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Maternal Circulating Angiogenic Factors in Twin and Singleton Pregnancies

Jessica M. FAUPEL-BADGER, Ph.D.^{1,2}, Thomas F. MCELRATH, M.D.³, Michele LAURIA, M.D.⁴, Lauren C. HOUGHTON, Ph.D.², Kee-Hak LIM, M.D.⁵, Samuel PARRY, M.D.⁶, David CANTONWINE, Ph.D.³, Gabriel LAI, Ph.D.², S. Ananth KARUMANCHI, M.D.⁷, Robert N. HOOVER, M.D.², and Rebecca TROISI, Sc.D.²

¹Division of Cancer Prevention, National Cancer Institute, Bethesda, MD

²Epidemiology and Biostatistics Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD

³Brigham and Women's Hospital, Harvard Medical School, Boston, MA

⁴Dartmouth Hitchcock Medical Center, Geisel School of Medicine, Lebanon, NH

⁵Harvard Medical School, Boston, MA; Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Beth Israel–Deaconess Medical Center, Boston, MA

⁶Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Pennsylvania Medical Center, Philadelphia, PA

⁷Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Abstract

Objective—The purpose of this study was to compare longitudinally sampled maternal angiogenic proteins between singleton and twin pregnancies.

Study Design—Placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and soluble endoglin (sEng) from healthy pregnant women were quantified at 10, 18, 26 and 35 weeks' gestation (n=91), and during the third trimester (31–39 weeks) and at delivery (33–41 weeks; n=41). Geometric means and 95% confidence intervals were calculated for gestational age adjusted angiogenic protein concentrations and compared between matched twin and singleton pregnancies.

Corresponding Author and Requests for Reprints: Rebecca Troisi, Sc.D., M.A., National Cancer Institute, troisir@mail.nih.gov.

Condensation: This longitudinal study found higher maternal anti-angiogenic proteins in twin than singleton pregnancies with implications for diseases associated with multiple gestations.

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Results—Maternal sFlt-1 concentrations and the sFlt-1/PlGF ratio were higher in twins than singletons across pregnancy and at delivery, with the greatest differences at week 35 [sFlt-1: 36916 vs. 10151 pg/mL; $p < 0.0001$; sFlt-1/PlGF: 168.4 vs. 29.0; $p < 0.0001$]. Maternal concentrations of s-endoglin also were higher in the third trimester and delivery. Maternal PlGF concentrations were lower in twin than singleton pregnancies at week 35 only [219.2 vs. 350.2 pg/mL; $p < 0.0001$]. Placental weight appeared to be inversely correlated with maternal sFlt-1/PlGF ratio at the end of the pregnancy in both twins and singletons.

Conclusions—Higher maternal anti-angiogenic proteins in twin than singleton pregnancies does not appear to be due to greater placental mass in the former, and may be one explanation for the increased risk of preeclampsia in women carrying multiple gestations. Determining whether women with a history of multiple gestations have an altered cardiovascular disease and breast cancer risk, like those with a history of preeclampsia, is warranted.

Keywords

twins; singletons; angiogenic balance; pregnancy; sFlt-1; endoglin

Introduction

Women carrying twins or other higher order multiples are at 2–3 times the risk of developing preeclampsia, a common cause of maternal and fetal morbidity,¹ than women with singleton pregnancies.^{2,3} Preeclampsia is marked by shallow trophoblast invasion into the maternal endometrium resulting in a less extensive vascular network supporting the pregnancy.⁴ Alterations in angiogenic proteins, as well as inflammatory cytokines and other immune-modulating molecules have been demonstrated in preeclamptic pregnancies,^{5–13} with elevations in soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (s-endoglin), two anti-angiogenic proteins, typically preceding the clinical manifestation of maternal disease.^{14–17}

Maternal angiogenic factors also appear altered in pregnancies involving multiples compared with singletons, with elevated concentrations of sFlt-1 in the former.^{18–20} The timing of these changes in angiogenic balance may provide insight into the mechanism whereby preeclampsia risk is elevated in women carrying multiple gestations. Therefore, we followed women longitudinally through pregnancy and delivery to examine circulating maternal concentrations of placental growth factor (PlGF), sFlt-1, and s-endoglin in twin and singleton pregnancies.

Materials and Methods

Study subjects

The data for the analysis derive from two sources: the BIRTH cohort,²¹ and a study of twins at The Geisel School of Medicine (Dartmouth College). The study protocols were approved by institutional review boards at each institution and at the U.S. National Cancer Institute, and written informed consent was obtained from all participating women.

BIRTH Cohort—Participants were enrolled at three U.S. tertiary care academic centers between October 2007 and June 2009. Eligible women initiated routine prenatal care at <15 weeks' gestation, were >18 years of age, and planned to deliver at the enrolling institution. Women who developed preeclampsia in the index pregnancy or a prior one (gestational hypertension defined as a blood pressure elevation of >140/90 on two occasions with concomitant proteinuria defined as positive urine protein test result >300 mg/24 hours or protein/creatinine >0.20) were excluded from the present analysis. A total of 2,230 singleton and 93 twin gestations were enrolled, and 2,193 and 91 singletons and twins, respectively, met the inclusion criteria for analysis.

Geisel School of Medicine Twin Study—Eligible were pregnant women 18 years of age who intended to deliver at the facility. Women carrying twin gestations and presenting for prenatal care or hospitalized for antenatal surveillance from 2003 to 2007 were approached in the third trimester of pregnancy and informed consent was obtained. The next singleton pregnancy that met the eligibility criteria and could be matched to the twin pregnancy on gestational age (within 1 week), parity (nulliparous/parous) and maternal age (+/- 5 years), in that order, was recruited for the study (prenatal controls; n = 40). Another group of women with singleton pregnancies were recruited at admission for labor and delivery (labor controls) and matched to twin mothers according to the criteria above. Five twin pregnancies and three singleton pregnancies were excluded because they developed preeclampsia after enrollment, leaving 41 twins and a total of 62 singleton controls (40 with blood samples in the third trimester and 52 with blood samples at labor and delivery). Placentas were routinely examined by the pathology department.

Biospecimen collection and processing

BIRTH cohort—Maternal blood samples were obtained at the following median (interquartile range) weeks of gestation: 9.7 (8.4–11.6), 17.8 (16.8–18.7), 25.9 (24.8–28.1), and 35.1 (34.6–35.9). Approximately 10 mL of blood was drawn in EDTA plasma tubes; the samples were kept at 4°C until processing for storage within four hours of venipuncture. The specimens were centrifuged for 20 minutes and stored at -80°C. Samples were shipped in batches on dry ice to Abbott Diagnostics (Abbott Park, IL) where they were stored at -80°C.

Geisel School of Medicine Twin Study—Blood samples were collected in the third trimester (31–39 weeks) and at the earliest possible time after admission for labor and before any administration of medication (33–41 weeks). A 10-mL red-top tube of whole blood was collected from the mother. After allowing samples to clot at room temperature, they were centrifuged and the sera were stored at -70°C. Samples were shipped on dry ice to a biorepository in Rockville, MD, where they were stored at -80°C.

Laboratory assays

BIRTH cohort—PlGF and sFlt-1 were measured with prototype ARCHITECT immunoassays (Abbott Laboratories, Abbott Park, IL). The PlGF immunoassay measures the free form of PlGF-1, with a lower limit of detection of 1 pg/mL, and a range up to 1500 pg/mL. The sFlt-1 immunoassay measures both free and bound sFlt-1, with a lower limit of

detection of 0.10 ng/mL and a range up to 150 ng/mL. The combined intra- and inter-assay coefficients of variation reported by the laboratory were <7% for PIGF and sFlt-1.

Geisel School of Medicine Twin Study—Serum levels of sFlt-1 and PIGF were determined in a blinded fashion using commercially available ELISA kits (R & D systems, MN) as described elsewhere.⁵ Interassay CVs for the sFlt1 kit ranged from 7.0–8.1% and for PIGF ranged from 10.9–11.8%. S-endoglin was also measured using commercially available ELISA kits (R&D systems, MN) as described elsewhere.⁵ Interassay CVs for the s-endoglin kit ranged from 6.3–6.7%.

In a subset of BIRTH Cohort mothers with singleton pregnancies, PIGF and sFlt-1 concentrations were measured using both the ARCHITECT immunoassay (used for study samples in the BIRTH cohort) and the R&D systems assay (used for study samples in the Geisel School of Medicine Twin Study) at each time point. Pearson's correlation was used to describe the concordance between logarithm-transformed values, as well as Cronbach's alpha, a measure of inter-rater reliability. PIGF and sFlt-1 values measured by the different assays showed high concordance (Table 1). Because absolute values for the angiogenic factors differ between assays, levels between studies could not be directly compared.

Clinical data

BIRTH Cohort—Maternal age, parity, and conception by assisted reproductive technologies (ART), and baby's birth anthropometrics were abstracted from medical records. The participants completed a brief questionnaire that ascertained information on race/ethnicity, medical history, and history of preeclampsia in a previous pregnancy. Gestational age was confirmed by ultrasound scanning at <15 weeks' gestation.

Geisel School of Medicine Twin Study—Data on mother's age, race/ethnicity, parity, and ART conception, and baby's sex and birth anthropometrics were abstracted from medical records and a form completed at delivery. The pathology report provided data on chorionicity of the twins and placental weight. Gestational age was confirmed by ultrasound.

Statistical methods

Clinical characteristics were compared between twin and singleton pregnancies using Student's t-test for continuous variables, and chi-square analysis for categorical variables. Z-scores were developed for birth weight values to account for fetal sex and gestational age using an external standard.²² Angiogenic protein values and their ratio were adjusted for gestational week in models with logarithm-transformed angiogenic factor as the dependent variable; geometric means and 95% confidence intervals (CI) were calculated by taking the exponent of the logarithm-transformed mean. Geometric mean values for the angiogenic factors and the ratio of sFlt1/PIGF and 95% confidence intervals (CI) from an analysis of variance model (PROC MIXED) that adjusted for weeks of gestation were plotted by gestational week at blood collection. Statistical analyses were performed using SAS version 9.2.

Results

Mothers of twins were slightly older on average than mothers of singletons in both studies (Table 2). The majority of women in the BIRTH cohort were white as were nearly all mothers in the Dartmouth Twin Study. In the BIRTH cohort, white mothers contributed a greater proportion of twin than singleton pregnancies (68.8% vs. 59%), and African-American mothers contributed a higher proportion of singleton than twin pregnancies (21.0% vs. 9.7%). As expected, gestational week at delivery was earlier among the twin pregnancies in both studies. Approximately a third of the women in both studies were nulliparous, and ART were more likely to have been used among the mothers of twins (BIRTH cohort 68.8%; Geisel School of Medicine study 39.0%) than among the mothers of singletons (6.2% and 8.1%, respectively). Birth weight was lower in the twin pregnancies, even when accounting for gestational age and fetal sex.

Table 3 shows the values for angiogenic factors at the four time points in pregnancy among women in the BIRTH Cohort. In singleton and twin mothers, PIGF concentrations increased from early to mid-pregnancy and then declined by the third trimester (as noted in the fourth time point), while s-Flt1 concentrations increased throughout the pregnancy. Accordingly, the s-Flt1/PIGF ratio decreased through mid-pregnancy but increased in the third trimester. In twins, maternal PIGF concentrations were higher than in singleton pregnancies until the third trimester, when values became lower than those in singleton pregnancies (Table 3). Maternal sFlt-1 concentrations and the sFlt-1/PIGF ratio were higher in twins compared with singletons throughout the pregnancy, with the greatest differences demonstrated in the third trimester (Table 3). Results for mean concentrations from repeated measures models which accounted for the correlations among angiogenic factors over the pregnancy were similar but the statistical significance of the differences, particularly for PIGF, were attenuated (Figures 1–3). Results were similar with additional adjustment for maternal age, race/ethnicity and parity (data not shown).

In the Geisel School of Medicine Twin Study, maternal concentrations of sFlt-1, s-endoglin and the sFlt-1/PIGF ratio were higher in twins than singletons in the third trimester and at delivery, while PIGF concentrations did not differ (Table 4). Results were similar when adjusted for the matching factors (gestational age, as well as maternal age and parity; data not shown).

Information on chorionicity of the twin pregnancy as well as twins' sex was available in the Geisel School of Medicine Twin Study. Thirty-six of the twin pregnancies were dichorionic and 5 were monochorionic. Opposite sex twins accounted for 17 of the twin pregnancies, whereas 8 were female/female and 16 were male/male. Placental weight was higher in dichorionic pregnancies than in monochorionic pregnancies (820 g (SD 158) vs. 743 g (SD 161)). Maternal prenatal concentrations of sFlt-1 (10,721 vs. 6,169 pg/ml, respectively; $p=0.03$), s-endoglin (26.6 vs. 12.3 ng/ml; $p=0.001$) and the sFLT-1:PIGF ratio (47.0 vs. 15.7 pg/ml; $p=0.03$) were higher in monochorionic than dichorionic twins after adjustment for gestational age. At labor, only maternal s-endoglin concentrations (45.8 vs. 23.0 ng/ml, respectively; $p=0.005$) remained higher in monochorionic than dichorionic twins. There were generally no associations between angiogenic factors and sex of the twins, except for

higher prenatal s-endoglin concentrations in same-sex than opposite sex twins (data not shown). Placental weight was positively correlated with maternal PIGF in the third trimester and at delivery in singleton pregnancies ($r=0.17$ and 0.28 , respectively) and in twin pregnancies ($r=0.20$ and 0.12 , respectively), but not with sFlt-1 ($r=-0.01$ at both time points in singleton pregnancies, and $r=0.001$ at both time points in twin pregnancies). There was an inverse correlation between placental weight and maternal sFlt-1/PIGF ratio in the third trimester and at delivery in singletons ($r=-0.11$ and $r=-0.22$, respectively), and in twins ($r=-0.14$ and -0.12).

Comment

Analysis of angiogenic factors throughout the pregnancy showed elevated maternal concentrations of sFlt-1, as well as the ratio of sFlt-1 to PIGF among twin compared with singleton pregnancies in the BIRTH Cohort. Analysis of the Geisel School of Medicine data confirmed these findings and showed higher s-endoglin concentrations, as well as elevated sFlt-1 and the sFlt-1 to PIGF ratio in twins compared to singletons at the end of pregnancy and at admission for labor and delivery. PIGF concentrations demonstrated a less consistent pattern in both cohorts with elevated concentrations among twins until mid-pregnancy, followed by higher values in singleton pregnancies in the third trimester, but not at delivery. In addition, angiogenic factor balance was associated with chorionicity, although these results were based on small numbers.

The elevated sFlt-1 concentrations and anti-angiogenic ratio in women carrying twins compared with singletons that we observed are consistent with previous smaller studies with measurements in the first,¹⁹ second²³ and third trimesters.^{19,23} While we showed findings for maternal s-endoglin similar to those for sFlt-1 at the end of pregnancy, another study found no differences in the first trimester.²⁰ The etiology of the increases in sFlt1 has been hypothesized to be related to increased placental mass in twins as the placenta is thought to be the major source of sFlt.²⁴ Although the placenta is also the major source of PIGF, dramatic changes in PIGF are not seen with increased placental mass as the commercial assays to measure PIGF only measure the unbound form or free PIGF.¹⁴ Our data on placental weight showed no correlation with maternal sFlt-1, a weak positive correlation with maternal PIGF, and a weak inverse correlation with the sFlt-1/PIGF ratio suggesting that greater placental mass does not explain the more antiangiogenic profile in twin compared with singleton pregnancies. The lack of correlation between placental weight and sFlt-1 should be evaluated in other studies. Hypoxia from reduced placental perfusion has been shown to stimulate sFlt-1 production in an animal model.²⁴ The possibility of chronic hypo-perfusion in the placentas of twin pregnancies could also be hypothesized to explain the increased sFlt-1 observed in twin compared with singleton pregnancies.

Our data demonstrating a more anti-angiogenic profile in mothers of twins than singletons are consistent with a few possible explanations. Twinning may share pathological mechanisms hypothesized to occur in preeclampsia: shallow trophoblast invasion of the maternal endometrium and unconverted spiral arteries, resulting in endothelial damage and changes in anti-angiogenic factor concentrations. Preeclampsia cases were excluded from our analysis to determine whether angiogenic factors were altered with twinning itself and

not its sequelae. Alternatively, differences in angiogenic balance in mothers of twins and singletons may be due to maternal host factors that result in spontaneous twin pregnancies. Host factors such as age and race/ethnicity did not influence our results, though we cannot exclude the possibility of other, unmeasured or unknown risk factors.

An anti-angiogenic protein balance, i.e. greater concentrations of anti-angiogenic proteins such as sFlt-1 and s-endoglin, is established in preeclampsia. Previous studies comparing women who subsequently develop preeclampsia to those who do not have shown similar or lower first trimester sFlt-1 levels, and increased levels at term.^{21, 25} In twin pregnancies, however, patterns of higher sFlt-1 concentrations emerged early in the pregnancy and persisted for the remainder of the gestation. Maternal PIGF in preeclamptic pregnancies is similar or lower in the first trimester and ends the pregnancy lower than levels in uncomplicated pregnancies.²¹ Low levels of maternal PIGF appeared later in the twin pregnancies in our data, specifically between weeks 26–34. Therefore, greater values for the sFlt-1:PIGF ratio seem to appear earlier in twin compared with preeclamptic pregnancies. A third-trimester increase in sFlt-1 alters angiogenic balance in preeclamptic pregnancies, whereas a decrease in PIGF during the third trimester contributes to this phenomenon in twin pregnancies. These findings support the hypothesis that anti-angiogenic balance plays a causative role in endothelial cell injury and that in most pregnancies, this imbalance is most apparent during the third trimester. Although the placenta is central to the process, it remains unclear; however, what causes sFlt-1 to increase and PIGF to decrease in women with preeclampsia or multiple gestation.²⁶ This study and others^{27,28} demonstrate that women with twins can have much higher sFlt-1/PIGF levels (compared to singletons) and not develop preeclampsia. It is unclear why but it probably points to the multifactorial aspect of this disease. There are other pro-angiogenic molecules produced by the placenta such as VEGF, and it is possible that in twins, the production of these molecules is also increased. This would counter the effect of sFlt-1 but would not be reflected in the sFlt-1/PIGF ratio.

Pregnancy provides a ‘stress test’ to identify risk for health conditions that occur later in a woman’s life, with investigation of pregnancy characteristics allowing refinement of biological hypotheses. For example, comparing angiogenic factor profiles between singleton, preeclamptic pregnancies and twin pregnancies, with further comparison of pregnancy conditions with risk of diseases that occur later in the mother may help determine a common etiology, or elucidate how pregnancy mediates preexisting host factors. Preeclamptic pregnancies are associated with an increased cardiovascular disease risk²⁹ and continued endothelial dysfunction. The subtle anti-angiogenic balance that persists in women with a history of preeclampsia could shed light on this association;²⁶ it would be interesting to determine if greater sFlt-1, endoglin and sFlt-1/PIGF concentrations persist in women with a twin history as well. Similar anti-angiogenic profiles in preeclamptic and twin pregnancies argue for determining whether women with a history of twin gestations have a similarly elevated cardiovascular disease risk.

Women with a history of preeclampsia may have an altered breast cancer risk as well, as studies have shown some protection,^{30,319} and there is some suggestion of a reduced maternal breast cancer risk after twin pregnancies.³² The biological mechanism to explain protection afforded by a history of preeclampsia is unknown but there has been recent

speculation regarding the involvement of angiogenic response.³³ Namely, women who mount an anti-angiogenic response when pregnant (as in preeclampsia and multiple gestation) may be more likely to respond similarly in response to tumorigenesis.

Most monozygotic twin gestations result in a monochorionic, diamniotic pregnancy with a single placental mass, while about 20–25% of the time, early post-conception cleavage of the morula will result in a dichorionic, diamniotic pregnancy, with separate placental masses. Maternal anti-angiogenic protein concentrations were greater, as was sFlt-1:PIGF ratio, in monochorionic compared with dichorionic pregnancies in our study. These results are consistent with another small study (n=19) which found some evidence that monochorionic twins had a higher sFlt-1:PIGF ratio. The observation that monochorionic twins have higher anti-angiogenic balance, seems contrary to the hypothesis that sFlt-1 increases with greater placental mass,¹⁹ because there are fewer placentas in the former, although one large placenta could have greater mass than two small ones. However, this is consistent with the lack of correlation between placental weight and sFlt-1 demonstrated in our data.

The strengths of our study include its relatively large sample size compared with previous investigations and the inclusion of two independent data sources - one which included longitudinal measurements throughout the pregnancy and women of various ethnic/racial backgrounds, and the other which included an additional time point (labor and delivery), an additional anti-angiogenic protein, s-endoglin which is implicated in preeclampsia, and information on chorionicity and sex of the twins.

In summary, maternal anti-angiogenic factors, as in preeclampsia, were elevated in twin pregnancies compared with singleton pregnancies. These data could indicate a shared physiology, but other explanations cannot be excluded. Determining whether women with a history of twin gestations are at an elevated cardiovascular disease risk, as are women with a history of preeclampsia is warranted.

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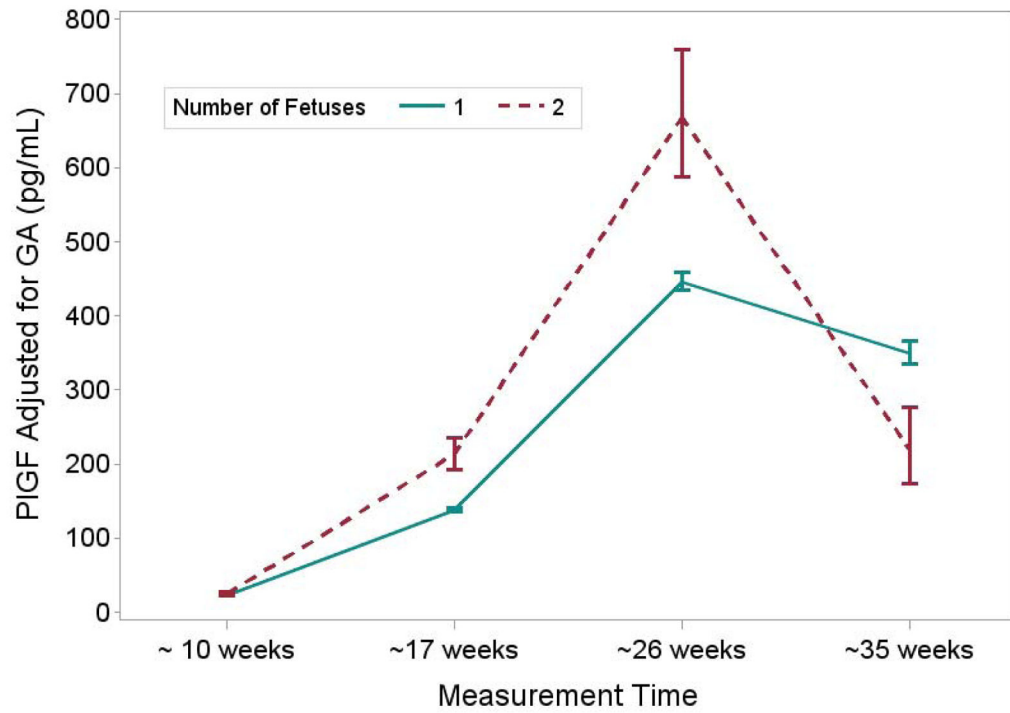


Figure 1. graph of mean (95% CI) PIGF in twins vs. singletons in BIRTH cohort adjusted for gestational weeks at blood collection and by week of gestation

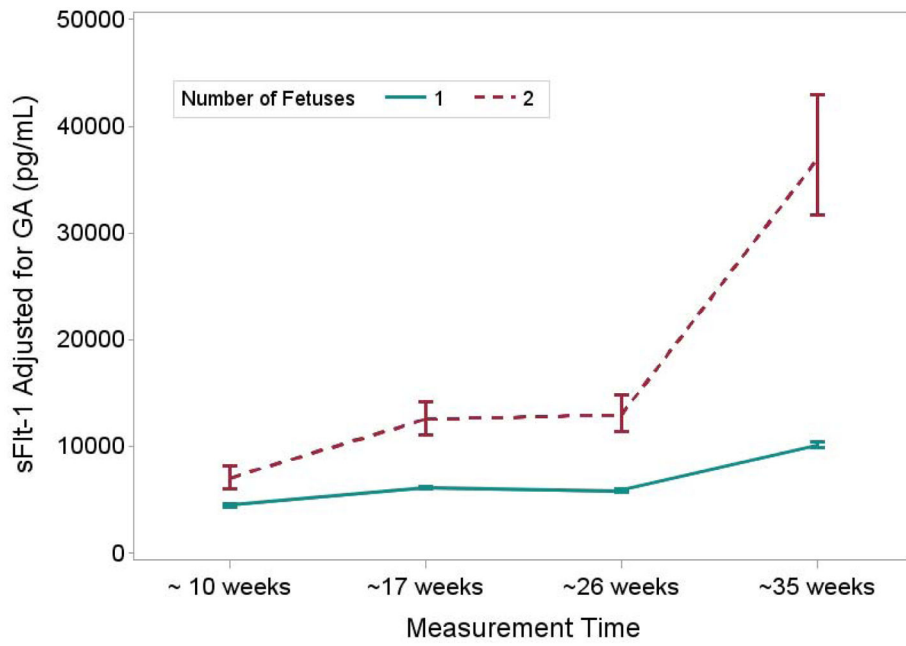


Figure 2. graph of mean (95% CI) sFlt-1 in twins vs. singletons in BIRTH cohort adjusted for gestational weeks at blood collection and by week of gestation

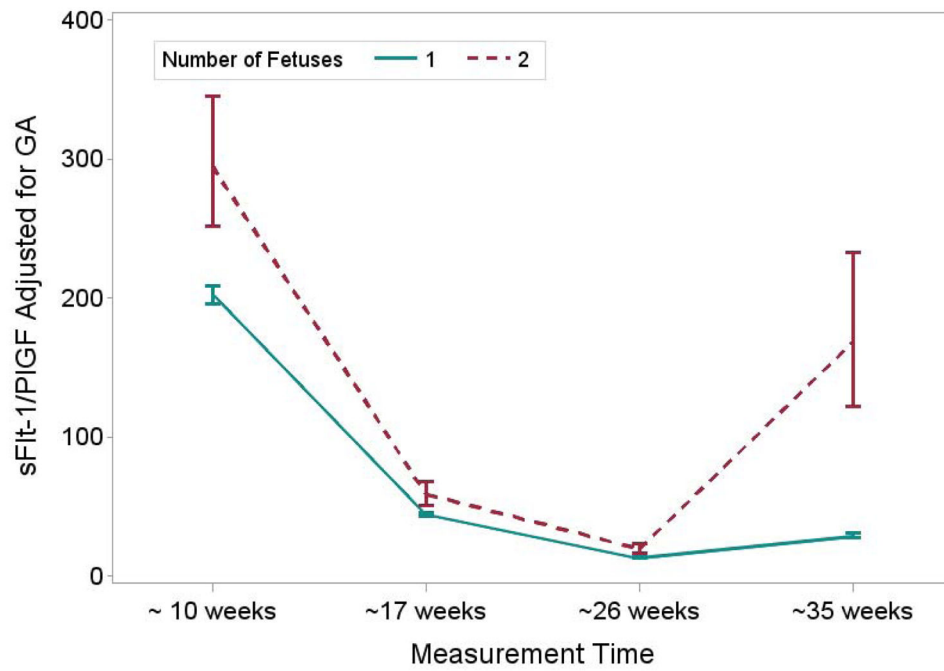


Figure 3. graph of mean (95% CI) sFlt-1/PlGF ratio in twins vs. singletons in BIRTH cohort adjusted for gestational weeks at blood collection and by week of gestation

Table 1

Correlations and Cronbach's Alpha Values between R&D and ARCHITECT Measures of sFlt-1 (soluble fms-like tyrosine kinase-1) and PIGF (placental growth factor)

	Assay	N	Pearson Correlation	Cronbach's Alpha
Time 1	PIGF	639	0.79	0.88
	sFlt-1	632	0.86	0.93
Time 2	PIGF	608	0.91	0.96
	sFlt-1	607	0.87	0.93
Time 3	PIGF	602	0.92	0.96
	sFlt-1	602	0.86	0.93
Time 4	PIGF	568	0.95	0.98
	sFlt-1	568	0.90	0.95

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Table 2
Clinical Characteristics¹ of Twin and Singleton Pregnancies in the BIRTH Cohort and Geisel School of Medicine Twin Study

	Birth Cohort		Geisel School of Medicine Twin Study	
	Singletons n=2193	Twins n=91	Singletons n=62	Twins n=41
Maternal and Gestational				
Maternal age in years (SD)	31.4 (5.7)	35.1 (5.8)	31.0 (5.7)	33.4 (5.9)
Gestational weeks (SD) at delivery	39.0 (1.8)	36.3 (2.3)	38.5 (1.8)	37.1 (2.1)
Nulliparous (%)	648 (29.1)	27 (29.0)	21 (33.9)	12 (29.3)
Artificial reproductive technologies used (%)	138 (6.2)	64 (68.8)	5 (8.1)	16 (39.0)
Race (%)				
White	1318 (59.1)	64 (68.8)	58 (95.1)	40 (97.6)
Black	469 (21.0)	9 (9.7)		
Asian	145 (6.5)	9 (9.7)		
Hispanic	213 (9.6)	9 (9.7)		
Other/mixed/unknown	85 (3.8)	2 (2.2)	3 (4.9)	1 (2.4)
Infant²				
Birth weight (g)	3312 (538)	2418 (214)	3257 (597)	2635 (412)
Z-score for birth weight	0.09 (0.97)	-0.84 (0.70)	0.20 (1.1)	-0.72 (0.57)

¹ Means are presented for continuous variables, percentages for categorical variables. P-values are from t-tests and chi-square tests, respectively, except for race among the Geisel School of Medicine Study women, which is based on Fisher exact test.

² Values are based on mean of twins' birth weights.

Table 3
Maternal serum angiogenic protein concentrations* at four time points during gestation; BIRTH Cohort

Gestational weeks mean (range)	9.7 (8.4–11.6)			17.8 (16.8–18.7)		
	Singleton n=2193	Twin n=91	p-value	Singleton n=2058	Twin n=89	p-value
PlGF (pg/ml)*	22.8 (22.4 – 23.2)	24.9 (23.0 – 26.9)	0.0323	138.4 (135.6 – 141.3)	213.5 (193.3 – 235.8)	< 0.0001
sFlt-1 (pg/ml)*	4485 (4347 – 4628)	7037 (6031 – 8212)	< 0.0001	6131 (5974 – 6291)	12543 (11074 – 14207)	< 0.0001
sFlt-1:PlGF ratio	202.4 (196.0 – 209.0)	294.6 (251.5 – 335.5)	< 0.0001	44.3 (42.9 – 45.7)	58.7 (50.6 – 68.2)	0.0003

Gestational weeks mean (range)	25.9 (24.8–28.1)			35.1 (34.6–35.9)		
	Singleton n=2089	Twin n=91	p-value	Singleton n=2064	Twin n=68	p-value
PlGF (pg/ml)*	445.9 (434.1 – 458.1)	668.0 (587.2 – 759.9)	< 0.0001	350.2 (335.6 – 365.5)	219.2 (173.2 – 277.3)	< 0.0001
sFlt-1 (pg/ml)*	5898 (5736 – 6065)	12968 (11347 – 14821)	< 0.0001	10151 (9875 – 10435)	36916 (31714 – 42971)	< 0.0001
sFlt-1:PlGF ratio	13.2 (12.7 – 13.7)	19.4 (16.2 – 23.2)	< 0.0001	29.0 (27.3 – 30.7)	168.4 (121.9 – 232.8)	< 0.0001

* means (95% confidence intervals). Means are adjusted for gestational age from models with logarithm-transformed angiogenic factor as the outcome; presented are geometric means derived from taking the exponent of the adjusted mean. sFlt-1 (soluble fms-like tyrosine kinase-1; PlGF (placental growth factor)

Table 4

Maternal serum angiogenic protein concentrations* in the third trimester and at delivery; Geisel School of Medicine Twin Study

	Prenatal			Delivery		
	Singleton	Twin	p-value	Singleton	Twin	p-value
Gestational weeks mean (range)	32.3 (31.3–38.7)	32.3 (31.0–33.1)		38.2 (33.4–41.0)	37.1 (32.6–40.4)	
N	40	41		52	41	
PlGF (pg/ml)*	467.3 (375.7–581.3)	386.2 (312.4–477.6)	0.24	152.4 (121.7–190.7)	147.5 (111.9–194.4)	0.86
sFlt-1 (pg/ml)*	2108 (1821–2440)	6129 (5317–7065)	<0.0001	7278 (5860–9039)	15899 (12179–20756)	<0.0001
s-endoglin (ng/ml)	4.61 (3.81–5.57)	13.0 (10.8–15.7)	<0.0001	14.5 (11.2–18.6)	24.8 (18.2–33.8)	0.01
sFlt-1/PlGF ratio	4.51 (3.37–6.04)	15.9 (12.0–21.1)	<0.0001	47.8 (34.7–65.7)	107.8 (72.9–159.5)	0.003

* Means (95% confidence intervals); means are adjusted for gestational age from models with logarithm-transformed angiogenic factor as the outcome; presented are geometric means derived from taking the exponent of the adjusted mean. sFlt-1 (soluble fms-like tyrosine kinase-1; PlGF (placental growth factor)