Published in final edited form as:

Placenta. 2013 December ; 34(12): . doi:10.1016/j.placenta.2013.09.015.

Evidence of sexual dimorphism in the placental function with severe preeclampsia

Sribalasubashini Muralimanoharan¹, Alina Maloyan¹, and Leslie Myatt^{*}

¹Center for Pregnancy and Newborn Research, Dept of OB/GYN, University of Texas Health Science Center, San Antonio, TX, USA, 78229

Abstract

Preeclampsia (PE) affects 5-8% of pregnancies and is responsible for 18% of maternal deaths in the US, and for long-term complications in mother and child. PE is an inflammatory state and may influence placental function in a sex-specific manner. We determined if there is a sexual dimorphism in the placental inflammatory and apoptotic responses in preeclamptic pregnancies. Placentas were collected from normotensive and preeclamptic pregnancies with either male or female fetuses (MPE and FPE respectively) after c-section at term with no labor. Expression patterns of markers of inflammation measured by ELISA, as well as hypoxia, apoptosis and angiogenesis markers measured by Western blotting were determined in the placenta. Consistent with previous studies, an increase in inflammation, hypoxia, and apoptotic cell death was observed in PE compared to normotensive pregnancies. Levels of TNF α , IL-6 and IL-8, and HIF-1 α were significantly greater, whereas the angiogenic marker VEGF was significantly reduced in MPE vs. FPE. Sexual dimorphism was also observed in the activation of cell death: the number of TUNEL-positive cells, and the expression pro-apoptotic markers PUMA and Bax being higher in MPE vs. FPE. We also found an increase in the levels of protein and DNA-binding activity of NF κ B p65 in MPE vs. FPE. In summary, we show here that in preeclamptic pregnancies the placentas of males were associated with significantly higher expression of inflammatory, hypoxia and apoptotic molecules but reduced expression of a pro-angiogenic marker compared to placentas of female fetuses. We propose that the transcription factor NF κ Bp65 might, at least partially, be involved in sexual dimorphism during PE.

Keywords

Preeclampsia; sexual dimorphism; apoptosis; inflammation; NF κ Bp65

Introduction

The syndrome of preeclampsia (PE) is defined by hypertension and significant proteinuria developed at or after 20 weeks of gestation in previously normotensive women and which resolves postpartum (1). It is a multisystem disorder, which complicates 5-8% of pregnancies in the United States (1). The incidence of PE has increased over past decades in association with increasing maternal age, and the incidence of diabetes, obesity and multiple

© 2013 Elsevier Ltd. All rights reserved.

^{*}Corresponding author: Leslie Myatt, PhD, Professor, Director, Center for Pregnancy and Newborn Research, Dept of Ob/GYN, University of Texas Health Science Center San Antonio, San Antonio, Texas, 78229, myattl@uthscsa.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

births (2). Several mechanisms have been suggested to play a role in the etiopathogenesis of PE including an abnormal immune response, defective placentation, relative placental hypoxia or ischemia and oxidative/nitrative stress (3). This leads to an exaggerated maternal inflammatory response (4) and generalized maternal endothelial cell activation, the causes of which are still uncertain but thought to be triggered by angiogenic or other factors released from the placenta (5).

Sexual dimorphism is now increasingly recognized as a factor in placental function and placental disorders. Microarray analysis has shown distinct sexual dimorphism in gene expression in the human placenta, in particular immune genes were expressed at higher level in female placenta compared to male (6). Gene expression in the placenta also responds to maternal inflammatory status in a sex-dependent manner (7). Expression of 59 genes were changed in the placenta of women with asthma vs. no asthma with a female fetus compared to only 6 genes changed in those with asthma but a male fetus (8). Some of these genes were associated with growth, inflammatory and immune pathways. Changes in diet provide distinctive signature of sexually dimorphic genes in placenta with expression generally higher in female than male placentas (9). The male placenta has higher TLR4 expression and a greater production of TNF α in response to LPS than the female placenta, which may underlie the propensity to preterm birth in males (10).

A male fetus is more at a risk of poor outcome than the female fetus in association with complications such as placental insufficiency, preeclampsia, infection, IUGR and preterm delivery (11). A Norwegian population-based study of 1.7 million singleton births has clearly identified that preterm delivery and perinatal mortality and morbidity are dominated by the male sex (12). While some reports suggest that preeclampsia is more prevalent with male fetuses, the Norwegian study shows an increased incidence of PE at <37 weeks gestation with a female fetus (13), which may reflect the fact that male fetuses are delivered earlier due to other problems and may not therefore stay in utero to allow the mother to develop PE (14). The male fetus is also associated with more vasoconstricted state in the maternal microcirculation and greater endothelial dysfunction of preeclamptic women compared to those with female fetus (15). Despite all these data, the mechanism responsible for a sexual dimorphic effect in preeclampsia remains unknown. The aim of present study was to determine if there was a sexual dimorphism in the placenta from pregnancies complicated by PE in expression of markers for inflammation, hypoxia, apoptosis and angiogenesis.

Materials and Methods

Ethical Approval and Study Participants

Placentas were collected from the Labor and Delivery Unit at University Hospital under a protocol approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio with informed consent from patients. Placentas were collected immediately following delivery by c-section in the absence of labor from normotensive (CTRL, n=10, 5 male/5 female) and severe preeclamptic pregnancies (PE, n=10, 5 male/5 female). Women with chronic hypertension, diabetes, renal disease, multifetal gestations or any other medical complication together with smokers were also excluded from the study. Severe preeclampsia was defined as hypertension (systolic blood pressure >160 mmHg and/or diastolic blood pressure >110 mmHg on two occasions 2 - 240 hr apart), and proteinuria (≥ 2 protein on dipstick) occurring after 20 weeks of gestation in a previously normotensive woman (16).

Materials

TUNEL assay kit was obtained from Roche Diagnostics, EMSA kit from Pierce, Thermo Scientific. Antibodies were purchased from BD Biosciences (HIF-1 α), Santa Cruz (VEGF), Cell Signaling (NF κ B p65 caspase-3, caspase-9, Bcl-2, p53, PUMA and BAX), and Sigma (β -actin). ELISA kits for TNF α , IL-6 and IL-8 were purchased from R&D Systems.

Tissue processing and sampling

A random sampling technique was used to collect tissue from 5 sites around the circumference of the placenta at least one inch from the periphery (17) Villous tissue was dissected out from beneath the chorionic plate, avoiding the basal plate, flash frozen and stored at -80°C . Tissues were homogenized by bead beater (Biospec Products, USA) in lysis buffer as described (18). Total protein in the homogenates was estimated using Bradford's reagent (BioRad).

ELISA

Fresh placental villous tissue homogenates were used to measure the levels of TNF α , IL-6 and IL-8 by ELISA by the manufacturer's protocol (R&D Systems).

TUNEL Assay

Frozen sections from CTRL and PE placentas were analyzed by terminal deoxynucleotidyl transferase mediated nick end labeling (TUNEL) according to the manufacturer's protocol. Nuclei were counterstained by DAPI (Invitrogen). Image acquisition was performed by fluorescence microscope (Nikon).

Western blotting

Proteins were separated on 4-20% gradient precast gels (Bio-Rad), transferred onto nitrocellulose membranes and blocked with 5% nonfat milk in 0.1% Tween, 20 mM Tris (pH7.5)-buffered saline (TTBS) (w/v) for 1 h. Blots were probed with primary antibody in 1% nonfat milk powder/TTBS overnight at 4°C and were detected using HRP-conjugated secondary antibody in 5% nonfat milk/TTBS for 1 h. Products were visualized by chemiluminescent HRP substrate (Millipore). Band intensity was measured in a G:Box using Gene Snap and Gene Tools software (Syngene).

EMSA

For high salt extraction of nuclear proteins, frozen tissue (~ 50 -100 mg) was homogenized in 1 ml of low salt buffer A (10 mM HEPES-KOH, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, and 1 mM dithiothreitol) containing 0.1% NP-40 (Sigma) and protease inhibitors (Roche), followed by centrifugation at $16,000 \times g$ for 5 min at 4°C pellet was resuspended in 50 μl of high salt buffer B (20 mM HEPES-KOH, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl₂, and 0.2 mM EDTA) and further incubated on ice for 30 min for high salt extraction. The samples were centrifuged at $16,000 \times g$ for 30 min at 4°C , and the supernatant were transferred to a pre-chilled tube. Binding of p65 to consensus DNA sequence was performed using the chemiluminescent nucleic acid detection module according to the manufacturer's protocol (Thermo Scientific). 5'-Biotin labeled consensus sequence (sense: 5AGTTGAGGGGACTTTCCAGGC3' and antisense: 5'GCCTGGGAAAGTCCCTCAACT3' was obtained from the nucleic acid core facility at UTHSCSA.

Statistical Analysis

Data are reported as mean $\pm$ SEM. Comparisons between two groups were performed with Student's t-test. One-way Analyses of Variance (ANOVA) with Tukey's post hoc test were used where appropriate. $P < 0.05$ was considered as significant.

Results

Clinical data

The clinical and demographic characteristics of the patients involved in the present study are listed in Table 1. Systolic and diastolic blood pressures were significantly greater in the preeclamptic group than the controls. No difference in BMI, maternal age and gestational age were found between normotensive controls and preeclampsics with a female fetus. Among the groups with a male fetus, the gestational age of preeclampsics was slightly but significantly smaller than of normotensive pregnancies with no difference in other characteristics. Birth weight percentile did not differ between the groups. Placental weight is not routinely measured at University Hospital and was therefore not available.

Cytokine and chemokine production in preeclamptic placentas

Release of inflammatory cytokines by the placenta in response to hypoxia/ischemia may lead to increased levels of them in maternal circulation and endothelial dysfunction during preeclampsia (19). Among the pro-inflammatory cytokines, placental TNF α and IL-6, as well as the chemokine IL-8, were previously reported to be elevated during preeclampsia (20). As shown in Figure 1, we found significantly higher levels of TNF α , IL-8 and IL-6 in both male and female placentas of preeclamptic women compared to normotensive controls. While there was no difference between male and female placentas in normotensive women, in preeclampsia, the male PE placenta had significantly higher levels of TNF α , IL-8 and IL-6 compared to female PE placenta.

Expression of markers for angiogenesis and hypoxia

It is well-known that preeclampsia is associated with altered production of angiogenic peptides caused by relative hypoxia in the placental tissue (21). We measured expression of VEGF protein as a marker for angiogenesis and HIF-1 α as a hypoxia marker. We found significantly reduced expression of VEGF in male preeclampsia (MPE) compared to the male CTRL (Figure 2) but with no differences seen in female placentas. Previously we have already shown an increase in HIF1 α levels in preeclamptic placentas (18). In this study, we have separated the PE samples by fetal sex and found that the protein expression of HIF-1 α was significantly higher in male PE placentas compared to female PE placentas. Consistent with previous report, the activity of HIF-1 α was reduced in placentas with preeclampsia (18). However, no fetal sex-specific differences were observed (not shown).

Activation of cell death in the placenta with preeclampsia

Activation of apoptosis has been previously reported in preeclamptic placentas (22). Here we determined whether there are fetal sex dependent differences in activation of cell death. The TUNEL staining was significantly increased in both cyto- and syncytiotrophoblasts from preeclamptic placentas compared to normotensive controls; however, in the MPE it was more than twice higher ($p < 0.05$) than in FPE (Figure 3). Protein expression of activated caspase-3, caspase-9, p53, PUMA and Bax, proapoptotic members of Bcl-2 family, were significantly increased in both MPE and FPE compared to their normotensive controls (Figure 4A- D, F and G). In contrast, expression of the antiapoptotic protein Bcl-2 was significantly lower in both male and female preeclamptic tissue compared to control (Figure

4A and E). Again, there was sexual dimorphism with expression of PUMA and Bax being significantly higher and Bcl-2 significantly lower in MPE when compared to FPE.

Activation of NF κ B signaling in PE placentas

Earlier studies have demonstrated the role of NF κ B in other inflammatory conditions, such as cancer and diabetes (23). Since we observed fetal-sex dependent differences in the expression of inflammatory, hypoxia and apoptosis markers, we measured expression of the p65 subunit of NF κ B in normotensive and preeclamptic placentas of male and female fetuses (Figure 5). We found significantly higher levels of p65 in preeclamptic placentas from both males and females compared to normotensive placentas. However, the expression of p65 in the male preeclamptic was significantly higher than in the corresponding female group (Figure 5A). Since NF κ B p65 is a transcription factor, we wished to determine whether its accumulation would correspond with an increase in DNA-binding activity. The binding of p65 to consensus DNA sequence in PE and normotensive placentas was measured by EMSA and found to be higher in the PE placentas compared to the controls. Male preeclamptic placentas showed significantly higher activation of NF κ B compared to the female preeclamptic placentas (Figure 5B).

Discussion

Preeclampsia is a “disease of the theories” because of its unknown etiology (24). Recently, excessive inflammation and the angiogenic imbalance have been highlighted as underlying the syndrome (25). mRNA expression of cytokines TNF- α , IL- β , IL-6, IL-5 and IL-8 vary in the placenta with severity of asthma and fetal sex (26). All cytokines were increased in female carrying placentas of pregnancies complicated by asthma relative to female control placenta whereas the male fetus had no significant alterations in placental cytokine expression in the presence of maternal asthma (27). In this study, we found elevated levels of TNF- α , IL-6 and IL-8 in preeclamptic placentas compared to the normotensive controls, and for the first time we report a sexual dimorphism in expression of cytokines with male PE placenta showing much higher levels of cytokines and cell death than female PE placentas. The mechanisms underlying the dimorphism remain unknown, however, recent reports link sex differences to gonadal steroids. Women with preeclampsia were shown to have increased plasma testosterone levels compared to healthy pregnant women, with significantly higher levels in male- than in female-bearing preeclamptic pregnancies (28). Additionally, placental levels of aromatase, a rate-limiting enzyme converting androgens to estrogens, varied depending on fetal sex: being much higher in the preeclamptic placentas with female than male fetuses (29).

Extensive research has been dedicated to placental ischemia/hypoxia and its possible role in preeclampsia. Hypoxia is known to stimulate expression of number of angiogenic proteins including endothelin, VEGF and Flt-1 (30). The transcription factor HIF-1 α regulates VEGF, and increased HIF-1 α is found with preeclampsia in the placenta (31). We have previously shown that the protein but not mRNA levels of HIF1 α were increased during preeclampsia, however, despite stabilization, the binding of HIF-1 α to the consensus hypoxia response element (HRE), was decreased causing relatively lower expression of VEGF (18). Syncytiotrophoblast secretes many bioactive factors that are significantly altered in preeclampsia. Of most interest is the soluble receptor for VEGF, sFlt-1, which, when present in excess, as in preeclampsia, binds to and inactivates VEGF (32). In this study, we observed that the levels of HIF-1 α were significantly higher in male preeclamptic placentas compared to both female preeclamptics and to normotensive male and female controls. Correspondingly, the VEGF protein levels were significantly lower in preeclamptic placenta and still lower in male preeclamptic compared to female.

Increased trophoblast apoptosis has been observed in placentas from pregnancies complicated by preeclampsia and cited as a contributing factor to the pathogenesis of this condition (22). Some early studies found no difference in Bcl-2, Bcl-xL, Bax and Bak expression in placental villi of preeclamptic vs. normotensive placentas (33), but others reported the expression of Bcl-2 to be less abundant in syncytiotrophoblast from severe preeclamptic placentas (34). The increased trophoblast apoptosis with preeclampsia is thought to be the result of placental oxidative stress, which may in part be triggered by hypoxia (35). Hypoxia induces apoptosis in term trophoblast by decreasing the expression of Bcl-2 (anti-apoptotic) while increasing the expression of p53 and Bax (pro-apoptotic) (36). In our study, we observed an increase in apoptosis by TUNEL staining in preeclamptic placenta, which was significantly greater in the placentas of males compared to females, again indicating sexual dimorphism. We observed an increase in TUNEL-positive nuclei both in cyto- and syncytiotrophoblast. Longtine et al., have reported that apoptosis is limited only to cytotrophoblast, but on the other hand they have suggested that, apoptotic cytotrophoblasts and/or cytotrophoblast-derived vesicles can be localized within the syncytiotrophoblast cytoplasm (37). However, the mechanism by which this occurs is unclear. One possibility is that the syncytiotrophoblast may act as a phagocyte and engulf the apoptotic cytotrophoblast, which can be potentially detected by TUNEL staining.

The increased expression of the apoptotic proteins p53 and p53 upregulated modulator of apoptosis (PUMA), as well as Bax, activated caspase-3 and caspase-9 together with significantly lower expression of Bcl-2 in preeclamptic placentas are consistent with the increased apoptosis. In addition, we have found that PUMA, Bax and Bcl-2 were changed in a fetal sex-dependent manner with significantly greater expression in male preeclamptic placentas vs. female preeclamptic placentas.

In vitro studies on placental explants and trophoblasts have shown that hypoxia can activate a sequence of events starting with upregulation of HIF-1 α and eventually lead to apoptotic cell death (19). Since we observed apoptosis in preeclamptic placentas, we suggest that it can be caused by relative hypoxia during preeclampsia. There is a growing body of evidence, however, suggesting that HIF-1 α , can also be activated through inflammation-related factors that include cytokines (IL-1 β and TNF α) with NF κ B as key link that drives cytokine cellular signaling (38). Studies have demonstrated the ability of NF κ B to upregulate expression of HIF-1 α under normoxic conditions (39). There is a crosstalk between hypoxia and inflammation in placenta: it was reported that HIF-1 α activates NF κ B, that NF κ B controls HIF-1 α transcription, and that HIF-1 α activation may be concurrent with inhibition of NF κ B (39).

NF κ B is a redox-sensitive transcription factor regulating a battery of inflammatory genes, and has a variety of different effects in numerous pathological states (40). Activation of NF κ B binding and increased caspase-3 both affects the endothelial cells under hypoxic conditions (41). In most cells, NF κ B is found in the cytoplasm in its inactive form, bound to inhibitory proteins. Many extracellular stimuli, including bacterial lipopolysaccharide, viruses, oxidants, inflammatory cytokines, and immune stimuli, can activate NF κ B. Once activated, it binds to regulatory DNA elements in the promoter regions of inflammatory and immune response genes, such as those encoding pro-inflammatory cytokines, chemokines, enzymes relevant for inflammation, and adhesion molecules (41). Aban et al. have reported elevated NF κ B immunostaining in placentas complicated by growth restriction and preeclampsia along with apoptotic markers (42). Vaughan and Walsh have shown a marked increase in NF κ B activity in preeclamptic placentas as well as in cultured trophoblasts exposed to either hypoxia or inflammation or both (43). We also found an increase in NF κ B activity in preeclamptic placentas vs. normotensive placentas. In addition to this, we show that expression and activation of NF κ B were changed in fetal-sex dependent manner.

In conclusion, for the first time, we report sexual dimorphism in pro-inflammatory cytokine production and apoptosis in the placenta in the setting of preeclampsia. We also found an increase in the expression and DNA binding activity of NF κ B p65 in the preeclamptic placentas compared to normotensive placentas with much higher levels in placentas of males compared to females. We propose that increased inflammation and trophoblast cell death observed in the placenta of preeclamptic pregnancies are, at least partially, induced by NF κ B p65, further emphasizing the role of inflammation in the etiology of preeclampsia. We hypothesize that, the sex differences in the placental inflammatory response and subsequent pathological changes might affect these fetuses as they reach adulthood. It was suggested by Nicolette et al. (44), that inflammation might compromise the development of the fetal innate immune response, supporting hypothesis of *in utero* origins of neonatal and childhood inflammatory disease. Further studies are required to understand the mechanism(s) underlying the sexual dimorphism in inflammatory responses and the involvement of NF κ B. Preeclampsia has a long-term impact on maternal health. Several studies have pointed the association between preeclampsia and later cardiovascular disease of the mother(45). Whether having a male fetus will have a greater effect on maternal health remains to be investigated.

References

1. Vitoratos N, Vrachnis N, Iavazzo C, Kyrgiou M. Preeclampsia: molecular mechanisms, predisposition, and treatment. *J Pregnancy*. 2012; 2012:145487. Editorial Introductory. [PubMed: 22690336]
2. Catov JM, Ness RB, Kip KE, Olsen J. Risk of early or severe pre-eclampsia related to pre-existing conditions. *Int J Epidemiol*. 2007 Apr; 36(2):412–9. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't. [PubMed: 17255351]
3. Myatt L, Miodovnik M. Prediction of preeclampsia. *Semin Perinatol*. 1999 Feb; 23(1):45–57. [Review]. [PubMed: 10102170]
4. Redman CW, Sargent IL. Preeclampsia and the systemic inflammatory response. *Semin Nephrol*. 2004 Nov; 24(6):565–70. Review. [PubMed: 15529291]
5. Staff AC, Benton SJ, von Dadelszen P, Roberts JM, Taylor RN, Powers RW, et al. Redefining preeclampsia using placenta-derived biomarkers. *Hypertension*. 2013 May; 61(5):932–42. Research Support, Non-U.S. Gov't. [PubMed: 23460278]
6. Sood R, Zehnder JL, Druzin ML, Brown PO. Gene expression patterns in human placenta. *Proc Natl Acad Sci U S A*. 2006 Apr 4; 103(14):5478–83. [PubMed: 16567644]
7. Scott NM, Hodyl NA, Murphy VE, Osei-Kumah A, Wyper H, Hodgson DM, et al. Placental cytokine expression covaries with maternal asthma severity and fetal sex. *Immunol*. 2009 Feb 1; 182(3):1411–20.
8. Osei-Kumah A, Smith R, Jurisica I, Caniggia I, Clifton VL. Sex-specific differences in placental global gene expression in pregnancies complicated by asthma. *Placenta*. 2011 Aug; 32(8):570–8. [PubMed: 21641640]
9. Mao J, Zhang X, Sieli PT, Falduto MT, Torres KE, Rosenfeld CS. Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. *Natl Acad Sci U S A*. 2010 Mar 23; 107(12):5557–62.
10. Yeganegi M, Leung CG, Martins A, Kim SO, Reid G, Challis JR, et al. Lactobacillus rhamnosus GR-1 stimulates colony-stimulating factor 3 (granulocyte) (CSF3) output in placental trophoblast cells in a fetal sex-dependent manner. *Biol Reprod*. 2011 Jan; 84(1):18–25. [PubMed: 20811016]
11. Clifton VL. Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta*. 2010 Mar.31 Suppl:S33–9. Research Support, Non-U.S. Gov't Review. [PubMed: 20004469]
12. Vatten LJ, Skjaerven R. Offspring sex and pregnancy outcome by length of gestation. *Early Hum Dev*. 2004 Jan; 76(1):47–54. [PubMed: 14729162]

13. Makhseed M, Musini VM, Ahmed MA. Association of fetal gender with pregnancy-induced hypertension and pre-eclampsia. *Gynaecol Obstet*. 1998 Oct; 63(1):55–6.
14. Challis J, Newnham J, Petraglia F, Yeganegi M, Bocking A. Fetal sex and preterm birth. *Placenta*. 2013 Feb; 34(2):95–9. Research Support, Non-U.S. Gov't. [PubMed: 23261268]
15. Stark MJ, Dierckx L, Clifton VL, Wright IM. Alterations in the maternal peripheral microvascular response in pregnancies complicated by preeclampsia and the impact of fetal sex. *Soc Gynecol Investig*. 2006 Dec; 13(8):573–8.
16. Myatt L, Clifton RG, Roberts JM, Spong CY, Hauth JC, Varner MW, et al. First-Trimester Prediction of Preeclampsia in Nulliparous Women at Low Risk. *Obstet Gynecol*. 2012 Jun; 119(6):1234–42. [PubMed: 22617589]
17. Mayhew TM. Taking tissue samples from the placenta: an illustration of principles and strategies. *Placenta*. 2008 Jan; 29(1):1–14. Research Support, Non-U.S. Gov't Review. [PubMed: 17658596]
18. Muralimanoharan S, Maloyan A, Mele J, Guo C, Myatt LG, Myatt L. MIR-210 modulates mitochondrial respiration in placenta with preeclampsia. *Placenta*. 2012 Oct; 33(10):816–23. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't. [PubMed: 22840297]
19. Ahn H, Park J, Gilman-Sachs A, Kwak-Kim J. Immunologic characteristics of preeclampsia, a comprehensive review. *American journal of reproductive immunology*. 2011 Apr; 65(4):377–94. Review. [PubMed: 20825381]
20. Benyo DF, Smarason A, Redman CW, Sims C, Conrad KP. Expression of inflammatory cytokines in placentas from women with preeclampsia. *The Journal of clinical endocrinology and metabolism*. 2001 Jun; 86(6):2505–12. Research Support, U.S. Gov't, P.H.S. [PubMed: 11397847]
21. Tuuli MG, Longtine MS, Nelson DM. Review: Oxygen and trophoblast biology--a source of controversy. *Placenta*. 2011 Mar; 32(2):S109–18. Research Support, N.I.H., Extramural Review. [PubMed: 21216006]
22. DiFederico E, Genbacev O, Fisher SJ. Preeclampsia is associated with widespread apoptosis of placental cytotrophoblasts within the uterine wall. *The American journal of pathology*. 1999 Jul; 155(1):293–301. Research Support, U.S. Gov't, P.H.S. [PubMed: 10393861]
23. Karin M, Lin A. NF-kappaB at the crossroads of life and death. *Nat Immunol*. 2002 Mar; 3(3):221–7. Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review. [PubMed: 11875461]
24. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet*. 2005 Feb 26; Mar 26; 365(9461):785–99. Review. [PubMed: 15733721]
25. Redman CW, Tannetta DS, Dragovic RA, Gardiner C, Southcombe JH, Collett GP, et al. Review: Does size matter? Placental debris and the pathophysiology of pre-eclampsia. *Placenta*. 2012 Feb; 33(S48-54) Review.
26. Scott NM, Hodyl NA, Murphy VE, Osei-Kumah A, Wyper H, Hodgson DM, et al. Placental cytokine expression covaries with maternal asthma severity and fetal sex. *Immunol*. 2009 Feb 1; 182(3):1411–20. Comparative Study Research Support, Non-U.S. Gov't.
27. Clifton VL. Sexually dimorphic effects of maternal asthma during pregnancy on placental glucocorticoid metabolism and fetal growth. *Cell Tissue Res*. 2005 Oct; 322(1):63–71. Review. [PubMed: 16052336]
28. Steier JA, Ulstein M, Myking OL. Human chorionic gonadotropin and testosterone in normal and preeclamptic pregnancies in relation to fetal sex. *Obstet Gynecol*. 2002 Sep; 100(3):552–6. [PubMed: 12220777]
29. Sathishkumar K, Balakrishnan M, Chinnathambi V, Chauhan M, Hankins GD, Yallampalli C. Fetal sex-related dysregulation in testosterone production and their receptor expression in the human placenta with preeclampsia. *J Perinatol*. 2011 Sep 8.
30. Nevo O, Soleymanlou N, Wu Y, Xu J, Kingdom J, Many A, et al. Increased expression of sFlt-1 in in vivo and in vitro models of human placental hypoxia is mediated by HIF-1. *Am J Physiol Regul Integr Comp Physiol*. 2006 Oct; 291(4):R1085–93. In Vitro Research Support, Non-U.S. Gov't. [PubMed: 16627691]
31. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*. 1992 Oct 29; 359(6398):843–5. Research Support, Non-U.S. Gov't. [PubMed: 1279431]

32. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med*. 2006 Sep 7;355(10):992–1005. Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural. [PubMed: 16957146]
33. Allaire AD, Ballenger KA, Wells SR, McMahon MJ, Lessey BA. Placental apoptosis in preeclampsia. *Obstet Gynecol*. 2000 Aug; 96(2):271–6. [PubMed: 10908776]
34. Ishihara N, Matsuo H, Murakoshi H, Laoag-Fernandez JB, Samoto T, Maruo T. Increased apoptosis in the syncytiotrophoblast in human term placentas complicated by either preeclampsia or intrauterine growth retardation. *Am J Obstet Gynecol*. 2002 Jan; 186(1):158–66. Research Support, Non-U.S. Gov't. [PubMed: 11810103]
35. Kilani RT, Mackova M, Davidge ST, Guilbert LJ. Effect of oxygen levels in villous trophoblast apoptosis. *Placenta*. 2003 Sep-Oct;24(8-9):826–34. Comparative Study Research Support, Non-U.S. Gov't. [PubMed: 13129679]
36. Levy R, Smith SD, Chandler K, Sadovsky Y, Nelson DM. Apoptosis in human cultured trophoblasts is enhanced by hypoxia and diminished by epidermal growth factor. *Am J Physiol Cell Physiol*. 2000 May; 278(5):C982–8. Research Support, U.S. Gov't, P.H.S. [PubMed: 10794672]
37. Longtine MS, Chen B, Odibo AO, Zhong Y, Nelson DM. Villous trophoblast apoptosis is elevated and restricted to cytotrophoblasts in pregnancies complicated by preeclampsia, IUGR, or preeclampsia with IUGR. *Placenta*. 2012 May; 33(5):352–9. [PubMed: 22341340]
38. Taylor CT, Cummins EP. The role of NF-kappaB in hypoxia-induced gene expression. *Ann N Y Acad Sci*. 2009 Oct;1177:178–84. [PubMed: 19845620]
39. Carbia-Nagashima A, Gerez J, Perez-Castro C, Paez-Pereda M, Silberstein S, Stalla GK, et al. RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1alpha during hypoxia. *Cell*. 2007 Oct 19; 131(2):309–23. [PubMed: 17956732]
40. Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB (Review). *Mol Med*. 1999 Sep; 4(3):223–30. Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review.
41. Matsushita H, Morishita R, Nata T, Aoki M, Nakagami H, Taniyama Y, et al. Hypoxia-induced endothelial apoptosis through nuclear factor-kappaB (NF-kappaB)-mediated bcl-2 suppression: in vivo evidence of the importance of NF-kappaB in endothelial cell regulation. *Circulation research*. 2000 May 12; 86(9):974–81. Research Support, Non-U.S. Gov't. [PubMed: 10807870]
42. Aban M, Cinel L, Arslan M, Dilek U, Kaplanoglu M, Arpaci R, et al. Expression of nuclear factor-kappa B and placental apoptosis in pregnancies complicated with intrauterine growth restriction and preeclampsia: an immunohistochemical study. *Tohoku J Exp Med*. 2004 Nov; 204(3):195–202. [PubMed: 15502418]
43. Vaughan JE, Walsh SW. Activation of NF-kappaB in placentas of women with preeclampsia. *Hypertens Pregnancy*. 2012; 31(2):243–51. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't. [PubMed: 22380486]
44. Hodyl NA, Stark MJ, Osei-Kumah A, Clifton VL. Prenatal programming of the innate immune response following in utero exposure to inflammation: a sexually dimorphic process? *Expert Rev Clin Immunol*. 2011 Sep; 7(5):579–92. Research Support, Non-U.S. Gov't. [PubMed: 21895471]
45. Thornburg KL, O'Tierney PF, Louey S. Review: The placenta is a programming agent for cardiovascular disease. *Placenta*. 2010 Mar; 31(1):S54–9. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review. [PubMed: 20149453]

Abbreviations

BMI	Body mass index
TNFα	Tumor necrosis factor alpha
IL-8	Interleukin-8
IL-6	Interleukin 6

PE	preeclampsia
HIF-1α	hypoxia inducible factor-1alpha
NFκB	factor kappa B
VEGF	vascular endothelial growth factor
PUMA	p53 upregulated modulator of apoptosis

Figure 1. Production of cytokines and chemokines by male and female placentas of normotensive and preeclamptic pregnancies

(A) TNF α , (B) IL-8 and. (C) IL-6 levels in CTRL and PE placentas. N=5 in each group. * p<0.05 vs. FCTRL and MCTRL. # p<0.05 compared to FPE.

Figure 2. Expression of HIF-1 α and VEGF in the placental villous tissue of normotensive and preeclamptic pregnancies

(A) Representative Western blots showing expression of HIF-1 α and VEGF. Actin expression was used as loading control. (B) Scatter plot showing the quantification of HIF-1 α and VEGF. N=5 in each group. * $p < 0.05$ compared to FCTRL and MCTRL. # $p < 0.05$ compared to FPE.

Figure 3. DNA Fragmentation in normotensive and preeclamptic pregnancies

Left panel, Representative images of TUNEL staining in cryosections from CTRL and PE placental villous tissue (green). The nuclei were counterstained with DAPI (blue). Right panel, quantitation of TUNEL positive nuclei normalized to total number of nuclei. N=5 in each group *, $p < 0.05$ compared to FCTRL and MCTRL; # $p < 0.05$ compared to FPE.

Figure 4. Apoptosis in normotensive and preeclamptic pregnancies

(A), Representative Western blots and (B-G), quantification data showing expression of pro-apoptotic (p53, PUMA, Bax, Caspase-3 and Caspase-9) and anti-apoptotic (Bcl-2) markers. Beta-actin expression was used as loading control. N=5 in each group. * $p < 0.05$ compared to FCTRL and MCTRL. # $p < 0.05$ compared to FPE.

Figure 5. NFκBp65 expression and binding to DNA in placentas from normotensive and preeclamptic pregnancies

(A) Representative Western blots and (B) quantification bars showing expression of NFκB p65 expression in nuclear fraction of CTRL and PE villous tissue. Expression of histone H3 was used as loading control. (C) EMSA showing binding of nuclear protein extract to the consensus DNA binding site of p65. N=5 in each group. *, $p < 0.05$ compared to FCTRL and MCTRL. #, $p < 0.05$ compared to FPE.

Table 1

Clinical and demographic characteristics of patients

Values are expressed as mean $\pm$ SEM. * $p < 0.05$ vs. CTRL.

	Normotensive Control		Preeclampsia		FCTRL vs FPE		MCTRL vs MPE	
	Female (n=5)	Male (n=5)	Female (n=5)	Male (n=5)	P	P	P	P
Maternal Age (Years)	24.3 $\pm$ 2.0	27.6 $\pm$ 1.9	27.0 $\pm$ 2.7	27.0 $\pm$ 3.3	0.45		0.8	
Nulliparity (n)	3	1	1	2				
Smoking (n)	0	0	0	0				
BMI (kg/m ²)	25.3 $\pm$ 3.1	29.0 $\pm$ 2.2	28.8 $\pm$ 1.2	29.6 $\pm$ 2.2	0.57		0.18	
Gestational age (weeks)	39.9 $\pm$ 0.5	40.0 $\pm$ 0.4	37.7 $\pm$ 2.6	37.2 $\pm$ 2.2 *	0.28		0.01	
Birth weight (g)	3124 $\pm$ 87	3535 $\pm$ 77	2870 $\pm$ 422	3123 $\pm$ 273	0.38		0.85	
SGA <10 percentile	0	0	1	1				
AGA 25 - 90 percentile	5	5	4	4				
Systolic Blood Pressure (mm/Hg)	98 $\pm$ 9	110 $\pm$ 10	153 $\pm$ 4 *	159 $\pm$ 4 *	0.02		0.01	
Diastolic Blood Pressure (mm/Hg)	70 $\pm$ 6	73 $\pm$ 4	99 $\pm$ 9 *	98 $\pm$ 8 *	0.01		0.01	
Proteinuria (Dipstick)	Not done	Not done	2-3	2-3				
Ponderal Index of fetus	2.4 $\pm$ 0.2	2.5 $\pm$ 0.2	2.9 $\pm$ 0.9	2.5 $\pm$ 0.4	0.30		0.85	

N=5 in each group. Values are mean $\pm$ SD, One-way Analyses of Variance (ANOVA) with Tukey's post hoc test

* $p < 0.05$ vs normotensive control.