OBSTETRICS

Angiogenic markers in pregnancies conceived through in vitro fertilization

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OBJECTIVE: Pregnancies that have been conceived through in vitro fertilization (IVF) have been associated with higher rates of preeclampsia and other complications that are associated with placental dysfunction. We evaluated whether IVF pregnancies, when compared with those conceived spontaneously, would be associated with alterations in serum angiogenic markers.

STUDY DESIGN: This was a retrospective cohort study from 3 US academic institutions (2006-2008). Women with singleton pregnancies who conceived via IVF or spontaneously were included. Placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured at 4 time points throughout gestation. Pregnancy outcomes that included diagnosis of preeclampsia or other obstetric complications were ascertained from the medical record. The relationship among IVF status, PlGF, and sFlt-1 were modeled over gestation and stratified by clinical pregnancy outcome.

RESULTS: Of the included 2392 singleton pregnancies, 4.5% (108 pregnancies) were conceived though IVF. IVF pregnancies were significantly more likely to be complicated by preeclampsia (15.7% vs 7.7%). IVF pregnancies had significantly higher levels of sFlt-1 at 18, 26, and 35 weeks of gestation ($P = .04$, $P = .004$, $P < .0001$, respectively) and lower levels of PlGF at 18 and 35 weeks of gestation ($P = .007$ and .0006, respectively). These differences persisted even after being controlled for maternal comorbidities or obstetric outcomes such as preeclampsia.

CONCLUSION: Pregnancies conceived via IVF were found to have an increased antiangiogenic profile (elevated sFlt-1 and decreased PlGF) at multiple time points throughout gestation when compared with spontaneously conceived pregnancies. Alterations in the angiogenic profile persisted even after we controlled for maternal comorbidities of clinically evident disorders of abnormal placentation such as preeclampsia. The increased antiangiogenic profile suggests fundamentally aberrant placentation related to in vitro fertilization, which may warrant closer fetal surveillance in these pregnancies.

Key words: angiogenic marker, in vitro fertilization, placental growth factor, preeclampsia, soluble fms-like tyrosine kinase-1

Cite this article as: Lee MS, Cantonwine D, Little SE, et al. Angiogenic markers in pregnancies conceived through in vitro fertilization. Am J Obstet Gynecol 2015;213:212.e1-8.

Since the birth of Louise Brown over 35 years ago, there has been a steady rise in the number of women who use in vitro fertilization (IVF) to conceive.[1] Infertility now affects approximately 12% of the reproductive-aged population in the United States.[2] In 2007, 142,415 IVF cycles were performed, which accounts for the birth of 57,564 infants; more recent data have demonstrated that IVF is increasingly popular.[2,3] In spite of the advancements in technology and increases in better perinatal outcomes reported with the use of IVF for conception, many studies still report that, when compared with spontaneous conceptions, pregnancies conceived through IVF carry a higher risk for obstetric complications. Such outcomes include, but are not limited to, preeclampsia and pregnancy-induced hypertension, placenta previa, placental abruption, preterm labor, small-for-gestational-age (SGA) infants, intrauterine growth restriction, and preterm delivery.[4-10]

The precise cause of the difference in obstetric outcomes between IVF and spontaneous pregnancies is yet unknown, although it has been suggested that aberrant placentation may be an initial pathologic step. Increasing data are establishing IVF placentas to be

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Received Dec. 2, 2014; revised Feb. 16, 2015; accepted March 17, 2015.

Supported by an unrestricted grant from Abbott Diagnostics Division (9MZ-04-06N03).

The authors report no conflict of interest.

Presented, in part, in poster format at the 30th annual meeting of the Society for Maternal-Fetal Medicine, Chicago, IL, Feb. 1-6, 2010.

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0002-9378/$36.00 • http://dx.doi.org/10.1016/j.ajog.2015.03.032

morphologically different; in a murine model, placentas in the IVF-exposed group were shown to have impaired amino acid and nutrient transport mechanisms.[11] IVF placentas have also been shown to have altered glucocorticoid metabolism[12] and expression of placenta genes[13,14] when compared with spontaneous conceptions, which further suggests that abnormal placentation partly may explain the difference in observed pregnancy outcomes.

An evolving body of literature also has linked the development of placental dysfunction with the expression of abnormal quantities of placental angiogenic proteins. Previous research has demonstrated that preeclampsia and other pregnancy-induced hypertensive disorders are associated with alterations in angiogenesis, which is detected by changes in the levels of circulating angiogenic and antiangiogenic proteins.[15] Vascular endothelial growth factor (VEGF) is a protein that is expressed at high levels in areas of active vascular proliferation. The soluble form of VEGF receptor-1, known as soluble fms-like tyrosine kinase-1 (sFlt-1), is known to inhibit VEGF activity by sequestering it from signaling receptors, thus acting as a natural inhibitor of VEGF action in vivo.[16,17] SFlt-1 is also known to bind and antagonize placental growth factor (PlGF), an isoform of VEGF, that results in abnormal uteroplacental blood flow.[18] Increased levels of sFlt-1 and reduced levels of PlGF (anti- and proangiogenic proteins, respectively) have been purported to predict the subsequent development of preeclampsia,[19] although previous longitudinal studies have failed to show a high positive predictive value in otherwise normal pregnancies.[20] This study investigates whether abnormal angiogenesis, as measured by altered sFlt-1 and PlGF concentrations, in part may be explained by the placental dysfunction that is seen in pregnancies that are conceived through IVF.

Although the body of research surrounding abnormal placentation in relation to serum angiogenic markers is growing and although IVF is a known risk factor for placental dysfunction, there is little known about the angiogenic profile of specifically IVF pregnancies. We hypothesized that IVF pregnancies, as compared with spontaneous conceptions, have altered levels of PlGF and sFlt-1. Specifically, we investigated whether these levels were different, even in the absence of clinically apparent obstetric complications, and may represent subclinical alterations in angiogenesis that present a broader range of risks for abnormal pregnancy outcomes because of aberrant placentation in IVF pregnancies.

Methods and Materials

Study subjects

Participants were enrolled at 3 tertiary care academic centers: Brigham & Women's Hospital (BWH) and Beth Israel Deaconess Medical Center in Boston, MA, and the Hospital of the University of Pennsylvania in Philadelphia, PA. Women who were eligible for enrollment received routine prenatal care at <15 weeks of gestation, were >18 years old, and planned to deliver at the enrolling institution. The protocol was approved by institutional review boards at each institution, and written informed consent was obtained from all participating women.

A total of 2636 gestations with delivery at ≥ 24 weeks of gestation were enrolled at the 3 study sites between October 2007 and June 2009. All subjects were enrolled prospectively in the first trimester. Among the 3 sites, BWH contributed the most (48%) participants; Beth Israel Deaconess Medical Center and Hospital of the University of Pennsylvania contributed 29% and 23%, respectively. This analysis excluded multiple gestations ($n = 148$; 5.6%) and the use of any other form of assisted reproductive technology ($n = 96$; 3.6%). Of the remaining 2393 participants 108 women (4.5%) underwent IVF. Study visits occurred at the following median (interquartile range) weeks' gestation: 10.0 (4.4–16.7), 17.8 (12.6–22.7), 26.0 (19.6–30.9), and 35.3 (31.3–39.4) weeks.

Biospecimen collection and processing

Maternal blood and urine samples were obtained at the 4 visits during the pregnancy. Approximately 10 mL of blood was drawn in EDTA plasma tubes at each study visit, and the samples were kept at $+4^{\circ}$C until processing for storage within 4 hours of venipuncture. The specimens were centrifuged for 20 minutes, aliquoted, and stored at -80°C. Samples were shipped in batches on dry ice to Abbott Diagnostics where they were stored at -80°C until analysis.[20]

Laboratory assays

PlGF and sFlt-1 were measured in maternal plasma with the use of prototype ARCHITECT immunoassays (Abbott Laboratories, Abbott Park, IL). The PlGF immunoassay measures the free form of PlGF-1. The assay has a detection range of 1-1,1500 pg/mL. The sFlt-1 immunoassay measures both free and bound sFlt-1. The assay has a detection range of 0.10-150 ng/mL. The combined intra- and interassay coefficients of variation are $<7\%$ for PlGF and sFlt-1.

Questionnaire and clinical data

Participants completed a brief questionnaire that ascertained information on race/ethnicity, tobacco use before and during the index pregnancy, medical history, and history of preeclampsia in previous pregnancies. Information on the index pregnancy and neonate were abstracted from the medical record and supplemented with data that were collected specifically for the study. The diagnosis of preeclampsia was made with the use of standard criteria that are available from the American College of Obstetrics and Gynecology. All cases of hypertensive disease were deidentified and reviewed by a panel of study principle investigators. Gestational age was confirmed by ultrasound scanning at <15 weeks of gestation.

Statistical methods

We first examined the sociodemographic and clinical characteristics of the study population for those participants who conceived spontaneously and those who conceived via IVF. Differences by mode of conception were tested by with Wilcoxon's rank sum or χ^2 tests for quantitative and categoric variables, respectively.