Endocrine, *vol. 19, no. 1, 113–125, October 2002* 0969–711X/02/19:113–125/$20.00

Pathophysiology and Maternal Biologic Markers of Preeclampsia

Jacques Massé,[1,2] Yves Giguère,[1,2] Abdelaziz Kharfi,[1] Joël Girouard,[1] and Jean-Claude Forest[1,2]

[1]Perinatology Research Unit, [2]Center for the Development, the Evaluation and the Rational Implantation of New Diagnostic Technologies (CEDERINDT), Hôpital Saint-François d'Assise Research Center, CHUQ, Quebec City, Quebec, Canada

Preeclampsia—increased blood pressure and proteinuria appearing after the twentieth week of pregnancy—is a major cause of materal and neonatal morbidity, leading to iatrogenic prematurity. Several lines of evidence suggest that the disorder is owing to diminished invasion of spiral arteries by trophoblastic cells, followed by reduced perfusion of the fetoplacental unit and oxidative stress. These alterations, in the presence of maternal predisposition, lead to endothelial dysfunction and occurrence of the clinical syndrome of preeclampsia (multisystemic lesions). Although the pathophysiology of preeclampsia is still unknown, progress has been made during the past 10 yr, and the early identification of at-risk women with the use of biochemical; ultrasonographic; and, more recently, genetic susceptibility markers has been the subject of intense research. In the present review, markers of maternal predisposition, placental implantation, oxidative stress, vasomotor regulation, and endothelial dysfunction are investigated as candidate markers in the early prediction of preeclampsia. Unfortunately, at the present time, no marker has been proven to have a clinically useful predictive performance in the general pregnant population, and, therefore, more research in that area is warranted.

Key Words: Preeclampsia; screening; endothelium; insulin resistance; oxidation; placenta.

Introduction

Pregnancy is characterized by adaptive physiologic changes reflected in variations in the homeostasis of blood volume, blood pressure, and immune response, among others. Diseases presenting during pregnancy are associated with various alterations in these physiologic responses. The various mediators involved in these biologic mechanisms, and in particular those detectable in maternal blood, either directly or by way of more stable metabolites are logical candidates for the early detection of pregnancy pathologies, in the hope of applying targeted preventive measures. To be clinically useful, the candidate marker must be measurable in noninvasively obtained specimens (usually maternal blood or urine). The technology necessary for its measurement must be widely available and relatively cheap to be applicable for the general pregnant population. Abnormal levels must be present before the development of clinically detectable disease (to still be considered a screening test and to allow the implementation of preventive measures before full installation of the pathologic manisfestations). In populations with low incidence of the disease, the marker must be relatively specific to maintain an acceptable positive predictive value (in particular if invasive, potentially harmful, or costly preventive measures are envisaged after a positive screening test).

Preeclampsia is a disease limited to the female gender during pregnancy. Its major manifestation is hypertension, as well as variable involvement of various organs (kidney, liver, brain), generally appearing after the twentieth week of pregnancy *(1–3)*. The disease's prevalence using current classifications *(3,4)* varies from 3 to 8% in developed countries *(5–9)*. It is a leading cause of maternal mortality in developed countries and increases perinatal mortality fivefold *(10)*. The only definitive treatment of preeclampsia *(11)* is delivery of the infant; thus, this disease is a major cause of iatrogenic prematurity. It is estimated that 15% of preterm births are secondary to delivery for preeclampsia *(12)*. The goal of obstetricians caring for women with preeclampsia is thus to control the disease as long as possible to diminish neonatal morbidity. The etiology of the syndrome is still unknown and probably has many different origins *(13)*. A better knowledge of the pathophysiology of preeclampsia would help to identify clinically useful markers of at-risk women, to manage patients with established disease, and possibly to discover better-targeted preventive interventions.

Pathophysiology of Preeclampsia

The woman with overt preeclampsia is vasoconstricted, has multiple organ dysfunction secondary to reduced perfusion, has evidence of activation of the coagulation cascade, and has a loss of endothelial integrity. These profound derangements make it difficult to discriminate pathophysiologic causes and effects in women with preeclampsia. Studies

Received July 26, 2002; Accepted July 26, 2002.

Author to whom all correspondence and reprint requests should be addressed: Dr. Jean-Claude Forest, Hôpital Saint-François d'Assise Research Center, 10, rue de l'Espinay, Quebec City, Quebec, Canada G1L 3L5. E-mail: Jean-Claude.Forest@bcx.ulaval.ca

of pathophysiologic changes before manifest disease are thus especially pertinent to understanding the disorder. Increased sensitivity to pressors *(7,14,15)*, platelet activation and increased turnover *(16–19)*, reduced plasma volume *(20)*, increased atrial natriuretic factor (ANF) *(21,22)*, and several indicators of endothelial activation *(23)* are all evident weeks to months prior to manifestation of preeclampsia.

The glomerular and pathophysiologic changes of preeclampsia, especially those before clinically evident disease, suggest that altered endothelial function is responsible for many of the changes in the syndrome *(23–29)*. This hypothesis is supported by abundant evidence of endothelial dysfunction in women with preeclampsia. Women with preeclampsia show increased circulating markers of endothelial activation von Willebrand factor *(30–32)*, cellular fibronectin (cFN) *(33–35)*, thrombomodulin *(36–40)*, endothelin *(27,41–44)*, and VCAM *(45,46)*, and increased growth factor activity *(47,48)*. The endothelial prostanoid, prostacyclin (prostaglandin I), is reduced in women with preeclampsia *(49–51)* whereas thromboxane, released from activated platelets, is increased *(52)*. Vessels removed from women with preeclampsia manifest reduced endothelial-mediated vasodilator function *(53–57)*. Several in vitro assays indicate that serum or plasma from women with preeclampsia can alter endothelial function *(23)*. These alterations include increased release of cFN *(58)*, prostanoid *(59–62)*, and VCAM *(63)*; increase generation of nitric oxide *(64,65)*; increase uptake of fatty acids; and increase expression of platelet-derived growth factor by endothelial cells incubated with serum or plasma from women with preeclampia *(66)*. Many of these activities are not only greater in the blood of women with preeclampsia compared with healthy women, but, as with the syndrome, disappear shortly after delivery *(48,67)*.

Epidemiologic data support the possibility that immunologic interaction between mother and fetus accounts for the abnormal placental implantation in preeclampsia. These data suggest that exposure to paternal antigen is protective against preeclampsia *(68)*. Preeclampsia is largely a disease of the first full-term pregnancy, two thirds of cases occurring in first pregnancies *(10)*. It is proposed that the normal fetal-maternal transfusion associated with delivery exposes the mother to products of the fetal (and hence paternal) genome, protecting her in subsequent pregnancies. In keeping with this concept, the protective effect of first pregnancy is partially lost if a woman has a child with a new partner *(69)*. Similarly, the risk of preeclampsia is less as the duration of time the woman has had sexual contact with the father of the baby increases *(70,71)*. In keeping with the protective effect of exposure to the paternal genome through semen, barrier contraception prior to first pregnancy increases the risk of preeclampsia *(72)*. As would be predicted, women inseminated with sperm that is not from their husband have an increased risk of preeclampsia *(73)*.

Since all pregnant women have placentas and only about 5% develop preeclampsia, there must be something different about the placenta of women with the disorder. Sixty years ago, Page *(74)* concluded that reduced perfusion was the placental feature that led to the development of preeclampsla. The abnormal implantation and reduced vascular invasion characteristic of preeclampsia support this hypothesis. Additionally, medical conditions associated with microvascular disease such as diabetes *(75)*, hypertension *(76)*, and collagen vascular diseases *(77)* all increase the risk of preeclampsia. In addition to the increased trophoblastic tissue present with hydatidiform mole, other conditions with large placentas including multiple gestations increase the risk of preeclampsia *(78)*. The contribution of the large placenta to the genesis of preeclampsia is posited to be secondary to a relative reduction in placental perfusion. Normal uterine blood flow is inadequate to perfuse the large placenta. Direct measurements of intervillous blood flow in preeclamptic women indicate that blood flow to the placenta is reduced in preeclampsia *(79)*. Finally, although animal models of preeclampsia have been difficult and not reproducible, there are several animal models in which reducing uterine or placental blood flow produces a preeclampsia-like syndrome *(80–84)*. Thus, it is generally believed that the first stage in the development of preeclampsia is the reduction in placental blood flow, frequently because of abnormal implantation.

The second stage of preeclampsia would be the transduction of reduced placental perfusion to systemic maternal pathophysiology. Reduced placental perfusion results in the production of mediators that can act systemically to alter endothelial function and reduce organ perfusion *(85)*. Many years ago, Assali et al. *(86)* demonstrated that ganglionic blockade had minimal effects on blood pressure in preeclampsia, leading to the concept that humoral factors were responsible for the increased vasoconstriction (and in more recent models of endothelial dysfunction) of preeclampsia. The search for this factor led to the identification of numerous cytokines *(45,87–91)* and growth factors *(48,92,93)* that are increased in the circulation of women with preeclampsia. Additionally, factors associated with the ability of serum or plasma to alter endothelial function in vitro have been partially characterized *(94)*.

The panoply of differences demonstrable in preeclamptic women has prompted a more directed approach based on clinical features of the disorder and the phenotype of the preeclamptic woman. One of the first conclusions reached with this approach is that reduced placental perfusion is not sufficient to explain preeclampsia. Fetal growth restriction, indicating reduced perfusion and delivery of nutrients, occurs in many pregnancies without the systemic manifestations of preeclampsia. Abnormal implantation is also not uniquely associated with preeclampsia. Infants with intrauterine growth restriction *(95)* and one third of infants with preterm birth *(96)* manifest abnormal features of implantation identical to those seen in preeclampsia. Interestingly, only one third of infants of preeclamptic women are growth restricted *(97)*.

Furthermore, conditions associated with large infants such as obesity *(98–102)* and gestational diabetes *(103–105)* increase the risk of preeclampsia. Thus, it appears that although reduced placental perfusion may be necessary for preeclampsia, it is certainly not sufficient. It is proposed that the abnormal implantation must interact with maternal factors to result in the preeclampsia syndrome. Thus, genetic, behavioral, and environmental factors would predispose a woman to preeclampsia. These "constitutional" factors, likely influenced by the unique physiologic changes of pregnancy, interact with fetal placental factors induced by reduced placental perfusion to bring about the pathophysiologic changes of preeclampsia *(106)*.

Maternal-Fetal Interactions in Genesis of Preeclampsia

Maternal Constitutional Factors

The concept that maternal factors contribute to the genesis of preeclampsia intimates that the disorder is heterogeneous, not all women developing it for the same reason. Studies of candidate genes support this concept. An angiotensinogen variant is associated with preeclampsia in Utah and Japan *(107,108)* but not in Great Britain *(109)*. Similarly, a variant of methylene tetrahydrofolate reductase, a homocysteine-metabolizing enzyme, is associated with preeclampsia in Japan *(110)* and Italy *(111)* but not in the United States *(112)*.

Preeclampsia is a syndrome of increased vascular resistance, and there have been studies of typical alterations in uterine artery waveform and velocity measurements by Doppler during the second trimester *(113)*. Albaiges et al. *(114)* studied the performance of early diastolic notch and mean resistance index (RI) (unilateral notch RI > 0.65 or bilateral notch RI > 0.55). With these criteria, they obtained 65% sensitivity at a false positive rate of 11%. In a prospective study of 1311 women, we assessed the performance of four Doppler abnormalities of uterine artery waveform (protodiastolic notch, peak systolic over protodiastolic velocities (A:C ratio > 2.5), peak systolic over diastolic velocities (A:B ratio > 90th percentile), and RI (RI ≥ 0.58) in predicting preeclampsia, low birth weight, and prematurity *(115)*. The performance of Doppler measurements at 26 wk of pregnancy was better than for those performed at 18 wk, but even if all Doppler abnormalities were associated with preeclampsia, sensitivities (26–34%) and positive predictive values (7–28%) were low, suggesting that they are not reliable screening tests for preeclampsia, at least in unselected populations. It is now established that Doppler studies of the uterine artery are not of sufficient diagnostic accuracy to predict at-risk pregnant women from the general population *(116)*.

Inspection of the risk factors for preeclampsia—hypertension, diabetes, obesity *(98–102)*, insulin resistance *(103–105)*, African American race *(100,117–119)*, and hyperhomocysteinemia *(120–123)*—supports this diversity. Interestingly, it also reveals risks identical to those for atherosclerosis. This concept of common risk factors for the two disorders is supported by the relationship of preeclampsia to cardiovascular disease later in life. The work of Chesley et al. *(124)* and Sibai et al. *(125)* indicated that well-defined preeclampsia, occurring only in first pregnancies, is not associated with excess cardiovascular morbidity in later life when women are compared with matched control subjects with unknown pregnancy outcome. This is not the case if preeclampsia occurs recurrently. With recurrent preeclampsia, there is an excess of cardiovascular disease and mortality in later life. This has been interpreted to indicate that preeclampsia does not cause cardiovascular disease but, rather, is caused by the same factors that contribute to cardiovascular disease later in life. Thus, women who have been pregnant but never developed preeclampsia have a lower risk of cardiovascular disease than the general female population *(126)*.

In addition to common risk factors, there are striking similarities in the pathophysiology of preeclampsia and atherosclerosis. Endothelial cells are intimately involved in the pathogenesis of both disorders *(23,127,128)*. Oxidative stress is proposed as a cause of the endothelial injury of atherosclerosis *(129)*. It has been postulated that the most important consequence of the dyslipidermia associated with atherosclerosis is the genesis of a variant of low-density lipoprotein (LDL)—small, dense LDL—that has a greater susceptibility to oxidative modification. Small, dense LDL particles have preferential access and reside longer in the subendothelial space, where they are sequestered from circulating antioxidants. This sequestration, worsened by the fact that the LDL variant is inherently more easily oxidized *(130)*, results in the accelerated formation of oxidized LDL (ox-LDL). ox-LDL causes local endothelial dysfunction and activates selectins, which recruit monocytes to the endothelial surface. Monocytes move through the endothelium to release free radicals and take up ox-LDL, eventually forming foam cells. The form of dyslipidemia found in preeclampsia is similar to that often associated with atherosclerosis. Triglycerides are increased *(131)*, high-density lipoprotein (HDL) is reduced *(132)*; and there is an increased prevalence of small, dense LDL *(133,134)*. In addition, as with atherosclerosis, there is evidence for oxidative stress in preeclampsia with increased plasma *(135–139)* and tissue concentrations *(140,141)* of markers of oxidative stress and increased antibodies to ox-LDL *(142,143)*.

This similarity to atherosclerosis is intriguing and raises numerous questions, in particular because the risk factors common to atherosclerosis and preeclampsia are present before and after pregnancy. Why do the endothelial functional abnormalities, which take years to develop in atherosclerosis, become rapidly manifest during pregnancy and resolve soon after the termination of pregnancy? Pregnancy is a time of major metabolic and physiologic modifications. For many of the changes described, the differences between nonpregnant and healthy women are more striking than the

differences between healthy pregnant women and those with preeclampsia. For example, insulin resistance is a recognized feature of normal pregnancy and is only slightly more prominent in preeclampsia. Furthermore, triglycerides double in normal pregnancy and increase another 70–100% in preeclampsia *(131)*. Even small, dense LDL is a feature of normal pregnancy *(144)*.

It seems likely that normal pregnancy changes could interact with the insults produced by abnormal placental perfusion and maternal predisposing factors to produce endothelial dysfunction and the preeclampsia syndrome. A recent observation supports this concept. Inflammatory markers were quantified on the surface of circulating leukocytes from nonpregnant women and from those with normal pregnancy, preeclampsia, and septic conditions. Surface markers of activation were strikingly increased in normal pregnancy (similar to sepsis) with some of the markers minimally higher in preeclampsia *(145,146)*. It is possible that inflammatory activation of the endothelium, occurring as a usual component in pregnancy response, sensitizes endothelium to insults, which would usually take years to cause injury. With the removal of this sensitization at pregnancy termination, the insult persists but the endothelium is no longer hypersensitive and no clinically apparent manifestations are observed.

Oxidative stress provides a plausible explanation for the endothelial dysfunction and subsequent pathophysiologic changes of preeclampsia *(147)*. The hypothesis proposes that reduced placental perfusion results in the generation of reactive oxygen species (ROS) *(23,106,147)*. Placental blood flow is known to be reduced by posture, activity, and uterine contractions and to return to normal with the termination of these stimuli. It is suggested that adaptive mechanisms prevent this from resulting in perfusion-reperfusion-mediated oxidative stress. Conversely, in the setting of the reduced placental perfusion characteristic of preeclampsia, these same stimuli are believed to reduce oxygen delivery sufficiently to result in oxidative stress. Evidence for this concept is the presence of stable metabolites generated by oxidative stress in the placenta of women with preeclampsia *(148)*. Perhaps even more pertinent is that the induction of xanthine oxidase activity and formation of nitrotyrosines are direct evidence of local oxidative stress, in the placenta of women with preeclampsia *(149)*.

The impact of this postulated generation of oxidative stress would be influenced by the maternal constitution that could also directly generate oxidative stress. For example, Hubel et al. *(150)* have recently demonstrated in their population that 20% of women with first pregnancy preeclampsia have a function-perturbing variant of lipoprotein lipase. Abnormalities of lipid metabolism could result in oxidative stress by the generation of ox-LDL with consequent endothelial injury. Alternatively, a diet deficient in antioxidants could eliminate a protective effect while inadequate intake of folate could increase circulating homocysteine, a contributor to oxidative stress. Other genetic abnormalities or metabolic factors could likewise contribute to the generation or amplification of oxidative stress. An important concept is that these factors rarely act alone. Rather, it is the culmination of varying degrees of reduced perfusion and maternal constitutional factors that summate to produce the syndrome.

Several hypotheses are advanced to explain the linkage of reduced placental perfusion to maternal systemic endothelial injury. First, stable metabolites of lipid peroxidation such as malondialdehyde are increased in the circulation of women with preeclampsia *(147)*. These metabolites could be produced in the placenta and injure the endothelium distally. Second, leukocytes from women with preeclampsia release more ROS *(151)*. This could occur by activation of these cells by oxidative stress as they traverse the intervillous space. The interaction of these activated leukocytes with endothelium would then result in the release of ROS and endothelial injury *(152)*. Third, increased release of syncytiotrophoblast microvillous fragments has been demonstrated in preeclampsia *(153)*. These fragments can alter endothelial function in vitro *(154,155)* and may contribute to the endothelial dysfunction of preeclampsia. It is possible that the increased release of these fragments is secondary to membrane alterations by oxidative stress. Finally, other candidate molecules including cytokines are produced in response to placental hypoxia. Thus, several hypotheses provide plausible mechanisms by which reduced placental perfusion could result in endothelial dysfunction.

Preeclampsia and the Fetus

The effect of preeclampsia on the fetus may extend beyond reduced placental perfusion and iatrogenic prematurity. There is evidence of endothelial activation in fetuses of women with preeclampsia *(156)*. For example, cFN is increased in the cord blood of these fetuses *(156)*. Furthermore, when corrected for gestational age, fetal cFN is positively correlated with ponderal index of the baby, suggesting that fetal endothelial activation increases the adiposity of these neonates *(157)*. One interpretation of these data would be that preeclampsia is "good for babies." This is consistent with several observations that the risk of cerebral palsy and intraventricular hemorrhage *(158)* is reduced in infants of women with preeclampsia when compared with control subjects matched for either gestational age or weight *(159–163)*. Part of this protective effect may reflect the association of spontaneous pre-term birth with subclinical infection *(164)* and the postulated association of this infection with cerebral palsy in the fetus *(165)*. Additionally, there is evidence that magnesium, frequently used for the treatment of preeclampsia, has an independent effect to reduce cerebral palsy *(166)*. However, the striking reduction in risk of cerebral palsy also raises the possibility of a fetal protective effect of preeclampsia. Haig *(167)* has extensively explored the concept that fetal adaptive responses may be evolutionarily

appropriate, even if not in the maternal best interest. This relationship could explain how preeclampsia, that has no evident maternal survival value, persists as an inherited disease *(168)*. A view of preeclampsia stimulated by this concept is that it may be a result of a normal fetal adaptive response to reduced placental perfusion. Abnormal implantation could result in preterm birth or intrauterine growth restriction.

Alternatively, the fetal-placental unit could launch a successful response to alter maternal metabolism to increase substrate delivery to overcome the unfavorable environment of reduced perfusion. In most cases, this would successfully overcome the deficit and increase fetal growth. On rare occasions, the maternal response to this addition could result in preeclampsia. Recent data examining cord and maternal leptin are consistent with this hypothesis. The human placenta contains leptin mRNA *(169)*. Leptin increases in maternal blood during pregnancy and rapidly returns to normal in the postpartum period *(170–172)*. Under ordinary circumstances, maternal and infant leptin are not correlated *(173)*. Rather, maternal leptin correlates with maternal fat mass *(171)* and neonatal leptin with infant fat mass *(174)*. In preeclampsia, placental leptin mRNA is increased as in circulating maternal blood while there are no changes in the leptin levels in the infant *(172,175)*. In contrast to normal pregnancy, leptin concentrations in mothers with preeclampsia and in their infants are strongly correlated *(172)*. One potential explanation is that placental leptin released into the maternal compartment alters the mother's metabolism in a manner that increases lipolysis and glucose turnover to provide sufficient substrate to influence fetal fat accrual. Since fetal leptin is driven by fetal fat mass, maternal and fetal leptins are correlated. This would have obvious implications regarding the therapeutic strategies to manage the maternal syndrome. Any therapeutic intervention for preeclampsia must take into consideration the fetal effects of the therapy, both beneficial and adverse.

In summary, elucidation of the pathophysiology of preeclampsia will help to develop early detection through the identification of predictive markers, and to define preventive and therapeutic interventions to achieve the most optimal outcome for both the mother and her infant.

Biochemical Markers of Pathophysiology of Preeclampsia

Maternal Predisposition Markers

As for atherosclerosis, preeclampsia has been shown to be associated with insulin resistance *(176,177)*. Insulin resistance in preeclampsia has been associated with a cluster of anomalies including hypertension, unfavorable lipid profile alterations, and obesity. This same combination of anomalies is also observed, outside pregnancy, with atherosclerosis (syndrome X *[178]*). Normal pregnancy is characterized by a state of insulin resistance *(179)* favoring the utilization of glucose by the fetus while the mother increases her reliance on lipolysis and protein catabolism as a source of energy. The normal daily insulin requirement during late pregnancy is increased by 30% in comparison with the nonpregnant state. Pregnancy thus can be considered a "physiologic stress test" of the pancreatic β-cell reserve. Most women with gestational diabetes present an adequate insulin sensitivity after delivery, if they do not gain weight *(180)*. However, the cumulative incidence rate for development of diabetes type II is approx 50% after 5 yr *(181,182)*. Obese women *(5,100,101,105)* and women with gestational diabetes *(105,183,184)* present an increased incidence of preeclampsia. Navajo Indians, who are at increased risk of hypertension, obesity, and diabetes, are also predisposed to develop preeclampsia *(185)*, suggesting some common pathophysiologic factors. Even for women with normal glucose tolerance in the third trimester, higher fasting and postload plasma insulin levels are associated with the development of preeclampsia *(186)*. Women who presented with preeclampsia show evidence of hyperinsulinemia at short- and long-term follow-up *(187–189)*.

The lipid profile in preeclampsia is markedly different from that in normal pregnancy; it is altered before the onset of overt illness and is similar to the lipid profile predisposing to atherosclerosis. Compared to normal pregnancies, triglycerides in preeclampsia are increased up to twofold *(131,132,190,191)*; HDL cholesterol is reduced *(134,192, 193)*; and free fatty acids are increased *(190,194–196)* as well as the concentration of small, dense LDL *(193)*.

Homocysteine can promote vascular disease by several mechanisms including direct cytotoxic effects on the endothelium, increased adhesiveness of the platelets, and effects on clotting factors *(197)*. Elevated levels of homocysteine have been linked to an increased risk of carotid stenosis *(198)*, vascular disease *(199)*, myocardial infarction in young women *(200)*, and mortality among patients with coronary disease *(201)*. During normal pregnancy, homocysteine levels decrease by about 50% *(202)*. In a recent study *(123)*, women with preeclampsia were shown to have higher fasting total homocysteine, and the levels were significantly correlated to cFN, a marker of endothelial activation. In another study *(203)*, hyperhomocysteinemia was shown to be inversely related to insulin sensitivity as measured by an iv glucose tolerance test. Hyperhomocysteinemia in women with preeclampsia has been corroborated by a number of studies *(120,122,204,205)*.

The most widely studied pathway for LDL oxidation involves metal ions *(206)*. Iron in micromolar concentrations, in the presence of smooth muscle cells, increases LDL oxidation *(207)*. Although still controversial, higher serum iron or body iron stores have been associated with an increased risk of cardiovascular diseases *(208–210)*. High serum iron levels and transferrin saturation have been reported in women with preeclampsia *(211–213)*.

Chesley et al. *(214)*, using sisters-in-law as control subjects, confirmed preliminary studies on heredity as a determinant of preeclampsia. Different genetic models of transmission have been proposed, such as monogenic autosomal recessive with or without the involvment of the fetus *(215–217)*, and autosomal dominant with incomplete penetrance or a more complex genetic susceptibility *(168)*. Twin studies investigating the heritability of preeclampsia were inconclusive *(218–220)*, but a recent large-scale study of 2000 twin pairs of women suggested a heritability estimate of 0.54 *(220)*. As is the case for many multifactorial diseases (e.g., hypertension, diabetes type II, asthma, cancers, osteoporosis), genetic predisposition to preeclampsia probably results from a combination of genetic and epigenetic factors (complex mode of inheritance) involving many genes with modest effect, which is why linkage analysis studies were often disappointing.

The potential role of human leukocyte antigen locus on chromosome 6 has been the subject of many investigations, but results are contradictory *(221–227)*, suggesting a modest, if any, effect. Since Jeunemaitre et al. *(228)* found an association between the M235T polymorphism of the angiotensinogen (*AGT*) gene and hypertension, others have studied the potential association between the M235T polymorphism of the *AGT* gene and preeclampsia in different populations. Some observed an association *(107,108,229)* whereas others did not *(231–233)*.

Because preeclampsia is associated with thrombophilic complications, potential associations between factor V Leiden (resistance to activated protein) *(111,234–243)* and the C677T polymorphism of the methylenetetrahydrofolate reductase (*MTHFR*) *(110–112,237,241–249)* gene and preeclampsia have also been studied, but the results are contradictory. Although factor V Leiden may be a good candidate in some populations *(111,234–236,240,241)*, the C677T polymorphism of the *MTHFR* gene is probably not a significant determinant, at least individually, since the majority of studies were unable to find any association *(112,237,241–249)*.

Polymorphisms at other candidate genes have also been studied, such as the angiotensin II type I receptor *(250)*, prothrombin *(238,251–254)*, plasma activator inhibitor I *(255,256)*, nitric oxide synthase-3 *(257,258)*, cytochrome P-450 1A1 and glutathione S-transferase family *(259)*, tumor necrosis factor-α *(260)*, apolipoprotein E *(261,262)*, and lipoprotein lipase *(150,263)* genes, but their contribution to the pathogenesis of preeclampsia is still undefined.

Discrepancies among studies on genetic susceptibility to preeclampsia may be owing to artifactual associations or genetic stratification in some populations, but they may also be the result of differences in study design, as well as in differences in genetic and environmental backgrounds between populations. In any case, in view of the expected, modest, individual effect of predisposing genes, the study of genetic susceptibility factors will need larger sample size to allow adequate power to test complex gene-gene and gene-environment interactions, and to study subgroups with extreme phenotypes. In addition, the study of haplotypes instead of individual single nucleotide polymorphisms (SNP) as well as the investigation of paternal and fetal genetic contribution may allow a better assessment of genetic susceptibility to preeclampsia.

Markers of Implantation

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the syncytiotrophoblast cells of the placenta. Its blood concentration is related to placental mass being increased in molar and twin pregnancies. Higher levels have been found in preeclampsia *(264–266)*. The increase may be owing to abnormal placental invasion, abnormal placental immunity, or the trophoblastic response to hypoxia. The availability of second-trimester hCG samples for prenatal Down syndrome screening has allowed the study of hCG as a potential predictive marker of preeclampsia. If studies agree that increased hCG is a predictive marker of the risk of preeclampsia, they disagree regarding its accuracy and its positive predictive value, which ranges from 1.9 to 15.1% *(264,267–272)*. Differences among studies may be explained, at least in part, by differences in gestational age at the time of sampling, selection of cutoff levels, characteristics of the study populations, and assay characteristics. When the false positive rate is within clinically acceptable limits (≤l 0%), no more than a third of the cases of women with preeclampsia would be identified by hCG measurement.

Activins are homo- and heterodimers of the inhibin β_A- and β_B-subunits, which form activin A, activin B, and activin AB *(273)*. The principal source of activin A during pregnancy is likely to be the fetoplacental unit. Activin A is involved in the control of trophoblast cell differentiation *(274)*. It has been isolated from the placenta *(275)*, and higher concentrations are found in multiple pregnancies *(276)*. Higher concentrations have been found in preeclampsia *(277–281)*. Inhibin is a dimer of two subunits designated α and β, of which the latter has two forms, β_A and β_B. The dimers are termed inhibin A and B *(282)*. Higher concentrations of inhibin A have been reported by some groups *(277–281)* but not by another *(283)*. It seems that inhibin A and activin A are increased secondary to increased placental production *(284)*. Interestingly, activin A levels are increased in women with preeclampsia but not in those with chronic hypertension or pregnancy-induced hypertension without proteinuria, suggesting that it may be a useful diagnostic and prognostic marker for the differential diagnosis of preeclampsia in hypertensive pregnancies *(277,279)*. Larger studies showed that inhibin A is elevated several weeks before the onset of preeclampsia *(285–289)*. Aquilina et al. *(286)* measured inhibin A levels between 15 and 19 wk of gestation in unselected women and found that those with a multiple of the median (a mean allowing the comparison of maternal serum marker levels even if they vary with gestational age *[290]*) >2 were at increased risk to

deliver small-for- gestational- age babies, to have stillbirth, and to develop preeclampsia. Inhibin A appears to be a better predictor of early onset than later-onset preeclampsia *(287)*, particularly for the prediction of preeclampsia occurring before 34 wk of gestation *(288)*. Still, the value of activin A and inhibin A is limited in the clinical setting (positive predictive values in the 10–35% range); therefore, other studies will be necessary to determine the utility of activin A and inhibin A measurements, possibly in combination with other markers, in the prediction of preeclampsia.

Another protein secreted by trophoblastic cells, plasma-associated plasma protein A (PAPP-A), when measured between 8 and 14 wk of gestation, has recently been reported as being decreased in women who later developed preeclampsia *(291,292)*. PAPP-A has been identified as a protease of insulin-like growth factor binding protein (IGFBP) *(293)*. Low levels of PAPP-A are thus expected to lead to higher IGFBP levels and lower free IGF, thus affecting fetal growth *(294)*. However, the overlap with normal pregnancies is large, indicating that PAPP-A is not a promising screening marker.

Oxidative Stress Markers

Malondialdehyde, ox-LDL, and copper-generated antigen are generated during the oxidation of lipids. Since they are produced by different mechanisms, the validity of an observed difference would be strengthened if more than one marker were abnormal. For example, malondialdehyde is not specific of lipid peroxidation alone *(295)*. The copper ox-LDL antibody assay does not detect only one specific antigenic epitope of ox-LDL, but, rather, it identifies a wide range of different antigens produced during the process of LDL oxidation *(296)*. Isoprostanes are prostaglandin isomers that are primarily generated from free-radical oxidation of arachidonic acid *(297)*. Levels of isoprostanes are increased in human atherosclerotic lesions *(298)*. High levels have also been found in the blood of women with preeclampsia *(299)*. Protein carbonyls are the product of protein oxidation, and they also have been found in increased quantities in atherosclerotic lesions *(300)*. This marker has the advantage of being more stable during storage. Higher levels have been reported in the placenta and maternal blood of pregnancies associated with preeclampsia *(301,302)*.

Endothelial Activation Markers

Cellular fibronectins are large glycoproteins synthesized by fibroblasts, endothelial cells, and other cells that are involved in cell adhesion, migration, growth, and differentiation *(303)*. One variant (EDI+) is found predominantly in large-vessel endothelial cells *(304)* and is soluble and thus measurable in blood. Increased concentrations have been reported in preeclampsia *(34,35)*. VCAM-1 and intracellular adhesion molecule-1 (ICAM-l) modulate the inflammatory response by allowing the adhesion of lymphocytes, monocytes, and eosinophils to the activated endothellum *(305)*. They are expressed in the decidua *(306)*. Several studies have reported high blood levels of sVCAM-1 in preeclampsia *(307–311)* but not of sICAM-I *(311,312)*. However, since no differences in sVCAM-1 levels were found during the second trimester of pregnancy *(311)*, it may not be an adequate early predictive marker of preeclampsia.

Placental growth factor is another member of the endothelial growth factor family. It is secreted solely by placental tissue and endothelial cells. Decreased levels have been reported in preeclamptic pregnancies *(313–315)*, but other studies indicate that this decrease may appear only late in pregnancy, invalidating its use as a screening marker *(316,317)*.

Markers of Vasomotor Regulation

Women with hypertensive pregnancies show increased response to angiotensin II. Compared with matched control subjects, they show decreased concentrations of active renin, angiotensin I, angiotensin II, aldosterone, and a lower activity of angiotensin-converting enzyme *(318)*. The angiotensin sensivity test was initially proposed to estimate vasopressor activity in pregnant women, but the assay is cumbersome and suffers from low sensitivity *(319)*. It is possible, however, to assess the activity of the renin-angiotensin system by measuring platelet angiotensin II binding (platelet angiotensin II binding density) during the second trimester of pregnancy. In a prospective study, we assessed the binding capacity of platelet angiotensin II receptors from nulliparous women during their second and third trimesters, as well as from nonpregnant women and women in the peripartum period *(320)*. We did not find any difference in the second or third trimester of pregnancy between normotensive, transiently hypertensive, preeclampsia, and/or chronically hypertensive women, and we could not demonstrate an improvement in positive predictive values of 20% with a power (1 -β) of 90% *(320)*. We developed a method based on the measurement of angiotensin II binding using Scatchard plot from suspensions of platelet concentrates. In a prospective study, we evaluated angiotensin II binding in 801 women for each trimester, but this platelet angiotensin II receptor method was also not a clinically useful predictive marker of preeclampsia *(5)*.

The atrial natriuretic peptide is a vasoactive peptide produced by the placenta and may be involved in preeclampsia. During the third trimester of pregnancy, Pouta et al. *(271)* found increased plasma levels of the N-terminal peptide of proatrial natriuretic peptide in women with preeclampsia compared with those with normal pregnancy. Furthermore, pregnant women with severe preeclampsia and Doppler abnormalities had higher levels than those with mild preeclampsia *(271)*, but these observations were, unfortunately, not confirmed in the second trimester *(321)*.

Other vasoactive molecules secreted by the placenta such as endothelin-1 *(322)* neurokinin B *(323)*, and kallikreins *(324)* have been studied in preeclampsia to various extents, but results are still too sparse or not convincing to be yet considered as potential clinical predictive markers.

Conclusion

Preeclampsia is a leading cause of iatrogenic prematurity. No preventive or definitive therapeutic (except delivery) intervention is available. Preterm delivery and the resulting low birth weight are associated with increased childhood morbidity, including respiratory illnesses, impaired postnatal growth, and neurodevelopmental problems *(325)*. Fortunately, preeclampsia seems to confer a "protective effect," and the premature infants from these pregnancies are less severely affected *(326–329)* than those born from women with other pathologies. It is thus important to appropriately appraise preventive or therapeutic interventions. Although they could be effective in diminishing some disease manifestations in the mother, they could also worsen neonate outcomes by mitigating adaptive mechanisms that normally protect the fetus.

A better knowledge of the pathophysiology of preeclampsia would help in targeting the most effective interventions that do not worsen fetal outcome. Biologic markers should have adequate high sensitivity and specificity and show high positive predictive values to identify at-risk women from the general population or, alternately, high negative predictive values to eliminate the risk of preeclampsia among pregnant women in high-risk subgroups. So far, none of the biologic markers studied have been adequate to find clinical application. Meanwhile, diagnosis and management of preeclampsia still rely on blood pressure and urinary protein measurements. Further studies on biologic markers based on the pathophysiology of preeclampsia using specific study designs allowing the evaluation of test performance regarding early and late-onset preeclampsia and its complications, and using serial measurements in both the general population of pregnant women and in at-risk groups, are necessary. Potential markers must be studied thoroughly, and new ones must be seeked. The discovery of a predictive test (or a combination of tests *[330]*) would help in the management of the affected pregnancies by allowing earlier intervention (even if it is limited to expectant management with close monitoring of the mother and fetus *[331]*). It could also help in the selection of women for future studies of preventive measures or to a better understanding of the pathophysiology of the disease.

References

1. Forest, J. C., Massé, J., Moutquin, J. M., and Radouco-Thomas, M. (1989). *Clin. Biochem.* **22,** 483–489.
2. Dekker, G. A. and Sibai, B. (1998). *Am. J. Obstet. Gynecol.* **179,** 1359–1375.
3. Helewa, M. E., Burrows, R. F., Smith, J., Williams, K., Brain, P., and Rabkln, S. W. (1997). *CMAJ* **157,** 715–725.
4. Anonymous. (1990). *Am. J. Obstet. Gynecol.* **163,** 1691–1712.
5. Massé, J., Forest, J. C., Moutquin, J .M., Degrandpre, P., and Forest, V. I. (1998). *Clin. Biochem.* **31,** 251–255.
6. Sibai, B. M., Caritis, S. N., Thom, E., et al. (1993). *N. Engl. J. Med.* **329,** 1213–1218.
7. Kyle, P.M., Buckley, D., Kissane, J., de Swiet, M., and Redman, C. W. (1995). *Am. J. Obstet. Gynecol.* **173,** 865–872.
8. Carroli, G., Duley, L., Belizan, M., and Villar, J. (1994). *Br. J. Obstet. Gynaecol.* **101,** 753–758.
9. Hauth, J. C., Goldenberg, R. L., Parker, C.R. Jr., et al. (1993). *Am. J. Obstet. Gynecol.* **168,** 1083–1091.
10. Roberts, J. M. (1998). In: *Maternal fetal medicine.* Creasy, R. K. and Resnik, R. (eds.). W. B. Saunders: Philadelphia.
11. Sibai, B. M.(1998). *Am. J. Obstet. Gynecol.* **179,** 1275–1278.
12. Meis, P. J., Goldenberg, R. L., Mercer, B. M., et al. (1998). *Am. J. Obstet. Gynecol.* **178,** 562–567.
13. Kliman, H. J. (2000). *Am. J. Pathol.* **157,** 1759–1768.
14. Gant, N. F., Daley, G. L., Chand, S., Whalley, P. J., and MacDonald, P. C. (1973). *J. Clin. Invest.* **52,** 2682–2689.
15. Wallenburg, H. C., Dekker, G. A., Makovitz, J. W., and Rotmans, N. (1991). *Am. J. Obstet. Gynecol.* **164,** 1169–1173.
16. Walker, J. J., Cameron, A. D., Bjornsson, S., Singer, C. R., and Fraser, C. (1989). *Am. J. Obstet. Gynecol.* **161,** 676–679.
17. Hutt, R., Ogunniyi, S. O., Sullivan, M. H., and Elder, M. G. (1994). *Obstet. Gynecol*. **83,** 146–149.
18. Calvert, S. M., Tuffnell, D. J., and Haley, J. (1996). *Eur. J. Obstet. Gynecol. Reprod. Biol.* **64,** 179–184.
19. Redman, C. W., Bonnar, J., and Beilin, L. (1978). *Br. Med. J.* **1,** 467–469.
20. Gallery, E. D., Saunders, D. M., Boyce, E. S., and Gyory, A. Z. (1980). *Pregnancy hypertension.* Bonnar, M. A., MacGillivray, I., and Symonds, M. S.(eds.). University Park Press: Baltimore.
21. Adam, B., Malatyalioglu, E., Alvur, M., Kokcu, A., and Bedir, A. (1998). *J. Matern. Fetal Invest*. **8,** 85–88.
22. Malee, M. P., Malee, K. M., Azuma, S. D., Taylor, R. N., and Roberts, J. M. (1992). *J. Clin. Endocrinol. Metab.* **4,** 1095–1100.
23. Roberts, J. M. (1998). *Semin. Reprod. Endocrinol.* **16,** 5–15.
24. Roberts, J. M., Taylor, R. N., Musci, T. J., Rodgers, G. M., Hubel, C. A., and McLaughlin, M. K. (1989). *Am. J. Obstet. Gynecol.* **161,** 1200–1204.
25. de Groot, C. J. and Taylor, R. N. (1993). *Ann. Med.* **25,** 243–249.
26. Ferris, T. F. (1991). *N. Engl. J. Med.* **325,** 1439–1440.
27. Kraayenbrink, A. A., Dekker, G. A., van Kamp, G. J., and van Geijn, H. P. (1993). *Am. J. Obstet. Gynecol.* **169,** 160–165.
28. Dekker, G. A. (1994). *Zentralbl. Gynakol.* **116,** 57–60.
29. Friedman, S. A., Schiff, E., Emeis, J. J., Dekker, G. A., and Sibai, B. M. (1995). *Am. J. Obstet. Gynecol.* **172,** 202, 203.
30. Redman, C. W., Denson, K. W., Beilin, L. J., Bolton, F. G., and Stirrat, G. M. (1977). *Lancet* **2,** 1249–1252.
31. Calvin, S., Corrigan, J., Weinstein, L., and Jeter, M. (1988). *Am. J. Perinatol.* **5,** 29–32.
32. Thorp, J. M. Jr., White, G. C., Moake, J. L., and Bowes, W. A. Jr. (1990). *Obstet. Gynecol.* **75,** 163–167.
33. Friedman, S. A., de Groot, C. T., Taylor, R. N., Golditch, B. D., and Roberts, J. M. (1994). *Am. J. Obstet. Gynecol.* **170,** 838–841.
34. Taylor, R. N., Crombleholme, W. R., Friedman, S. A., Jones, L. A., Casal, D. C., and Roberts, J. M. (1991). *Am. J. Obstet. Gynecol.* **165,** 895–901.
35. Lockwood, C. J. and Peters, J. H. (1990). *Am. J. Obstet. Gynecol.* **162,** 358–362.
36. Boffa, M. C., Valsecchi, L., Fausto, A., et al. (1998). *Thromb. Haemost.* **79,** 1092–1095.
37. de Moerloose, P., Mermillod, N., Amiral, J., and Reber, G. (1998). *Thromb. Haemost.* **79,** 554–556.
38. Hsu, C. D., Iriye, B., Johnson, T. R., Witter, F. R., Hong, S. F., and Chan, D. W. (1993). *Am. J. Obstet. Gynecol.* **169,** 148, 149.
39. Hsu, C. D., Copel, J. A., Hong, S. F., and Chan, D. W. (1995). *Obstet. Gynecol.* **86,** 897–899.
40. Minakami, H., Takahashi, T., Izumi, A., and Tamada, T. (1993). *Gynecol. Obstet. Invest.* **36,** 208–210.

41. Taylor, R. N., Varma, M., Teng, N. N., and Roberts, J. M. (1990). *J. Clin. Endocrinol. Metab.* **71,** 1675–1677.
42. Schiff, E., Ben Baruch, G., Peleg, E., et al. (1992). *Am. J. Obstet. Gynecol.* **166,** 624–628.
43. Nova, A., Sibai, B. M., Barton, J. R., Mercer, B. M., and Mitchell, M. D. (1991). *Am. J. Obstet. Gynecol.* **165,** 724–727.
44. Mastrogiannis, D. S., O'Brien, W. F., Krammer, J., and Benoit, R. (1991). *Am. J. Obstet. Gynecol.* **165,** 1711–1716.
45. Budak, E., Madazli, R., Aksu, M. F., et al. (1998). *Int. J. Gynaecol. Obstet.* **63,** 115–121.
46. Higgins, J. R., Papayianni, A., Brady, H. R., Darling, M. R., and Walshe, J. J. (1998). *Am. J. Obstet. Gynecol.* **179,** 464–469.
47. Taylor, R. N., Heilbron, D. C., and Roberts, J. M. (1990). *Am. J. Obstet. Gynecol.* **163,** 1839–1844.
48. Musci,T. J., Roberts, J. M., Rodgers, G. M., and Taylor, R. N. (1988). *Am. J. Obstet. Gynecol.* **159,** 1446–1451.
49. Fitzgerald, D. J., Entman, S. S., Mulloy, K., and FitzGerald, G. A. (1987). *Circulation* **75,** 956–963.
50. Walsh, S. W. (1999). *Reprod. Endocrinol. Infertil. Genet.* **5,** 1–21.
51. Mills, J. L., DerSimonian, R., Raymond, E., et al. (1999). *JAMA* **282,** 356–362.
52. Goeschen, K., Henkel, E., and Behrens, O. (1993). *J. Perinat. Med.* **21,** 481–489.
53. McCarthy, A. L., Woolfson, R. G., Raju, S. K., and Poston, L. (1993). *Am. J. Obstet. Gynecol.* **168,** 1323–1330.
54. Pascoal, I. F., Lindheimer, M. D., Nalbantian-Brandt, C., and Umans, J. G. (1998). *J. Clin. Invest.* **101,** 464–470.
55. Ashworth, J. R., Warren, A.Y., Baker, P. N., and Johnson, I. R. (1996). *Am. J. Obstet. Gynecol.* **175,** 1307–1312.
56. Cockell, A. P. and Poston, L. (1997). *Hypertension* **30,** 247–251.
57. Knock, G. A. and Poston, L. (1996). *Am. J. Obstet. Gynecol.* **175,** 1668–1674.
58. Roberts, J. M., Edep, M. E., Goldfien, A., and Taylor, R. N. (1992). *Am. J. Reprod. Immunol.* **27,** 101–108.
59. de Groot, C. J., Davidge, S. T., Friedman, S. A., McLaughlin, M. K., Roberts, J. M., and Taylor, R. N. (1995). *Am. J. Obstet. Gynecol.* **172,** 976–985.
60. Baker, P. N., Davidge, S. T., Barankiewicz, J., and Roberts, J. M. (1996). *Hypertension* **27,** 56–61.
61. Branch, D. W., Dudley, D. J., LaMarche, S., and Mitchell, M. D. (1992). *Prostaglandins Leukot. Essent. Fatty Acids* **45,** 191–195.
62. Wellings, R. P., Brockelsby, J. C., and Baker, P. N. (1998). *J. Soc. Gynecol. Invest.* **5,** 31–37.
63. Endresen, M. J., Morris, J. M., Nobrega, A. C., Buckley, D., Linton, E. A., and Redman, C. W. (1998). *Am. J. Obstet. Gynecol.* **179,** 665–670.
64. Baker, P. N., Davidge, S. T., and Roberts, J. M. (1995). *Hypertension* **26,** 244–248.
65. Davidge, S. T., Baker, P. N., and Roberts, J. M. (1995). *Am. J. Physiol* **269,** H1106–H1112.
66. Taylor, R. N., Musci, T. J., Rodgers, G. M., and Roberts, J. M. (1991). *Am. J. Reprod. Immunol.* **25,** 105–108.
67. Rodgers, G. M., Taylor, R. N., and Roberts, J. M. (1988). *Am. J. Obstet. Gynecol.* **159,** 908–914.
68. Taylor, R. N. 1997). *Am. J. Reprod. Immunol.* **37,** 79–86.
69. Trupin, L. S., Simon, L. P., and Eskenazi, B. (1996). *Epidemiology* **7,** 240–244.
70. Robillard, P. Y., Hulsey, T. C., Perianin, J., Janky, E., Miri, E. H., and Papiernik, E. (1994). *Lancet* **344,** 973–975.
71. Robillard, P. Y., Dekker, G. A., and Hulsey, T. C. (1998). *Aust. NZ J. Obstet. Gynaecol.* **38,** 284–287.
72. Klonoff-Cohen, H. S., Savitz, D. A., Cefalo, R. C., and McCann, M. F. (1989). *JAMA* **262,** 3143–3147.
73. Smith, G. N., Walker, M., Tessier, J. L., and Millar, K. G. (1997). *Am. J. Obstet. Gynecol.* **177,** 455–458.
74. Page, E. W. (1939). *Am. J. Obstet. Gynecol.* **35,** 291–293.
75. Rudge, M. V., Calderon, I. M., Ramos, M. D., Peracoli, J. C., and Pim, A. (1997). *Gynecol. Obstet. Invest.* **44,** 11–15.
76. Livingston, J. C. and Sibai, B. M. (2001). *Obstet. Gynecol. Clin. North Am.* **28,** 447–463.
77. Petri, M. (1998). *Baillieres Clin. Rheumatol.* **12,** 449–476.
78. Coonrod, D. V, Hickok, D. E., Zhu, K., Easterling, T. R., and Daling, J. R. (1995). *Obstet. Gynecol.* **85,** 645–650.
79. Rauramo, I. and Forss, M. (1988). *Acta Obstet. Gynecol. Scand.* **67,** 15–20.
80. Hodgkinson, C. P., Hodari, A., and Bumpus, F. M. (1967). *Obstet. Gynecol.* **30,** 371–380.
81. Cavanagh, D., Rao, P. S., Knuppel, R. A., Desai, U., and Balis, J. U. (1985). *Am. J. Obstet. Gynecol.* **151,** 987–999.
82. Abitbol, M. M., Pirani, C. L., Ober, W. B., Driscoll, S. G., and Cohen, M. W. (1976). *Obstet. Gynecol.* **48,** 537–548.
83. deGroot, C. J. M ., Merrill, D. C., Taylor, R. N., Kitzmiller, J. L., Goldsmith, P. C., and Roberts, J. M. (1997). *Hypertens. Pregnancy* **16,** 177–185.
84. Combs, C. A., Katz, M. A., Kitzmiller, J. L., and Brescia, R. J. (1993). *Am. J. Obstet. Gynecol.* **169,** 215–223.
85. Roberts, J. M. (1995). *Hosp. Pract. (Off. Ed.)* **30,** 25, 26.
86. Assali, N. S., Vergon, J. M., Tada, Y., and Garber, S. T. (1952). *Am. J. Obstet. Gynecol.* **63,** 978–988.
87. Daniel, Y., Kupferminc, M. J., Baram, A., et al. (1998). *Am. J. Reprod. lmmunol.* **39,** 376–380.
88. Eneroth, E., Remberger, M., Vahlne, A., and Ringden, O. (1998). *Acta Obstet. Gynecol. Scand.* **77,** 591–593.
89. Conrad, K. P., Miles, T. M., and Benyo, D. F. (1998). *Am. J. Reprod. Immunol.* **40,** 102–111.
90. Conrad, K. P. and Benyo, D. F. (1997). *Am.J. Reprod. Immunol.* **37,** 240–249.
91. Kupferminc, M. J., Peaceman, A. M., Aderka, D., Wallach, D., and Socol, M. L. (1996). *Obstet. Gynecol.* **88,** 420–427.
92. Furugori, K., Kurauchi, O., Itakura, A., et al. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2726–2730.
93. Kupferminc, M. J., Daniel,Y., Englender, T., et al. (1997). *Am. J. Reprod. Immunol.* **38,** 302–306.
94. Davidge, S. T., Signorella, A. P., Hubel, C. A., Lykins, D. L., and Roberts, J. M. (1996). *Hypertension* **28,** 758–764.
95. Khong, T. Y., De Wolf, F., Robertson, W. B., and Brosens, J. (1986). *Br. J. Obstet. Gynaecol.* **93,** 1049–1059.
96. Arias, F., Rodriquez, L., Rayne, S. C., and Kraus, F. T. (1993). *Am. J. Obstet. Gynecol.* **168,** 585–591.
97. Eskenazi, B., Fenster, L., Sidney, S., and Elkin, E. P. (1993). *Am. J. Obstet. Gynecol.* **169,** 1112–1118.
98. Abi-Said, D., Annegers, J. F., Combs-Cantrell, D., Frankowski, R. F., and Willmore, L. J. (1995). *Am. J. Epidemiol.* **142,** 437–441.
99. Efiong, E. I. (1975). *Br. J. Obstet. Gynaecol.* **82,** 903–906.
100. Eskenazi, B., Fenster, L., and Sidney, S. (1991). *JAMA* **266,** 237–241.
101. Sibai, B. M., Gordon, T., Thom, E., et al. (1995). *Am. J. Obstet. Gynecol.* **172,** 642–648.
102. Stone, J. L., Lockwood, C. J., Berkowitz, G. S., Alvarez, M., Lapinski, R., and Berkowitz, R. L. (1994). *Obstet. Gynecol.* **83,** 357–361.
103. Khan, K. S., Hashmi, F. A., and Rizvi, J. H. (1995). *JPMA J. Pak. Med. Assoc.* **45,** 176–179.
104. Sibai, B. M., Ewell, M., Levine, R. J., et al. (1997). *Am. J. Obstet. Gynecol.* **177,** 1003–1010.
105. Joffe, G. M., Esterlitz, J. R., Levine, R. J., et al. (1998). *Am. J. Obstet. Gynecol.* **179,** 1032–1037.
106. Ness, R. B. and Roberts, J. M. (1996). *Am. J. Obstet. Gynecol.* **175,** 1365–1370.

107. Ward, K., Hata, A., Jeunemaitre, X., et al. (1993). *Nat. Genet.* **4,** 59–61.
108. Kobashi, G., Hata, A., Shido, K., et al. (1999). *Am. J. Med. Genet.* **86,** 232–236.
109. Morgan, L., Baker, P., Pipkin, F. B., and Kalsheker, N. (1995). *Br. J. Obstet. Gynaecol.* **102,** 489, 490.
110. Sohda, S., Arinami, T., Hamada, H., Yamada, N., Hamaguchi, H., and Kubo, T. (1997). *J. Med. Genet.* 34, 525, 526.
111. Grandone, E., Margaglione, M., Colaizzo, D., et al. (1997). *Thromb. Haemost.* **77,** 1052–1054.
112. Powers, R. W., Minich, L. A., Lykins, D. L., Ness, R. B., Crombleholme, W. R., and Roberts, J. M. (1999). *J. Soc. Gynecol. Invest.* **6,** 74–79.
113. Campbell, S., Pearce, J. M., Hackett, G., Cohen-Overbeek, T., and Hernandez, C. (1986). *Obstet. Gynecol.* **68,** 649–653.
114. Albaiges, G., Missfelder-Lobos, H., Lees, C., Parra, M., and Nicolaides, K. H. (2000). *Obstet. Gynecol.* **96,** 559–564.
115. Irion, O., Massé, J., Forest, J. C., and Moutquin, J. M. (1998). *Br. J. Obstet. Gynaecol.* **105,** 422–429.
116. Chien, P. F., Arnott, N., Gordon, A., Owen, P., and Khan, K. S. (2000). *Br. J. Obstet. Gynaecol.* **107,** 196–208.
117. Irwin, D. E., Savitz, D. A., Hertz-Picciotto, I., and St. Andre, K. A. (1994). *Am. J. Public Health* **84,** 1508–1510.
118. Mittendorf, R., Lain, K. Y., Williams, M. A., and Walker, C. K. (1996). *J. Reprod. Med.* **41,** 491–496.
119. Knuist, M., Bonsel, G. J., Zondervan, H. A., and Treffers, P. E. (1998). *Obstet. Gynecol.* **92,** 174–178.
120. Dekker, G. A., de Vries, J. I., Doelitzsch, P. M., et al. (1995). *Am. J. Obstet. Gynecol.* **173,** 1042–1048.
121. Cotter, A. M., Molloy, A. M., Scott, J. M., and Daly, S. F. (2001). *Am. J. Obstet. Gynecol.* **185,** 781–785.
122. Rajkovic, A., Catalano, P. M., and Malinow, M. R. (1997). *Obstet. Gynecol.* **90,** 168–171.
123. Powers, R. W., Evans, R. W., Majors, A. K., et al. (1998). *Am. J. Obstet. Gynecol.* **179,** 1605–1611.
124. Chesley, L. C., Annitto, J. E., and Cosgrove, R.A. (1976). *Am. J. Obstet. Gynecol.* **124,** 446–459.
125. Sibai, B. M., el Nazer, A., and Gonzalez-Ruiz, A. (1986). *Am. J. Obstet. Gynecol.* **155,** 1011–1016.
126. Fisher, K. A., Luger, A., Spargo, B. H., and Lindheimer, M. D. (1981). *Medicine (Baltimore)* **60,** 267–276.
127. Ferri, C., Desideri, G., Baldoncini, R., et al. (1998). *Diabetes* **47,** 660–667.
128. Segrest, J. P. and Anantharamaiah, G. M. (1994). *Curr. Opin. Cardiol.* **9,** 404–410.
129. Holvoet, P. and Collen, D. (1998). *Atherosclerosis* **137 (Suppl.),** S33–S38.
130. Chait, A., Brazg, R. L., Tribble, D. L., and Krauss, R. M. (1993). *Am. J. Med.* **94,** 350–356.
131. Hubel, C. A., McLaughlin, M. K., Evans, R. W., Hauth, B. A., Sims, C. J., and Roberts, J. M. (1996). *Am. J. Obstet. Gynecol.* **174,** 975–982.
132. Kaaja, R., Tikkanen, M. J., Viinikka, L., and Ylikorkala, O. (1995). *Obstet. Gynecol.* **85,** 353–356.
133. Hubel, C. A., Lyall, F., Weissfeld, L., Gandley, R. E., and Roberts, J. M. (1998). *Metabolism* **47,** 1281–1288.
134. Sattar, N., Bendomir, A., Berry, C., Shepherd, J., Greer, I. A., and Packard, C. J. (1997). *Obstet. Gynecol.* **89,** 403–408.
135. Hubel, C. A., Kagan, V. E., Kisin, E. R., McLaughlin, M. K., and Roberts, J. M. (1997). *Free Radic. Biol. Med.* **23,** 597–609.
136. Cargnoni, A., Gregorini, G., Ceconi, C., Maiorca, R., and Ferrari, R. (1994). *Adv. Exp. Med. Biol.* **366,** 420, 421.
137. Uotila, J. T., Kirkkola, A. L., Rorarius, M., Tulmala, R. J., and Metsa-Ketela, T. (1994). *Free Radic. Biol. Med.* **16,** 581–590.
138. Hubel, C. A., Roberts, J. M., Taylor, R. N., Musci, T. J., Rogers, G. M., and McLaughlin, M. K. (1989). *Am. J. Obstet. Gynecol.* **161,** 1025–1034.
139. Maseki, M., Nishigaki, I., Hagihara, M., Tomoda, Y., and Yagi, K. (198). *Clin. Chim. Acta* **115,** 155–161.
140. Cester, N., Staffolani, R., Rabini, R. A., et al. (1994). *Mol. Cell Biochem.* **131,** 151–155.
141. Garzetti, G. G., Tranquilli, A. L., Cugini, A. M., Mazzanti, L., Cester, N., and Romanini, C. (1993). *Obstet. Gynecol.* **81,** 337–340.
142. Branch, D. W., Mitchell, M. D., Miller, E., Palinski, W., and Witztum, J. L. (1994). *Lancet* **343,** 645, 646.
143. Uotila, J., Solakivi, T., Jaakkola, O., Tuimala, R., and Lehtimaki, T. (1998). *Br. J. Obstet. Gynaecol.* **105,** 1113–1117.
144. Sattar, N., Greer, I. A., Louden, J., et al. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2483–2491.
145. Sacks, G. P., Studena, K., Sargent, K., and Redman, C. W. (1998). *Am. J. Obstet. Gynecol.* **179,** 80–86.
146. Redman, C. W., Sacks, G. P., and Sargent, I. L. (1999). *Am. J. Obstet. Gynecol.* **180,** 499–506.
147. Hubel, C. A. (1997). *Fetal Matern. Med. Rev.* **9,** 73–101.
148. Walsh, S. W., Vaughan, J. E., Wang, Y., and Roberts, L. J. (2000). *FASEB J* **14,** 1289–1296.
149. Many, A., Hubel, C. A., Fisher, S. J., Roberts, J. M., and Zhou, Y. (2000). *Am. J. Pathol.* **156,** 321–331.
150. Hubel, C. A., Roberts, J. M., and Ferrell, R. E. (1999). *Clin. Genet.* **56,** 289–296.
151. Tsukimori, K., Maeda, H., Ishida, K., Nagata, H., Koyanagi, T., and Nakano, H. (1993). *Obstet. Gynecol.* **81,** 536–540.
152. Palluy, O., Morliere, L., Gris, J. C., Bonne, C., and Modat, G. (1992). *Free Radic. Biol. Med.* **13,** 21–30.
153. Knight, M., Redman, C. W., Linton, E. A., and Sargent, I. L. (1998). *Br. J. Obstet. Gynaecol.* **105,** 632–640.
154. Smarason, A. K., Sargent, I. L., Starkey, P. M., and Redman, C. W. (1993). *Br. J. Obstet. Gynaecol.* **100,** 943–949.
155. Cockell, A. P., Learmont, J. G., Smarason, A. K., Redman, C. W., Sargent, I. L., and Poston, L. (1997). *Br. J. Obstet. Gynaecol.* **104,** 235–240.
156. Davidge, S. T., Signorella, A. P., Lykins, D. L., Gilmour, C. H., and Roberts, J. M. (1996). *Am. J. Obstet. Gynecol.* **175,** 1301–1306.
157. Gilmour, C. H., Patrick, T. E., and Roberts, J. M. (1999). *J. Soc. Gynecol. Invest.* **6,** 264–267.
158. Martin, D., Gonzalez, J. L., Gardner, M. O., Izquierdo, L. A., Tobey, K., and Curet, L. B. (1998). *Prenat. Neonat. Med.* **3,** 250–254.
159. Kuban, K. C., Leviton, A., Pagano, M., Fenton, T., Strassfeld, R., and Wolff, M. (1992). *J. Child Neurol.* **7,** 70–76.
160. Nelson, K. B. (1996). *JAMA* **276,** 1843, 1844.
161. Gray, P. H., O'Callaghan, M. J., Mohay, H. A., Burns, Y. R., and King, J. F. (1998). *Arch. Dis. Child Fetal. Neonat. Ed.* **79,** F88–F93.
162. O'Shea, T.M., Klinepeter, K. L., and Dillard, R. G. (1998). *Am. J. Epidemiol.* **147,** 362–369.
163. Murphy, D. J., Sellers, S., MacKenzie, I. Z., Yudkin, P. L., and Johnson, A.M. (1995). *Lancet* **346,** 1449–1454.
164. Goldenberg, R. L. and Rouse, D. J. (1998). *N. Engl. J. Med.* **339,** 313–320.
165. Yoon, B. H., Jun, J. K., Romero, R., et al. (1997). *Am. J. Obstet. Gynecol.* **177,** 19–26.
166. Nelson, K. B. and Grether, J. K. (1995). *Pediatrics* **95,** 263–269.
167. Haig, D. (1993). *Q. Rev. Biol.* **68,** 495–532.
168. Arngrimsson, R., Bjornsson, H., and Geirsson, R. T. (1995) *Hypertens. Pregnancy* **14,** 27–38.
169. Senaris, R., Garcia-Caballero, T., Casabiell, X., et al. (1997). *Endocrinology* **138,** 4501–4504.
170. Yura, S., Sagawa, N., Mise, H., et al. (1998). *Am. J. Obstet. Gynecol.* **178,** 926–930.
171. Highman, T. J., Friedman, J. E., Huston, L. P., Wong, W. W., and Catalano, P. M. (1998). *Am. J. Obstet. Gynecol.* **178,** 1010–1015.
172. McCarthy, J. F., Misra, D. N., and Roberts, J. M. (1999). *Am. J. Obstet. Gynecol.* **180,** 731–736.

173. Schubring, C., Kiess, W., Englaro, P., et al. (1997). *J. Clin. Endocrinol. Metab* .**82,** 1480–1483.
174. Harigaya, A., Nagashima, K., Nako, Y., and Morikawa, A. (1997). *J. Clin. Endocrinol. Metab.* **82,** 3281–3284.
175. Mise, H., Sagawa, N., Matsumoto, T., et al. (1998). *J. Clin. Endocrinol. Metab.* **83,** 3225–3229.
176. Berkowitz, K. M. (1998). *Clin. Perinatol.* **25,** 873–885.
177. Seely, E. W. (1999). *J. Clin. Endocrinol. Metab.* **84,** 1858–1861.
178. Reaven, G. M. (1988). *Diabetes* **37,** 1595–1607.
179. Buchanan, T. A., Metzger, B. E., Freinkel, N., and Bergman, R. N. (1990). *Am. J. Obstet. Gynecol.* **162,** 1008–1014.
180. Peters, R. K., Kjos, S. L., Xiang, A., and Buchanan, T. A. (1996). *Lancet* **347,** 227–230.
181. O'Sullivan, J. B. (1989). In: *Carbohydrate metabolism in pregnancy and the newborn.* Sutherland, H. W., Stowers, J. M., and Pearson, D. W. M. (eds.). Springer-Verlag: London.
182. Metzger, B. E., Cho, N. H., Roston, S. M., and Radvany, R. (1993). *Diabetes Care* **16,** 1598–1605.
183. Rudge, M. V., Calderon, T. M., Ramos, M. D., Peracoli, J. C., and Pim, A. (1997). *Gynecol. Obstet. Invest.* **144,** 11–15.
184. Greco, P., Loverro, G., and Selvaggi, L. (1994). *Gynecol. Obstet. Invest.* **7,** 242–245.
185. Levy, M. T., Jacober, S. T., and Sowers, J. R. (1994). *Arch. Intern. Med.* **154,** 2181–2183.
186. Martinez, A. E., Gonzalez, O. M., Quinones, G. A., and Ferrannini, E. (1996). *Am. J. Hypertens.* **9,** 610–614.
187. Sowers, J. R., Standley, P. R., Jacober, S., Niyogi, T., and Simpson, L. (1993). *Am. J. Hypertens.* **6,** 302–307.
188. Fuh, M. M., Yin, C. S., Pei, D., et al. (1995). *Am. J. Hypertens.* **8,** 768–771.
189. Laivuori, H., Tikkanen, M. J., and Ylikorkala, O. (1996). *J. Clin. Endocrinol. Metab.* **81,** 2908–2911.
190. Lorentzen, B., Endresen, M. J., Clausen, T., and Henriksen, T. (1994). *Hypertens. Pregnancy* **13,** 103–109.
191. Gratacos, E., Casals, E., Sanllehy, C., Cararach,V., Alonso, P. L., and Fortuny, A. (1996). *Acta Obstet. Gynecol. Scand.* **75,** 896–901.
192. Rosing, U., Samsioe, G., Olund, A., Johansson, B., and Kallner, A. (1989). *Horm. Metab. Res.* **21,** 376–382.
193. Sattar, N., Bendomir, A., Berry, C., Shepherd, J., Greer, I. A., and Packard, C. J. (1997). *Obstet. Gynecol.* **89,** 403–408.
194. Endresen, M. T., Tosti, E., Heimli, H., Lorentzen, B., and Henriksen, T. (1994). *Scand. J. Clin. Lab. Invest.* **4,** 549–557.
195. Lorentzen, B., Drevon, C. A., Endresen, M. T., and Henriksen, T. (1995). *Br. J. Obstet. Gynaecol.* **102,** 530–537.
196. Endresen, M. J., Morris, J. M., Nobrega, A. C., Buckley, D., Linton, E. A., and Redman, C. W. (1998). *Am. J. Obstet. Gynecol.* **179,** 665–670.
197. Mayer, E. L., Jacobsen, D. W., and Robinson, K. (1996). *J. Am. Coll. Cardiol.* **27,** 517–527.
198. Selhub, J., Jacques, P. F., Bostom, A. G., et al. (1995). *N. Engl. J. Med.* **332,** 286–291.
199. Graham, I. M., Daly, L. E., Refsum, H. M., et al. (1977). *JAMA* **277,** 1775– 1781.
200. Schwartz, S. M., Siscovick, D. S., Malinow, M. R., et al. (1997). *Circulation* **96,** 412–417.
201. Nygard, O., Nordrehaug, J. E., Refsum, H., Ueland, P. M., Farstad, M., and Vollset, S. E. (1997). *N. Engl. J. Med.* **337,** 230–236.
202. Andersson, A., Hultberg, B., Brattstrom, L., and Isaksson, A. (1992). *Eur. J. Clin. Chem. Clin. Biochem.* **30,** 377–379.
203. Laivuori, H., Kaaja, R., Turpeinen, U., Viinikka, L., and Ylikorkala, O. (1999). *Obstet. Gynecol.* **93,** 489–493.
204. de Vries, J. I., Dekker, G. A., Huijgens, P. C., Jakobs, C., Blomberg, B. M., and van Geijn, H. P. (1997). *Br. J. Obstet. Gynaecol.* **104,** 1248–1254.
205. Leeda, M., Riyazi, N., de Vries, J. I., Jakobs, C., van Geijn, H. P., and Dekker, G. A. (1998). *Am. J. Obstet. Gynecol.* **179,** 135–139.
206. Heinecke, J. W. (1998). *Atherosclerosis* **141,** 1–15.
207. Heinecke, J. W., Rosen, H., and Chait, A. (1984). *J. Clin. Invest.* 7**4,** 1890–1894.
208. Tuomainen, T. P., Punnonen, K., Nyyssonen, K., and Salonen, J. T. (1998) *Circulation* **97,** 1461–1466.
209. Tuomainen, T. P., Kontula, K., Nyyssonen, K., Lakka, T. A., Helio, T., and Salonen, J. T. (1999). *Circulation* **100,** 1274–1279.
210. Salonen, J. T., Nyyssonen, K., Korpela, H., Tuomilehto, J., Seppanen, R., and Salonen, R. (1992). *Circulation* **86,** 803–811.
211. Hubel, C. A., Kozlov, A. V., Kagan, V. E., et al. (1996). *Am. J. Obstet. Gynecol.* **175,** 692–700.
212. Entman, S. S., Kambam, J. R., Bradley, C. A., and Cousar, J. B. (1987). *Am. J. Obstet. Gynecol.* **156,** 1169–1173.
213. Samuels, P., Main, E. K., Mennuti, M. T., and Gabbe, S. G. (1987). *Am. J. Obstet. Gynecol.* **157,** 721–725.
214. Chesley, L. C., Annitto, J. E., and Cosgrove, R. A. (1968). *Obstet. Gynecol.* **32,** 303–311.
215. Cooper, D. W. and Liston, W. A. (1979). *J. Med. Genet.* **16,** 409–416.
216. Cooper, D. W., Hill, J. A., Chesley, L. C., and Bryans, C. I. (1988). *Br. J. Obstet. Gynaecol.* **95,** 644–653.
217. Liston, W. A. and Kilpatrick, D. C. (1991). *Br. J. Obstet. Gynaecol.* **98,** 1079–1086.
218. Thornton, J. G. and Onwude, J. L. (199l). *BMJ* **303,** 1241, 1242.
219. Thornton, J. G. and Macdonald, A. M. (I 999). *Br. J. Obstet. Gynaecol.* **106,** 570–575.
220. Salonen, R. H., Lichtenstein, P., Lipworth, L., and Cnattingius, S. (2000). *Am. J. Med. Genet.* **91,** 256–260.
221. Simon, P., Fauchet, R., Pilorge, M., et al. (1988). *Kidney Int. Suppl.* **25,** S125–S128.
222. Kilpatrick, D. C., Liston, W. A., Gibson, F., and Livingstone, J. (1989). *Lancet* **2,** 1063–1065.
223. De Luca Brunori, I., Battini, L., Simonelli, M., et al. (2000). *Hum. Reprod.* **15,** 1807–1812.
224. Honda, K., Takakuwa, K., Hataya, M., Yasuda, M., Kurabayashi, T., and Tanaka, K. (2000). *Obstet. Gynecol.* **96,** 385–389.
225. Humphrey, K. E., Harrison, G. A., Cooper, D. W., Wilton, A. N., Brennecke, S. P., and Trudinger, B. J. (1995). *Br. J. Obstet. Gynaecol.* **102,** 707–710.
226. Wilton, A. N., Cooper, D. W., Brennecke, S. P., Bishop, S. M., and Marshall, P. (I 990). *Lancet* **336,** 653–657.
227. Hayward, C., Livingstone, J., Holloway, S., Liston, W. A., and Brock, D. T. (1992) *Am. J. Hum. Genet.* **50,** 749–757.
228. Jeunemaitre, X., Soubrier, F., Kotelevtsev, Y. V., et al. (1992). *Cell* **71,** 169–180.
229. Kobashi, G., Shido, K., Hata, A., et al. (2001). *Semin. Thromb. Hemost.* **27,** 143–147.
230. Guo, G., Wilton, A. N., Fu, Y., Qiu, H., Brennecke, S. P., and Cooper, D. W. (1997). *Electrophoresis* **18,** 1646–1649.
231. Morgan, L., Crawshaw, S., Baker, P. N., Broughton, P. F., and Kalsheker, N. (1999). *Br. J. Obstet. Gynaecol.* **106,** 244–251.
232. Suzuki, Y., Tanemura, M., Suzuki, Y., Murakami, I., and Suzumori, K. (1999). *Hypertens. Pregnancy* **18,** 261–271.
233. Bashford, M. T., Hefler, L. A., Vertrees, T. W., Roa, B. B., and Gregg, A. R. (2001). *Am. J. Obstet. Gynecol.* **184,** 1345–1351.
234. Dizon-Townson, D. S., Nelson, L. M., Easton, K., and Ward, K. (1996). *Am. J. Obstet. Gynecol.***175,** 902–905.
235. Nagy, B., Toth, T., Rigo, J. Jr., Karadi, J., Romics, L., and Papp, Z. (1998). *Clin. Genet.* **53,** 478–481.
236. Kupferminc, M. J., Eldor, A., Steinman, N., et al. (1999). *N. Engl. J. Med.* **340,** 9–13.
237. O'Shaughnessy, K. M., Fu, B., Ferraro, F., Lewis, I., Downing, S., and Morris, N. H. (1999). *Hypertension* **33,** 1338–1341.
238. de Groot, C. J., Bloemenkamp, K. W., Duvekot, E.J., et al. (1999). *Am. J. Obstet. Gynecol.* **181,** 975–980.
239. Kobashi, G., Yamada, H., Asano, T., et al. (1999). *Semin. Thromb. Hemost.* **25,** 487–489.

240. Mello, G., Parretti, E., Martini, E., et al. (1999). *Haemostasis* **29,** 197–203.
241. Rigo, J. Jr., Nagy, B., Fintor, L., et al. (2000). *Hypertens. Pregnancy* **19,** 163–172.
242. Livingston, J. C., Barton, J. R., Park, V., Haddad, B., Phillips, O., and Sibai, B. M. (2001). *Am. J. Obstet. Gynecol.* **185,** 153–157.
243. Kim,Y. J., Williamson, R. A., Murray, J. C., et al. (2001). *Am. J. Obstet. Gynecol.* **184,** 1211–1217.
244. Rajkovic, A., Mahomed, K., Rozen, R., Malinow, M. R., King, I. B., and Williams, M. A. (2000). *Mol. Genet. Metab.* **69,** 33–39.
245. Kobashi, G., Yamada, H., Asano,T., et al. (2000). *Am. J. Med. Genet.* **93,** 122–125.
246. Laivuori, H., Kaaja, R., Ylikorkala, O., Hiltunen, T., and Kontula, K. (2000). *Obstet. Gynecol.* **96,** 277–280.
247. Raijmakers, M. T., Zusterzeel, P. L., Steegers, E. A., and Peters, W. H. (2001). *Eur. J. Obstet. Gynecol. Reprod. Biol.* **95,** 226~228.
248. Zusterzeel, P. L., Visser, W., Blom, H. J., Peters, W. H., Heil, S. G., and Steegers, E. A. (2000). *Hypertens. Pregnancy* **19,** 299–307.
249. Kaiser, T., Brennecke, S. P., and Moses, E. K. (2001) *Hum. Hered.* **51,** 20–22.
250. Morgan, L., Crawshaw, S., Baker, P. N., Edwards, R., Broughton, P. F., and Kalsheker, N. (1997). *J. Hypertens.* **15,** 1389–1396.
251. Poort, S. R., Rosendaal, F. R., Reitsma, P. H., and Bertina, R. M. (1996). *Blood* **88,** 3698–3703.
252. Martinelli, I., Sacchi, E., Landi, G., Taioli, E., Duca, F., and Mannucci, P. M. (1998). *N. Engl. J. Med.* **338,** 1793–1797.
253. Grandone, E., Margaglione, M., Colaizzo, D., et al. (1999). *Thromb. Haemost.* **81,** 349–352.
254. Higgins, J. R., Kaiser, T., Moses, E. K., North, R., and Brennecke, S. P. (2000). *Gynecol. Obstet. Invest.* **50,** 254–257.
255. Yamada, N., Arinami, T., Yamakawa-Kobayashi, K., et al. (2000). *J. Hum. Genet.* **45,** 138–141.
256. Glueck, C. J., Phillips, H., Cameron, D., et al. (2000). *Metabolism* **49,** 845–852.
257. Guo, G., Lade, J. A., Wilton, A. N., et al. (1999). *Hum. Genet.* **105,** 641–647.
258. Lewis, I., Lachmeijer, G., Downing, S., et al. (1999). *Am. J. Hum. Genet.* **64,** 310–313.
259. Zusterzeel, P. L., Visser, W., Peters,W. H., Merkus,H. W., Nelen, W. L., and Steegers, E. A. (2000). *Obstet. Gynecol.* **96,** 50–54.
260. Chen, G., Wilson, R., Wang, S. H., Zheng, H. Z., Walker, J. J., and McKillop, J. H. (1996). *Clin. Exp. Immunol.* **104,** 154–159.
261. Makkonen, N., Heinonen, S., Hiltunen, M., Helisalmi, S., Mannermaa, A., and Kirkinen, P. (2001). *J. Clin. Pathol.* **54,** 652–654.
262. Nagy, B., Rigo, J. Jr., Fintor, L., Karadi, I., and Toth, T. (1998). *J Clin. Pathol.* **51,** 324, 325.
263. Kim, Y. J., Williamson, R. A., Chen, K., Smith, J. L., Murray, J. C., and Merrill, D. C. (2001). *Hypertension* **38,** 992–996.
264. Vaillant, P., David, E., Constant, I., et al. (1996). *Nephron* **72,** 557–563.
265. Vaillant, P., David, E., Lemay, C., et al. (1992). *Presse Med.* **21,** 1413–1418.
266. Sorensen, T. K., Williams, M. A., Zingheim, R. W., Clement, S. J., and Hickok, D. E. (1993). *Am. J. Obstet. Gynecol.* **169,** 834–838.
267. Muller, F., Savey, L., Le Fiblec, B., et al. (1996). *Am. J. Obstet. Gynecol.* **175,** 37–40.
268. Onderoglu, L. S. and Kabukcu, A. (1997). *Int. J. Gynaecol. Obstet.* **56,** 245–249.
269. Ashour, A. M., Lieberman, E. S., Haug, L. E., and Repke, J. T. (1997). *Am. J. Obstet. Gynecol.* **176,** 438–442.
270. Morssink, L. P., Heringa, M. P., Beekhuis, J. R., De Wolf, B. T., and Mantingh, A. (1997). *Obstet. Gynecol.* **89,** 666–670.
271. Pouta, A. M., Hartikainen, A. L., Vuolteenaho, O. J., Ruokonen, A. O., and Laahkainen, T. J. (1998). *Obstet. Gynecol.* **91,** 940–944.
272. Wald, N. J. and Morris, J. K. (2001). *J. Med. Screen.* **8,** 65–68.
273. O'Connor, A. E., McFarlane, J. R., Hayward, S., et al. (1999). *Hum. Reprod.* **14,** 827–832.
274. Caniggia, I., Lye, S. J., and Cross, J. C. (1997). *Endocrinology* **138,** 3976–3986.
275. Yokoyama, Y., Nakamura,, T., Nakamura, R., Irahara, M., Aono, T., and Sugino, H. (1995). *J. Clin. Endocrinol. Metab.* **80,** 915–921.
276. Lockwood, G. M., Ledger, W. L., Barlow, D. H., Groome, N. P., and Muttukrishna, S. (1997). *Biol. Reprod.* **57,** 1490–1494.
277. Muttukrishna, S., Knight, P. G., Groome, N. P., Redman, C. W., and Ledger, W. L. (1997). *Lancet* **349,** 1285–1288.
278. Silver, H. M., Lambert-Messerlian, G. M., Star, J. A., Hogan, J., and Canick, J. A. (1999). *Am. J. Obstet. Gynecol.* **180,** 1131–1137.
279. Petraglia, F., Aguzzoli, L., Galfinelli, A., et al. (1995). *Placenta* **16,** 447–454.
280. Laivuori, H., Kaaja, R., Turpeinen, U., Stenman, U. H., and Ylikorkala, O. (1999). *Br. J. Obstet. Gynaecol.* **106,** 1298–1303.
281. D'Antona, D., Reis, F. M., Benedetto, C., et al. (2000). *J. Endocrinol.* **165,** 157–162.
282. Robertson, D., Burger, H. G., Sullivan, J., et al. (1996). *J. Clin. Endocrinol. Metab.* **81,** 669–676.
283. Raty, R., Koskinen, P., Alanen, A., Irjala, K., Matinlauri, I., and Ekblad, U. (1999). *Prenat. Diagn.* **19,** 122–127.
284. Jackson, N., Biddolph, S. C., Ledger, W., Groome, N. P., and Manek, S.. (2000). *Int. J. Gynecol. Pathol.* **19,** 219–224.
285. Cuckle, H., Sehmi, I., and Jones, R. (1998). *Br. J. Obstet. Gynaecol.* **105,** 1101–1103.
286. Aquilina, J., Barnett, A., Thompson, O., and Harrington, K. (1999). *Am. J. Obstet. Gynecol.* **181,** 131–136.
287. Lambert-Messerlian, G. M., Silver, H. M., Petraglia, F., et al. (2000). *J. Soc. Gynecol. Invest.* **7,** 170–174.
288. Muttukrishna, S., North, R. A., Morris, J., et al. (2000). *Hum. Reprod.* **15,** 1640–1645.
289. Sebire, N. J., Roberts, L., Noble, P., Wallace, E., and Nicolaides, K. H. (2000). *BJOG* **l 07,** 795–797.
290. Benn, P. A., Clive, J. M., and Collins, R. (1997). *Clin. Chem.* **43,** 333–337.
291. Ong, C.Y., Liao, A. W., Spencer, K., Munim, S., and Nicolaides, K. H. (2000). *BJOG* **107,** 1265–1270.
292. Smith, G. C., Stenhouse, E. J., Crossley, J. A., Aitken, D. A., Cameron, A. D., and Connor, J. M. (2002). *J. Clin. Endocrinol. Metab.* **87,** 1762–1767.
293. Lawrence, J. B., Oxvig, C., Overgaard, M. T., et al. (1999). *Proc. Natl. Acad. Sci. USA* **96,** 3149–3153.
294. van Kleffens, M., Groffen, C., Lindenbergh-Kortleve, D. J., et al. (1998). *Mol. Cell Endocrinol.* **140,** 129–135.
295. Janero, D. R. (1990). *Free Radic. Biol. Med.* **9,** 515–540.
296. Palinski, W., Horkko, S., Miller, E., et al. (1996). *J. Clin. Invest.* **98,** 800–814.
297. Witztum, J. L. and Berliner, J. A. (1998). *Curr. Opin. Lipidol.* **19,** 44l–448.
298. Gniwotta, C., Morrow, J. D., Roberts, L. J., and Kuhn, H. (1997). *Arteriosclr. Thromb. Vasc. Biol.* **17,** 3236–3241.
299. Barden, A., Beilin, L. J., Ritchie, J., Croft, K. D., Walters, B. N., and Michael, C. A. (1996). *Clin. Sci.(Colch.)* **91,** 711–718.
300. Miyata, T., Inagi, R., Asahi, K., et al. (1998). *FEBS Lett.* **437,** 24–28.
301. Zusterzeel, P. L., Rutten, H., Roelofs, H. M., Peters, W. H., and Steegers, E. A. (2001). *Placenta* **22,** 213–219.

302. Zusterzeel, P. L., Mulder, T. P., Peters, W. H., Wiseman, S. A., and Steegers, E. A. (2000). *Free Radic. Res.* **33,** 471–476.
303. Brubaker, D. B., Ross, M. G., and Maninoff, D. (1992). *Am. J. Obstet. Gynecol.* **166,** 526–531.
304. Vartio, T., Laitinen, L., Narvanen, O., et al. (1987). *J. Cell. Sci.* **88(Pt. 4),** 419–430.
305. Jang, Y., Lincoff, A. M., Plow, E. F., and Topol, E. T. (1994). *J. Am. Coll. Cardiol.* **24,** 1591–1601.
306. Burrows, T. D., King, A., and Loke, Y. W. (1994). *Placenta* **15,** 21–33.
307. Heyl, W., Handt, S., Reister, F., Gehlen, J., Mittermayer, C., and Rath, W. (1999). *Obstet. Gynecol.* **180,** 68–72.
308. Krauss, T., Kuhn, W., Lakoma, C., and Augustin, H. G. (1997). *Am. J. Obstet. Gynecol.* **177,** 443–449.
309. Lyall, F., Greer, I. A., Boswell, F., Macara, L. M., Walker, J. J., and Kingdom, J. C. (1994). *Br. J. Obstet. Gynaecol.* **101,** 485 487.
310. Budak, E., Madazli, R., Aksu, M. F., et al. (1998). *Int. J. Gynaecol. Obstet.* **63,** 115–121.
311. Phocas, I., Rizos, D., Papoulias, J., Xyni, K., Sarandakou, A., and Salamalekis, E. (2000). *J. Perinatol.* **20,** 114–119.
312. Djurovic, S., Schjetlein, R., Wisloff, F., Haugen, G., and Berg, K. (1997). *Br. J. Obstet. Gynaecol.* **104,** 466–470.
313. Livingston, J. C., Chin, R., Haddad, B., McKinney, E. T., Ahokas, R., and Sibai, B. M. (2000). *Am. J. Obstet. Gynecol.* **183,** 1554–1557.
314. 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.
315. Tidwell, S. C., Ho, H. N., Chiu, W. H., Torry, R. J., and Torry, D. S. (2001). *Am. J. Obstet. Gynecol.* **184,** 1267–1272.
316. Livingston, J. C., Haddad, B., Gorski, L. A., et al. (2001). *Am. J. Obstet. Gynecol.* **184,** 1218–1220.
317. Ong, C. Y., Liao, A. W., Cacho, A. M., Spencer, K., and Nicolaides, K. H. (2001). *Obstet. Gynecol.* **98,** 608–611.
318. Langer, B., Grima, M., Coquard, C., Bader, A. M., Schlaeder, G., and Imbs, J. L. (1998). *Obstet. Gynecol.* **91,** 196–202.
319. Kyle, P. M., Buckley, D., Kissane, J., de Swiet, M., and Redman, C. W. (1995). *Am. J. Obstet. Gynecol.* **173,** 865–872.
320. Pouliot, L., Forest, J. C., Moutquin, J. M., Coulombe, N., and Massé, J. (1998). *Obstet. Gynecol.* **91,** 591–595.
321. Pouta, A. M., Hartikainen, A. L., Vuolteenaho, O. J., Ruokonen, A. O., and Laatikainen, T. J. (1998). *Obstet. Gynecol.* **91,** 940–944.
322. Paarlberg, K. M., de Jong, C. L., van Geijn, H. P., van Kamp, G. J., Heinen, A. G., Dekker, G. A. (1998). *Am. J. Obstet. Gynecol.* **179,** 1559–1564.
323. Page, N. M., Woods, R. J., Gardiner, S. M., et al. (2000). *Nature* **405,** 797–800.
324. Millar, J. G., Campbell, S. K., Albano, J. D., Higgins, B. R., and Clark, A. D. (1996). *Br. J. Obstet. Gynaecol.* **103,** 421–426.
325. Hack, M. and Merkatz, I. R. (1995). *N. Engl. J. Med.* **333,** 1772–1774.
326. Spinillo, A., Capuzzo, E., Stronati, M., Iasci, A., Ometto, A., and Solerte, L. (1995). *J. Perinat. Med.* **23,** 175–181.
327. Kandler, C., Kevekordes, B., Zenker, M., et al. (1998). *J. Perinat. Med.* **26,** 486–490.
328. Spinillo, A., Capuzzo, E., Cavallini, A., Stronati, M., De Santolo, A., and Fazzi, E. (1998). *Eur. J. Obstet. Gynecol. Reprod. Biol.* **77,** 151–155.
329. Piper, J. M., Langer, O., Xenakis, E. M., McFarland, M., Elliott, B. D., and Berkus, M. D. (1996). *Obstet. Gynecol.* **88,** 194–199.
330. Massé, J., Forest, J. C., Moutquin, J. M., Marcoux, S., Brideau, N. A., and Belanger, M. (1993). *Am. J. Obstet. Gynecol.* **169,** 501–508.
331. Sibai, B. M., Mercer, B. M., Schiff, E., and Friedman, S. A. (1994). *Am. J. Obstet. Gynecol.* **171,** 818–822.