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Research Article

ASSESSMENT OF PLACENTAL OXIDATIVE STRESS PARAMETERS IN PRE-ECLAMPTIC AND NORMAL PREGNANT WOMEN

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ABSTRACT

Objective: Oxidative stress occurs when cellular levels of reactive oxygen species exceed antioxidant capabilities and has been implicated in the pathogenesis of pre-eclampsia. In this study, we examined the tissue levels of endogenous antioxidant enzymes or proteins (superoxide dismutase [SOD], glutathione peroxidise, reduced glutathione, catalase, and thioredoxin) and the levels of lipid peroxides, protein carbonyls, hydrogen peroxide, and nitrosative biomarkers in the placental samples from normal and pre-eclamptic pregnancies.

Results: Pre-eclamptic tissue homogenates demonstrated significantly increased levels of lipid peroxidation (21.61±0.18 vs. 5.695±0.46) and a trended increase in protein carbonyls (245.95±4.05 vs. 203.48±3.65) concentration when compared to controls. The levels and activities of the antioxidant proteins; SOD (365.2±2.915 vs. 205.6±3.76), thioredoxin (100.64±3.38 vs. 80.89±3.37), glutathione peroxidase (340.88±6.16 vs. 164.46±3.03), catalase (5.26±0.02 vs. 4.62±0.11), and reduced glutathione (46.99±0.508 vs. 28.19±0.178) were all found to be significantly reduced when comparing pre-eclamptic placental tissue homogenates to gestational age matched control placentae from non pre-eclamptic pregnancies.

Conclusion: The results of this study demonstrate a decreased enzymatic antioxidant capacity and increased oxidation in placental tissue from pre-eclamptic women, which may contribute to the pathogenesis of this complex disorder.

Keywords: Oxidative stress, Reactive oxygen species, Superoxide dismutase, Glutathione peroxidise, Reduced glutathione, Catalase, Thioredoxin, Lipid peroxides and protein carbonyls.

INTRODUCTION

Pre-eclampsia is an abnormal condition of pregnancy, clinically diagnosed according to the Australian Society for the study of hypertension in pregnancy defines as the onset of acute hypertension and maternal organ dysfunction after 20 weeks gestation in women with no known history of hypertension or renal disease, and whole blood pressure was normal in the first half of pregnancy. Diagnosis can be made when hypertension arises after 20 weeks gestation - confirmed on 2 or more occasions. Accompanied by random urine protein/creatinine ratio ≥30 mg/mmol, serum or plasma creatinine ≥90 mmol/L or Oliguria, raised transaminases, severe epi-gastric or right upper quadrant pain, severe headache persistent visual disturbances, hyperreflexia with sustained clonus convulsions, stroke pulmonary edema, intrauterine fetal growth restriction, placental abruption.[1,2]. A longer-term burden also exist, as women who develop pre-eclampsia, are 2½ times more likely to display ischemic heart disease later in life [3], and infants born to pre-eclampsia mothers are at a higher risk of developing respiratory diseases and experiencing long-term neurological morbidity [4-6].

The economic significance of pre-eclampsia is substantial when considering the requirement of regular antenatal monitoring for symptoms, the preservation of maternal health and care of premature or small for gestational age infants whose incidence of acute neonatal morbidity is increased. A longer-term burden also exist, as women who develop pre-eclampsia, are two and a half times more likely to display ischemic heart disease later in life [7], and infants born to pre-eclampsia mothers are at a higher risk of developing respiratory diseases and experiencing long-term neurological morbidity [8].

Pre-eclampsia complicates 8-10% of all pregnancies and affects more than one million women each year. 12% of total maternal deaths can be attributed to hypertensive disorders of pregnancy, in particular, the progression of pre-eclampsia to eclampsia. Pre-eclampsia is not only a leading cause of maternal morbidity and mortality in the southern world, it claims the lives more than 2000 Indian babies annually, making it single highest trigger for neonatal intensive care and peri-natal mortality [6,7].

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Oxidative stress defined as a imbalance between the cellular generation of reactive oxygen species (ROS) and the capacity of antioxidants to prevent oxidative damage, oxidative stress has been implicated in a wide variety of disease and degenerative states including: Cancer, rheumatoid arthritis, cardiovascular disease, aging [10] and most importantly for this investigation pre-eclampsia [11].

Apoptosis is the programed death of cells and occurs during normal tissue turnover to preserve homeostasis. During pregnancy, apoptosis may be a normal part of a tissue to embryonic development and placental functioning, or the response of tissue to exogenous stimuli such as cytotoxic agents, oxidative stress, or hypoxia [12]. The mechanism that controls apoptosis is complex and to some degree remains obscure. The role of oxidative stress in this progress is suggested by finding that ROS appear in many forms of apoptosis, and the exogenous slow dose administration of oxidants can initiate the apoptotic process [13]. Oxidative stress plays a vital role in the pathogenesis of pre-eclampsia, despite the fact that the precise mechanism is yet to be elucidated [13].

Placental hypoxia resulting from deficient trophoblast invasion has been suggested as a primary cause of placental oxidative stress in pre-eclampsia.

Aim and objectives

Assessment of placental oxidative stress parameters in normal and pre-eclamptic pregnancies and to examine lipid peroxides and protein carbonyls as measures of placental oxidative state in tissue homogenates from age-matched pre-eclamptic and normal pregnancies. To quantitate and compare the endogenous antioxidant capacity of reduced glutathione, Vitamin A, Vitamin C, Vitamin E, superoxide dismutase (SOD), glutathione peroxidise, and catalase in tissue homogenates of aged-matched pre-eclamptic and normal placentae.

Plan of work

The present study was conducted in in-patient wards of Chanda Kanthaiah Memorial (CKM) Government Maternity Hospital, which is having 100 beds, 15 nurses, 17 doctors, 21 paramedical staff along with 2 ultrasound machines, which is located in Warangal, AP, India. The study is a prospective interventional study. This study has been carried out for a period of 9-month from February to November 2014. Patients were: (1) Aged between 18 and 41-year-old pregnant women, (2) pregnant women willing to give informed consent, (3) pregnant women with pre-eclampsia visiting the inpatient Department of CKM and Warangal Hospital, Warangal. Pregnant women, if they were younger than 18 years, if co-morbidities are present, pregnant women not willing to give informed consent form were excluded from the study.

Source of data

All the relevant and necessary data were be collected by taking: (1) Patient's case notes, (2) treatment charts, (3) medication administration records, (4) interviewing patients or patients caretakers, (5) interviewing healthcare professionals (especially nursing staff), and (6) pregnant women cord blood and placenta sample.

Human Ethical Committee approval for the collection and study of human placental tissues was obtained from the superintendent of CKM hospital and Warangal Hospital, Warangal with fully informed consent obtained from all patients prior to obstetric delivery.

Sample collection

The obstetric and midwifery members of each respective hospital were responsible for the collection of placental tissues and the assignment of correct pregnancy and labor status on delivery, placental samples were snap frozen in liquid nitrogen, and stored at −80°C until analysis.

Biochemical analysis

Tissue protein extracts were performed on all placental and blood samples, with subsequent protein estimations performed on extracts. The activity of endogenous antioxidant proteins (SOD, glutathione peroxidise, glutathione reduced, catalase, and thioredoxin) and the levels of lipid peroxides, protein carbonyls, hydrogen peroxide, and nitrosative biomarkers in the placental samples from normal and pre-eclamptic pregnancies.

Data analysis

All data were processed using the Graph Pad Prism version 5.0 statistical package with $p<0.05$ considered significant unpaired t-tests and one-way ANOVA were used for the analysis of data. All data are presented as mean±standard deviation.

METHODS

Materials

Unless otherwise stated, all reagents were obtained and were of analytical reagent grade.

Tissue processing

Prior to dissection, tissue was perfused with a phosphate buffered saline solution, pH 7.4. Containing 0.16 mg/ml heparin to remove any red blood cells and clots and homogenized in 5-10 ml cold buffer (i.e., 50 mM potassium phosphate, pH 7.5. 1 mM EDTA) per gram tissue, finally centrifuge at 100,000 ×*g* for 15 minutes at 4°C. The supernatant was removed for assay and stored in ice. If not assayed on the same day, freeze the sample at −80°C. The sample will be stable for at least 1 month.

Protein estimations were done with bicinchoninic acid (BCA) protein assay reagent kit (Pierce, Rockford, USA) BCA is used for colorimetric detection and quantitation of total protein concentration, as the resultant chromophore demonstrates a linear increase in absorbance at 540 nm for increasing protein concentration.

Antioxidant biomarkers estimated include: Glutathione reduced, ascorbic acid, Vitamin A, catalase assay, SOD, and glutathione peroxidase.

Table 1: Characteristics of the normal pregnant women and patients with pre-eclampsia

S. No.	Characteristics	Normal pregnancy (n=200)	Pre-eclamptic pregnancy (n=120)
1.	Maternal age (years)	23.42±0.082	23.4±0.108
2.	Gestational age (weeks)	39.26±0.076	33.35±0.08
3.	Blood pressure (mmHg)		
	Systolic	115.12±0.47	159.64±0.53
	Diastolic	79.51±0.56	94.5±0.7
4.	Maternal weight (kg)	51.55±0.69	54.19±0.81
5.	Proteinurea (mg/24 hrs)	-	2255.02±32.74
6.	Gestational age at delivery (weeks)	37.31±0.095	35.13±0.13
7.	Birth weight (kg)	3.53±0.01	2.41±0.055

Table 2: Laboratory investigations with pre-eclampsia and normal pregnant women

S. No.	Characteristics	Normal range	Normal pregnancy (n=200)	Pre-eclamptic pregnancy (n=120)
1.	Serum creatinine (µmol/L)	70-150	106.3±1.40	210.75±2.423
2.	Trigylcerides (nmol/L)	0.5-1.7	1.27±0.013	2.19±0.1305
3.	Total cholesterol (g/L)	4-10	7.1±0.087	8.09±0.184
4.	Uric acid (mmol/L)	2.5-6.7	4.58±0.07	12.7±0.219
5.	Platelets (10^9/L)	150-450	323.37±4.54	125.75±1.573
6.	RBC (10^{12}/L)	3.8-5.8	4.85±0.027	3.195±0.017
7.	WBC (10^9/L)	4-11	6.930.093	2.89±0.07
8.	Hemoglobin (g/dL)	11.5-16.5	11.19±0.093	9.7±0.1028
9.	Hematocrit (%)	0.37-0.47	0.42±0.0013	0.205±0.007
10.	AST (u/L)	3-35	20.85±0.39	112.83±2.51
11.	ALT (u/L)	3-35	18.4±0.41	113.2±2.43
12.	LDH (u/L)	30-150	80.4±1.70	200.16±2.09
13.	Total bilirubin (g/L)	3-17	8.12±0.2003	7.008±0.138

RBC: Red blood cell, WBC: White blood cell, LDH: Lactate dehydrogenase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

Oxidative biomarkers estimated include: Lipid peroxide, protein carbonyls, and nitric oxide assay.

RESULTS

Placental tissue and placental blood samples from 200 normal and 120 pre-eclamptic patients matched for gestational age were analyzed to assess placental oxidative state and compare the levels of glutathione reduced catalase, Vitamin A, Vitamin C, Vitamin E, SOD, glutathione peroxidase, thioredoxin, and glutathione peroxidase. The oxidative state of pre-eclamptic and normal placental tissues were measured via malonaldehyde, protein carbonyls, lipid peroxides, and nitric oxide concentrations.

Table 3: List of parameters and their mean, SEM and p values

S. No.	Parameters	Groups	Mean±SEM	p value
1.	GSH	Control	46.99±0.508	<0.001***
		Test	28.19±0.178	
2.	Catalase	Control	5.26±0.028	<0.001***
		Test	4.62±0.11	
3.	SOD	Control	365.2±2.915	<0.001***
		Test	205.6±3.76	
4.	Vitamin A	Control	1.06±0.01294	<0.001***
		Test	0.607±0.01	
5.	Vitamin C	Control	1.182±0.01552	<0.001***
		Test	0.894±0.009	
6.	Vitamin E	Control	3.098+0.02509	<0.001***
		Test	3.25±0.05	
7.	Protein carbonyls	Control	203.48+3.658	>0.05**
		Test	247.15±4.05	
8.	Thioredoxin	Control	100.64±3.386	<0.001***
		Test	80.89±3.37	
9.	GPx	Control	340.88±6.162	<0.001***
		Test	164.46±3.03	
10.	MDA	Control	2.878±0.06673	<0.001***
		Test	6.91±0.19	
11.	LPO	Control	5.695±0.03322	<0.001***

MDA: Malondialdehyde, GSH: Glutathione, GPx: Glutathione peroxidase, LP: Lipid peroxidation, SOD: Superoxide dismutase, SEM: Standard error of mean, **: Moderate, ***: Highly significant

Table 4: MDA

S. No.	Group	Mean±SEM (µM/mg)
1.	Control	2.878±0.066
2.	Test	6.91±0.19

MDA: Malondialdehyde, SEM: Standard error of mean

Table 5: Protein carbonyls

S. No.	Group	Mean±SEM (µM/mg)
1.	Control	203.48±3.658
2.	Test	247.15±4.05

SEM: Standard error of mean

Table 6: LPO

S. No.	Group	Mean±SEM (µM/mg)
1.	Control	5.695±0.03322
2.	Test	21.61±0.19

SEM: Standard error of mean, LPO: Lipid peroxidation

Table 7: GSH

S. No.	Group	Mean±SEM (mg/dl)
1.	Control	46.99±0.508
2.	Test	28.19±0.178

SEM: Standard error of mean, GSH: Glutathione

DISSCUSSION

Despite its prevalence and severity, the pathophysiology of pre-eclampsia is still not completely understood. It would appear that maternal endothelium dysfunction and a hypersensitivity reaction to placental debris are central to the changes occurring within the mother, and linked to placental apoptosis [14]. Several reports have indicated increased apoptosis and shedding of placental fragments into the maternal circulation during pre-eclamptic pregnancy [15]. However, the underlying cause of increased apoptosis is not defined. Increased oxidative stress and antioxidant disequilibria have been shown to promote syncytiotrophoblast apoptosis with maternal exposure to shed membrane fragments responsible for the initiation of the systemic inflammation that plays a defining role in the development of pre-eclampsia. In this study, we observed an increased state of biological oxidation in pre-eclamptic placentae when compared to normal controls, which is accordance with previous reports [16]. There was an increase in the concentrations of lipid peroxides and H_2O_2 pre-eclamptic toxaemia placentae. Lipid peroxides play a significant role in the oxidative stress characteristic of pre-eclampsia

Table 8: Catalase

S. No.	Group	Mean±SEM (mmol/min/mg)
1.	Control	5.26±0.028
2.	Test	4.62±0.11

SEM: Standard error of mean

Table 9: Vitamin C

S. No.	Group	Mean±SEM (mg/ml)
1.	Control	1.182±0.015
2.	Test	0.894±0.009

SEM: Standard error of mean

Table 10: Vitamin A

S. No.	Group	Mean±SEM (mg/ml)
1.	Control	1.06±0.012
2.	Test	0.607±0.01

SEM: Standard error of mean

Table 11: Vitamin E

S. No.	Group	Mean±SEM (mg/ml)
1.	Control	3.098±0.025
2.	Test	3.25±0.05

SEM: Standard error of mean

Table 12: SOD

S. No.	Group	Mean±SEM (U/L)
1.	Control	340.88±6.162
2.	Test	164.46±3.03

SEM: Standard error of mean, SOD: Superoxide dismutase

Table 13: GPx

S. No.	Group	Mean±SEM (U/L)
1.	Control	340.88±6.162
2.	Test	164.46±3.03

GPx: Glutathione peroxidase, SEM: Standard error of mean

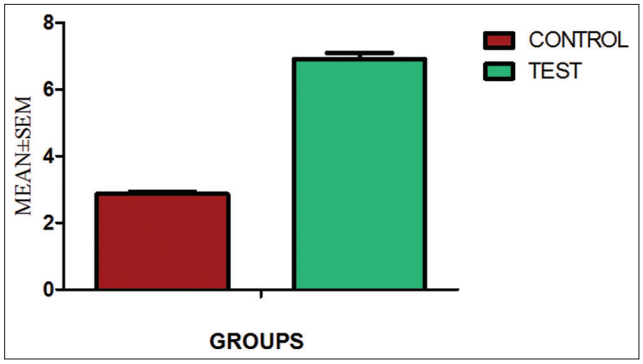

Fig. 1: Malondialdehyde

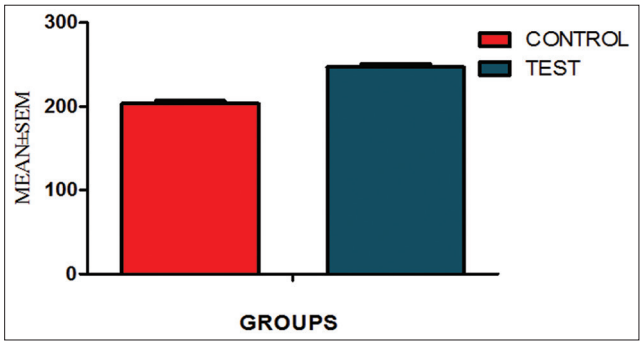

Fig. 2: Protein carbonyls

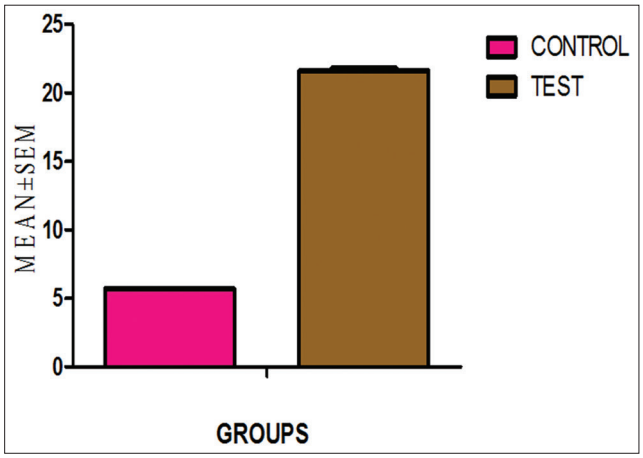

Fig. 3: Lipid peroxidation

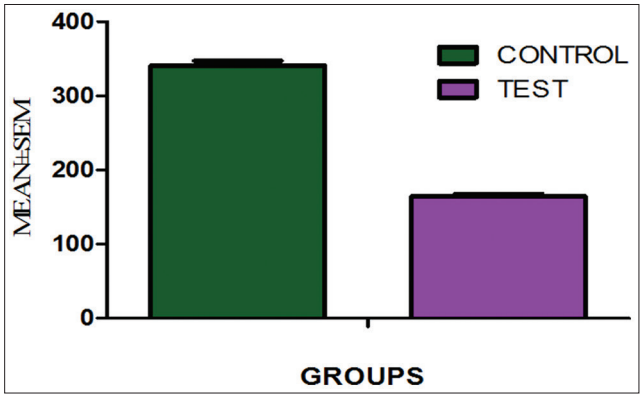

Fig. 4: Glutathione peroxidase

by stimulating the cyclooxygenase, increasing endothelial monolayer permeability, thrombin formation, the IXA_2/prostaglandin A2 ratio, and the production of vasoconstrictors while reducing the levels of antithrombin III and the production of vasodilatory prostacyclin and nitric oxide (NO) [17]. These changes contribute to the clinical feature of pre-eclampsia including increased platelet aggregation, maternal hypertension, proteinuria, edema, thrombus formation, and reduced uteroplacental blood flow in normal pregnancy, increasing gestational age promoted low levels of placental peroxidation and increased antioxidant status. Conversely, increased vasoconstriction and hypoxia cause significantly higher lipid peroxidation products [18] and reduced levels of antioxidants such as Vitamin C, Vitamin A, SOD, glutathione peroxidase, etc., to be associated with pre-eclampsia. The increasing protein and lipid oxidation demonstrated in this investigation indicate that the pre-eclamptic placenta is experiencing oxidative stress that may be due to either excessive generation of ROS or a deficient antioxidant capacity [19].

CONCLUSION

By early medical intervention, we can prevent progression of pre-eclampsia from HELLP syndrome to eclampsia, where neurological problems such as hyperreflexia with an abnormal pattern of neuromuscular activity, severe headache, scotomas, clonic-tonic seizures, coma, renal and hepatic failure, cerebral hemorrhage, lung edema, and liver hemorrhage. By administrating the antioxidants such as vitamins along with antihypertensive drugs at the early stage of pre-eclampsia, we can prevent the progression of the disease to HELLP syndrome and eclampsia and also from the detrimental effects of ROS (oxidative stress).The state of hypoxia-reoxygenation established in the placenta as a consequence of the increase in oxygen tension exposes the placenta to the oxidative stress. The delicate balance of oxidative control by antioxidant proteins is crucial to the healthy progression of pregnancy and disequilibria in compensatory antioxidant control are proposed as a causative mechanism in the pathophysiology of pre-eclampsia. The results of investigations included in this study confirm the involvement of increased oxidative stress and decreased antioxidant enzyme capacity in the progression of pre-eclampsia. The decrease in placental antioxidants observed in this study may be due to decreased production as a result of reduced mRNA expression. Conversely, the increased placental oxidation observed may be responsible for the reduction in antioxidant enzyme activity through the inhibition of sulfhydryl repair mechanisms and so may be a consequence of the disease. Either way, the decreased endogenous activity of key antioxidant enzymes leads to augmentation of the oxidative stress produced as a consequence of hypoxia reoxygenation within the placenta and may contribute to the increased apoptosis, which is characteristic of the pre-eclampsia.

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REFERENCES

1. Brown MA, Hague WM, Higgins J. The Detection, Investigation and Management of Hypertension in Pregnancy: Executive Summary Council of the Australian Society of Hypertension in Pregnancy; 2000.
2. National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000;183(1):S1-22.
3. Lowe SA, Brown MA, Dekker G, Gatt SM, McMahon L, Mangos G, *et al*. Guidelines for the management of hypertensive disorders of pregnancy. Soc Obstet Med Aust New Z 2008;49(3):242-6.
4. Fagermo N, Makonnen E,Debella A,Levinson,Jawetz E,Tsai CC. Hypertensive Disorders of Pregnancy. Queensland: Statewide Maternity and Neonatal Clinical Guidelines - Government of Queensland; 2010.
5. Available from: http://www.en.wikipedia.org/wiki/Pre-eclampsia#Epidemiology.

6. van Beck E, Peeters LL. Pathogenesis of preeclampsia: A comprehensive model. Obstet Gynecol Surv 1998;53:233-9.
7. Wang Y, Alexander JS. Placental pathophysiology in preeclampsia. Pathophysiology 2000;6:261-70.
8. Jónsdóttir LS, Arngrímsson R, Geirsson RT, Sigvaldason H, Sigfússon N. Death rates from ischemic heart disease in women with a history of hypertension in pregnancy. Acta Obstet Gynecol Scand 1995;74:772-6.
9. VanWijk MJ, Kublickiene K, Boer K, VanBavel E. Vascular function in preeclampsia. Cardiovasc Res 2000;47:38-48.
10. Dekker GA. Risk factors for preeclampsia. Clin Obstet Gynecol 1999;42:422-35.
11. Available from: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001900/, http://www.en.wikipedia.org/wiki/Pre-eclampsia.
12. Hung TH, Skepper JN, Charnock-Jones DS, Burton GJ. Hypoxia-reoxygenation: A potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. Circ Res 2002;90:1274-81.
13. Redman CW, Sargent IL. The pathogenesis of pre-eclampsia. Gynecol Obstet Fertil 2001;29(7-8):518-22.
14. Ohlsson R, Glaser A, Holmgren L, Franklin G. The molecular biology of placental development. In: Redman C, Sargent I, Starkey PM, editors. The Human Placenta. Oxford: Blackwell Scientific Publications; 1993. p. 33-81.
15. Seeley R, Stephens T, Tate T. Anatomy and Physiology. St. Louis: Mosby; 1995.
16. Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M, *et al.* Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. J Clin Invest 2004;114:744-54.
17. Genbacev O, Joslin R, Damsky CH, Polliotti BM, Fisher SL. Hypoxia alters early gestation human cytotrophoblast differentiation/invasion *in vitro* and models the placental defects that occur in preeclampsia. J Clin Invest 1996;97:540-50.
18. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: Implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod 2003;69:1-7.
19. Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, *et al.* Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? J Clin Invest 1997;99:2139-51. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to a mimic a vascular adhesion phenotype one cause of defective endovascular invasion in this syndrome? J Clin Invest 1997b;99:2152-64.