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Role of Placenta in Preeclampsia

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Preeclampsia, which manifests itself as hypertension, proteinuria, and edema in pregnancy, requires the presence of trophoblast tissue but not a fetus. It is characterized by abnormal trophoblast invasion of the spiral arteries of the decidua and myometrium leading to a failure to establish an adequate uteroplacental blood flow and, therefore, is thought to give rise to relatively hypoxic trophoblast tissue. This, in turn, may promote an exaggerated state of oxidative stress in the placenta. This hypoxia/oxidative stress may then further attenuate trophoblast invasion but also alters placental villous angiogenesis leading to a poorly developed fetoplacental vasculature with abnormal reactivity. Oxidative stress *per se* may also affect vascular reactivity, blood flow, and oxygen and nutrient delivery to the fetus, which ultimately may be compromised. The synthetic and transport functions of the syncytiotrophoblast may also be altered, and there is an increased rate of trophoblast apoptosis. The linkage among abnormal trophoblast invasion, trophoblast dysfunction, and the maternal disease remains unidentified. The presumptive humoral factor that is released by the preeclamptic placenta to cause maternal disease remains elusive. Current therapies to prevent preeclampsia aim toward preventing the maternal syndrome, not preventing the primary pathophysiology.

Key Words: Preeclampsia; placenta; trophoblast; oxidative stress; hypoxia.

Introduction

Preeclampsia is a disease of pregnancy, thought to be unique to humans, that manifests itself as a clinical syndrome of hypertension, proteinuria, and edema after 20 wk of gestation in a previously normotensive individual. The syndrome resolves after delivery, a fact that points to a prime role of the placenta in the etiology of the disease. Indeed, the presence of a fetus is not necessary for preeclampsia to develop—it occurs in molar pregnancy and removal of trophoblast resolves the disease. Preeclampsia is the leading cause of maternal morbidity and mortality in underdeveloped countries. However, in developed countries, such as the United States, with access to advanced health-care facilities, prompt delivery of women with preeclampsia limits maternal morbidity and mortality. The morbidity and mortality associated with preeclampsia in the United States is from iatrogenic preterm delivery of the neonate of a woman with preeclampsia.

Preeclampsia affects 7% of the low-risk nulliparous population *(1)* but rates of 15–20% are found in certain groups of patients, including those with multifetal gestations, chronic hypertension, pregestational diabetes, and previous preeclampsia *(2)*. This gives some clues to the etiology of the disease but also suggests that any individual's risk of developing preeclampsia is contributed to placental and maternal interactions *(3)*. The presence of a placenta is necessary and greater placental mass (multifetal gestations) increases risk, but individuals with preexisting maternal vascular disease (diabetes, previous preeclampsia, chronic hypertension) are at greater risk. Indeed, while pregnancy *per se* is a stress test for the maternal cardiovascular system, women who develop severe preeclampsia in pregnancy are at greater risk of developing hypertensive disorders later in life *(4)*.

Role of Placenta in Pregnancy

The placenta fulfills several critical roles in pregnancy. For example, as the interface between the fetus and mother, it serves to prevent rejection of the fetal allograft. In addition to its immune barrier function, the placenta, with its specialized epithelium, functions to transport gases, nutrients, and waste products. It also functions as a major synthetic organ for a variety of peptide and steroid hormones that regulate placental, fetal, and maternal systems. As stated, the presence of the placenta is necessary to cause preeclampsia. This suggests either that the placenta secretes a humoral factor that causes the maternal syndrome of preeclampsia (i.e., hypertension, proteinuria, and edema) or that maternal adaptation to the placenta sometimes results in preeclampsia. Dogma states that there is abnormal trophoblast invasion of the decidual and myometrial spiral arteries in preeclampsia that is directly related to the pathophysiology. The cause of this abnormal trophoblast invasion is still under active investigation. Correspondingly, this abnormal invasion may have consequences to overall placental development.

What is the role of the placenta in preeclampsia? In this review, I concentrate on first describing the events of nor-

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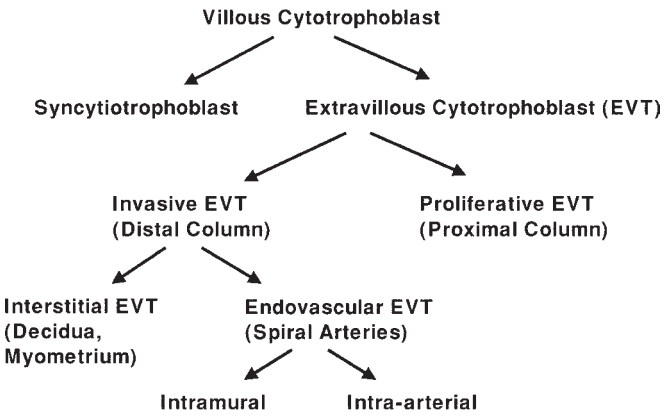


Fig. 1. Pathways of trophoblast differentiation.

mal trophoblast invasion and villous vasculogenesis and then the changes that are observed in preeclampsia, which may be related to the pathophysiology. Finally, I discuss the effect of these changes on placental function.

Trophoblast Invasion in Pregnancy

At the placental-maternal interface, villous cytotrophoblast stem cells differentiate toward extravillous trophoblast that invades the maternal decidua and myometrium (Fig. 1). The extravillous cytotrophoblast is found within the lumen of the spiral arteries (endovascular trophoblast), where it replaces the endothelium; around vascular structures (perivascular trophoblast); and throughout the decidua and myometrium (interstitial trophoblast). The source of endovascular trophoblast cells and route of invasion is disputed *(5)*. One view favors intravasation, in which spiral arteries are infiltrated by interstitial and perivascular trophoblast. An opposing view is of extravasation, in which endovascular trophoblast enters the spiral arteries from the intervillous space and migrates against the blood flow to displace the endothelium and infiltrate the vessel wall. The net result of endovascular invasion is conversion of the spiral arteries to flaccid low-resistance uteroplacental arteries. In preeclamptic pregnancies, a defect in endovascular trophoblast invasion of the spiral arteries has been clearly described *(6–9)* such that some vessels are not invaded at all, with others being superficially invaded, leading to a lack of the normal adaptation or physiologic change of uteroplacental vessels and a corresponding lack of the normal increase in blood flow to the intervillous space. However, interstitial trophoblast density and the approach of noncritical vascular structures by perivascular trophoblast has been found to be similar in preeclampsia and normal pregnancy *(10)*.

Regulation of Trophoblast Invasion

Invasion of the decidua and myometrium by extravillous trophoblast occurs in a carefully choreographed manner in which these cells express specific antigens defining their stage in the differentiation process and their role in invasion, including integrin cell–extracellular matrix (ECM) antigens *(11)*; matrix metalloproteinases (MMPs) *(12)*; signal transduction proteins such as focal adhesion kinase *(13)*; transforming growth factor-β 3 (TGF-β3) *(14)*; vascular endothelial growth factor (VEGF) and VEGF receptors *(15)*; and insulin like-growth factor-2 (IGF-2) *(16)*, which interacts with decidual IGF-binding protein-1. The critical role of some of these molecules in the invasive process has been demonstrated in vitro by manipulation, resulting in knockdown or loss of function *(11–13)*. These manipulations also mimic the supposed placental defect in preeclampsia, that of abnormal or shallow trophoblast invasion of the decidua, myometrium, and spiral arteries, where alterations in expression of integrins *(17,18)* and overexpression of TGF-β3 *(14)* have been found. This strongly suggests that defects in mechanisms regulating trophoblast differentiation/invasion are critical to the etiology of preeclampsia.

Role of Integrins and Cell Adhesion Molecules

Extravillous trophoblast cells secrete large amounts of heterogeneous ECM along the invasive pathway including fibronectin isoforms, collagen IV, laminin, vitronectin, and heparan sulfate. The extravillous trophoblast cells in this ECM then express specific ECM receptors or integrins *(19)*. The integrins are heterodimers that act as receptors for ECM molecules, and their critical role in invasion has mostly been gleaned by loss-of-function experiments. Proliferating trophoblast expresses mainly $\alpha_6\beta_1$ integrin, the receptor for laminin, and is found in the proximal parts of cell columns. Invasive trophoblast expresses $\alpha_5\beta_1$, a fibronectin receptor, and $\alpha_1\beta_1$, a receptor for laminin and collagens I and IV *(20)*. The switch from $\alpha_6\beta_4$ to $\alpha_5\beta_1$ and $\alpha_1\beta_1$ is characteristic of the switch from proliferative to invasive phenotype of extravillous trophoblast; it may be involved in regulation of trophoblast invasion; and, if disturbed, it may lead to abnormal trophoblast invasion *(11)*.

Cell adhesion molecules (CAMs) are necessary for cell-to-cell contact. E-cadherin is expressed by proliferative extravillous trophoblast in proximal cell columns but is turned off with invasion *(21)*. Neural CAM is responsible for cell-to-cell contact and matrix adhesion. It is restricted to proximal proliferative extravillous trophoblast. No changes in adhesion molecules, including intracellular CAM, vascular CAM, and R-, E-, or L-selectin, have been reported in the placental bed in preeclampsia *(22)*.

Role of Proteases

To invade the decidua and myometrium, the extravillous trophoblast must degrade the ECM *(23)* by employing proteases, particularly the MMPs *(12,23)*, including MMP-1 (interstitial collagenase), MMP-2 (72-kDa type IV collagenase, gelatinase A), MMP-3 (stromelysin), MMP-7 (matrilysin), MMP-9 (92-kDa type IV collagenase, gelatinase B),

MMP-11 (stromelysin 3), MMP-14, and MMP-15. The activity of these metalloproteinases is regulated by their tissue inhibitors (tissue inhibitors of metalloproteinases [TIMPs]), of which TIMP-1, an inhibitor of all MMPs, and TIMP-2, an inhibitor of MMP-2, have been found in both decidual cells and extravillous trophoblast. TIMP-3, an inhibitor of MMP-9, is also found in invasive trophoblast. In vitro TIMP-1 and TIMP-2 will completely inhibit trophoblast invasion *(24)*.

Factors Regulating Trophoblast Invasion

As the repertoire of biochemical changes involved in trophoblast differentiation/invasion has been elucidated, so the search for factors that might stimulate or inhibit trophoblast invasion has grown. Oxygen obviously appears to be a major regulatory factor. Hepatocyte growth factor (HGF) has been shown to stimulate trophoblast invasion, possibly via acting through the met receptor and induction of 92-kDa collagenase *(25)*. Trophoblast from preeclamptic placentas produces less HGF than normal placentas, supporting a role for HGF in trophoblast invasion. Anti-HGF antibody inhibited the effect of HGF on trophoblast invasion in vitro *(26)*.

The isoprostane 8-iso-$PGF_{2\alpha}$, which is a marker of oxidative stress, is found in increased concentrations in preeclamptic gestational tissue; 8-iso-$PGF_{2\alpha}$ reduced MMP-2, MMP-9, and collagenase type IV activity in conditioned medium from JAR cells and reduced their invasive capacity *(27)*. This finding provides evidence linking oxidative stress to trophoblast invasion.

Apoptosis of Trophoblast and Preeclampsia

Differentiation of cytotrophoblast to syncytiotrophoblast starts trophoblast down the apoptotic cascade *(28,29)*. Increased apoptosis of villous trophoblast has been reported in preeclampsia and/or intrauterine growth restriction, consistent with increased cytotrophoblast proliferation and trophoblast turnover *(30–33)*. Prostanoids mediate cell differentiation, and altered prostanoid metabolism from prostacyclin toward thromboxane A_2 inhibited biochemical and morphologic differentiation of cultured trophoblast and increased trophoblast apoptosis *(34)*.

Apoptosis also occurs in the extravillous trophoblast in preeclampsia in association with abnormal invasion. Little apoptosis of trophoblast is seen in the placental bed of normal pregnancies *(35,36)*, but 15–50% of cells were apoptotic and failed to stain for the survival factor bcl-2 in preeclampsia. The reduced trophoblast invasion into uteroplacental spiral arteries is also associated with an excess of macrophages in and around these arteries. In vitro these macrophages cause apoptosis of extravillous trophoblast via tumor necrosis factor-α secretion and tryptophan depletion *(37)*.

The microvilli of syncytiotrophoblast are shed into the maternal circulation throughout gestation perhaps as a result of apoptosis or necrosis of syncytiotrophoblast. The amount of microvilli of syncytiotrophoblast shed is significantly increased in preeclampsia *(38)*. It is thought that microvilli of syncytiotrophoblast may contribute to the maternal endothelial dysfunction underlying preeclampsia *(39,40)* and may provoke an inflammatory response in the mother *(41)*.

Oxygenation of Intervillous Circulation in Early Pregnancy

Until recently, it was assumed that maternal blood reached the intervillous space and was in contact with villous trophoblast from early in the first trimester and throughout gestation. However, critical review of early work, coupled with novel observations of the placental intervillous circulation with color Doppler ultrasound imaging *(42,43)*, has convincingly shown that before 12 wk of gestation the villous tissue is separated from maternal blood by a continuous layer of trophoblast cells. Hence, the villous placenta develops by histiotrophic nutrition in a hypoxic environment in the first trimester *(44)*. At 10–12 wk, this trophoblastic shell is disrupted and the maternal circulation is established into the intervillous space *(42)*. This increase in blood flow and hence oxygen tension in the intervillous space at 10–12 wk *(45)* of gestation subjects the placenta to oxidative stress and the potential for complications *(46)* (discussed later). This switch in oxygen tension at 10–12 wk also has implications for trophoblast invasion. Prior to 10–12 wk, the low oxygen tension in the placenta appears to prevent trophoblast invasion toward the invasive extravillous trophoblast cell type. Expression of HIF-1α and TGF-β3, an inhibitor of trophoblast invasion, parallels the change in oxygen tension, being high in the first trimester and falling at 10–12 wk of gestation *(47)*, when Po_2 increases. It was found that antisense inhibition of HIF-1α inhibited TGF-β3 expression and stimulated extravillous trophoblast outgrowth from villous explants in vitro. Explants from preeclamptic placentas (in which there is defective invasion) show increased TGF-β3 expression, and antisense inhibition of TGF-β3 restored the invasive capacity of these explants *(14)*. In a mouse model, the central role of HIF-mediated hypoxic responses in determining placental cell differentiation has been clearly shown *(48)*. Animals with placentas null for Arnt, a HIF-1 subunit, show reduced labyrinthe and spongiotrophoblast layers but increased numbers of trophoblast giant cells.

The defective trophoblast invasion of preeclampsia is also thought to give rise to a relatively hypoxic placenta. Hence, hypoxia-inducible transcription factors have come under scrutiny for their role in regulation of trophoblast invasion. In placental tissues collected at term, protein expression of HIF-2α, but not HIF-1α or HIF-1β, was found to be increased in the preeclamptic placenta *(49)*. The relationship of this finding to events earlier in pregnancy when trophoblast invasion is occurring is unclear. However, hypoxia is reported to alter trophoblast differentiation/invasion in vitro and mimic the changes seen in preeclampsia *(50)* (Fig. 2).

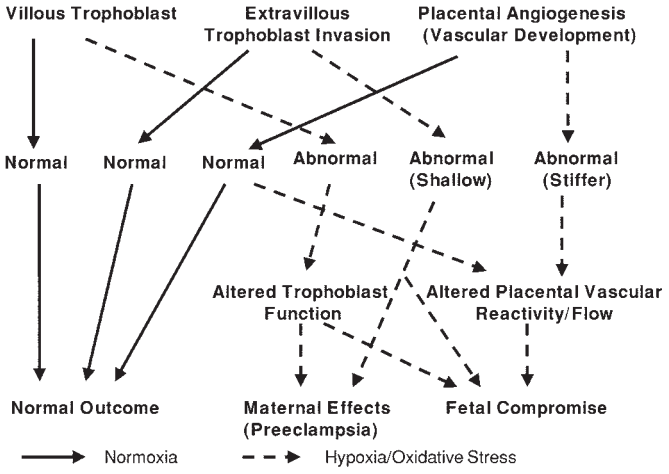


Fig. 2. Efects of normoxia or hypoxia/oxidative stress on development and function of villous and extravillous trophoblast and on placental angiogenesis.

Oxidative Stress in the Placenta

Oxidative stress is described as an imbalance in the production of reactive oxygen species (ROS) and the ability of antioxidant defenses to scavenge them. It can arise from increased production of ROS and/or a decrease in antioxidant capacity. The ROS superoxide can be synthesized by several enzymatic routes in tissues including cyclooxygenases, NADPH oxidases, and xanthine oxidase. Interestingly, ischemia/reperfusion injury and hypoxia can lead to oxidative stress *(51)*. Pregnancy is characterized as a state of oxidative stress in the maternal circulation and the placenta, and this oxidative stress seems to be exacerbated in preeclampsia. Evidence for oxidative stress in the placenta in preeclampsia includes elevated concentrations of isoprostanes and malondialdehyde *(52,53)*; lipid peroxides *(54,55)*; increased activity of xanthine oxidase in invasive cytotrophoblasts *(51)*; increased protein carbonyls *(56)*; and immunostaining for nitrotyrosine residues, a marker for formation and action of the powerful prooxidant peroxynitrite in villous tissue *(57–59)*, and invasive cytotrophoblasts *(51)*. Antioxidant defenses are widespread in the placenta but enhanced levels of protein thiol/disulfide oxidoreductases, which regenerate oxidatively damaged proteins and eliminate ROS, have been described *(60)*. Decreased superoxide dismutase mRNA expression and enzyme activity is also seen in the preeclamptic placentas, suggesting reduced antioxidant defenses *(61)*. Whether the decrease in antioxidant levels is a primary event leading to oxidative stress or is the result of consumption in reducing ROS is unknown.

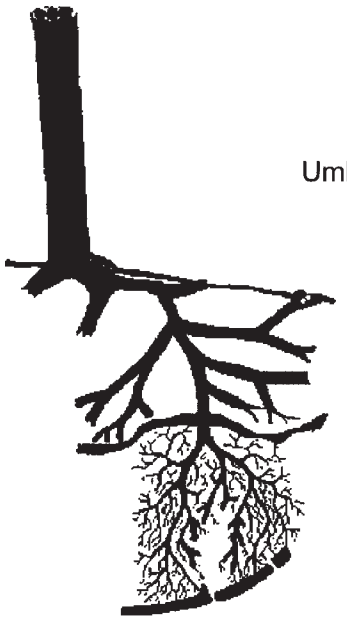
The concept of preeclampsia being a state of oxidative stress has gained credence as a unifying hypothesis that may explain the maternal vascular disease and provide a link to placental dysfunction *(62)*. It has also led to clinical trials with antioxidants to prevent preeclampsia. Indeed, a small-scale study of antioxidant therapy with vitamins C and E in women at high risk of developing preeclampsia showed a positive effect in reducing the incidence of maternal disease *(63)*. Several large-scale trials are now under way or about to commence.

Control of Villous Vascular Development

In addition to being a major regulator of extravillous trophoblast invasion, oxygen is also perhaps the major regulator of development of the villous vascular tree and villous trophoblast proliferation. In all pathologic conditions in which the placenta is hypoxic, the amount of villous cytotrophoblast is increased. By contrast, strong oxygenation of villi reduces the rate of proliferation *(64)*. The villous vasculature also responds to hypoxia with hypercapillarization (resulting from branching angiogenesis) *(64)* with similar findings from placentas at high altitude and with maternal anemia. The angiogenic factors that may mediate the hypoxic signal include platelet-derived growth factor, acidic and basic fibroblast growth factor, VEGF, placental growth factor (PLGF), and angiopoietin-1 (Ang-1) and Ang-2. Knockout of the VEGF-R2 (KDR) in mice has highlighted the role of the VEGF system in formation of fetoplacental capillaries *(65)*. VEGF is found in villous trophoblast and macrophage *(66,67)*, and PLGF is produced in villous syncytiotrophoblast and the media of large villous vessels *(68,69)*. The receptors for both growth factors, VEGF-R1 (FLT) and VEGF-R2 (KDR), are found in villous endothelium *(70)*. VEGF acting via FLT-1 and KDR is thought to be involved in branching angiogenesis (early pregnancy) and PLGF acting via FLT-1 in nonbranching angiogenesis (last trimester) *(5)*.

Oxygen may regulate the balance between VEGF and PLGF, with hypoxia upregulating expression of VEGF and its receptors but downregulating PLGF *(69)*, suggesting that changing oxygen tissues throughout gestation controls the switch from VEGF to PLGF effects.

The mRNAs for Ang-1 and Ang-2 and the endothelial cell receptor Tie-2 are found in the placenta, with Ang-2 abundant on syncytiotrophoblast. Placentae from women with preeclampsia had reduced Ang-2 mRNA with no difference in Ang-1. In vitro hypoxia has opposite effects on the molecules, increasing Ang-2 mRNA but reducing the stability of Ang-1 mRNA *(71)*. This again highlights the potential role of oxygen tension in regulating placental angiogenesis. In preeclampsia—either decreased *(72)*, increased *(73)*, or no change *(74)*—placental VEGF mRNA with reduced PLGF mRNA have been reported. These differences in findings may represent the heterogeneity of fetoplacental angiogenesis seen in preeclampsia *(64)*. Similar discrepancies have been reported among different studies of maternal serum VEGF and PLGF levels in preeclampsia compared to normotensive pregnancies *(75–77)*. Indeed, there has been considerable debate whether maternal PLGF can *(78–80)* or cannot *(81,82)* predict the subsequent onset of preeclampsia.



	Constriction	Dilation
Umbilical Vessels	O2 Et-1 5-HT Bk AII TxA2 OT VP NPY AVP	PGI2 NO
Chorionic Plate Vessels	5-HT	NO CGRP
Villous Tree	AII Et-1 TxA2 ET-3 Big ET-1 LTC4 LTD4 Bk Purine	NO PTHrP BNP CRH CGRP ANP CO Urocortin PGI2 Hist Subst P VIP Purine PTH

Fig. 3. Regional actions of autacoids in umbilical placental circulation.

Vascular Reactivity in Placenta

There is evidence for both abnormal placental vascular structure and abnormal vascular reactivity in the placenta in preeclampsia. Doppler ultrasound waveform analysis has provided evidence for increased vascular resistance in the fetoplacental circulation in preeclampsia *(83,84)*. Recent studies have shown an increase in elastic tissue fibers in blood vessel walls of placental stem villi, and the number of thick stem villous vessels was increased in preeclampsia *(85)*. Whether this is a response to placental hypoxia or an adaptation to altered resistance or flow remains to be established (Fig. 2).

In the absence of autonomic innervation, vascular resistance in the fetoplacental circulation is controlled by autocrine/paracrine agents or humoral factors *(86)*. There are regional variations throughout the fetoplacental vasculature (umbilical cord, chorionic plate vessels, and villous vessels) in response to the different families of autocoids *(86)* (Fig. 3). Important vascular regulators in the human placenta include the renin-angiotensin system *(87,88)*, endothelin *(89,90)*, nitric oxide (NO) *(91–93)*, carbon monoxide *(94)*, histamine, serotonin *(88,95,96)*, prostaglandins *(81,82)*, natriuretic peptides *(97–99)*, parathyroid hormone (PTH), PTHrp and calcitonin gene–related peptide *(100)*, adrenomedullin *(101)*, urocortin *(102)*, and corticotropin-releasing hormone (CRH) *(103,104)*. There is abundant evidence that there are alterations in both production and response to many of these factors in preeclampsia. It is now generally accepted that synthesis of the vasodilator NO in the placenta is increased in preeclampsia, perhaps as an adaptive response to increased vascular resistance *(105–108)*. Carbon monoxide is a vasodilator in the placenta, being synthesized by heme oxygenase (HO). There is little of the HO-1 isoform in the placenta *(94,109–111)*, whereas HO-2 is found in vascular endothelium as well as villous and extravillous trophoblast. A reduction in HO-2 expression was found in endothelial cells in preeclampsia *(111)*. In vitro the vasoconstrictive responses to serotonin but not histamine in umbilical and placental vessels are reduced with preeclampsia *(95)*. While there are no significant differences in placental abundance of pro-atrial natriuretic peptide mRNA in preeclampsia *(112)*, the vasodilation response to ANP and urocortin is reportedly greater in preeclampsia *(113)*. Expression of adrenomedullin, determined by immunohistochemistry, was significantly lower in the syncytiotrophoblast of preeclamptic placentas compared with normal controls *(114)*. However, there was no difference in relaxatory response to adrenomedullin in stem villous arteries in vitro between preeclampsia and control *(101)*.

mRNA levels of endothelin-1 were not different in the placentas of women with preeclampsia; however, mRNA for the endothelin-A receptor, but not endothelin-B receptor, was significantly reduced *(115)*, suggesting that the vasoconstrictor response to endothelin might be reduced. Real-time polymerase chain reaction analysis has shown an upregu-

lation of angiotensinogen mRNA, and angiotensin-1 (AT-1) receptor mRNA was found in preeclamptic placentas *(116)*. Western blot analysis confirmed this upregulation of AT-1, which was localized to syncytiotrophoblast and villous capillaries. This upregulation of AT-1 mRNA may be an adaptive response to the reported downregulation of AT-1 ligand-binding sites previously reported to occur in the placenta in preeclampsia *(117)*. The activity of angiotensin-converting enzyme, together with protein and mRNA expression, was high in the preeclamptic placenta *(118)*, an effect thought to be regulated by hypoxia.

Oxidative Stress and Placental Vascular Reactivity

While there is appreciable indirect evidence for oxidative stress in the placenta in preeclampsia and, as stated earlier, evidence for altered vascular reactivity, data or evidence that shows a direct cause-and-effect relationship or mechanistic link between the two (Fig. 2) is only just being accumulated. A product of oxidative stress and lipid peroxidation, 8-iso-$PGF_{2\alpha}$ is a vasoconstrictor, albeit less potent than thromoxane B_2. The maximal vasoconstrictor effect to 8-iso-$PGF_{2\alpha}$ was significantly reduced in placental resistance arteries from pregnancies complicated by preeclampsia *(119)*.

NO is an active vasodilator in the placenta. However, under conditions of oxidative stress with increased production of superoxide, superoxide and NO can interact to produce peroxynitrite, a powerful prooxidant that causes lipid peroxidation and nitrates tyrosine residues on proteins, altering protein structure and function and disrupting tyrosine kinase–mediated signal transduction pathways. My laboratory has provided evidence for increased peroxynitrite formation and action in the preeclamptic placenta by immunohistochemical staining for nitrotyrosine residues *(57,58)*. To demonstrate a cause-and-effect relationship, we determined vascular reactivity of the placenta before and following perfusion of the placenta with authentic peroxynitrite *(120)*. Peroxynitrite treatment, which resulted in formation of nitrotyrosine residues, attenuated vascular responses to both vasoconstrictors and vasodilators, resulting in reduced vascular compliance. Interestingly, the vascular responses seen following peroxynitrite treatment were similar to those seen in placentas from pregnancies complicated by preeclampsia *(120)*, i.e., attenuated responses to both vasoconstrictors and vasodilators. The search is now on to identify the protein targets that are modified by nitration by peroxynitrite.

In addition to the effects of peroxynitrite on the placental vasculature in preeclampsia, peroxynitrite produced in the placenta may act on other placental cells including trophoblast, and thus affect placental functions including transport *(121)*.

Placental Peptide Expression in Preeclampsia

The placenta synthesizes and releases a wide variety of peptide hormones during gestation mainly from the syncytiotrophoblast. Therefore, alterations in the amount of syncytiotrophoblast or its function as seen in preeclampsia may be reflected in alterations in production of these peptide hormones, which can then be measured in maternal serum to potentially predict the disease. Activin and inhibin are markers of the TGF-β superfamily, and both activin A and inhibin A are produced by the placenta in increasing amounts throughout gestation. Maternal serum concentrations of activin A and inhibin A are high in preeclamptic pregnancies *(122–125)*. Longitudinal studies are needed to determine whether elevated activin or inhibin levels can be used to predict who may develop preeclampsia. In the placenta, increased concentrations of activin A and the Alk-2 receptor protein, but decreased amounts of the Act RIIB receptor were found *(123)* although localization was not different. Inhibin-α- and inhibin-βA-subunits were both localized to the cytoplasm of syncytiotrophoblast with weak staining in intermediate trophoblast *(124)*. The intensity of staining for inhibin-α was significantly greater in preeclampsia while the staining for inhibin-βA, which was stronger than that of the α-subunit, was also greater in preeclampsia. These data, together with the measurements in maternal serum, are evidence for trophoblast dysfunction in preeclampsia.

CRH is also synthesized in syncytiotrophoblast *(126)* and may exert a paracrine vasodilator effect on the fetoplacental vasculature *(103,104,127)* in addition to effects on adrenocorticotropic hormone and glucocorticoid synthesis. Greater CRH1–41 and pro-CRH125–151 immunoreactivity was found in the preeclamptic placenta than in placenta from normal pregnancies *(128)*. Correspondingly, higher maternal serum CRH1–41 levels were found in women with preeclampsia. The syncytiotrophoblast is also the site of synthesis of neurokinin B (NKB) *(129,130)*, previously thought to be solely a neuropeptide. Maternal NKB concentrations increase in pregnancy and are significantly increased in preeclampsia. The initial claim that excessive NKB was the cause of preeclampsia *(129)* has not been substantiated, however, but the data are additional evidence for trophoblast dysfunction in preeclampsia.

Other Examples of Trophoblast Dysfunction in Preeclampsia

As already reported, there is now abundant evidence that villous trophoblast function is altered in preeclampsia. Whether this is a cause or consequence, e.g., as a result of hypoxia/oxidative stress (Fig. 2), remains to be proven. Recently, the human endogenous defective retrovirus HERV-W-derived protein syncytin has been shown to be highly expressed in syncytiotrophoblast *(131)* and was demonstrated to play a role in cytotrophoblast fusion and hence placental morphogenesis. Subsequent evidence has been found *(132, 133)* for reduced expression of syncytin in the placenta in preeclampsia. Morever, in preeclampsia, syncytin appears to be localized at the apical microvillous membrane rather

than the basal membrane of syncytiotrophoblast. Whether this is a cause or consequence of abnormal trophoblast function remains to be determined, but syncytin is necessary for syncytialization.

Expression of the very low-density or low-density lipoprotein receptor mRNAs was found to be significantly lower in third-trimester preeclamptic placenta compared with normal third-trimester placenta *(134)*, perhaps leading to abnormal fetomaternal lipid transport. By contrast, enhanced activity of villous trophoblast dypeptidyl peptidase IV (CD26) activity was found in preeclampsia *(135)*. The Ca–adenosine triphosphatase activity of plasma membranes of trophoblast was reported to be decreased in preeclampsia *(136)*. The activity of the enzyme 11-β hydroxysteroid dehydrogenase, which is also localized in syncytiotrophoblast, was significantly lower in the placenta of preeclamptic pregnancies compared with normal *(137)* and corresponded to a higher level of cortisol in umbilical cord blood. This may then have an effect on fetal growth and on fetal blood pressure. The overall or generalized nature of trophoblast dysfunction in preeclampsia was also recently illustrated by a report that mitogen-activated protein kinase signaling pathways were downregulated in the placentas of women with preeclampsia *(138)*. Such an effect may potentially have effects in many signal transduction pathways and cellular processes.

In preeclampsia, there is excessive fibrin deposition in the placenta, particularly perivillous fibrinoid deposits, as a result of an imbalance in placental coagulation vs fibrinolysis. In normal pregnancy, the plasminogen activators tPA and uPA increase while the inhibitor PAI-1 increases *(139)*. An increase in PAI-1 is found in preeclampsia *(139)*. Similarly, PAI-2 has been found to be higher in the placenta in preeclampsia *(140)*, with a negative correlation between PAI-2 and thrombomodulin. Thus, placental antifibrinolytic activity is increased in preeclampsia and may contribute to the prothrombotic state and increased perivillous fibrin deposition that are seen. Expression of syndecan1, a cell-surface heparan sulfate proteoglycan that binds growth factors and antithrombin III, is also severely reduced in the placenta in preeclampsia *(141)*.

Conclusion

Although it is clear that the presence of the placenta is necessary to cause the maternal syndrome of preeclampsia, the cause-and-effect relationship is still unclear. The factor(s) that are potentially released by the placenta (to give the maternal effect) or the derangement of the normal maternal-placental interaction is still not identified. There are many indications that placental function is changed at the time disease is manifest and that these changes have deleterious effects on placental-fetal interactions and hence fetal well-being. Again, whether these changes are a cause or a consequence of the initiating pathologic event that occurs early in gestation remains to be determined. There is a large weight of evidence suggesting that abnormal trophoblast invasion is occurring in the first trimester of pregnancy and that this may contribute to a relative placental hypoxia and oxidative stress that may further exacerbate placental pathology. Although many data have recently gathered on the molecular control of trophoblast invasion, better model systems (animal) are needed to test therapeutic approaches. Similarly, empirical approaches to treating or preventing maternal disease (low-dose aspirin, calcium, antioxidants) may be treating the consequence and not the cause of the disease. Longitudinal studies measuring biochemical markers of trophoblast invasion and trophoblast function, together with markers for maternal vascular function, may give clues as to the etiology of the disease.

References

1. Sibai, B. M., Caritis, S. N., Thom, E., et al. (1993). *N. Engl. J. Med.* **329,** 1213–1218.
2. Caritis, S., Sibai, B., Hauth, J., Lindheimer, M. D., Klebanoff, M., Thom, E., VanDorsten, P., Landon, M., Paul, R., Miodovnik, M., Meis, P., and Thurnau, G. (1998). *N. Engl. J. Med.* **338,** 701–705.
3. Myatt, L. and Miodovnik, M. (1999). *Semin. Perinatol.* **23,** 45–57.
4. Sibai, B. M., el-Nazer, A., and Gonzalez-Ruiz, A. (1986). *Am. J. Obstet. Gynecol.* **155,** 1011–1016.
5. Benirschke, K. and Kaufmann, P. (1999). *Pathology of the human placenta*, 4th ed. Springer-Verlag: New York.
6. Brosens, I. A., Robertson, W. B., and Dixon, H. G. (1970). *J. Pathol.* **101,** vi.
7. Brosens, I. A., Robertson, W. B., and Dixon, H. G. (1972). *Obstet. Gynecol. Ann.* **1,** 177–191.
8. Sheppard, B. L. and Bonnar, J. (1976). *Br. J. Obstet. Gynaecol.* **83,** 948–959.
9. Bonnar, J. and Sheppard, B. L. (1976). *J. Physiol.* **263,** 282P.
10. Pijnenborg, R., Vercruysse, L., Verbist, L., and Van Assche, F. A. (1998). *Placenta* **19,** 569–575.
11. Lim, K. H., Zhou, Y., Janatpour, M., McMaster, M., Bass, K., Chun, S. H., and Fisher, S. J. (1997). *Am. J. Pathol.* **151,** 1809–1818.
12. Graham, C. H. and Lala, P. K. (1992). *Biochem. Cell. Biol.* **70,** 867–874.
13. Ilic, D., Genbacev, O., Jin, F., Caceres, E., Almeida, E. A., Bellingard-Dubouchaud, V., Schaefer, E. M., Damsky, C. H., and Fisher, S. J. (2001). *Am. J. Pathol.* **159,** 93–108.
14. Caniggia, I., Grisaru-Gravnosky, S., Kuliszewsky, M., Post, M., and Lye, S. J. (1999). *J. Clin. Invest.* **103,** 1641–1650.
15. Zhou, Y., McMaster, M., Woo, K., Janatpour, M., Perry, J., Karpanen, T., Alitalo, K., Damsky, C., and Fisher, S. J. (2002). *Am. J. Pathol.* **160,** 1405–1423.
16. Irwin, J. C., Suen, L. F., Martina, N. A., Mark, S. P., and Giudice, L. C. (1999). *Hum. Reprod.* **14(Suppl. 2),** 90–96.
17. Zhou, Y., Damsky, C. H., and Fisher, S. J. (1997). *J. Clin. Invest.* **99,** 2152–2164.
18. Zhou, Y., Damsky, C. H., Chiu, K., Roberts, J. M., and Fisher, S. J. (1993). *J. Clin. Invest.* **91,** 950–960.
19. Fisher, S. J. and Damsky, C. H. (1993). *Semin. Cell Biol.* **4,** 183–188.
20. Damsky, C. H., Librach, C., Lim, K. H., Fitzgerald, M. L., McMaster, M. T., Janatpour, M., Zhou, Y., Logan, S. K., and Fisher, S. J. (1994). *Development* **120,** 3657–3666.
21. Reuss, B., Hellmann, P., Dahl, E., Traub, O., Butterweck, A., Grummer, R., and Winterhager, E. (1996). *Dev. Dyn.* **205,** 172–182.

22. Jaakkola, K., Jokimaa, V., Kallajoki, M., Jalkanen, S., and Ekholm, E. (2000). *Placenta* **21,** 133–141.
23. Graham, C. H. and Lala, P. K. (1991). *J. Cell. Physiol.* **148,** 228–234.
24. Librach, C. L., Werb, Z., Fitzgerald, M. L., Chiu, K., Corwin, N. M., Esteves, R. A., Grobelny, D., Galardy, R., Damsky, C. H., and Fisher, S. J. (1991). *J. Cell. Biol.* **113,** 437–449.
25. Kauma, S. W., Bae-Jump, V., and Walsh, S. W. (1999). *J. Clin. Endocrinol. Metab.* **84,** 4092–4096.
26. Dokras, A., Gardner, L. M., Seftor, E. A., and Hendrix, M. J. (2001). *Biol. Reprod.* **65,** 1278–1288.
27. Staff, A. C., Ranheim, T., Henriksen, T., and Halvorsen, B. (2000). *Hypertension* **35,** 1307–1313.
28. Huppertz, B., Frank, H. G., Reister, F., Kingdom, J., Korr, H., and Kaufmann, P. (1999). *Lab. Invest.* **79,** 1687–1702.
29. Huppertz, B., Frank, H. G., Kingdom, J. C., Reister, F., and Kaufmann, P. (1998). *Histochem. Cell. Biol.* **110,** 495–508.
30. Leung, D. N., Smith, S. C., To, K. F., Sahota, D. S., and Baker, P. N. (2001). *Am. J. Obstet. Gynecol.* **184,** 1249–1250.
31. Ishihara, N., Matsuo, H., Murakoshi, H., Laoag-Fernandez, J. B., Samoto, T., and Maruo, T. (2002). *Am. J. Obstet. Gynecol.* **186,** 158–166.
32. Huppertz, B. and Hunt, J. S. (2000). *Placenta* **21(Suppl. A),** S74–S76.
33. Huppertz, B., Rote, N. S., Nelson, D. M., Reister, F., Black, S., and Hunt, J. S. (2001). *Placenta* **22(Suppl. A),** S101–S103.
34. Yusuf, K., Smith, S. D., Levy, R., Schaiff, W. T., Wyatt, S. M., Sadovsky, Y., and Nelson, D. M. (2001). *Pediatr. Res.* **50,** 203–209.
35. Genbacev, O., DiFederico, E., McMaster, M., and Fisher, S. J. (1999). *Hum. Reprod.* **14(Suppl. 2),** 59–66.
36. DiFederico, E., Genbacev, O., and Fisher, S. J. (1999). *Am. J. Pathol.* **155,** 293–301.
37. Reister, F., Frank, H. G., Kingdom, J. C., Heyl, W., Kaufmann, P., Rath, W., and Huppertz, B. (2001). *Lab. Invest.* **81,** 1143–1152.
38. Knight, M., Redman, C. W., Linton, E. A., and Sargent, I. L. (1998). *Br. J. Obstet. Gynaecol.* **105,** 632–640.
39. Kertesz, Z., Hurst, G., Ward, M., Willis, A. C., Caro, H., Linton, E. A., Sargent, I. L., and Redman, C. W. (1999). *Placenta* **20,** 71–79.
40. Redman, C. W. and Sargent, I. L. (2000). *Placenta* **21,** 597–602.
41. Benyo, D. F., Smarason, A., Redman, C. W., Sims, C., and Conrad, K. P. (2001). *J. Clin. Endocrinol. Metab.* **86,** 2505–2512.
42. Jauniaux, E. (1996). *Ultrasound Obstet. Gynecol.* **8,** 73–76.
43. Jaffe, R., Jauniaux, E., and Hustin, J. (1997). *Am. J. Obstet. Gynecol.* **176,** 695–705.
44. Burton, G. J., Hempstock, J., and Jauniaux, E. (2001). *Placenta* **22(Suppl. A),** S70–S77.
45. Jauniaux, E., Watson, A., Ozturk, O., Quick, D., and Burton, G. (1999). *Hum. Reprod.* **14,** 2901–2904.
46. Jauniaux, E., Watson, A. L., Hempstock, J., Bao, Y. P., Skepper, J. N., and Burton, G. J. (2000). *Am. J. Pathol.* **157,** 2111–2122.
47. Caniggia, I., Winter, J., Lye, S. J., and Post, M. (2000). *Placenta* **21(Suppl. A),** S25–S30.
48. Adelman, D. M., Gertsenstein, M., Nagy, A., Simon, M. C., and Maltepe, E. (2000). *Genes Dev.* **14,** 3191–3203.
49. Rajakumar, A., Whitelock, K. A., Weissfeld, L. A., Daftary, A. R., Markovic, N., and Conrad, K. P. (2001). *Biol. Reprod.* **64,** 499–506.
50. Genbacev, O., Joslin, R., Damsky, C. H., Polliotti, B. M., and Fisher, S. J. (1996). *J. Clin. Invest.* **97,** 540–550.
51. Many, A., Hubel, C. A., Fisher, S. J., Roberts, J. M., and Zhou, Y. (2000). *Am. J. Pathol.* **156,** 321–331.
52. Walsh, S., Vaughan, J., Wang, Y., and Roberts, L. N. (2000). *FASEB J.* **14,** 1289–1296.
53. Skoczylas-Pietrzyk, M., Stryjecka-Zimmer, M., and Oleszczuk, J. (1998). *Ginekol. Pol.* **69,** 902–908.
54. Gratacos, E., Casals, E., Deulofeu, R., Gomez, O., Cararach, V., Alonso, P. L., and Fortuny, A. (1999). *Hypertens. Pregnancy* **18,** 139–146.
55. Gratacos, E., Casals, E., Deulofeu, R., Cararach, V., Alonso, P. L., and Fortuny, A. (1998). *Am. J. Obstet. Gynecol.* **178,** 1072–1076.
56. Zusterzeel, P. L., Rutten, H., Roelofs, H. M., Peters, W. H., and Steegers, E. A. (2001). *Placenta* **22,** 213–219.
57. Myatt, L., Rosenfield, R. B., Eis, A. L., Brockman, D. E., Greer, I., and Lyall, F. (1996). *Hypertension* **28,** 488–493.
58. Lyall, F., Gibson, J. L., Greer, I. A., Brockman, D. E., Eis, A. L., and Myatt, L. (1998). *Diabetes Care* **21,** 1753–1758.
59. Stanek, J., Eis, A. L., and Myatt, L. (2001). *Placenta* **22 (Suppl. A),** S56–S62.
60. Shibata, E., Ejima, K., Nanri, H., Toki, N., Koyama, C., Ikeda, M., and Kashimura, M. (2001). *Placenta* **22,** 566–572.
61. Wiktor, H. and Kankofer, M. (1998). *Ginekol. Pol.* **69,** 915–918.
62. Hubel, C. A. (1999). *Proc. Soc. Exp. Biol. Med.* **222,** 222–235.
63. Chappell, L. C., Seed, P. T., Briley, A. L., Kelly, F. J., Lee, R., Hunt, B. J., Parmar, K., Bewley, S. J., Shennan, A. H., Steer, P. J., and Poston, L. (1999). *Lancet* **354,** 810–816.
64. Kingdom, J. C. and Kaufmann, P. (1997). *Placenta* **18,** 613–621.
65. Shalaby, F., Rossant, J., Yamaguchi, T. P., Gertsenstein, M., Wu, X. F., Breitman, M. L., and Schuh, A. C. (1995). *Nature* **376,** 62–66.
66. Charnock-Jones, D. S., Sharkey, A. M., Boocock, C. A., Ahmed, A., Plevin, R., Ferrara, N., and Smith, S. K. (1994). *Biol. Reprod.* **51,** 524–530.
67. Ahmed, A., Li, X. F., Dunk, C., Whittle, M. J., Rushton, D. I., and Rollason, T. (1995). *Growth Factors* **12,** 235–243.
68. Khaliq, A., Li, X. F., Shams, M., Sisi, P., Acevedo, C. A., Whittle, M. J., Weich, H., and Ahmed, A. (1996). *Growth Factors* **13,** 243-250.
69. Shore, V. H., Wang, T. H., Wang, C. L., Torry, R. J., Caudle, M. R., and Torry, D. S. (1997). *Placenta* **18,** 657–665.
70. Clark, D. E., Smith, S. K., Sharkey, A. M., and Charnock-Jones, D. S. (1996). *Hum. Reprod.* **11,** 1090–1098.
71. Zhang, E. G., Smith, S. K., Baker, P. N., and Charnock-Jones, D. S. (2001). *Mol. Med.* **7,** 624–635.
72. Cooper, J. C., Sharkey, A. M., Charnock-Jones, D. S., Palmer, C. R., and Smith, S. K. (1996). *Br. J. Obstet. Gynaecol.* **103,** 1191–1196.
73. Ahmed A, W. M. and Khaliq A. (1997). *J. Soc. Gynecol. Invest.* **4,** A663.
74. Ranheim, T., Staff, A. C., and Henriksen, T. (2001). *Acta Obstet. Gynecol. Scand.* **80,** 93–98.
75. Reuvekamp, A., Velsing-Aarts, F. V., Poulina, I. E., Capello, J. J., and Duits, A. J. (1999). *Br. J. Obstet. Gynaecol.* **106,** 1019–1022.
76. Helske, S., Vuorela, P., Carpen, O., Hornig, C., Weich, H., and Halmesmaki, E. (2001). *Mol. Hum. Reprod.* **7,** 205–210.
77. Livingston, J. C., Chin, R., Haddad, B., McKinney, E. T., Ahokas, R., and Sibai, B. M. (2000). *Am. J. Obstet. Gynecol.* **183,** 1554–1557.
78. Tidwell, S. C., Ho, H. N., Chiu, W. H., Torry, R. J., and Torry, D. S. (2001). *Am. J. Obstet. Gynecol.* **184,** 1267–1272.
79. Su, Y. N., Lee, C. N., Cheng, W. F., Shau, W. Y., Chow, S. N., and Hsieh, F. J. (2001). *Obstet. Gynecol.* **97,** 898–904.
80. Tjoa, M. L., van Vugt, J. M., Mulders, M. A., Schutgens, R. B., Oudejans, C. B., and van Wijk, I. J. (2001). *Obstet. Gynecol.* **98,** 600–607.
81. Livingston, J. C., Haddad, B., Gorski, L. A., Neblett, P., Ahokas, R. A., Ramsey, R., and Sibai, B. M. (2001). *Am. J. Obstet. Gynecol.* **184,** 1218–1220.

82. Ong, C. Y., Liao, A. W., Cacho, A. M., Spencer, K., and Nicolaides, K. H. (2001). *Obstet. Gynecol.* **98,** 608–611.
83. Trudinger, B. J., Giles, W. B., Cook, C. M., Bombardieri, J., and Collins, L. (1985). *Br. J. Obstet. Gynaecol.* **92,** 23–30.
84. Trudinger, B. J. and Ishikawa, K. (1990). *Clin. Diagn. Ultrasound* **26,** 119–137.
85. Wilhelm, D., Mansmann, U., Neudeck, H., Matejevic, D., Vetter, K., and Graf, R. (1999). *Anat. Embryol. (Berl.)* **200,** 597–605.
86. Myatt, L. (1992). *Placenta* **13,** 329–341.
87. Glance, D. G., Elder, M. G., Bloxam, D. L., and Myatt, L. (1984). *Am. J. Obstet. Gynecol.* **149,** 450–454.
88. Glance, D. G., Elder, M. G., and Myatt, L. (1985). *Am. J. Obstet. Gynecol.* **151,** 387–391.
89. Myatt, L., Langdon, G., Brewer, A. S., and Brockman, D. E. (1991). *Am. J. Obstet. Gynecol.* **165,** 1717–1722.
90. Myatt, L., Brewer, A. S., and Brockman, D. E. (1992). *Am. J. Obstet. Gynecol.* **167,** 1651–1656.
91. Myatt, L., Brewer, A., and Brockman, D. E. (1991). *Am. J. Obstet. Gynecol.* **164,** 687–692.
92. Myatt, L., Brockman, D. E., Langdon, G., and Pollock, J. S. (1993). *Placenta* **14,** 373–383.
93. Myatt, L., Brockman, D. E., Eis, A. L., and Pollock, J. S. (1993). *Placenta* **14,** 487–495.
94. Lyall, F., Barber, A., Myatt, L., Bulmer, J. N., and Robson, S. C. (2000). *FASEB J.* **14,** 208–219.
95. Bertrand, C. and St-Louis, J. (1999). *Am. J. Obstet. Gynecol.* **180,** 650–659.
96. Glance, D. G., Elder, M. G., and Myatt, L. (1986). *Br. J. Obstet. Gynaecol.* **93,** 488–494.
97. Holcberg, G., Kossenjans, W., Brewer, A., Miodovnik, M., and Myatt, L. (1995). *Am. J. Obstet. Gynecol.* **172,** 71–77.
98. Holcberg, G., Miodovnik, M., Rosenn, B., Kossenjans, W., and Myatt, L. (1995). *Obstet. Gynecol.* **85,** 428–432.
99. Holcberg, G., Kossenjans, W., Brewer, A., Miodovnik, M., and Myatt, L. (1995). *J. Soc. Gynecol. Invest.* **2,** 1–5.
100. Mandsager, N. T., Brewer, A. S., and Myatt, L. (1994). *J. Soc. Gynecol. Invest.* **1,** 19–24.
101. Jerat, S., Morrish, D. W., Davidge, S. T., and Kaufman, S. (2001). *Hypertension* **37,** 227–231.
102. Clifton, V. L., Gu, Q., Murphy, V. E., Schwartz, J., Madsen, G., Smith, R., and Qing, G. (2000). *Placenta* **21,** 782–788.
103. Clifton, V. L., Read, M. A., Leitch, I. M., Boura, A. L., Robinson, P. J., and Smith, R. (1994). *J. Clin. Endocrinol. Metab.* **79,** 666–669.
104. Clifton, V. L., Read, M. A., Leitch, I. M., Giles, W. B., Boura, A. L., Robinson, P. J., and Smith, R. (1995). *J. Clin. Endocrinol. Metab.* **80,** 2888–2893.
105. Lyall, F., Greer, I. A., Young, A., and Myatt, L. (1996). *Placenta* **17,** 165–168.
106. Myatt, L., Eis, A. L., Brockman, D. E., Greer, I. A., and Lyall, F. (1997). *Hum. Reprod.* **12,** 167–172.
107. Norris, L. A., Higgins, J. R., Darling, M. R., Walshe, J. J., and Bonnar, J. (1999). *Obstet. Gynecol.* **93,** 958–963.
108. Shaamash, A. H., Elsonosy, E. D., Zakhari, M. M., Radwan, S. H., and El-Dien, H. M. (2001). *Int. J. Gynaecol. Obstet.* **72,** 127–133.
109. McLean, M., Bowman, M., Clifton, V., Smith, R., and Grossman, A. B. (2000). *J. Clin. Endocrinol. Metab.* **85,** 2345–2349.
110. Yoshiki, N., Kubota, T., and Aso, T. (2000). *Biochem. Biophys. Res. Commun.* 276, 1136–1142.
111. Barber, A., Robson, S. C., Myatt, L., Bulmer, J. N., and Lyall, F. (2001). *FASEB J.* **15,** 1158–1168.
112. Gude, N. M., Stebbing, P. N., Wang, L., Xue, J., Brennecke, S. P., and Lim, A. T. (2000). *Gynecol. Obstet. Invest.* **49,** 114–118.
113. Szukiewicz, D., Szukiewicz, A., Maslinska, D., and Markowski, M. (1999). *Arch. Gynecol. Obstet.* **263,** 37–41.
114. Kanenishi, K., Kuwabara, H., Ueno, M., Sakamoto, H., and Hata, T. (2000). *Pathol. Int.* **50,** 536–540.
115. Faxen, M., Nisell, H., and Kublickiene, K. R. (2000). *Arch. Gynecol. Obstet.* **264,** 143–149.
116. Leung, P. S., Tsai, S. J., Wallukat, G., Leung, T. N., and Lau, T. K. (2001). *Mol. Cell. Endocrinol.* **184,** 95–102.
117. Knock, G. A., Sullivan, M. H., McCarthy, A., Elder, M. G., Polak, J. M., and Wharton, J. (1994). *J. Pharmacol. Exp. Ther.* **271,** 1007–1015.
118. Ito, M., Itakura, A., Ohno, Y., Nomura, M., Senga, T., Nagasaka, T., and Mizutani, S. (2002). *J. Clin. Endocrinol. Metab.* **87,** 1871–1878.
119. Kwek, K., Read, M. A., Khong, T. Y., Bisits, A. T., and Walters, W. A. (2001). *Placenta* **22,** 526–533.
120. Kossenjans, W., Eis, A., Sahay, R., Brockman, D., and Myatt, L. (2000). *Am. J. Physiol. Heart Circ. Physiol.* **278,** H1311–H1319.
121. Ayuk, P. T., Theophanous, D., D'Souza, S. W., Sibley, C. P., and Glazier, J. D. (2002). *J. Clin. Endocrinol. Metab.* **87,** 747–751.
122. Muttukrishna, S., Knight, P. G., Groome, N. P., Redman, C. W., and Ledger, W. L. (1997). *Lancet* **349,** 1285–1288.
123. Manuelpillai, U., Schneider-Kolsky, M., Dole, A., and Wallace, E. M. (2001). *J. Endocrinol.* **171,** 57–64.
124. Jackson, N., Biddolph, S. C., Ledger, W., Groome, N. P., and Manek, S. (2000). *Int. J. Gynecol. Pathol.* **19,** 219–224.
125. Gratacos, E., Casals, E., Gomez, O., Aibar, C., Cararach, V., Alonso, P. L., and Fortuny, A. (2000). *Hypertens. Pregnancy* **19,** 315–321.
126. Riley, S. C., Walton, J. C., Herlick, J. M., and Challis, J. R. (1991). *J. Clin. Endocrinol. Metab.* **72,** 1001–1007.
127. Clifton, V. L., Owens, P. C., Robinson, P. J., and Smith, R. (1995). *Eur. J. Endocrinol.* **133,** 591–597.
128. Ahmed, I., Glynn, B. P., Perkins, A. V., Castro, M. G., Rowe, J., Morrison, E., and Linton, E. A. (2000). *J. Clin. Endocrinol. Metab.* **85,** 755–764.
129. Page, N. M., Woods, R. J., Gardiner, S. M., Lomthaisong, K., Gladwell, R. T., Butlin, D. J., Manyonda, I. T., and Lowry, P. J. (2000). *Nature* **405,** 797–800.
130. Page, N. M., Woods, R. J., and Lowry, P. J. (2001). *Regul. Pept.* **98,** 97–104.
131. Mi, S., Lee, X., Li, X., Veldman, G. M., Finnerty, H., Racie, L., LaVallie, E., Tang, X. Y., Edouard, P., Howes, S., Keith, J. C. Jr., and McCoy, J. M. (2000). *Nature* **403,** 785–789.
132. Knerr, I., Beinder, E., and Rascher, W. (2002). *Am. J. Obstet. Gynecol.* **186,** 210–213.
133. Lee, X., Keith, J. C. Jr., Stumm, N., Moutsatsos, I., McCoy, J. M., Crum, C. P., Genest, D., Chin, D., Ehrenfels, C., Pijnenborg, R., van Assche, F. A., and Mi, S. (2001). *Placenta* **22,** 808–812.
134. Murata, M., Kodama, H., Goto, K., Hirano, H., and Tanaka, T. (1996). *Am. J. Obstet. Gynecol.* **175,** 1551–1556.
135. Neudeck, H., Joncic, M., Schuster, C., Bisson, S., Hildebrandt, R., Oney, T., Stiemer, B., Hopp, H., and Graf, R. (1997). *Am. J. Reprod. Immunol.* **37,** 449–458.
136. Casart, Y., Proverbio, T., Marin, R., and Proverbio, F. (2001). *Gynecol. Obstet. Invest.* **51,** 28–31.
137. McCalla, C. O., Nacharaju, V. L., Muneyyirci-Delale, O., Glasgow, S., and Feldman, J. G. (1998). *Steroids* **63,** 511–515.
138. Hannke-Lohmann, A., Pildner von Steinburg, S., Dehne, K., Benard, V., Kolben, M., Schmitt, M., and Lengyel, E. (2000). *Obstet. Gynecol.* **96,** 582–587.
139. Gao, M., Nakabayashi, M., Sakura, M., and Takeda, Y. (1996). *J. Obstet. Gynaecol. Res.* **22,** 9–16.
140. Kanfer, A., Bruch, J. F., Nguyen, G., He, C. J., Delarue, F., Flahault, A., Nessmann, C., and Uzan, S. (1996). *Lab. Invest.* **74,** 253–258.
141. Jokimaa, V. I., Kujari, H. P., Ekholm, E. M., Inki, P. L., and Anttila, L. (2000). *Am. J. Obstet. Gynecol.* **183,** 1495–1498.