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Increased Apoptosis, Altered Oxygen Signaling, and Antioxidant Defenses in First-Trimester Pregnancies with High-Resistance Uterine Artery Blood Flow

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The mechanisms of deficient placentation in the first trimester remain poorly understood, although apoptosis, hypoxia, and oxidative stress have been implicated. High uterine artery Doppler resistance indexes (RIs) are predictive of placental complications of pregnancy, such as preeclampsia, fetal growth restriction, and stillbirth. We provide evidence that even in the first trimester, pregnancies with high uterine artery Doppler RI demonstrate alterations in placental gene and protein expression. Apoptosis was significantly higher in high RI placental tissue, as determined by Western blot analysis of cleaved poly (ADP-ribose) polymerase and caspase 3. Protein expression of the trophoblast survival factor insulin-like growth factor-2 was significantly lower. Both high and normal RI placentas showed evidence of hypoxia and oxidative stress with expression of hypoxia-inducible factors 1α and 2α, heat shock protein 70, presence of nitrotyrosine residues, and lipid peroxidation. We observed no exaggerated placental hypoxia or oxidative stress associated with high RI pregnancies. High RI placental tissue demonstrated an altered balance of antioxidant enzyme activity. Hypoxia and oxidative stress appear to be a physiological state in early pregnancy; our data did not support the hypothesis that they are associated with deficient placentation in the first trimester. Higher levels of apoptosis, reduced insulin-like growth factor-2 expression, and altered antioxidant defenses may contribute to abnormal placentation and the later development of pregnancy complications, such as preeclampsia, fetal growth restriction, and stillbirth. *(Am J Pathol 2015, 185: 2731–2741; http://dx.doi.org/10.1016/j.ajpath.2015.06.020)*

Successful outcome of pregnancy is dependent on placental sufficiency, namely successful implantation and remodeling of the maternal uterine spiral arteries in early pregnancy. Disorders of pregnancy related to deficient placentation include preeclampsia (PE), fetal growth restriction (FGR), abruption, and stillbirth.[1–3] The pathophysiology of these placental obstetric disorders remains unclear, although advances in our understanding have been made in recent years.[4,5] PE, in particular, is a major cause of maternal morbidity and mortality worldwide and occurs in 2% to 5% of pregnancies.[6]

Evidence of oxidative stress, hypoxia, and altered antioxidant defenses have been demonstrated in placental studies of pregnancies affected by PE.[7,8] Apoptosis, which may result from increased cell stress, has also been implicated in the pathogenesis of deficient placentation, with higher levels of placental apoptosis in PE and FGR.[9,10] However, study of the placenta after delivery has limited value because by that

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time the disease process has progressed to the point that delivery is indicated. These studies, although highly suggestive that pathways involving placental hypoxia, oxidative stress, and apoptosis are involved in diseases of placental origin, are unable to answer the question of whether they occur as a response to the disease or are truly causal in the pathogenesis. If we are to institute treatments to ameliorate, or ideally prevent, the consequences of poor placentation, then an understanding of the pathophysiology in the first trimester is necessary.

Uterine artery Doppler (UtAD) ultrasound has been shown to be predictive of placental complications in pregnancy.[11,12] It has a greater ability to predict preterm PE and PE associated with FGR than term disease, which may not have such a clear placental origin.[13–15] High-resistance UtAD indices in the first trimester are associated with decreased endovascular trophoblast invasion[16] and an increased risk of placental complications of pregnancy.[11] We have previously shown functional differences in high-resistance pregnancies in trophoblast sensitivity to apoptotic stimuli and decidual natural killer cell function that would potentially have a detrimental impact on placentation.[17,18] We hypothesized that placentas from pregnancies with high-resistance UtAD flow would demonstrate alterations in apoptosis, oxidative stress, antioxidant defenses, and placental oxygen signaling.

Materials and Methods

First-Trimester UtAD and Pregnancy Outcome in Continuing Pregnancies

This was a prospective, observational study of women booking routine antenatal care. All women with a singleton pregnancy, attending for routine first-trimester nuchal translucency ultrasound assessment, were offered the option to participate in the study. Written informed consent was obtained from them, and the study was approved by the local ethics committee.

Transabdominal UtAD assessment was performed by the sonographer at the time of the nuchal translucency scan. UtAD indexes were measured as described previously.[16] In brief, the paracervical vascular plexus was identified and color Doppler was used to identify the uterine artery as it made its ascent to the uterine body. Pulsed-wave Doppler was used to obtain uterine artery waveforms. When three similar consecutive waveforms were obtained, the presence of a protodiastolic notch was recorded and the resistance index (RI) was measured. The RI was preferred to the pulsatility index (PI) in this study because it has demonstrably better intraobserver and interobserver measurement repeatability.[19] The patients and their clinicians were blinded to the results of the first-trimester UtAD assessment. Patient characteristics, including demographic details, and obstetric and medical histories were obtained at the first hospital visit and entered into our database. All pregnancy outcomes were obtained from the delivery suite database. Cases with fetal chromosomal or structural abnormalities, intrauterine infection, toxic insult (alcohol or drugs), medication (aspirin, heparin, antioxidants, or steroids), or concurrent maternal disease (eg, renal disease, connective tissue disease, malnutrition, cardiac disease, and diabetes) were excluded from the study. Patients who had a previous pregnancy affected by PE were included in the study; however, according to the exclusion criteria, none was routinely prescribed prophylactic therapy.

FGR was defined as a birth weight $<10^{th}$ percentile for gestational age (GA) with abnormal Doppler indexes (umbilical artery PI $>95^{th}$ percentile, middle cerebral artery PI <5th percentile, or ductus venosus PI $>95^{th}$ percentile for GA). Stillbirth was defined as intrauterine demise after 24 weeks' gestation. PE was defined after 20 weeks' gestation, according to the guidelines of the International Society for the Study of Hypertension in Pregnancy. This requires two recordings of diastolic blood pressure of 90 mmHg at least 4 hours apart in previously normotensive women, proteinuria of 300 mg or more in 24 hours, or two readings of at least 2+ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection is available.[20] Pregnancy outcomes from this data set have previously been published in several prospective series.[13,14,21–24] However, the results were analyzed with an emphasis on screening performance rather than the positive predictive value for placental complications; we, therefore, present the data with these results.

Doppler Ultrasound Characterization and Tissue Collection

Determination of uterine artery RI was performed in women attending a clinic for termination of pregnancy in the first trimester, as previously described,[16] at St. George's Hospital (London, UK). Ethical committee approval and full written consent were obtained (reference, 01.96.8 and 01.78.5). Inclusion criteria were singleton pregnancy, GA of 9 to 14 weeks by crown-rump length (assigned by transvaginal measurement in accordance with local unit clinical policy), normal fetal anatomy, and nuchal translucency with no known maternal medical condition or history of recurrent miscarriage. High-resistance cases were defined as a mean RI >95th percentile with bilateral diastolic notches. Normal-resistance cases had a mean RI of <95th percentile. Tissue obtained from first-trimester surgical terminations of pregnancy was collected and rinsed in ice-cold phosphate-buffered saline. Placental villous tissue was separated from the decidua by blunt dissection and was randomly sampled and divided. Approximately 100 mg of tissue was snap frozen in liquid nitrogen in two to three aliquots and stored at −70°C until use.

Protein Extraction

Placental villous tissue (approximately 100 mg) was placed in a lysing tube (Matrix D; MP Biomedicals, Santa Ana,