter[18]. Levels of ET-1 performed in early pregnancy have been reported to have low[112] or no predictive value[108,113] as to the later development of pre-eclampsia. Additionally, in experimental models of pregnancy hypertension, endothelin levels are often not elevated[104]. Yet its effect on blood pressure most likely is more accurately described by its local action at the endothelial and vascular level rather than its serum concentration. Therefore, the actions of endothelin in regulating blood pressure are no doubt correlated best with its effects as a paracrine or autocrine factor. Faxen *et al.*[114] noted no change in mRNA for ET-1 in myometrium or placenta of patients compared to normotensive pregnant patients. However, the expression of ETA-mRNA was significantly reduced in placenta, whereas that of ETB was unchanged. These data suggested that high circulating levels of ET-1 might have downregulated the ETA receptor. ET-1 levels have been determined by Singh *et al.*[109] to be significantly higher in placental tissues from women with pre-eclampsia than in normotensive pregnancies. Additionally, an ETA receptor antagonist has been reported to lower blood pressure in pregnant rats

in which hypertension was induced by chronic reductions in uterine blood flow[115]. Napolitano *et al.*[116] reported an increased ET-1 expression in cultured human placental trophoblastic cells obtained from pre-eclamptic pregnancies compared with those harvested from the placentas of patients with normal pregnancies. The expression of iNOS was decreased in their studies, whereas that of eNOS was increased. The authors postulated that interactions between the ET and NOS systems could represent an important pathogenetic mechanism in the development of the reduced uteroplacental blood flow associated with pre-eclampsia[116]. Additionally, we could show, that incubation of human umbilical vein endothelial cells (HUVEC) with serum from pre-eclamptic women results in increased ET-1 production[97], therefore suggesting, that serum from pre-eclamptic women contains a factor(s) that specifically stimulates ET-1 secretion. Finally, when incubating HUVEC's for 24 hours with ET-1 in different concentrations (0-1,000 pmol/L)[117], at lower concentrations (5-50 pmol/L), ET-1 increases the intracellular content of lipid peroxides (LPO), stimulates the secretion of thromboxane A_2 (TXA_2), but inhibits the secretion of prostacyclin (PGI_2). At higher concentrations (100-1,000 pmol/L), ET-1 increases the intracellular content of glutathione, but results in a decrease of LPO and an increase of PGI_2 back to control levels. ET-1 had no effect on NO secretion. Therefore ET-1 is able to induce oxidative stress and alter secretion of vasoactive substances in human endothelial cells. This observation supports the postulate that ET-1 is involved in the progression to a severe phase of the disease.

Prostaglandins :

The prostaglandins and thromboxane A_2 (TXA_2) are a series of biologically active compounds derived from arachidonic acid[118]. The former are vasodilatory, while the latter is a vasoconstrictor. The prostaglandins are considered to represent important mediators of the minute-to-minute tone of the vasculature acting to offset the vasoconstrictive influence of angiotensin II. The major vasodilator prostaglandin is prostacyclin (prostaglandin I_2 [PGI_2]). TXA_2 is the principal metabolite of arachidonic acid in platelets. This compound is only evanescently present in plasma (its half-life is approximately 30 seconds), so that its effects are largely a function of the microenvironment of its action. TXA_2 is an important contributor to platelet aggregation intravascularly, but also contracts the muscular layer of arteries. Prostacyclin, however, is an inhibitor of platelet aggregation and is also a major vasodilator.

Several lines of evidence suggest that changes in the prostaglandin system may play a role in mediating the renal dysfunction and increase in arterial pressure during pre-eclampsia[119]. Significant alterations in PGI_2 and TXA_2 production occur in women with pre-eclampsia[120]. Plasma and urine levels of TXA_2 are elevated in women with pre-eclampsia, whereas synthesis of prostaglandins, such as PGI_2, is reduced[120-122].

Additional evidence for a potential role of TXA_2 in pre-eclampsia derives from

a study demonstrating that short-term increases in systemic arterial pressure produced by acute reductions in uterine perfusion in pregnant dogs can be prevented by thromboxane receptor antagonism[123]. In addition, Wang and coworkers[122] reported that there is an abnormal increase of serum lipid peroxides in pre-eclamptic women. They postulated that these substances, which cause oxidative stress and thereby cellular damage, act by inhibiting prostaglandin synthase.

Further evidence of a potential role for TXA_2 is supported by studies in humans indicating that low-dose aspirin may attenuate the development of pre-eclampsia, but unfortunately, a meta-analysis revealed disappointing results[124]. This can be in part explained by the different settings of these studies. Recent evidence suggest that in women at high risk for the disease[125], low dose aspirin (100 mg/day) has a significant benefit, when started early in pregnancy (before week 16).

Renin-Angiotensin-Aldosterone System :

The renin-angiotensin-aldosterone system (RAAS) plays an important role in the long-term regulation of renal function and arterial pressure during a variety of physiological and pathophysiological conditions. It is a major contributor not only to the sodium and volume status of the intact organism, but also to the maintenance of the blood pressure[126]. Because of its importance in not only the day-to-day but also the minute-to-minute regulation of blood pressure, the RAAS in the pathogenesis of pre-eclampsia has been extensively investigated.

During normal pregnancy, plasma renin concentration, renin activity, angiotensin II (AT II) levels, and aldosterone are all elevated ; however, the vascular responsiveness to AT II appears to be reduced[9,127].

The importance of the RAAS in the regulation of renal function and arterial pressure during pre-eclampsia has not yet been fully elucidated. Plasma renin activity and AT II levels are usually lower than normal throughout pregnancy in patients with pre-eclampsia. Only in the third trimester does the aldosterone level increase in hypertensive pregnancy[127].

Although circulating levels of AT II may be normal during pre-eclampsia, it is possible that reducing uteroplacental perfusion pressure could increase the renal sensitivity to AT II through reductions in nitric oxide (NO) or prostacyclin synthesis or by enhanced formation of thromboxane.

This suggestion is confirmed by studies indicating enhanced vascular responsiveness to AT II in vessels from animals or humans with pre-eclampsia[120]. Furthermore, the pre-glomerular vessels of the renal circulation become extremely sensitive to the vasoconstrictor actions of AT II when the renal synthesis of NO or prostacyclin is reduced or when thromboxane synthesis is elevated[128]. Increased vascular AT II responsiveness during pre-eclampsia, however, does not prove AT II as an important endogenous mediator of the vasoconstriction or hypertension in experimental models of pre-eclampsia, because increased responsiveness may only reflect low endogenous AT II formation. Neither does the chronic blockade of the RAAS

system seem to play an important role in the hypertensive response to chronic reductions in uterine perfusion pressure in the rat[129]. In addition, the refractoriness to AT II is lost as early as the midtrimester in women who later develop pregnancy-induced hypertension[130].

Thus, while the RAAS seems to be responsible in a major way for regulating the cardiovascular adaptation that occurs in pregnancy, its role in the causation of pre-eclampsia remains unclear.

Atrial Natriuretic Peptide :

Atrial natriuretic peptide (ANP) has been originally discovered by de Bold *et al.*[131] as a natriuretic factor produced by the myocytes of the atrium. It is secreted from the atria following the splitting of the storage form, a 126-amino acid prohormone (pro-ANP), into an N-terminal moiety of 98 amino acids (N-ANP) and the biologically active hormone, in equimolar amounts[132]. ANP is diuretic, natriuretic, vasorelaxant and has antiproliferative properties[133]. It causes intravascular volume contraction, both because of its ability to induce natriuresis and diuresis, as well as because it causes a shift of fluid from the capillary bed to the interstitium[134], resulting in a reduction in both blood pressure and preload[135].

In normotensive pregnancy ANP levels trend downward as gestation proceeds from the eighth through the 32nd week, then increase significantly in the 36th week[136]. In hypertensive pregnant women, some investigators reported that ANP levels increase markedly in late pregnancy[136–138], but this increase is not a universal finding[139,140] and is counterintuitive given the plasma volume contraction seen in pre-eclamptic patients as term approaches[141]. These increments in plasma ANP may be secondary to some other factors, such as the release of increased amounts of angiotensin II (AT II), endothelin, or catecholamines, either into the circulation or locally in the affected tissues[142,143]. The enhanced ANP secretion could represent a defense against additional vasoconstriction and sodium retention in pre-eclamptic patients. ANP is also produced by a small population of human placental trophoblast-like cells[144], suggesting that ANP may be secreted locally or into the feto-placental circulation and that its effects occur as a result of paracrine or autocrine actions[144,145]. However, pro-ANP mRNA levels performed on placental tissue of pre-eclamptic patients do not differ from those of normal pregnant woman[141]. Therefore, production of ANP by the placenta is not altered at this pre-translational level in pre-eclampsia.

Adrenomedullin :

Adrenomedullin, a member of the calcitonin gene-related peptide family, is a 52-amino acid peptide, originally discovered in human pheochromocytoma tissue[146], that produces blood pressure reduction along with natriuresis and diuresis[147]. The latter effect is the result of an ability to increase glomerular filtration rate (GFR) as well as to inhibit distal tubular sodium reabsorption[147]. Its hypotensive action is

potent and long lasting[146].

Plasma levels of adrenomedullin progressively increase as pregnancy proceeds[138]. Whereas first trimester adrenomedullin levels did not differ from those of non-pregnant women in either the follicular or luteal phases of the menstrual cycle in studies performed by Minegishi *et al.*[138], third trimester plasma concentrations were significantly higher than first and second trimester levels. In contrast, Di Iorio *et al.*[148] could not find any significant difference of plasma adrenomedullin levels throughout gestation. Amniotic fluid adrenomedullin concentrations decreased after the first trimester (8-12 weeks of gestation) and were lowest at 13-20 weeks of gestation and then increased at 21-28 weeks of gestation. A further increase was found in samples collected after 37 weeks of gestation[148]. In the umbilical vein, adrenomedullin concentration was higher than in the umbilical artery, suggesting that adrenomedullin in the fetal circulation derives from the placenta[148]. These findings lead to the conclusion that adrenomedullin may have an important role in human reproduction, from implantation to delivery[148].

In pre-eclamptic patients, third trimester adrenomedullin levels did not differ significantly from those measured in pre-eclamptic patients at 28 to 40 weeks of pregnancy[138]. Additionally, Di Iorio and his co-workers[149] have reported that plasma levels of adrenomedullin from normotensive pregnant patients did not differ from those obtained from pre-eclamptic patients and Hata *et al.*[150] reported that mean levels of adrenomedullin in pre-eclamptic patients were lower than those obtained from normal pregnant women in the third trimester. Interestingly, levels in amniotic fluid and umbilical vein plasma were several-fold higher in pre-eclamptic patients than in normal pregnant women[149]. These data are supported by the findings that the umbilical veins in the intrauterine growth restriction (IUGR) group had significantly higher levels of growth restricted mean fetal adrenomedullin than control patients, whereas there was no difference in maternal plasma adrenomedullin levels of the two groups[151].

Additionally, adrenomedullin has been identified in human fetoplacental tissues[152,153], in which its presence, determined by immunohistochemical methods, seems to be greater in fetal membranes than in the placenta. The peptide was found to be localized to the amnion and to trophoblast cells[152]. Kanenishi *et al.*[153] report decreased immunohistochemical adrenomedullin expression in the placentas obtained from pre-eclamptic pregnancies, and most recently Knerr *et al.*[154] reported a significantly lower adrenomedullin mRNA expression in placental villi from pre-eclamptic compared with normotensive women.

Furthermore, Jerat *et al.*[155] reported no significant differences in the response of stem villous arteries taken from normal pregnant patients compared with those from pre-eclamptic patients with respect to their response to adrenomedullin.

Based upon the available data to date, it appears that adrenomedullin may be involved in the adaptation of the vascular system to pregnancy and in the regulation of placental vascular tone. However, controversy exists on the status of circulating

and placental adrenomedullin in pre-eclampsia and of the relative contribution of adrenomedullin to impaired fetoplacental circulation and fetal growth.

NON-VASOACTIVE PEPTIDES

β-human chorionic gonadotropin :

Human chorionic gonadotropin (hCG) is a glycoprotein composed of two non-covalently linked subunits, α and β, and is secreted from the blastocyst and early placental syncytiotrophoblast. Maternal serum level peaks at 8-10 weeks of gestation and then declines to reach a plateau at 18-20 weeks. The free β-hCG circulating in maternal serum corresponds to only about 0.3%-4% of total hCG[156].

In the case of β-hCG, there are several reports of an association with the incidence of pre-eclampsia[157,158]. There is general agreement that the placenta remains the main source of hCG in patients with pre-eclampsia, but whether the cause of the high circulating levels of the hormone is placental overproduction is still debated. Some advocate that hCG secretion may be increased as a consequence of abnormal placental invasion or placental immaturity[159]. It may also be linked to the trophoblast response to hypoxia with the development of a hypersecretory state[160]. Compared with normal pregnancies, the placentas of patients with unexplained elevated maternal hCG levels in the second trimester tend to be larger and to have an increased density of hCG-positive trophoblasts along with an increased intensity of hCG immunostaining within the placental villi[161]. However, in contrast to that, a small sample study found equivalent expression of β-hCG mRNA in normal and pre-eclamptic placental tissues[162].

On average, maternal hCG levels are already increased in the second trimester in pregnancies that subsequently develop pre-eclampsia[157,163-165]. Because the measurement of hCG levels during the second trimester for Down's syndrome screening has already been incorporated into clinical practice at many antenatal clinics worldwide, thousands of records of midtrimester hCG levels for women attending screening programs and their respective outcomes have permitted the investigation of whether the finding of elevated hCG concentrations in maternal serum is predictive of pre-eclampsia. There are accumulating data from studies that evaluated whether a single elevated hCG value (usually above 2.0 MoM) between 14 and 24 weeks of gestation is predictive of pre-eclampsia[158,163,166-172]. The results of these studies are convergent in suggesting that women with elevated hCG levels in the second trimester are at increased risk for pre-eclampsia, but there is divergence regarding the accuracy of this test and, by consequence, its predictive value. Many reasons contribute to the disagreement between the studies. The sensitivity and specificity of the test may change according to the method of assay, the clinical and epidemiological background of the subjects, the gestational age at which samples were collected, and the cutoff chosen to distinguish high from normal hCG levels. Most recent publications have suggested that hCG may be more

predictive for early than late onset pre-eclampsia[171,172].

But nevertheless, only when hCG was incorporated into a multifactorial model (including body mass index, parity, and age) did the sensitivity of the test prove effective with a specificity of 71%[173].

Inhibin A and activin A :

Inhibins are glycoproteins that were first isolated from ovarian follicular fluid and named after their ability to inhibit the pituitary secretion of follicle stimulating hormone (FSH). Inhibins A and B are heterodimers composed by an α subunit and a βA or βB subunit, respectively, linked by a disulfide bridge[174]. Inhibin-related proteins comprise activins, which are homodimers composed by the same β subunits of the inhibin molecule, and follistatin, a binding protein with affinity for inhibins and activins via the β subunit. Activins are peptides that act as growth and differentiation factors in many cells and tissues. Inhibins and activins are members of the transforming growth factor (TGF)-β superfamily, a group of structurally similar but functionally diverse growth factors[175].

Inhibin α and βA subunits are widely localized in the cytotrophoblast and syncytiotrophoblast[176], and the intensity of the hybridization signal for inhibin α and βA subunit mRNA increases throughout pregnancy, peaking in extracts prepared from term placentas[176]. Although the decidua[177], membranes[178], and fetus all produce inhibin, the placenta is the major source[179]. In consonance with placental expression, maternal serum inhibin A and activin A concentrations increase progressively during gestation, especially in the last trimester[180].

Maternal serum activin A and inhibin A levels are substantially increased in the presence of hypertensive disorders[181-185]. Although this might happen only because of hemoconcentration or decreased urinary clearance, activin A levels begin to rise significantly before the onset of hematological or renal manifestations of clinical disease[181,186,187]. The most probable mechanism for the high activin A and inhibin A concentrations in patients with pre-eclampsia is increased placental production[184,188]. This increased placental production is more likely to represent a placental response than a primary overproduction, but the mechanism which increases the activin A level in pre-eclampsia is yet unknown.

The above mentioned observations that inhibin A and activin A levels increase before onset of the disease[181,186,187] led to the theory that they might be diagnostic and prognostic markers of pre-eclampsia[181]. Silver and coworkers[183] found that inhibin A and activin A levels were higher in women with pre-eclampsia and observed that before 34 weeks of gestation there was a more pronounced difference in the average levels of both analytes between normal and complicated pregnancies. Studies have indicated that inhibin A is elevated several weeks before the onset of clinical signs of pre-eclampsia[164,187,189-191]. Women with an inhibin A concentration exceeding 2.0 MoM between 15 and 19 weeks of gestation were more likely to develop pre-eclampsia, to deliver a small-for-gestational-age infant, or to have a

stillbirth or neonatal death[190]. Lambert-Messerlian *et al.*[164] observed that inhibin A levels tended to be higher when the onset of pre-eclampsia occurred within a shorter interval after collection of the second trimester screening sample and suggested that second trimester inhibin A would be more effective in predicting early onset rather than late onset disease. Inhibin A has been reported to be particularly sensitive in predicting the occurrence of pre-eclampsia before 34 weeks, when the impact of the disease on maternal-fetal outcome is worse[187]. Compared with inhibin A, activin A seems to be a more sensitive marker at 21-25 weeks[187].

Altogether, the studies evaluating second trimester inhibin A and activin A measurements to predict pre-eclampsia suggest that these markers have limited sensitivity and low positive predictive value when applied to low risk populations, but it may add significant information when used in combination with other screening modalities such as Doppler ultrasound[192,193].

Leptin :

Leptin was initially introduced as an adipocyte-derived hormone that regulates energy metabolism via its hypothalamic receptor[194]. Subsequent studies revealed various physiologic functions of leptin. It plays an essential role especially in reproduction by regulating gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus[195].

Leptin is also produced by placental trophoblast and is secreted both in maternal and fetal circulation[196]. During pregnancy, leptin levels show marked changes, suggesting the placenta as a putative source of production of leptin in addition to adipose tissue. Maternal plasma leptin levels rise sharply during the first trimester[197,198] and decline back to normal values after delivery[199]. Serum leptin levels increase in the second and third trimester, and may contribute to the inhibition of increased food intake, body weight, and body fat[198].

Elevated maternal leptin levels have been described in women with pre-eclampsia in the third trimester[200-203] but not at delivery[203]. The rise in total leptin represents an increase of free leptin levels, as the bound fraction is paradoxically decreased[201]. The most probable mechanism of leptin increase in pre-eclampsia is increased placental production[204] (Fig. 4), with placental hypoxia and inflammatory mediators being important stimulators[202]. This explains why pre-eclampsia subverts the physiological relationship between adiposity and leptin levels in pregnant women[205].

A longitudinal study showed increased leptin levels beginning at 20 weeks of gestation in women who subsequently developed pre-eclampsia, suggesting that leptin may be an early marker of the disease[200].

Neurokinin B :

Recently, elevated levels of neurokinin B (NKB) have been reported to cause pre-eclampsia[206]. NKB belongs to the tachykinin family. These neuropeptides

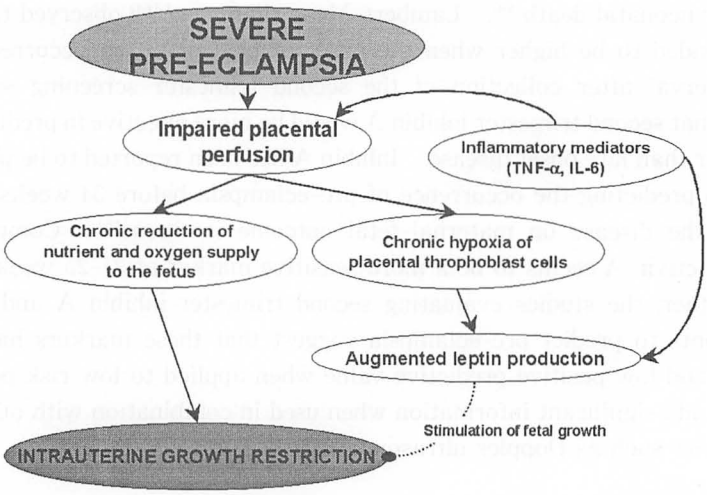

Fig. 4. Relationship between placental leptin production and intrauterine growth restriction in severe pre-eclampsia.

have been implicated in a variety of biological functions from smooth muscle contraction[207], vascular reactivity[208,209], pain transmission[210], neurogenic inflammation[211], and the activation of the immune system[212]. They are considered normally to be restricted to nervous tissue and to exert their effects peripherally only by release from nerve endings in the target tissue, activating neurokinin (NK) receptors, of which there are three. NKB binds preferentially to the NK_3-receptor, but in higher doses is also able to activate other neurokinin receptors.

In animal studies, NKB has been found to cause contraction of the hepatic portal vein[208], venoconstriction of the mesenteric bed[209], and increases in heart rate[213], which are all potentially hypertensive effects. A model of its physiological role in normal pregnancy and its potential involvement in pre-eclampsia[214] has been proposed: NKB is thought to play a physiological role in establishing the early trophoblast. It may be an important regulator of placental perfusion by serving to dilate the uterine spiral arteries. By causing vascular changes, NKB might not only increase maternal blood pressure but also shunts blood from certain organs, such as the liver and the mesenteric beds, to the uterus and placenta in order to maintain a sufficient blood supply to these organs. In pre-eclampsia, it is supposed that if the defective trophoblastic invasion does not rectify itself after the first trimester, then the placenta may start to secrete NKB into the maternal circulation in ever-increasing amounts. The incomplete invasion of the trophoblasts could lead to a situation where the uterine spiral arteries are unresponsive and cannot be dilated. Therefore an adequate blood supply to the placenta fails to occur. Raised levels of NKB may then lead to maternal hypertension and damage the kidneys and liver. At very elevated concentrations, peripheral NK1-receptors (on platelets, neutrophils or monocytes and in the brain) may be activated and in this way, NKB could also cause

the symptoms and complications usually found in pre-eclamptic patients.

In contrast to the results reported by Page *et al.*[206], we found lower levels of NKB in women with pre-eclampsia compared with normotensive pregnant women[215]. Our findings are supported by the results published by Li and co-workers[216], who found an increased expression of placental neutral endopeptidase 3.4.24.11 (NEP) in pre-eclampsia, suggesting that this enzyme may be involved in the pathophysiological changes of pre-eclampsia. As NKB is a substrate for endogenous tachykinin inhibitors, especially NEP[207], decreased NKB levels in pre-eclampsia could be due to changes in its inhibitor. Therefore, the role of NKB in pre-eclampsia, if any, remains to be determined.

OXIDATIVE STRESS AND LIPID METABOLISM

Oxidative stress and hyperlipidemia are common themes among many chronic disorders such as atherosclerosis, diabetes, neurodegenerative disorders, and cancer. Pre-eclampsia has also been reported to be associated with oxidative stress[60] and hyperlipidemia[217], as are risk factors for pre-eclampsia such as obesity, hypertension, and diabetes[18].

Oxidative stress may be defined as an imbalance between oxidant and antioxidant forces in favour of oxidation. Oxidative stress is considered to be an important final pathway in causing endothelial damage and is evident in the maternal circulation of pre-eclamptic women. The increased oxidative stress in pre-eclampsia may on one side be due to elevated free radical generation[218]. Circulating levels of lipid peroxides (LPO), as estimated by malondialdehyde (MDA) or conjugated dienes, are abnormally elevated and graded in proportion to the severity of the disorder[219]. Maternal plasma levels of isoprostanes, which are considered accurate markers of oxidative stress and lipid peroxidation, are increased[220]. Serum levels of iron are also elevated[221], which leads to oxidant stress because transition metals such as the ferrous ion (Fe^{2+}) initiate peroxidation of lipids according to the Fenton reaction. On the other hand, a decrease in levels of antioxidants and/or impaired regeneration of reduced forms of antioxidants[222–224] may lead to oxidative stress, although not all investigators find decreased levels of antioxidants[223,225,226].

In pre-eclampsia, maternal predispositions could also interact with the poorly perfused intervillous space to generate reactive oxygen species. It seems likely that pre-eclamptic women miss a protective factor X or mechanism, which normally protects the pregnant woman[227], making the endothelium more sensitive to possible noxes. Several suggestions have been advanced to explain the transfer of oxidative stress from the intervillous space to the systemic circulation. Activated neutrophils and monocytes are present in pre-eclampsia. These cells could be activated by oxidative stress in the intervillous space and then generate free radicals on contact with endothelium[220]. Again, the consequences of this interaction are defined by maternal factors (decreased antioxidants, sensitized endothelium, lipoproteins espe-

cially sensitive to oxidation). Transfer of oxidative stress could also be secondary to the formation of stable products of lipid peroxidation (e.g., malondialdehyde) or by oxidized fragments of syncytiotrophoblast entering the systemic circulation. Finally, the hypoxic placenta might produce cytokines with the potential to generate oxidative stress. Placentas of women with pre-eclampsia are characterized by oxidative stress, evidenced by abnormally elevated tissue levels and production rates of LPO, as well as an imbalance in the arachidonic acid metabolites, thromboxane, and prostacyclin[125,126,228]. Evidence indicates that the imbalance of increased thromboxane and decreased prostacyclin is caused by placental oxidant stress because oxidized lipids stimulate thromboxane synthesis while inhibiting prostacyclin synthesis. Thromboxane is a potent vasoconstrictor and prostacyclin is a potent vasodilator, so this imbalance restricts uteroplacental blood flow. Further evidence of placental oxidative stress is the finding that placental isoprostane levels are significantly increased in pre-eclampsia[229]. The cause of these placental abnormalities is not known yet.

Administration of antioxidants to women in early pregnancy decreases oxidative stress, endothelial activation, and the frequency of pre-eclampsia, which lends support to the potential role of oxidative stress in pre-eclampsia[230].

IMMUNE MARADAPTATION

In 1976, Drs. James R. Scott and Alan A. Beer[231] wrote in a review article titled "Immunologic aspects of pre-eclampsia", the following terse but incisive introduction to their review article: "The normal pregnant state represents the only natural and successful transplantation of living tissue from one person to another, but one has to look no farther than pregnancies complicated by Rh isoimmunization to realize that immunologic homeostasis between the mother and fetus is not always perfect." Dekker and Sibai[232] have championed the idea that "genuine" pre-eclampsia is a disease of first pregnancies. They point out the fact that the incidence of pre-eclampsia is low in women who have had a previously normal pregnancy. Interestingly, even a prior abortion may provide protection against this disease[233]. However, if a woman changes sexual partners, the immunity conferred by multiparity is lost[19,20,234,235]. Repeated exposure to sperm from the same individual may also be a preventive factor in the development of pre-eclampsia[20,22], and artificial insemination increases the risk of this disorder[20,236,237]. Although not well understood, the hypothesis propounded to explain these protective effects of sperm exposure is that T cells within the genital tract may recognize antigens without the need for binding to class I human leukocyte antigen (HLA) on antigen-presenting cells, allowing trophoblasts lacking classical HLA to be recognized[238]. In addition, a transient state of T lymphocyte hyporesponsiveness to paternal class I HLA has been reported, which may impact this immune reaction[239]. A lower level of messenger RNA for HLA-G has been noted in trophoblasts from pre-eclamptic

patients than from normal pregnant patients[240], but this could be the result of fewer trophoblast cells in pre-eclamptic patients[241].

Implantation and placentation present an immune challenge because of the semi-allogenic nature of the conceptus. Decidualization of the endometrium itself has features in common with an inflammatory response[64]. During decidualization, infiltration by uterine natural killer cells occurs, and these interact with the non-polymorphic HLA class I antigens expressed by invading extravillous trophoblast. Candidates for mediators of the immune maladaptation in pre-eclampsia include cytokines (especially tissue necrosis factor [TNF]-α and interleukin [IL]-2 and IL-6)[233]. Additionally, enzymes released by activated neutrophils, such as elastase and oxygen-free radicals, including LPO, have been thought to be implicated[233].

Redman *et al.*[64] hypothesized an interesting theory: They suggest that the endothelial dysfunction is a part of a more generalized intravascular inflammatory reaction involving intravascular leukocytes, as well as the clotting and complement systems, and proposed that such an inflammatory response is already well developed in normal pregnancy and that pre-eclampsia arises when a universal maternal intravascular inflammatory response to pregnancy decompensates in particular cases, which may occur because either the stimulus or the maternal response is too strong.

GENETICS OF PRE-DCLAMPSIA

The familial nature of preeclampsia-eclampsia has been appreciated since at least the 1800s. In the last decades numerous papers report on the familial nature of pre-eclampsia[67,242,243].

The inheritance patterns of pre-eclampsia have been described as Mendelian (autosomal recessive, autosomal dominant with incomplete penetrance), polygenic/multifactorial, or mitochondrial. Additionally, a unique type of inheritance has been postulated that involves an interaction between the genetic components of the mother and those of the father, manifesting through the fetal-placental unit, and possibly through imprinting. But until now, the exact inheritance pattern is still unknown[67,244], no single gene has been identified that explains a clear Mendelian inheritance, and genomic scans of women with pre-eclampsia have yielded varying results. No doubt pre-eclampsia is a syndrome with underlying genetic heterogeneity. It is most likely that genes or mutations in certain genes will predispose women to develop pre-eclampsia, and that these loci will vary in different populations. The most plausible genetic model to date postulates that maternal genes dictate a woman's susceptibility for the expression of the pre-eclamptic phenotype, whereas expression of the phenotype in a woman with a given genetic susceptibility might depend on the genetic load from the trophoblast and possibly on environmental factors[245,246]. This susceptibility is dictated by genes and their interaction with environment, but it will be transferred into biochemical and molecular changes to be

found in the syndrome of pre-eclampsia and perhaps in later life.

Fetal (paternal) contribution :

Theoretically, both mother and fetus (and therefore the father) may contribute to the risk. Pre-eclampsia may reflect problems in the close biological interaction between the two subjects[245]. Current knowledge on the epidemiology of pre-eclampsia, like the particularly high risk in first pregnancies, points primarily to an effect of maternal factors[19].

Although it is likely that the fetus may also contribute to the pathophysiology of the syndrome pre-eclampsia, few studies have focused on the contribution of fetal and/or paternal genes[247,248]. Lie *et al.*[247] suggested that paternal genes (as expressed in the fetus) contribute also strongly to the mother's risk of pre-eclampsia. Mothers who were pregnant by a partner who fathered a pre-eclamptic pregnancy in another woman had nearly twice the risk in their own pregnancy[247]. Esplin and co-workers[248] reported that both men and women who were the product of a pregnancy complicated by pre-eclampsia were significantly more likely than control men and women to have a child who was the product of a pregnancy complicated by pre-eclampsia.

Candidate Genes :

The search for genes that increase maternal susceptibility to pre-eclampsia is ongoing. Besides thrombophilic mutations a number of genes have been supposed to be involved in the pathogenesis of pre-eclampsia. Potential genes or chromosome locations are chosen for study based upon their pathophysiologic plausibility or their linkage to pre-eclampsia by genome-wide scanning[67].

Thrombophilia :

A characteristic feature of pre-eclampsia is the maternal hypercoagulable state and intravascular coagulation, which is evidenced by increased platelet consumption and reduced platelet lifespan. Several pro-coagulant factors like tissue plasminogen activator (tPA)[249,250], plasminogen avtivator inhibitor (PAI)[249–252], von-Willebrand factor[253] and anticardiolipin antibodies[29,30] are changed in pre-eclampsia. Several endothelial cell-associated anticoagulant proteins have also shown to be associated in pre-eclampsia. A decrease in antithrombin III (AT III) occurs in pre-eclampsia[25] and AT III deficiency is more frequent in pre-eclamptic women (odds ratio of 7.2)[28]. Protein C deficiency has been reported to be associated with pre-eclampsia compared with control women (odds ratio 21.5)[28]. Additionally, protein S deficiency is more frequent in pre-eclampsia (12.3% versus 0.6% in normal pregnancy, odds ratio 12.7)[28].

Thrombophilic mutations such as factor V Leiden, G20210A prothrombin mutation, and methylenetetrafolate reductase mutation have been reported to be underlying factors for the development of pre-eclampsia, although this association

has not shown up that clearly on a molecular level so far. In a Dutch study on women with severe early onset pre-eclampsia, more than 50% of women had at least one underlying thrombophilic disorder[25]. Kupferminc *et al.*[27] reported a combined prevalence (67%) of inherited and acquired thrombophilic disorders in women with severe pre-eclampsia. Women with thrombophilia delivered at an earlier gestational age, and their neonates' birth weights were lower compared with those of women without thrombophilia[27]. Van Pampus *et al.*[26] also showed a high prevalence of hemostatic abnormalities of about 40% in women with a history of severe pre-eclampsia. Recently, Alfirevic *et al.*[28] summarized in their review on the association between maternal thrombophilia and adverse pregnancy outcome that "women with adverse pregnancy outcomes are more likely to have a positive thrombophilia screen."

Some studies failed to find any association of thrombophilic mutations with pre-eclampsia[254-256], but this might be explained by the genetic heterogeneity in the different populations studied and/or the different diagnostic criteria used[28,257]. To summarize, screening for thrombophilic mutations seems to be justified in high risk women, but not as a general screening of all pregnant women[28].

Factor V Leiden Mutation :

Factor V Leiden deficiency has been associated with an increased risk of pre-eclampsia[25-27,31,32], whereby this mutation especially seems to be associated with HELLP syndrome[33-35]. The Leiden mutation of factor V (G1691A) was first described in a subgroup of individuals who had activated protein C deficiency[258]. The presence of the G1691A mutation is a relatively common missense mutation in which substitution of arginine in place of glutamine changes the factor V protein into one that resists inactivation by activated protein C. The prolonged procoagulant activity of factor V in the heterozygote state increases the carrier to thromboembolic complications. The factor V Leiden frequency is found in about 5% of Caucasians, whereas this mutation is only rarely, if at all, found in people of Asian or African ancestry[259].

G20210A prothrombin mutation :

Poort and co-workers[260] described in 1996 the G20210A prothrombin mutation. This guanine-to-adenine mutation at nucleotide 20210 in the 3'-untranslated region of the prothrombin gene is also associated with an increased risk of venous thromboembolism. In that case, factor Xa/Va-complex cannot convert prothrombin to thrombin[260].

The prevalence of this mutation is reported in about 2% of Caucasians[260,261]. Again, this mutation is rarely found in people with Asian or African ancestry[261].

Few studies focussed on the association of the G20210A prothrombin mutation with pre-eclampsia. Although the role of this mutation remains unclear, women who are found to be heterozygous carriers of that mutation are reported to have a

2-fold higher risk (odds ratio 2.4) for pre-eclampsia[28].

Methylenetetrafolate Reductase :

Mutations in the methylenetetrafolate reductase (MTHFR) gene in a non-pregnant population have been associated with a modest elevation in plasma homocysteine concentration. The latter has been implicated in vascular injury and an increased risk for cardiovascular disease[262]. Hyperhomocysteinemia has been reported to be involved in the pathogenesis of pre-eclampsia[25,26,263]. In women with pre-eclampsia, hyperhomocysteinemia can be found in 14.8% compared with 4.5% in control women (odds ratio 2.2)[28]. Because vascular resistance to uterine blood flow and resultant ischemia is thought to be causative in pre-eclampsia, investigators have targeted the MTHFR gene as a susceptibility gene for pre-eclampsia. Especially the association of a common missense mutation, a C to T substitution at nucleotide 677 (T677), in the MTHFR gene with pre-eclampsia has been investigated[28,624]. Heterozygous carrier frequency is estimated to be 42.2% in pre-eclamptic women compared with 38.6% in the controls (odds ratio 1.2)[28]. The homozygous carrier frequency is 13.4% in pre-eclampsia and 10.3% in normal pregnant women (odds ratio 1.7)[28].

Candidate genes involved in hemodynamic changes of pregnancy :

Beside thrombophilic mutations a variety of candidate genes have been targeted as susceptibility genes for pre-eclampsia[67].

Angiotensinogen :

Angiotensinogen (AGT) is a precursor to angiotensin II, a highly vasoactive compound that is important in the regulation of blood pressure and intravascular volume. Ward and colleagues[265] were the first to describe an association between pre-eclampsia and a molecular variant in the maternal AGT gene (T235). The substitution of threonine for methionine in the variant appears to be linked to an A(−6) promoter mutation that causes increased AGT expression, which in turn leads to higher levels of angiotensin II. This association was confirmed by Arngrimsson *et al.*[266], while others could not confirm this association[267]. Recently, it has been reported that the AGT T235 allele predisposes women toward abnormal physiologic change, potentially beginning the cascade of events leading to pre-eclampsia[268]. Finally it has been postulated that an angiotensin II type 1 receptor gene expression in the fetus may contribute to the etiology of pre-eclampsia. It was unclear whether susceptibility is conferred by the fetal genotype acting alone, or by allele sharing by mother and fetus[269].

Endothelial Nitric Oxide Synthase (eNOS) :

Another interesting candidate gene is the nitric oxide synthase (NOS) gene. By microsatellite amplification, Arngrimsson *et al.*[270] found evidence for a linkage of

pre-eclampsia with a microsatellite (D7S505) within intron 13 of chromosome 7q36 encoding the endothelial nitric oxide synthase (eNOS) gene in a population of Scottish and Icelandic origin. The linkage results reported by Guo *et al.*[271], supported the possibility that a susceptibility locus for pre-eclampsia resides in the 7q36 region, however, they failed to support the notion that the eNOS gene itself is responsible for susceptibility to pre-eclampsia. Yoshimura *et al.*[272] identified a G to T conversion at nucleotide position 894 within exon 7 of the eNOS gene resulting in replacement of glutamic acid with aspartic acid at codon 298 (Glu298Asp). Subsequently, they investigated the association of this mutation with pre-eclampsia[273] and found a higher frequency of this mutation in women with severe pre-eclampsia, which was recently confirmed by others[274,275]. Savvidou *et al.*[275] reported an association of the eNOS Glu298Asp polymorphism is related to differences in endothelium-dependent dilation at 12 weeks' gestation. The presence of this polymorphism was found to be correlated with lower flow-mediated dilation of the brachial artery, a vascular abnormality purported to predict pre-eclampsia. They suggested a role of this mutation in the normal vascular adaptation to pregnancy and pre-eclampsia. Tempfer and coworkers[276] reported that a polymorphism in the eNOS gene, segregating with lower NO metabolites, is associated with a six times greater risk of developing pre-eclampsia.

The association of the eNOS gene with pre-eclampsia, however, could not be confirmed by other studies[271,277]. Even Arngrimsson *et al.*[278] couldn't confirm their observation when publishing a genome-wide scan for pre-eclampsia using the same eNOS markers.

Candidate genes involved in oxidative stress :

Endothelial dysfunction is characteristic for the syndrome pre-eclampsia and oxidative stress has been implicated to cause endothelial damage. Marked dyslipidemia may contribute to the endothelial cell dysfunction in pre-eclampsia. A sequence variation in the lipoprotein (LPL) gene has been reported to be associated with pre-eclampsia[279]. Carriers of N291S or combined D9N/−93T → G mutations in the LPL gene which both predispose to dyslipidemia and cardiovascular disease, are at substantially increased risk of pre-eclampsia[279]. This finding could not be confirmed by Kim *et al.*[280], although in a small sub-group of patients, the *N291S* mutation was associated with an increased risk for nulliparous hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome.

Oxidative stress may be caused by toxic compounds such as lipid peroxides (LPO) and oxygen-free radicals. Therefore, enzymes that scavenge and detoxify substances were felt to possibly be involved. Epoxide hydrolase is a liver microsomal enzyme, involved in metabolism of endogenous and exogenous toxins. Genetic polymorphisms in the gene (EPHX) coding for this enzyme have been associated with the degree of its activity. Specifically, two polymorphisms, 113Tyr3His in exon 3, and 139His 3 Arg in exon 4 have been associated with

decreased activity. Zusterzeel *et al.*[281] found that those women with pre-eclampsia were nearly twice as likely as the healthy women (29% vs. 16%) to have the high activity variant of microsomal epoxide hydrolase. Furthermore, these polymorphisms did not seem to increase the risk for concurrent development of the syndrome of HELLP[281].

Recently, association of pre-eclampsia of the P1b-1b genotype of the glutathionine-S-transferase (GST) gene, which encodes a detoxificating biotransfromation enzyme, was studied in a Dutch study population[282]. This genotype, possibly resulting in a lower glutathione-S-transferase detoxification capacity of oxidative stress-related factors, was more frequent in pre-eclamptic patients than in controls[282].

Candidate genes involved in immunogenetics :

The HLA-G genotype is another interesting candidate gene, which may be associated with pre-eclampsia. Whereas previous studies couldn't find any association between HLA-G polymorphisms and pre-eclampsia[283,284], recently, O'Brien *et al.*[285] reported that the distribution of *HLA-G* polymorphisms was different between normal and pre-eclamptic cytotrophoblast samples.

Results on genes encoding pro-inflammatory cytokines and genes participating in the regulation of the immune response, like tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) are also controversial: Whereas Chen *et al.*[286] found an association between the TNF-T1 allele and pre-eclampsia, others did not find this association[287-289].

Additionally, recent study on polymorphisms in the IL-1 and interleukin-1 receptor antagonist (IL1ra) gene and pre-eclampsia or HELLP syndrome did not show association with either gene[290,291].

In summary, it is quite obvious that pre-eclampsia has a complex inheritance pattern, one similar to chronic illnesses such as diabetes, hypertension, and asthma. The genetics of chronic illness involve multiple disease susceptibility loci as well as environmental gene interactions. The potential of the paternal (fetal) component further complicates the elucidation of the inheritance mode of pre-eclampsia. Furthermore, imprinting or differences in methylation patterns may be involved. The finding of etiologic or susceptibility genes in pre-eclampsia will not only allow early identification of susceptible women, but may one day allow detection of unknown protein products that will direct future investigators to the elusive cause or causes of this disorder.

PRACENTAL ANGIOGENESIS

Research on the subject of pre-eclampsia has revolved around placental growth and angiogenesis, as both are central to the etiology of the disease. The formation of new vessels is crucial for many physiologic pathways in pregnancy and can be

divided into two major stages termed vasculogenesis and angiogenesis (Fig. 5)[292].

Vasculogenesis:

This earliest stage of vascular development, beginning at day 21 postconception, includes the differentiation, expansion, and coalescence of vascular endothelial cell precursors into the initial vascular network. The early vascular plexus forms from mesoderm by differentiation of angioblasts, which subsequently generate primitive blood vessels. Factors of the fibroblast growth factor (FGF) family are crucial to form angioblasts and hematopoetic cells. Vascular endothelial growth factor (VEGF)-receptors and sufficient levels of their ligands seem to be needed to maintain angioblast differentiation and therefore for the vasculogenic process. Induction of VEGF-receptor 2 (KDR=kinase insert domain containing receptor) may thus initiate angioblast differentiation, whereas the quantity and activity of VEGF ligands determine angioblast survival.

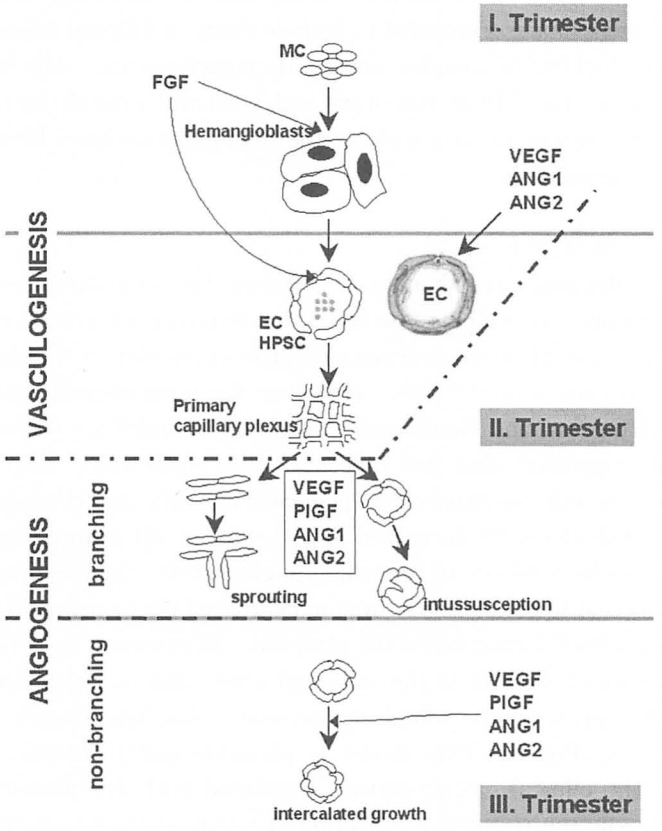

Fig. 5. Vasculo- and angiogenesis in pregnancy. Abbreviations: MC=mesodermal cell; FGF=fibroblast growth factor; EC=endothelial cell; HPSC=hematopoietic stem cell; VEGF=vascular endothelial growth factor; ANG1/2=angiopoitein-1/-2; PlGF=placenta growth factor.

Angiogenesis :

The primary plexus is then remodeled by a process referred to as angiogenesis. Further blood vessels are generated by branching and differential growth of the vessels, forming a more mature system with larger and smaller vessels. This process is termed *pruning*, as the resulting pattern resembles a tree. Beside the vascular endothelial growth factor (VEGF) family, angiopoietins as well as their receptors act during branching (true sprouting of capillaries from pre-existing vessels or intussusceptive growth) and non-branching (intercalated growth) angiogenesis. Branching angiogenesis occurs both in the yolk sac and the embryo and predominantly in the first and second trimester in situations of relative hypoxia. Non-branching angiogenesis occurs predominantly in the third trimester in situations of relative normoxia. The formed vasculature is further differentiated by recruitment of pericytes and smooth muscle cells and remodeled into a more major tree-like hierarchy containing vessels of different sizes.

From fibroblast growth factor (FGF) and VEGF as endothelial cell mitogens, the study of angiogenesis has expanded to include many additional agonists, receptors, and inhibitors working in complex and subtle menchanisms. The key factors are the VEGF family, the VEGF receptors and placental growth factor (PlGF). In addition, several newer factors such as the angiopoietins have been shown to be important in angiogenesis[72].

Regulation of angiogenesis :

Metabolic demands are thought to regulate the vascularisation of placenta, tissues, and tumors. It is a common belief that hypoxia is a crucial etiologic factor for late spontaneous abortion, intrauterine growth restriction (IUGR), pre-eclampsia, and abruptio placentae[68,73,74,293]. However, the term *placental hypoxia* might be too simple. Kingdom and Kaufmann[68] proposed a model for the origins of fetal hypoxia and suggested that *pre*-placental, *utero*-placental, and *post*-placental hypoxia should be differentiated: *Pre*-placental hypoxia (as occurs in high altitude, anemia, or smoking) with decreased PO_2 levels in all compartments (mother-placenta-fetus) which results in branching angiogenesis. In late onset *pre*-eclampsia, *utero*-placental hypoxia also results in decreased placental and fetal PO_2 levels and branching vessel formation in the placenta. In contrast, in IUGR with absent or reversed enddiastolic flow in the umbilical artery and in early onset pre-eclampsia, higher PO_2 levels can be found in placental tissue, resulting in non-branching vessel formation (Fig. 6). This model is plausible and substantiated in clinical practice. In the clinical circumstances associated with *pre*-placental and *utero*-placental hypoxia, the result is a reduction of villous oxygen content. Adaptation in the form of increased angiogenesis and trophoblast proliferation takes place. This produces greater amounts of highly vascularized terminal villi (branching angiogenesis), which lowers capillary-mediated impedance to blood flow. Opposite

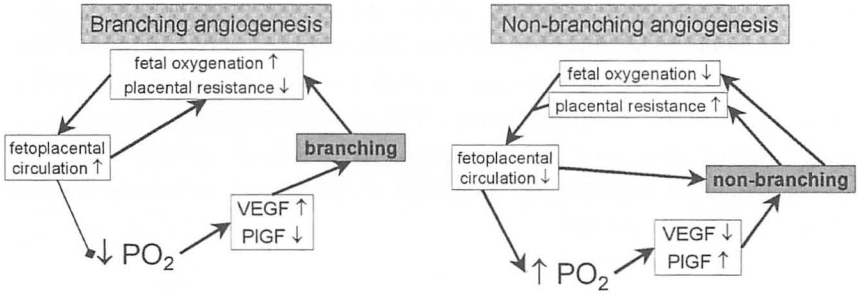

Fig. 6. Placental oxygen content, angiogenic growth factors, and angiogenesis.

to that is the situation of severe preterm IUGR and early onset pre-eclampsia. Here, normal terminal villi have not formed[294]. The feto-placental and utero-placental circulation is compromised. The result is a rising intraplacental oxygen content, which, in turn, results in a suppressed angiogenic drive to form terminal villi. Abnormal, non-branching angiogenesis results, conferring an increase, rather than the normal decrease in vascular impedance[294]. Oxygen extraction from these malformed villi is reduced[295]. This situation is what Kingdom and Kaufmann refer to as placental *hyperoxia*[68].

Angiogenic growth factors :

Of the variety of growth factors, the vascular endothelial growth factor (VEGF) family has been most extensively studied. Placental VEGF expression declines as pregnancy advances[296-298] and is upregulated under hypoxic conditions and in pre-eclampsia[75,297,298]. VEGF mediates its action via two tyrosine kinase receptors: fms-like tyrosine kinase (flt-1) and kinase insert domaine-containing receptor (KDR). When binding to flt-1, VEGF stimulates endothelial nitric oxide synthase (eNOS) and leads to inhibition of cytotrophoblast proliferation. Placental growth factor (PlGF) shares 53% homology with VEGF, is produced exclusively in the placenta, and acts only via flt-1[299]. In contrast to VEGF, placental PlGF expression is stimulated by oxygen and PlGF levels increase with advanced gestational age[297,298].

In pre-eclampsia maternal VEGF serum or plasma levels have been reported to be increased[71,300-301] and this increase correlates with the severity of the disease[301]. Other sources reported decreased VEGF levels in pre-eclampsia[69,70,302], which might be explained by the interference with binding proteins, different gestational age, or the fact that VEGF is bound to a soluble VEGF-receptor, which has been reported to be elevated in pre-eclampsia[76-78]. VEGF has shown to increase endothelial production of vasoactive substances and has vasodilative effects on resistance vessels[303-305].

Maternal PlGF is decreased in pre-eclampsia[69,70,78-80]. Recent studies indicate evidence that PlGF may be useful for the prediction of pre-eclampsia[306-308].

Recently, angiopoietin-1 and its natural antagonist, angiopoietin-2, as well as their vascular endothelial receptor tyrosine kinase TIE-2, have attracted attention. The angiopoietins are thought to play a major role in vessel maturation, acting downstream to VEGF. Maternal serum TIE levels decrease with gestational age and were further decreased in pre-eclampsia[309]. Geva *et al.*[72] reported and increase of placental angiopoietin-1 mRNA as pregnancy proceeds, whereas angiopoietin-2 mRNA decreased.

CONCLUSION AND OUTLOOK

Despite tremendous work, the enigma pre-eclampsia still exists and it is still a "disease of theories." It is obvious that a single mechanism responsible for the syndrome pre-eclampsia does not exist. It is more likely that some or all of the described mechanisms can act together and even multiply each other. Additionally, in different populations, different mechanisms may be more important due to the genetic heterogeneity. Several underlying risk factors and possible pathophysiologic mechanisms have been elucidated and, although small, possibilities of earlier detection and also prophylaxis have been developed.

The complicated etiology of pre-eclampsia calls for studies that take into account the following:

- Early onset and late onset of the disease, which both give rise to different villous morphologies and are two separate and distinct patient groups[310]
- The contribution from both the mother and the fetus (and therefore the father)

REFERENCES

1. Saftlas AF, Olson DR, Franks AL, Atrash HK, Pokras R. Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986. Am J Obstet Gynecol, **163**: 460-465, 1990.
2. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol, **183**: S1-S22, 2000.
3. Beinder E, Frobenius W. Die Praeeklampsie: Eine Endothelerkrankung? Deutsches Aerzteblatt, **97**: A2703-2706, 2000.
4. Harlow FH, Brown MA. The diversity of diagnoses of preeclampsia. Hypertens Pregnancy, **20**: 57-67, 2001.
5. Friedman SA, Taylor RN, Roberts JM. Pathophysiology of preeclampsia. Clin Perinatol, **18**: 661-682, 1991.
6. Cunningham FG, Twickler D. Cerebral edema complicating eclampsia. Am J Obstet Gynecol, **182**: 94-100, 2000.
7. Royburt M, Seidman DS, Serr DM, Mashiach S. Neurologic involvement in hypertensive disease of pregnancy. Obstet Gynecol Surv, **46**: 656-664, 1991.
8. Schwartz ML, Lien JM. Spontaneous liver hematoma in pregnancy not clearly associated with preeclampsia: a case presentation and literature review. Am J Obstet Gynecol, **176**: 1328-1332, 1997.
9. Lindheimer MB, Katz AI. Renal physiology and disease in pregnancy. *In*: Seldin DW, Giebisch G, ed. The Kidney: Physiology and Pathophysiology. 2nd ed. Raven Press,

New York, 3371-3431, 1992.
10. Norwitz ER, Hsu CD, Repke JT. Acute complications of preeclampsia. Clin Obstet Gynecol, **45**: 308-329, 2002.
11. Sciscione AC, Ivester T, Largoza M, Manley J, Shlossman P, Colmorgen GH. Acute pulmonary edema in pregnancy. Obstet Gynecol, **101**: 511-515, 2003.
12. McCrae KR, Samuels P, Schreiber AD. Pregnancy-associated thrombocytopenia: pathogenesis and management. Blood, **80**: 2697-2714, 1992.
13. Barron WM, Heckerling P, Hibbard JU, Fisher S. Reducing unnecessary coagulation testing in hypertensive disorders of pregnancy. Obstet Gynecol, **94**: 364-370, 1999.
14. Weinstein L. Syndrome of hemolysis, elevated liver enzymes, and low platelet count: a severe consequence of hypertension in pregnancy. Am J Obstet Gynecol, **142**: 159-167, 1982.
15. Martin JN Jr, May WL, Magann EF, Terrone DA, Rinehart BK, Blake PG. Early risk assessment of severe preeclampsia: admission battery of symptoms and laboratory tests to predict likelihood of subsequent significant maternal morbidity. Am J Obstet Gynecol, **180**: 1407-1414, 1999.
16. Maternal mortality-United States, 1982-1996. MMWR Morb Mortal Wkly Rep, **47**: 705-707, 1998.
17. Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. Lancet, **357**: 53-56, 2001.
18. Eskenazi B, Fenster L, Sidney S. A multivariate analysis of risk factors for preeclampsia. JAMA, **165**: 237-241, 1991.
19. Trupin LS, Simon LP, Eskenazi B. Change in paternity: a risk factor for preeclampsia in multiparas. Epidemiology, **7**: 240-244, 1996.
20. Dekker G. The partner's role in the etiology of preeclampsia. J Reprod Immunol, **57**: 203-15, 2002.
21. Skjaerven RS, Wilcox AJ, Lie RT. The interval between pregnancies and the risk of preeclampsia. N Engl J Med, **346**: 33-38, 2002.
22. Robillard PY, Hulsey TC, Perianin J, Janky E, Miri EH, Papiernik E. Association of pregnancy-induced hypertension with duration of sexual cohabitation before conception. Lancet, **344**: 973-975, 1994.
23. Rey E, Courturier A. The prognosis of pregnancy in women with chronic hypertension. Am J Obstet Gynecol, **171**: 410-416, 1994.
24. Dawson LM, Parfrey PS, Hefferton D, Dicks EL, Cooper MJ, Young D, Marsden PA. Familial risk of preeclampsia in Newfoundland: a population based study. J Am Soc Nephrol, **13**: 1901-1906, 2002.
25. Dekker GA, de Vries JIP, Doelitzsch PM, Huijgens PC, von Blomberg BME, Jakobs C, van Geijn HP. Underlying disorders associated with severe early-onset preeclampsia. Am J Obstet Gynecol, **173**: 1042-1048, 1995.
26. van Pampus MG, Dekker GA, Wolf H, Huijgens PC, Koopman MM, von Blomberg BM, Buller HR. High prevalence of hemostatic abnormalities in women with a history of severe preeclampsia. Am J Obstet Gynecol, **180**: 1146-1150, 1999.
27. Kupferminc MJ, Fait G, Many A, Gordon D, Eldor A, Lessing JB. Severe preeclampsia and high frequency of genetic thrombophilic mutations. Obstet Gynecol, **96**: 45-49, 2000.
28. Alfirevic Z, Roberts D, Martlew V. How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review. Eur J Obstet Gynecol Reprod Biol, **101**: 6-14, 2002.
29. Hayslett JP. The effect of systemic lupus erythematosus on pregnancy and pregnancy outcome. Am J Reprod Immunol, **28**: 199-204, 1992.
30. Heilmann L, von Tempelhoff GF, Pollow K. Antiphospholipid syndrome in obstetrics. Clin Appl Thromb Hemost, **9**: 143-150, 2003.
31. Dizon-Townson DS, Nelson LM, Easton K, Ward K. The factor V Leiden mutation

may predispose women to severe preeclampsia. Am J Obstet Gynecol, **175**: 902-905, 1996.
32. Bloomenthal D, Delisle MF, Tessier F, Tsang P. Obstetric implications of the factor V Leiden mutation: a review. Am J Perinatol, **19**: 37-47, 2002.
33. Krauss T, Augustin HG, Osmers R, Meden H, Unterhalt M, Kuhn W. Activated protein C resistance and factor V Leiden in patients with hemolysis, elevated liver enzymes, low platelets syndrome. Obstet Gynecol, **92**: 457-460, 1998.
34. Bozzo M, Carpani G, Leo L, Marcozzi S, Sacchi E, Moroni G, Pardi G. HELLP syndrome and factor V Leiden. Eur J Obstet Gynecol Reprod Biol, **95**: 55-58, 2001.
35. Schlembach D, Beinder E, Zingsem J, Wunsiedler U, Beckmann MW, Fischer T. Association of maternal and/or fetal factor V Leiden and G20210A prothrombin mutation with HELLP syndrome and intrauterine growth restriction. Clin Sci, **105**: 279-285, 2003.
36. Lindoff C, Ingemarsson I, Martinsson G, Segelmark M, Thysell H, Astedt B. Preeclampsia is associated with a reduced response to activated protein C. Am J Obstet Gynecol, **176**: 457-460, 1997.
37. Raijmakers MT, Zusterzeel PL, Steegers EA, Peters WH. Hyperhomocysteinaemia: a risk factor for preeclampsia? Eur J Obstet Gynecol Reprod Biol, **95**: 226-228, 2001.
38. Tyni T, Ekholm E, Pihko H. Pregnancy complications are frequent in long-chain 3-hydroxylacyl-coenzyme A dehydrogenase deficiency. Am J Obstet Gynecol, **178**: 603-608, 1998.
39. Ibdah JA, Dasouki MJ, Strauss AW. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. Variable expressivity of maternal illness during pregnancy and unusual presentation with infantile cholestasis and hypocalcaemia. J Inherit Metab Dis, **22**: 811-814, 1999.
40. Wetzka B, Winkler K, Kinner M, Friedrich I, Marz W, Zahradnik HP. Altered lipid metabolism in preeclampsia and HELLP syndrome: links to enhanced platelet reactivity and fetal growth. Semin Thromb Hemost, **25**: 455-462, 1999.
41. Sattar N, Greer IA. Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening? BMJ, **325**: 157-160, 2002.
42. Chesley LC, Annitto JE, Cosgrove RA. The remote prognosis of eclamptic women: sixth periodic report. Am J Obstet Gynecol, **124**: 446-459, 1976.
43. Fisher KA, Luger A, Spargo BH, Lindheimer MD. Hypertension in pregnancy: clinical-pathological correlations and remote prognosis. Medicine, **60**: 267-276, 1981.
44. Coonrod DV, Hickok DE, Zhu K, Easterling TR, Daling JR. Risk factors for preeclampsia in twin pregnancies: A population-based cohort study. Obstet Gynecol, **85**: 645-650, 1995.
45. Brittain PC, Bayliss P. Partial hydatidiform molar pregnancy presenting with severe preeclampsia prior to twenty weeks gestation: a case report and review of the literature. Mil Med, **160**: 42-44, 1995.
46. Choong S, Meagher S. Antenatal human parvovirus B19 infection and nonimmune hydrops fetalis presenting as severe preeclampsia. Aust NZ J Obstet Gynecol, **36**: 359-360, 1996.
47. Pryde PG, Nugent CE, Pridjian G, Barr MJr, Faix RG. Spontaneous resolution of nonimmune hydrops fetalis secondary to Parvovirus B19 infection. Obstet Gynecol, **79**: 869-871, 1992.
48. Bird IM, Zhang L, Magness RR. Possible mechanisms underlying pregnancy-induced changes in uterine artery endothelial function. Am J Physiol (Regul Integr Comp Physiol), **284**: R245-R528, 2003.
49. Myatt L. Role of Placenta in Preeclampsia. Endocrine, **19**: 103-111, 2002.
50. van Beck E, Peeters LH. Pathogenesis of preeclampsia: A comprehensive model. Obstet Gynecol Surv, **53**: 233-239, 1998.
51. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the path-

ogenesis of preeclampsia. Obstet Gynecol Annu, **1**: 177-191, 1972.
52. Zhou Y, Damsley CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype: one cause of defective endovascular invasion in this syndrome? J Clin Invest, **99**: 2152-2164, 1997.
53. Khong T.Y, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by preeclampsia and by small gestational age infants. Br J Obstet Gynaecol, **93**: 1049-1059, 1986.
54. Kraayenbrink AA, Dekker GA, van Kamp GJ, van Geijn HP. Endothelial vasoactive mediators in preeclampsia. Am J Obstet Gynecol, **169**: 160-165, 1993.
55. Paarlberg KM, de Jong CL, van Geijn HP, van Kamp GJ, Heinen AG, Dekker GA. Vasoactive mediators in pregnancy-induced hypertensive disorders: a longitudinal study. Am J Obstet Gynecol, **179**: 1559-1564, 1998.
56. Vural P. Nitric oxide/endothelin-1 in preeclampsia. Clin Chim Acta, **317**: 65-70, 2002.
57. Roberts JM. Endothelial dysfunction in preeclampsia. Sem Reprod Endocrinol, **16**: 5-15, 1998.
58. Davidge ST. Oxidative stress and altered endothelial cell function in preeclampsia. Semin Reprod Endocrinol, **16**: 65-73, 1998.
59. Walker JJ. Antioxidants and inflammatory cell response in preeclampsia. Semin Reprod Endocrinol, **16**: 47-55, 1998.
60. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exp Biol Med, **222**: 222-235, 1999.
61. Doering TP, Haller NA, Montgomery MA, Freeman EJ, Hopkins MP. The role of AT1 angiotensin receptor activation in the pathogenesis of preeclampsia. Am J Obstet Gynecol, **178**: 1307-1312, 1998.
62. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jupner A, Baur E, Nissen E, Vetter K, Neichel D, Dudenhausen JW, Haller H, Luft FC. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. J Clin Invest, **103**: 945-952, 1999.
63. Taylor RN. Review: immunology of preeclampsia. Am J Reprod Immunol, **37**: 79-86, 1997.
64. Redman CWG, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol, **180**: 499-506, 1999.
65. von Dadelszen P, Magee LA. Could an infectious trigger explain the differential maternal response to the shared placental pathology of preeclampsia and normotensive intrauterine growth restriction? Acta Obstet Gynecol Scand, **81**: 642-648, 2002.
66. Morgan T, Ward K. New insights into the genetics of preeclampsia Semin Perinatology, **23**: 14-23, 1999.
67. Lachmeijer AMA, Dekker GA, Pals G, Aarnoudse JG, ten Kate LP, Arngrimsson R. Searching for preeclampsia genes: the current position. Eur J Obstet Gynecol Reprod Biol, **105**: 94-113, 2002.
68. Kingdom JC, Kaufmann P. Oxygen and placental villous development: origins of fetal hypoxia. Placenta, **18**: 613-621, 1997.
69. Reuvekamp A, Velsing-Aarts FV, Poulina IE, Capello JJ, Duits AJ. Selective deficit of angiogenic growth factors characterises pregnancies complicated by pre-eclampsia. Br J Obstet Gynaecol, **106**: 1019-1022, 1999.
70. Livingston JC, Chin R, Haddad B, McKinney ET, Ahokas R, Sibai BM. Reductions of vascular endothelial growth factor and placental growth factor concentrations in severe preeclampsia. Am J Obstet Gynecol, **183**: 1554-1557, 2000.
71. Bosio PM, Wheeler T, Anthony F, Conroy R, O'herlihy C, McKenna P. Maternal plasma vascular endothelial growth factor concentrations in normal and hypertensive pregnancies and their relationship to peripheral vascular resistance. Am J Obstet Gynecol, **184**: 146-152, 2001.
72. Geva E, Ginzinger DG, Zaloudek CJ, Moore DH, Byrne A, Jaffe RB. Human placental

vascular development: vasculogenic and angiogenic (branching and nonbranching) transformation is regulated by vascular endothelial growth factor-A, angiopoietin-1, and angiopoietin-2. J Clin Endocrinol Metab, **87**: 4213-4224, 2002.
73. Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. Pathophysiology of preeclampsia: linking placental ischemia/hypoxia with microvascular dysfunction. Microcirculation, **9**: 147-160, 2002.
74. Hung TH, Skepper JN, Charnock-Jones DS, Burton GJ. Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. Circ Res, **90**: 1274-1281, 2002.
75. Zhou Y, McMaster M, Woo K, Janatpour M, Perry J, Karpanen T, Alitalo K, Damsky C, Fisher SJ. Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. Am J Pathol, **160**: 1405-1423, 2002.
76. Koga K, Osuga Y, Yoshino O, Hirota Y, Ruimeng X, Hirata T, Takeda S, Yano T, Tsutsumi O, Taketani Y. Elevated serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) levels in women with preeclampsia. J Clin Endocrinol Metab, **88**: 2348-2351, 2003.
77. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest, **111**: 649-658, 2003.
78. Schlembach D, Beinder E. Angiogenic factors in preeclampsia. J Soc Gynecol Invest, **10** (Suppl): 316A, 2003.
79. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. Am J Obstet Gynecol, **188**: 177-182, 2003.
80. Torry DS, Mukherjea D, Arroyo J, Torry RJ. Expression and function of placenta growth factor: implications for abnormal placentation. J Soc Gynecol Investig, **10**: 178-188, 2003.
81. Furchgott RI, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature, **288**: 373-376, 1980.
82. Loscalzo J, Welch G. Nitric oxide and its role in the cardiovascular system. Prog Cardiovasc Dis, **38**: 87-104, 1995.
83. Myatt L, Brewer A, Langdon G, Brockman DE. Attenuation of the vasoconstrictor effects of thromboxane and endothelin by nitric oxide in the human fetal-placental circulation. Am J Obstet Gynecol, **166**: 224-230, 1992.
84. Baylis C, Engels K. Adverse interactions between pregnancy and a new model of systemic hypertension produced by chronic blockade of endothelial-derived relaxing factor (EDRF) in the rat. Clin Exp Hypertens B, **11**: 117-129, 1992.
85. Granger JP, Alexander BT. Abnormal pressure-natriuresis in hypertension: Role of nitric oxide. Acta Physiol Scand, **168**: 161-168, 2000.
86. Baylis C, Suto T, Conrad K. Importance of nitric oxide in control of systemic and renal hemodynamics during normal pregnancy: studies in the rat and implications for preeclampsia. Hypertens Pregnancy, **15**: 47-169, 1996.
87. Yallampalli C, Garfield RE. Inhibition of nitric oxide synthesis in rats during pregnancy produces signs similar to those of preeclampsia. Am J Obstet Gynecol, **169**: 1316-1320, 1993.
88. Molnar M, Suto T, Toth T, Hertelendy F. Prolonged blockade of nitric oxide synthesis in gravid rats produces sustained hypertension, proteinuria, thrombocytopenia, and intrauterine growth retardation. Am J Obstet Gynecol, **170**: 1458-1466, 1994.
89. Xu DL, Martin PY, St John J, Tsai P, Summer SN, Ohara M, Kim JK, Schrier RW. Upregulation of endothelial and neuronal constitutive nitric oxide synthase in pregnant rats. Am J Physiol, **271**: R1739-R1745, 1996.

90. Cadnapaphornchai MA, Ohara M, Morris KG Jr, Knotek M, Rogachev B, Ladtkow T, Carter EP, Schrier RW. Chronic NOS inhibition reverses systemic vasodilation and glomerular hyperfiltration in pregnancy. Am J Physiol (Renal Physiol), **280**: F592-F598, 2001.
91. Alexander BT, Kassab SE, Miller MT, Abram SR, Reckelhoff JF, Bennett WA, Granger JP. Reduced uterine perfusion pressure during pregnancy in the rat is associated with increases in arterial pressure and changes in renal nitric oxide. Hypertension, **37**: 1191-1195, 2001.
92. Seligman SP, Buyon JP, Clancy RM, Young BK, Abramson SB. The role of nitric oxide in the pathogenesis of preeclampsia. Am J Obstet Gynecol, **171**: 944-948, 1994.
93. Choi JW, Im M.W, Pai SH. Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. Ann Clin Lab Sci, **32**: 257-263, 2002.
94. Davidge ST, Stranko CP, Roberts JM. Urine but not plasma nitric oxide metabolites are decreased in women with preeclampsia. Am J Obstet Gynecol, **174**: 1008-1013, 1996.
95. Beinder E, Mohaupt MG, Schlembach D, Fischer T, Sterzel RB, Lang N, Baylis C. Nitric oxide synthase activity and Doppler parameters in the fetoplacental and uteroplacental circulation in preeclampsia. Hypertens Pregnancy, **18**: 115-127, 1999.
96. von Mandach U, Lauth D, Huch R. Maternal and fetal nitric oxide production in normal and abnormal pregnancy. J Matern Fetal Neonatal Med, **13**: 22-27, 2003.
97. Scalera F, Schlembach D, Beinder E. Production of vasoactive substances by human umbilical vein endothelial cells after incubation with serum from preeclamptic patients. Eur J Obstet Gynecol Reprod Biol, **99**: 172-178, 2001.
98. Rowe J, Campbell S, Gallery ED. Nitric oxide production by decidual endothelial cells is not reduced in preeclampsia. Hypertens Pregnancy, **22**: 63-75, 2003.
99. Shaamash AH, Elsonosy ED, Zakhari MM, Radwan SH, El-Dien HM. Placental nitric oxide synthase (NOS) activity and nitric oxide (NO) production in normal pregnancy, pre-eclampsia and eclampsia. Int J Gynaecol Obstet, **72**: 127-133, 2001.
100. Stjernquist M. Endothelins, vasoactive peptides and growth factors. Cell Tissue Res, 292: 1-9, 1998.
101. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature, **332**: 411-415, 1988.
102. Hahn AW, Resink TJ, Scott-Burden T, Powell J, Dohi Y, Buhler FR. Stimulation of endothelin mRNA and secretion in rat vascular smooth muscle cells: a novel autocrine function. Cell Regul, **1**: 649-659, 1990.
103. Clozel M, Gray GA, Breu V, Loffler BM, Osterwalder R. The endothelin ETB receptor mediates both vasodilation and vasoconstriction in vivo. Biochem Biophys Res Commun, **186**: 867-873, 1992.
104. Schiffrin EL. Endothelin: potential role in hypertension and vascular hypertrophy. Hypertension, **25**: 1135-1143, 1995.
105. Taylor RN, Varma M, Teng NNH, Roberts JM. Women with preeclampsia have higher plasma endothelin levels than women with normal pregnancies. J Clin Endocrinol Metab, **71**: 1675-1677, 1990.
106. Dekker GA, Kraayenbrink AA, Zeeman GG, van Kamp GJ. Increased plasma levels of the novel vasoconstrictor peptide endothelin in severe preeclampsia. Eur J Obstet Gynecol Reprod Biol, **40**: 215-220, 1991.
107. Nova A, Sibai BM, Barton JR, Mercer BM, Mitchell MD. Maternal plasma level of endothelin is increased in preeclampsia. Am J Obstet Gynecol, **165**: 724-727, 1991.
108. Slowinski T, Neumayer HH, Stolze T, Gossing G, Halle H, Hocher B. Endothelin system in normal and hypertensive pregnancy. Clin Sci, **103** (Suppl 48): 446S-449S, 2002.
109. Singh HJ, Rahman A, Larmie ET, Nila A. Endothelin-1 in fetoplacental tissues from normotensive pregnant women and women with preeclampsia. Acta Obstet Gynecol

Scand, **80**: 99-103, 2001.
110. Khedun SM, Naicker T, Moodley J. Endothelin-1 activity in pregnancy. J Obstet Gynaecol, **22**: 590-593, 2002.
111. Carbonne B, Mignot TM, Papiernik E, Ferre F. Higher endothelin concentrations in the fetoplacental unit of pregnant women of African ancestry. Am J Obstet Gynecol, **178**: 491-492, 1998.
112. Shaarawy M, Abel-Magid AM. Plasma endothelin-1 and mean arterial pressure in the prediction of preeclampsia. Int J Gynaecol Obstet, **68**: 105-111, 2000.
113. Polliotti BM, Fry AG, Saller DN, Mooney RA, Cox C, Miller RK. Second-trimester maternal serum placental growth factor and vascular endothelial growth factor for predicting severe, early-onset preeclampsia. Obstet Gynecol, **101**: 1266-1274, 2003.
114. Faxen M, Nisell H, Kublickiene KR. Altered gene expression of endothelin-A and endothelin-B receptors, but not endothelin-1, in myometrium and placenta from pregnancies complicated by preeclampsia. Arch Gynecol Obstet, **264**: 143-149, 2000.
115. Alexander BT, Rinewalt AN, Cockrell KL, Massey MB, Bennett WA, Granger JP. Endothelin type. A receptor blockade attenuates the hypertension in response to chronic reductions in uterine perfusion pressure. Hypertension, **37**: 485-489, 2001.
116. Napolitano M, Miceli F, Calce A, Vacca A, Gulino A, Apa R, Lanzone A. Expression and relationship between endothelin-1 messenger ribonucleic acid (mRNA) and inducible/endothelial nitric oxide synthase mRNA isoforms from normal and preeclamptic placentas. J Clin Endocrinol Metab, **85**: 2318-2323, 2000.
117. Scalera F, Dittrich R, Beckmann MW, Beinder E. Effect of endothelin-1 on intracellular glutathione and lipid peroxide availability and on the secretion of vasoactive substances by human umbilical vein endothelial cells. Eur J Clin Invest, **32**: 556-562, 2002.
118. Oates JA, FitzGerald GA, Branch RA, Jackson EK, Knapp HR, Roberts LJ 2nd. Clinical implications of prostaglandins and thromboxane A2 formation. N Engl J Med, **319**: 689-698, 1988.
119. Friedman SA. Preeclampsia: a review of the role of prostaglandins. Obstet Gynecol, **71**: 122-137, 1988.
120. Roberts JM, Taylor RN, Goldfien A. Clinical and biochemical evidence of endothelial cell dysfunction in the pregnancy syndrome preeclampsia. Am J Hypertens, **4**: 700-708, 1991.
121. Walsh SW. Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. Am J Obstet Gynecol, 152: 335-340, 1985.
122. Wang Y, Walsh SW, Kay HH. Placental lipid peroxides and thromboxane are increased and prostacyclin is decreased in women with preeclampsia. Am J Obstet Gynecol, **167**: 946-949, 1992.
123. Woods LL. Importance of prostaglandins in hypertension during reduced uteroplacental perfusion pressure. Am J Physiol, **257**: R1558-R1561, 1989.
124. Knight M, Duley L, Henderson-Smart DJ, King JF. Antiplatelet agents for preventing and treating pre-eclampsia (Cochrane Review). *In*: *The Cochrane Library*, Issue 2, 2003.
125. Klockenbusch W, Rath W. Prevention of pre-eclampsia by low-dose acetylsalicylic acid—a critical appraisal. Z Geburtshilfe Neonatol, **206**: 125-130, 2002.
126. Hall JE, Guyton AC, Brands MW. Control of sodium excretion and arterial pressure by intrarenal mechanisms and the renin-angiotensin system. *In*: Laragh JH, Brenner BM, ed. Hypertension: Pathophysiology, Diagnosis and Management. 2nd ed. Raven Press, New York, 1451-1475, 1995.
127. Elsheikh A, Creatsas G, Mastorakos G, Milingos S, Loutradis D, Michalas S. The renin-aldosterone system during normal and hypertensive pregnancy. Arch Gynecol Obstet, **264**: 182-185, 2001.
128. Granger JP, Schnackenberg C. Renal mechanisms of angiotensin II-induced hypertension. Semin Nephrol, **20**: 417-425, 2000.

129. Alexander BT, Cockrell K, Cline FD, Llinas MT, Sedeek M, Granger JP. Effect of angiotensin II synthesis blockade on the hypertensive response to chronic reductions in uterine perfusion pressure in pregnant rats. Hypertension, **38**: 742-745, 2001.
130. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor responses throughout primigravid pregnancy. J Clin Invest, **52**: 2682-2689, 1973.
131. DeBold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci, **28**: 89-94, 1981.
132. Mathisen P, Hall C, Simonson S. Comparative study of atrial peptides ANF (1-98) and ANF (99-126) as diagnostic markers of atrial distension in patients with cardiac disease. Scand J Clin Lab Invest, **53**: 41-49, 1991.
133. Kleinert HD, Maack T, Atlas SA, Januszewicz A, Sealey JE, Laragh JH. Atrial natriuretic factor inhibits angiotensin, norepinephrine and potassium induced vascular contractility. Hypertension, **6**: I143-147, 1984.
134. Weidmann P, Hasler L, Gnadinger MP, Lang RE, Uehlinger DE, Shaw S, Rascher W, Reubi FC. Blood levels and renal effects of atrial natruiretic peptide in normal man. J Clin Invest, **77**: 734-742, 1986.
135. Charles CI, Espiner EA, Richards AM. Cardiovascular actions of ANF: Contributions of renal, neurohumoral and hemodynamic factors in sheep. Am J Physiol, **264**: R533-R538, 1993.
136. Zafirovska KG, Maleska VT, Bogdanovska SV, Lozance LA, Masin-Paneva J, Gerasimovska BD. Plasma human atrial natriuretic peptide, endothelin-1, aldosterone and plasma-renin activity in pregnancy-induced hypertension. J Hypertens, **17**: 1317-1322, 1999.
137. Eguchi K, Oguni N, Sawai T, Yonesawa M. Comparison of plasma concentrations of arginine vasopressin (AVP) and atrial natriuretic peptide (ANP) in normal and preeclamptic pregnancies. J Perinat Med, **24**: 437-443, 1996.
138. Minegishi T, Nakamura M, Abe K, Tano M, Andoh A, Yoshida M, Takagi T, Nishikimi T, Kojima M, Kangawa K. Adrenomedullin and atrial natriuretic peptide concentrations in normal pregnancy and preeclampsia. Mol Hum Reprod, **5**: 767-770, 1999.
139. Fievet P, Fournier A, de Bold A, el Esper N, Gregoire I, Westeel PF, Renaud H, Makdassi R. Atrial natriuretic factor in pregnancy-induced hypertension and preeclampsia: Increased plasma concentrations possibly explaining these hypovolemic states with paradoxical hyporeninism. Am J Hypertens, **1**: 16-21, 1988.
140. Frenkel Y, Blonder I, Masiach S, Weiss M. Atrial natriuretic peptide plasma level remains unchanged in various hypertensive disorders of pregnancy. Eur J Obstet Gynecol Reprod Biol, **59**: 97-200, 1995.
141. Senoz S, Sahin N, Ozcan T, Direm B, Gokmen O. The concentration of plasma atrial natriuretic peptide in normotensive and preeclamptic pregnancies. Eur J Obstet Gynecol Reprod Biol, **62**: 173-177, 1995.
142. McQueen J, Jardine A, Kingdom J, Templeton A, Whittle MJ, Connell JM. Interaction of angiotensin II and atrial natriuretic peptide in the human fetoplacental unit. Am J Hypertens, **3**: 641-644, 1990.
143. Kublickiene KR, Grunewald C, Kublickas M, Lindblom B, Lunell NO, Nisell H. Effects of atrial natriuretic peptide and cyclic guanosine monophosphate on isolated human myometrial arteries preconstricted by endothelin-1. Gynecol Obstet Invest, **40**: 190-194, 1995.
144. Lim AT, Gude NM. Atrial natriuretic factor production by the human placenta. J Clin Endocrinol Metab, **80**: 3091-3093, 1995.
145. Graham CH, Watson JD, Blumenfeld AJ, Pang SC. Expression of atrial natriuretic peptide by third-trimester placental cytotrophoblasts in women. Biol Reprod, **54**: 834-840, 1996.
146. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T.

Adrenomedullin : a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun, **192** : 553-560, 1993.
147. Jougasaki M, Wei CM, Aarhus LL, Heublein DM, Sandberg SM, Burnett JC Jr. Renal localization and actions of adrenomedullin : A natriuretic peptide. Am J Physiol, **268** : F657-F663, 1995.
148. Di Iorio R, Marinoni E, Letizia C, Villaccio B, Alberini A, Cosmi EV. Adrenomedullin production is increased in normal human pregnancy. Eur J Endocrinol, **140** : 201-206, 1999.
149. Di Iorio R, Marinoni E, Letizia C, Alo P, Villaccio B, Cosmi EV. Adrenomedullin, a new vasoactive peptide, is increased in preeclampsia. Hypertension, **32** : 758-763, 1998.
150. Hata T, Miyazaki K, Matsui K. Decreased circulating adrenomedullin in preeclampsia. Lancet, **350** : 1600, 1997.
151. Di Iorio R, Marinoni E, Letizia C, Gazzolo D, Lucchini C, Cosmi EV. Adrenomedullin is increased in the fetoplacental circulation in intrauterine growth restriction with abnormal umbilical artery waveforms. Am J Obstet Gynecol, **182** : 650-654, 2000.
152. Marinoni E, Di Iorio R, Letizia C, Villaccio B, Scucchi L, Cosmi EV. Immunoreactive adrenomedullin in human fetoplacental tissues. Am J Obstet Gynecol, **179** : 784-787, 1998.
153. Kanenishi K, Kuwabara H, Ueno M, Sato C, Sakamoto H, Hata T. Change of adrenomedullin concentrations in plasma and amniotic fluid, and human placental adrenomedullin expression with advancing gestation. Placenta, **22** : 244-250, 2001.
154. Knerr I, Dachert C, Beinder E, Metzler M, Dotsch J, Repp R, Rascher W. Adrenomedullin, calcitonin gene-related peptide and their receptors : evidence for a decreased placental mRNA content in preeclampsia and HELLP syndrome. Eur J Obstet Gynecol Reprod Biol, **101** : 47-53, 2002.
155. Jerat S, Morrish DW, Davidge ST, Kaufman S. Effect of adrenomedullin on placental arteries in normal and preeclamptic pregnancies. Hypertension, **37** : 227-231, 2001.
156. Spencer K. Evaluation of an assay of the free beta-subunit of choriogonadotropin and its potential value in screening for Down's syndrome. Clin Chem, **37** : 809-814, 1991.
157. Said ME, Campbell DM, Azzam ME, MacGillivray I. Beta-human chorionic gonadotrophin levels before and after the development of pre-eclampsia. Br J Obstet Gynaecol, **91** : 772-775, 1984.
158. Lee IS, Chung DY, Cole LA, Copel JA, Isozaki T, Hsu CD. Elevated serum nicked and urinary beta-core fragment hCG in preeclamptic pregnancies. Obstet Gynecol, **90** : 889-892, 1997.
159. Lieppman RE, Williams MA, Cheng EY, Resta R, Zingheim R, Hickok DE, Luthy DA. An association between elevated levels of human chorionic gonadotropin in the midtrimester and adverse pregnancy outcome. Am J Obstet Gynecol, **168** : 1852-1856, 1993.
160. Hsu CD, Chan DW, Iriye B, Johnson TR, Hong SF, Repke JT. Elevated serum human chorionic gonadotropin as evidence of secretory response in severe preeclampsia. Am J Obstet Gynecol, **170** : 1135-1138, 1994.
161. Liu DF, Dickerman LH, Redline RW. Pathologic findings in pregnancies with unexplained increases in midtrimester maternal serum human chorionic gonadotropin levels. Am J Clin Pathol, **111** : 209-215, 1999.
162. Petit A, Geoffroy P, Belisle S. Expression of G proteins in human placentas from pregnancies complicated by gestational hypertension. Life Sci, **60** : 953-960, 1997.
163. Muller F, Savey L, Le Fiblec B, Bussieres L, Ndayizamba G, Colau JC, Giraudet P. Maternal serum human chorionic gonadotropin level at fifteen weeks is a predictor for preeclampsia. Am J Obstet Gynecol, **175** : 37-40, 1996.
164. Lambert-Messerlian GM, Silver HM, Petraglia F, Luisi S, Pezzani I, Maybruck WM, Hogge WA, Hanley-Yanez K, Roberts JM, Neveux LM, Canick JA. Second-trimester levels of maternal serum human chorionic gonadotropin and inhibin a as predictors of

preeclampsia in the third trimester of pregnancy. J Soc Gynecol Investig, **7**: 170-174, 2000.
165. Davidson EJ, Riley SC, Roberts SA, Shearing CH, Groome NP, Martin CW. Maternal serum activin, inhibin, human chorionic gonadotrophin and alpha-fetoprotein as second trimester predictors of pre-eclampsia. Br J Obstet Gynaecol, **110**: 46-52, 2003.
166. Vaillant P, David E, Constant I, Athmani B, Devulder G, Fievet P, Gondry J, Boulanger JC, Fardelone P, Fournier A. Validity in nulliparas of increased beta-human chorionic gonadotrophin at mid-term for predicting pregnancy-induced hypertension complicated with proteinuria and intrauterine growth retardation. Nephron, **72**: 557-563, 1996.
167. Ashour AM, Lieberman ES, Haug LE, Repke JT. The value of elevated second-trimester beta-human chorionic gonadotropin in predicting development of preeclampsia. Am J Obstet Gynecol, **176**: 438-442, 1997.
168. Bahado-Singh RO, Oz U, Isozaki T, Seli E, Kovanci E, Hsu CD, Cole L. Midtrimester urine human chorionic gonadotropin beta-subunit core fragment levels and the subsequent development of pre-eclampsia. Am J Obstet Gynecol, **179**: 738-741, 1998.
169. Luckas M, Hawe J, Meekins J, Neilson J, Walkinshaw S. Second trimester serum free beta human chorionic gonadotrophin levels as a predictor of pre-eclampsia. Acta Obstet Gynecol Scand, **77**: 381-384, 1998.
170. Pouta AM, Hartikainen AL, Vuolteenaho OJ, Ruokonen AO, Laatikainen TJ, Midtrimester N-terminal proatrial natriuretic peptide, free beta hCG, and alpha-fetoprotein in predicting preeclampsia. Obstet Gynecol, **91**: 940-944, 1998.
171. Heikkila A, Makkonen N, Heinonen S, Kirkinen P. Elevated maternal serum hCG in the second trimester increases prematurity rate and need for neonatal intensive care in primiparous preeclamptic pregnancies. Hypertens Pregnancy, **20**: 99-106, 2001.
172. Shenhav S, Gemer O, Sassoon E, Volodarsky M, Peled R, Segal S. Mid-trimester triple test levels in early and late onset severe pre-eclampsia. Prenat Diagn, **22**: 579-582, 2002.
173. Lee LC, Sheu BC, Shau WY, Liu DM, Lai TJ, Lee YH, Huang SC. Mid-trimester beta-hCG levels incorporated in a multifactorial model for the prediction of severe pre-eclampsia. Prenat Diagn, **20**: 738-743, 2000.
174. Burger HG, Igarashi M. Inhibin: definition and nomenclature, including related substances. Endocrinology, **122**: 1701-1702, 1988.
175. Massague J. The transformimg growth factor-b family. Annu Rev Cell Biol, **6**: 597-641, 1990.
176. Petraglia F, Garuti GC, Calza L, Roberts V, Giardino L, Genazzani AR, Vale W, Meunier H. Inhibin subunits in human placenta: localization and messenger ribonucleic acid levels during pregnancy. Am J Obstet Gynecol, **165**: 750-758, 1991.
177. Petraglia F, Calza L, Garuti GC, Abrate M, Giardino L, Genazzani AR, Vale W, Meunier H. Presence and synthesis of inhibin subunits in human decidua. J Clin Endocrinol Metab, **71**: 487-492, 1990.
178. Petraglia F, Anceschi MM, Calza L, Garuti GC, Fusaro P, Giardino L, Genazzani AR, Vale W. Inhibin and activin in human fetal membranes: evidence for a local effect on prostaglandin release. J Clin Endocrinol Metab, **77**: 542-548, 1993.
179. Petraglia F, Sawchenko P, Lim AT, Rivier J, Vale W. Localization, secretion, and action of inhibin in human placenta. Science, **237**: 187-189, 1987
180. Muttukrishna S, George L, Fowler PA, Groome NP, Knight PG. Measurement of serum concentrations of inhibin-A (alpha-beta A dimer) during human pregnancy. Clin Endocrinol (Oxf), **42**: 391-397, 1995.
181. Petraglia F, Aguzzoli L, Gallinelli A, Florio P, Zonca M, Benedetto C, Woodruff K. Hypertension in pregnancy: changes in activin A maternal serum concentration. Placenta, **16**: 447-454, 1995.
182. Muttukrishna S, Knight PG, Groome NP, Redman CW, Ledger WL. Activin A and inhibin A as possible endocrine markers for pre-eclampsia. Lancet, **349**: 1285-1288,

1997.
183. Silver HM, Lambert-Messerlian GM, Star JA, Hogan J, Canick JA. Comparison of maternal serum total activin A and inhibin A in normal, preeclamptic, and nonproteinuric gestationally hypertensive pregnancies. Am J Obstet Gynecol, **180**: 1131-1137, 1999.
184. Florio P, Ciarmela P, Luisi S, Palumbo MA, Lambert-Messerlian G, Severi FM, Petraglia F. Pre-eclampsia with fetal growth restriction: placental and serum activin A and inhibin A levels. Gynecol Endocrinol, **16**: 365-372, 2002.
185. Keelan JA, Taylor R, Schellenberg JC, Groome NP, Mitchell MD, North RA. Serum activin A, inhibin A, and follistatin concentrations in preeclampsia or small for gestational age pregnancies. Obstet Gynecol, **99**: 267-274, 2002.
186. Grobman WA, Wang, EY. Serum levels of activin A and inhibin A and the subsequent development of preeclampsia. Obstet Gynecol, **96**: 390-394, 2000.
187. Muttukrishna S, North RA, Morris J, Schellenberg JC, Taylor RS, Asselin J, Ledger W, Groome N, Redman CW. Serum inhibin A and activin A are elevated prior to the onset of pre-eclampsia. Hum Reprod, **15**: 1640-1645, 2000.
188. Bersinger NA, Groome N, Muttukrishna S. Pregnancy-associated and placental proteins in the placental tissue of normal pregnant women and patients with pre-eclampsia at term. Eur J Endocrinol, **147**: 785-793, 2002.
189. Cuckle H, Sehmi I, Jones R. Maternal serum inhibin A can predict pre-eclampsia. Br J Obstet Gynaecol, **105**: 1101-1103, 1998.
190. Aquilina J, Barnett A, Thompson O, Harrington K. Second-trimester maternal serum inhibin A concentration as an early marker for preeclampsia. Am J Obstet Gynecol, **181**: 131-136, 1999.
191. Sebire NJ, Roberts L, Noble P, Wallace E, Nicolaides KH. Raised maternal serum inhibin A concentration at 10 to 14 weeks of gestation is associated with pre-eclampsia. Br J Obstet Gynaecol, **107**: 795-797, 2000.
192. Aquilina J, Thompson O, Thilaganathan B, Harrington K. Improved early prediction of pre-eclampsia by combining second-trimester maternal serum inhibin-A and uterine artery Doppler. Ultrasound Obstet Gynecol, **17**: 477-484, 2001.
193. Florio P, Reis FM, Pezzani I, Luisi S, Severi FM, Petraglia F. The addition of activin A and inhibin A measurement to uterine artery Doppler velocimetry to improve the early prediction of pre-eclampsia. Ultrasound Obstet Gynecol, **21**: 165-169, 2003.
194. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature, **372**: 425-432, 1994.
195. Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet, **12**: 318-320, 1996.
196. Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, Nishimura H, Yoshimasa Y, Tanaka I, Mori T, Nakao K. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat Med, **3**: 1029-1033, 1997.
197. Highman TJ, Friedman JE, Huston LP, Wong WW, Catalano PM. Longitudinal changes in maternal serum leptin concentrations, body composition, and resting metabolic rate in pregnancy. Am J Obstet Gynecol, **178**: 1010-1015, 1998.
198. Lage M, Garcia-Mayor RV, Tome MA, Cordido F, Valle-Inclan F, Considine RV, Caro JF, Dieguez C, Casanueva FF. Serum leptin levels in women throughout pregnancy and the postpartum period and in women suffering spontaneous abortion. Clin Endocrinol, **50**: 211-216, 1999.
199. Hardie L, Trayhurn P, Abramovich D, Fowler P. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. Clin Endocrinol, **47**: 101-106, 1997.
200. Anim-Nyame N, Sooranna SR, Steer PJ, Johnson MR. Longitudinal analysis of maternal plasma leptin concentrations during normal pregnancy and pre-eclampsia. Hum Reprod, **15**: 2033-2036, 2000.

201. Teppa RJ, Ness RB, Crombleholme WR, Roberts JM. Free leptin is increased in normal pregnancy and further increased in preeclampsia. Metabolism, **49**: 1043-1048, 2000.
202. Poston L. Leptin and preeclampsia. Semin Reprod Med, **20**: 131-138, 2002.
203. Laml T, Preyer O, Hartmann BW, Ruecklinger E, Soeregi G, Wagenbichler P. Decreased maternal serum leptin in pregnancies complicated by preeclampsia. J Soc Gynecol Investig, **8**: 89-93, 2001.
204. Mise H, Sagawa N, Matsumoto T, Yura S, Nanno H, Itoh H, Mori T, Masuzaki H, Hosoda K, Ogawa Y, Nakao K. Augmented placental production of leptin in preeclampsia: possible involvement of placental hypoxia. J Clin Endocrinol Metab, **83**: 3225-3229, 1998.
205. Williams MA, Havel PJ, Schwartz MW, Leisenring WM, King IB, Zingheim RW, Zebelman AM, Luthy DA. Pre-eclampsia disrupts the normal relationship between serum leptin concentrations and adiposity in pregnant women. Paediatr Perinat Epidemiol, **13**: 190-204, 1999.
206. Page NM, Woods RJ, Gardiner SM, Lomthalsong K, Gladwell RT, Butlin DJ, Manyonda IT, Lowry PJ. Excessive placental secretion of neurokinin B during the third trimester causes pre-eclampsia. Nature, **405**: 797-800, 2000.
207. Patak EN, Pennefather JN, Story ME. Proceedings of the Australian Physiological and Pharmacological Society Symposium Tachykinins: The Challenge Continues. Effects Of Tachykinins On Uterine Smooth Muscle. Clin Exp Pharmacol Physiol, **27**: 922-927, 2000.
208. Mastrangelo D, Mathison R, Huggel HJ, Dion S, D'Orleans-Juste P, Rhaleb NE, Drapeau G, Rovero P, Regoli D. The rat isolated portal vein: a preparation sensitive to neurokinins, particularly neurokinin-B. Eur J Pharmacol, **134**: 321-326, 1987.
209. D'Orleans-Juste P, Claing A, Telemaque S, Warner TD, Regoli D. Neurokinins produce selective venoconstriction via NK-3 receptors in the rat mesenteric vascular bed. Eur J Pharmacol, **204**: 329-334, 1991.
210. Willis WD. Role of neurotransmitters in sensitization of pain responses. Ann NY Acad Sci, **933**: 142-156, 2001.
211. Herbert MK, Holzer P. Neurogenic inflammation. I. Basic mechanisms, physiology and pharmacology. Anasthesiol Intensivmed Notfallmed Schmerzther, **37**: 314-325, 2002.
212. Joachim RA, Hildebrandt M, Oder J, Klapp BF, Arck PC. Murine stress-triggered abortion is mediated by increase of CD8+ TNF-alpha+ decidual cells via substance P. Am J Reprod Immunol, **45**: 303-309, 2001.
213. Thompson GW, Hoover DB, Ardell JL, Armour JA. Canine intrinsic cardiac neurons involved in cardiac regulation possess NK1, NK2, and NK3 receptors. Am J Physiol, **275**: R1683-1689, 1998.
214. Page NM, Woods RJ, Lowry PJ. A regulatory role for neurokinin B in placental physiology and pre-eclampsia. Regul Peptides, **98**: 97-104, 2001.
215. Schlembach D, Scalera F, Fischer T, Marx SG, Beinder E, Garfield RE. Neurokinin B peptide serum levels are higher in normotensive pregnant women than in preeclamptic pregnant women. Am J Obstet Gynecol (accepted for publication).
216. Li XM, Moutquin JM, Deschenes J, Bourque L, Marois M, Forest JC. Increased immunohistochemical expression of neutral metalloendopeptidase (enkephalinase, EC-3.4.24.11) in villi of the human placenta with preeclampsia. Placenta, **16**: 435-445, 1995.
217. Lorentzen B, Drevon CA, Endresen MJ, Henriksen T. Fatty acid pattern of esterified and free fatty acids in sera of women with normal and pre-eclamptic pregnancy. Br J Obstet Gynaecol, **102**: 530-537, 1995.
218. Kharb S, Gulati N, Singh V, Singh GP. Superoxide anion formation and glutathione levels in patients with preeclampsia. Gynecol Obstet Invest, **49**: 28-30, 2000.
219. Hubel CA, McLaughlin MK, Evans RW, Hauth BA, Sims CJ, Roberts JM.: Fasting serum triglycerides, free fatty acids, and malondialdehyde are increased in pre-eclamp-

sia, are positively correlated, and decrease within 48 hours post partum. Am J Obstet Gynecol, **174**: 975-982, 1996.
220. Barden A, Ritchie J, Walters B, Michael C, Rivera J, Mori T, Croft K, Beilin L. Study of plasma factors associated with neutrophil activation and lipid peroxidation in preeclampsia. Hypertension, **38**: 803-808, 2001.
221. Hubel CA, Kozlov AV, Kagan VE, Evans RW, Davidge ST, McLaughlin MK, Roberts JM. Decreased transferrin and increased transferrin saturation in sera of women with preeclampsia: implications for oxidative stress. Am J Obstet Gynecol, **175**: 692-700, 1996.
222. Mikhail MS, Anyaegbunam A, Garfinkel D, Palan PR, Basu J, Romney SL. Preeclampsia and antioxidant nutrients: decreased plasma levels of reduced ascorbic acid, alpha-tocopherol, and beta-carotene in women with preeclampsia. Am J Obstet Gynecol, **171**: 150-157, 1994.
223. Hubel CA, Kagan VE, Kisin ER, McLaughlin MK, Roberts JM. Increased ascorbate radical formation and ascorbate depletion in plasma from women with preeclampsia: implications for oxidative stress. Free Radic Biol Med, **23**: 597-609, 1997.
224. Madazli R, Benian A, Aydin S, Uzun H, Tolun N. The plasma and placental levels of malondialdehyde, glutathione and superoxide dismutase in pre-eclampsia. J Obstet Gynaecol, **22**: 477-480, 2002.
225. Schiff E, Friedman SA, Stampfer M, Kao L, Barrett PH, Sibai BM. Dietary consumption and plasma concentrations of vitamin E in pregnancies complicated by preeclampsia. Am J Obstet Gynecol, **175**: 1024-1028, 1996.
226. Ben-Haroush A, Harell D, Hod M, Bardin R, Kaplan B, Orvieto R, Bar J. Plasma levels of vitamin E in pregnant women prior to the development of preeclampsia and other hypertensive complications. Gynecol Obstet Invest, **54**: 26-30, 2002.
227. Scalera F, Fischer T, Schlembach D, Beinder E. Serum from healthy pregnant women reduces oxidative stress in human umbilical vein endothelial cells. Clin Sci, **103**: 53-57, 2002.
228. Walsh SW, Wang Y. Trophoblast and placental villous core production of lipid peroxides, thromboxane, and prostacyclin in preeclampsia. J Clin Endocrinol Metab, **80**: 1888-1893, 1995.
229. Walsh SW, Vaughan JE, Wang Y, Roberts LJ 2nd. Placental isoprostane is significantly increased in preeclampsia. FASEB J, **14**: 1289-1296, 2000.
230. Chappell LC, Seed PT, Kelly FJ, Briley A, Hunt BJ, Charnock-Jones DS, Mallet A, Poston L. Vitamin C and E supplementation in women at risk of preeclampsia is associated with changes in indices of oxidative stress and placental function. Am J Obstet Gynecol, **187**: 777-784, 2002.
231. Scott JR, Beer AA. Immunologic aspects of preeclampsia. Am J Obstet Gynecol, **125**: 418-427, 1976.
232. Dekker GA, Sibai BM. The immunology of preeclampsia. Semin Perinatology, **23**: 24-33, 1999.
233. Strickland DM, Guzick DS, Cox K, Gant NF, Rosenfeld CR. The relationship between abortion in the first pregnancy and development of pregnancy-induced hypertension in the subsequent pregnancy. Am J Obstet Gynecol, **154**: 146-148, 1986.
234. Need JA. Preeclampsia in pregnancies by different fathers. Br Med J, **11**: 548-549, 1975.
235. Robillard PY, Hulsey TC, Alexander GR, Keenan A, de Caunes F, Papiernik E. Paternity patterns and risk of preeclampsia in the last pregnancy in multiparae. J Reprod Immunol, **24**: 1-12, 1993.
236. Need JA, Bell B, Meffin E, Jones WR. Preeclampsia in pregnancies from donor inseminations. J Reprod Immunol, **5**: 329-338, 1983.
237. Smith GN, Walker M, Tessier JL, Millar KG. Increased incidence of preeclampsia in women conceiving by intrauterine insemination with donor versus partner sperm for

treatment of primary infertility. Am J Obstet Gynecol, **177**: 455-458, 1997.
238. Clark DA. Does immunological intercourse prevent preeclampsia? Lancet, **344**: 969-970, 1994.
239. Robertson SA, Mau VJ, Hudson SN, Tremellen KP. Cytokine-leukocyte networks and the establishment of pregnancy. Am J Reprod Biol, **37**: 438-442, 1997.
240. Colbern GT, Chiang MH, Main EK. Expression of the nonclassic histocompatibility antigen HLA-G by preeclamptic placenta. Am J Obstet Gynecol, **170**: 1244-1250, 1994.
241. Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH, Fisher SJ. Human cytotrophoblast differentiation/invasion is normal in preeclampsia. Am J Pathol, **151**: 1809-1818, 1997.
242. Arngrimsson R, Bjornsson S, Geirsson RT, Bjornsson H, Walker JJ, Snaedal G. Genetic and familial predisposition to eclampsia and pre-eclampsia in a defined population. Br J Obstet Gynaecol, **97**: 762-769, 1990.
243. Sutherland A, Cooper DW, Howie PW, Liston WA, MacGillivray I. Genetic and familial predisposition to eclampsia and pre-eclampsia in a defined population. Br J Obstet Gynaecol, **97**: 762-769, 1990.
244. Arngrimsson R, Bjornsson H, Geirsson RT. Analysis of different inheritance patterns in preeclampsia/eclampsia syndrome. Hypertens Pregnancy, **14**: 27-38, 1995.
245. Haig D. Genetic conflicts in human pregnancy. Q Rev Biol, **68**: 495-532, 1993.
246. Dekker GA, Robillard PY, Hulsey TC. Immune maladaptation in the etiology of preeclampsia: a review of corroborative epidemiologic studies. Obstet Gynecol Surv, **53**: 377-382, 1998.
247. Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie-Nielsen E, Irgens LM. Fetal and maternal contributions to risk of pre-eclampsia: population based study. BMJ, **316**: 1343-1347, 1998.
248. Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, Varner MW. Paternal and maternal components of the predisposition to preeclampsia. N Engl J Med, **344**: 867-872, 2001.
249. Gao M, Nakabayashi M, Sakura M, Takeda Y. The imbalance of plasminogen activators and inhibitor in preeclampsia. J Obstet Gynaecol Res, **22**: 9-16, 1996.
250. Bel L, Santos-Silva A, Rumley A, Lowe G, Pereira-Leite L, Quintanilha A, Rebelo I. Elevated tissue plasminogen activator as a potential marker of endothelial dysfunction in pre-eclampsia: correlation with proteinuria. Br J Obstet Gynaecol, **109**: 1250-1255, 2002.
251. Estelles A, Gilabert J, Grancha S, Yamamoto K, Thinnes T, Espana F, Aznar J, Loskutoff DJ. Abnormal expression of type 1 plasminogen activator inhibitor and tissue factor in severe preeclampsia. Thromb Haemost, **79**: 500-508, 1998.
252. Clausen T, Djurovic S, Reseland JE, Berg K, Drevon CA, Henriksen T. Altered plasma concentrations of leptin, transforming growth factor-beta(1) and plasminogen activator inhibitor type 2 at 18 weeks of gestation in women destined to develop pre-eclampsia. Circulating markers of disturbed placentation? Placenta, **23**: 380-385, 2002.
253. Deng L, Bremme K, Hansson LO, Blomback M. Plasma levels of von Willebrand factor and fibronectin as markers of persisting endothelial damage in preeclampsia. Obstet Gynecol, **84**: 941-945, 1994.
254. Kim YJ, Williamson RA, Murray JC, Andrews J, Pietscher JJ, Peraud PJ, Merrill DC. Genetic susceptibility to preeclampsia: roles of cytosineto-thymine substitution at nucleotide 677 of the gene for methylenetetrahydrofolate reductase, 68-base pair insertion at nucleotide 844 of the gene for cystathionine beta-synthase, and factor V Leiden mutation. Am J Obstet Gynecol, **184**: 1211-1217, 2001.
255. Livingston JC, Barton JR, Park V, Haddad B, Phillips O, Sibai BM. Maternal and fetal inherited thrombophilias are not related to the development of severe preeclampsia. Am J Obstet Gynecol, **185**: 153-157, 2001.
256. Morrison ER, Miedzybrodzka ZH, Campbell DM, Haites NE, Wilson BJ, Watson MS,

Greaves M, Vickers MA. Prothrombotic genotypes are not associated with pre-eclampsia and gestational hypertension: results from a large population-based study and systematic review. Thromb Haemost, **87**: 779-785, 2002.
257. Carmel R. Ethnic and racial factors in cobalamin metabolism and its disorders. Semin Haematol, **36**: 88-100, 1999.
258. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature, **369**: 64-67, 1994.
259. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet, **346**: 1133-1134, 1995.
260. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood, **88**: 3698-3703, 1996.
261. Rosendaal FR, Doggen CJ, Zivelin A, Arruda VR, Aiach M, Siscovick DS, Hillarp A, Watzke HH, Bernardi F, Cumming AM, Preston FE, Reitsma PH. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost, **79**: 706-708, 1998.
262. McCully KS. Homocysteine and vascular disease. Nat Med, **2**: 386-389, 1996.
263. Leeda M, Riyazi N, de Vries JI, Jakobs C, van Geijn HP, Dekker GA. Effects of folic acid and vitamin B6 supplementation on women with hyperhomocysteinemia and a history of preeclampsia or fetal growth restriction. Am J Obstet Gynecol, **179**: 135-139, 1998.
264. Powers RW, Minich LA, Lykins DL, Ness RB, Crombleholme WR, Roberts JM. Methylenetetrahydrofolate reductase polymorphism, folate, and susceptibility to preeclampsia. J Soc Gynecol Investig, **6**: 74-79, 1999.
265. Ward K, Hata A, Jeunemaitre X, Helin C, Nelson L, Namikawa C, Farrington PF, Ogasawara M, Suzumori K, Tomoda S, et al. A molecular variant of angiotensinogen associated with preeclampsia. Nat Genet, **4**: 59-61, 1993.
266. Arngrimsson R, Purandare S, Connor M, Walker JJ, Bjornsson S, Soubrier F, Kotelevtsev YV, Geirsson RT, Bjornsson H. Angiotensinogen. A candidate gene involved in preeclampsia? Nat Genet, **4**: 114-115, 1993.
267. Morgan L, Crawshaw S, Baker PN, Broughton Pipkin F, Kalsheker N. Maternal and fetal angiotensinogen gene allele sharing in pre-eclampsia. Br J Obstet Gynaecol, **106**: 244-251, 1999.
268. Morgan T, Craven C, Lalouel JM, Ward K. Angiotensinogen Thr235 variant is associated with abnormal physiologic change of the uterine spiral arteries in first-trimester decidua. Am J Obstet Gynecol, **180**: 95-102, 1999.
269. Morgan L, Crawshaw S, Baker PN, Brookfield JF, Broughton Pipkin F, Kalsheker N. Distortion of maternal-fetal angiotensin II type 1 receptor allele transmission in pre-eclampsia. J Med Genet, **35**: 632-636, 1998.
270. Arngrimsson R, Hayward C, Nadaud S, Baldursdottir A, Walker JJ, Liston WA, Bjarnadottir RI, Brock DJ, Geirsson RT, Connor JM, Soubrier F. Evidence for a familial pregnancy-induced hypertension locus in the eNOS-gene region. Am J Hum Genet, **61**: 354-362, 1997.
271. Guo G, Lade JA, Wilton AN, Moses EK, Grehan M, Fu Y, Qiu H, Cooper DW, Brennecke SP. Genetic susceptibility to pre-eclampsia and chromosome 7q36. Hum Genet, **105**: 641-647, 1999.
272. Yoshimura M, Yasue H, Nakayama M, Shimasaki Y, Sumida H, Sugiyama S, Kugiyama K, Ogawa H, Ogawa Y, Saito Y, Miyamoto Y, Nakao K. A missense Glu298Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. Hum Genet, **103**: 65-69, 1998.
273. Yoshimura T, Yoshimura M, Tabata A, Shimasaki Y, Nakayama M, Miyamoto Y, Saito Y, Nakao K, Yasue H, Okamura H. Association of the missense Glu298Asp variant of the endothelial nitric oxide synthase gene with severe preeclampsia. J Soc Gynecol

Investig, **7**: 238-241, 2000.
274. Kobashi G, Yamada H, Ohta K, Kato E, Ebina Y, Fujimoto S. Endothelial nitric oxide synthase gene (NOS3) variant and hypertension in pregnancy. Am J Med Genet, **103**: 241-244, 2001.
275. Savvidou MD, Vallance PJT, Nicolaides KH, Hingorani AD. Endothelial nitric oxide synthase gene polymorphism and maternal vascular adaptation to pregnancy. Hypertension, **38**: 1289-1293, 2001.
276. Tempfer CB, Dorman K, Deter RL, O'Brien WE, Gregg AR. An endothelial nitric oxide synthase gene polymorphism is associated with preeclampsia. Hypertens Pregnancy, **20**: 107-118, 2001.
277. Lewis I, Lachmeijer G, Downing S, Dekker G, Glazebrook C, Clayton D, Morris NH, O'Shaughnessy KM. Failure to detect linkage of preeclampsia to the region of the NOS3 locus on chromosome 7q. Am J Hum Genet, **64**: 310-314, 1999.
278. Arngrimsson R, Sigurardottir S, Frigge ML, Bjarnadottir RI, Jonsson T, Stefansson H, Baldursdottir A, Einarsdottir AS, Palsson B, Snorradottir S, Lachmeijer AM, Nicolae D, Kong A, Bragason BT, Gulcher JR, Geirsson RT, Stefansson K. A genome-wide scan reveals a maternal susceptibility locus for pre-eclampsia on chromosome 2p13. Hum Mol Genet, **8**: 1799-1805, 1999.
279. Hubel CA, Roberts JM, Ferrell RE. Association of pre-eclampsia with common coding sequence variations in the lipoprotein lipase gene. Clin Genet, **56**: 289-296, 1999.
280. Kim YJ, Williamson RA, Chen K, Smith JL, Murray JC, Merrill DC. Lipoprotein lipase gene mutations and the genetic susceptibility of preeclampsia. Hypertension, **38**: 992-996, 2001.
281. Zusterzeel PL, Peters WH, Visser W, Hermsen KJ, Roelofs HM, Steegers EA. A polymorphism in the gene for microsomal epoxide hydrolase is associated with pre-eclampsia. J Med Genet, **38**: 234-237, 2001.
282. Zusterzeel PL, Visser W, Peters WH, Merkus HW, Nelen WL, Steegers EA. Polymorphism in the glutathione S-transferase P1 gene and risk for preeclampsia. Obstet Gynecol, **96**: 50-54, 2000.
283. Humphrey KE, Harrison GA, Cooper DW, Wilton AN, Brennecke SP, Trudinger BJ. HLA-G deletion polymorphism and pre-eclampsia/eclampsia. Br J Obstet Gynaecol, **102**: 707-710, 1995.
284. Aldrich C, Verp MS, Walker MA, Ober C. A null mutation in HLA-G is not associated with preeclampsia or intrauterine growth retardation. J Reprod Immunol, **47**: 41-48, 2000.
285. O'Brien M, McCarthy T, Jenkins D, Paul P, Dausset J, Carosella ED, Moreau P. Altered HLA-G transcription in pre-eclampsia is associated with allele specific inheritance: possible role of the HLA-G gene in susceptibility to the disease. Cell Mol Life Sci, **58**: 1943-1949, 2001.
286. Chen G, Wilson R, Wang SH, Zheng HZ, Walker JJ, McKillop JH. Tumour necrosis factor-alpha (TNF-alpha) gene polymorphism and expression in pre-eclampsia. Clin Exp Immunol, **104**: 154-159, 1996.
287. Dizon-Townson DS, Major H, Ward K. A promoter mutation in the tumor necrosis factor alpha gene is not associated with preeclampsia. J Reprod Immunol, **38**: 55-61, 1998.
288. Livingston JC, Park V, Barton JR, Elfering S, Haddad B, Mabie WC, Quasney M, Sibai BM. Lack of association of severe preeclampsia with maternal and fetal mutant alleles for tumor necrosis factor alpha and lymphotoxin alpha genes and plasma tumor necrosis factor alpha levels. Am J Obstet Gynecol, **184**: 1273-1277, 2001.
289. Lachmeijer AM, Crusius JB, Pals G, Dekker GA, Arngrimsson R, ten Kate LP. Polymorphisms in the tumor necrosis factor and lymphotoxin-alpha gene region and preeclampsia. Obstet Gynecol, **98**: 612-619, 2001.
290. Hefler LA, Tempfer CB, Gregg AR. Polymorphisms within the interleukin-1 beta gene

cluster and preeclampsia. Obstet Gynecol, **97**: 664-668, 2001.
291. Lachmeijer AM, Nosti-Escanilla MP, Bastiaans EB, Pals G, Sandkuijl LA, Kostense PJ, Aarnoudse JG, Crusius JB, Pena AS, Dekker GA, Arngrimsson R, ten Kate LP. Linkage and association studies of IL1B and IL1RN gene polymorphisms in preeclampsia. Hypertens Pregnancy, **21**: 23-38, 2002.
292. Risau W. Mechanisms of angiogenesis. Nature, **386**: 671-674, 1997.
293. Caniggia I, Winter J, Lye SJ, Post M. Oxygen and placental development during the first trimester: implications for the pathophysiology of pre-eclampsia. Placenta, **21** (Suppl A): S25-S30, 2000.
294. Krebs C, Macara LM, Leiser R, Bowman AW, Greer IA, Kingdom JC. Intrauterine growth restriction with absent end-diastolic flow velocity in the umbilical artery is associated with maldevelopment of the placental terminal villous tree. Am J Obstet Gynecol, **175**: 1534-1542, 1996.
295. Steiner H, Staudach A, Spitzer D, Schaffer KH, Gregg A, Weiner CP. Growth deficient fetuses with absent or reversed umbilical artery end-diastolic flow are metabolically compromised. Early Hum Dev, **41**: 1-9, 1995.
296. Cooper JC, Sharkey AM, Charnock-Jones DS, Palmer CR, Smith SK. VEGF mRNA levels in placentae from pregnancies complicated by pre-eclampsia. Br J Obstet Gynaecol, 1191-1196, 1996.
297. Khaliq A, Dunk C, Jiang J, Shams M, Li XF, Acevedo C, Weich H, Whittle M, Ahmed A. Hypoxia down-regulates placenta growth factor, whereas fetal growth restriction up-regulates placenta growth factor expression: molecular evidence for "placental hyperoxia" in intrauterine growth restriction. Lab Invest, **79**: 151-170, 1999.
298. Ahmed A, Dunk C, Ahmad S, Khaliq A. Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF) and soluble Flt-1 by oxygen - a review. Placenta, **21** (Suppl A): S16-S24, 2000.
299. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, Lammoglia R, Charnock-Jones DS. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. Biol Reprod, **59**: 1540-1548, 1998.
300. Baker PN, Krasnow J, Roberts JM, Yeo KT. Elevated serum levels of vascular endothelial growth factor in patients with preeclampsia. Obstet Gynecol, **86**: 815-821, 1995.
301. Kupferminc MJ, Daniel Y, Englender T, Baram A, Many A, Jaffa AJ, Gull I, Lessing JB. Vascular endothelial growth factor is increased in patients with preeclampsia. Am J Reprod Immunol, **38**: 302-306, 1997.
302. Lyall F, Young A, Boswell F, Kingdom JC, Greer IA. Placental expression of vascular endothelial growth factor in placentae from pregnancies complicated by pre-eclampsia and intrauterine growth restriction does not support placental hypoxia at delivery. Placenta, **18**: 269-276, 1997.
303. Wu HM, Huang Q, Yuan Y, Granger HJ. VEGF induces NO-dependent hyperpermeability in coronary venules. Am J Physiol, **271**: H2735-H2739, 1996.
304. Hayman R, Brockelsby J, Kenny L, Baker P. Preeclampsia: the endothelium, circulating factor(s) and vascular endothelial growth factor. J Soc Gynecol Investig, **6**: 3-10, 1999.
305. Brockelsby JC, Anthony FW, Johnson IR, Baker PN. The effects of vascular endothelial growth factor on endothelial cells: a potential role in preeclampsia. Am J Obstet Gynecol, **182**: 176-183, 2000.
306. Ong CY, Liao AW, Cacho AM, Spencer K, Nicolaides KH. First-trimester maternal serum levels of placenta growth factor as predictor of preeclampsia and fetal growth restriction. Obstet Gynecol, **98**: 608-611, 2001.
307. Tidwell SC, Ho HN, Chiu WH, Torry RJ, Torry DS. Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia. Am J Obstet Gynecol, **184**: 1267-1272, 2001.
308. Tjoa ML, van Vugt JM, Mulders MA, Schutgens RB, Oudejans CB, van Wijk IJ. Plasma

placental growth factor levels in midtrimester pregnancies. Obstet Gynecol, **98**: 600-607, 2001.

309. Vuorela P, Matikainen MT, Kuusela P, Ylikorkala O, Alitalo K, Halmesmaki E. Endothelial tie receptor antigen in maternal and cord blood of healthy and preeclamptic subjects. Obstet Gynecol, **92**: 179-183, 1998.
310. Rasmussen S, Irgens LM. Fetal growth and body proportion in preeclampsia. Obstet Gynecol, **101**: 575-583, 2003.