Published in final edited form as:
J Matern Fetal Neonatal Med. 2010 September ; 23(9): 960–972. doi:10.3109/14767050903410664.

FETAL DEATH : A CONDITION WITH A DISSOCIATION IN THE CONCENTRATIONS OF SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 BETWEEN THE MATERNAL AND FETAL COMPARTMENTS

Tinnakorn Chaiworapongsa, MD[1,2], **Juan Pedro Kusanovic, MD**[1,2], **Zeynep Alpay Savasan, MD**[1,2], **Shali Mazaki-Tovi, MD**[1,2], **Sun Kwon Kim, MD**[1], **Edi Vaisbuch, MD**[1,2], **Adi L Tarca, PhD**[1,3], **Pooja Mittal, MD**[1,2], **Giovanna Ogge, MD**[1], **Ichchha Madan, MD**[1,2], **Zhong Dong, MD**[1], **Lami Yeo, MD**[1,2], **Sonia S Hassan, MD**[1,2], and **Roberto Romero, MD**[1,2,4]

[1]Perinatology Research Branch, NICHD, NIH, DHHS, Bethesda, MD and Detroit, MI, USA

[2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA

[3]Department of Computer Science, Wayne State University, Detroit, MI

[4]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA

Abstract

OBJECTIVE—An anti-angiogenic state has been implicated in the pathophysiology of preeclampsia, fetal growth restriction and fetal death. Vascular endothelial growth factor (VEGF), an indispensible angiogenic factor for embryonic and placental development exerts its angiogenic properties through the VEGF receptor (VEGFR)-2. A soluble form of this protein (sVEGFR-2) has been recently detected in maternal blood. The aim of this study was to determine if fetal death was associated with changes in the concentrations of sVEGFR-2 in maternal plasma and amniotic fluid.

STUDY DESIGN—Maternal plasma was obtained from patients with fetal death (n=59) and normal pregnant women (n=134). Amniotic fluid was collected from 36 patients with fetal death and the control group consisting of patients who had an amniocentesis and delivered at term (n=160). Patients with fetal death were classified according to the clinical circumstances into the following groups: 1) unexplained; 2) preeclampsia and/or placental abruption; and 3) chromosomal and/or congenital anomalies. Plasma and amniotic fluid concentrations of sVEGFR-2 were determined by ELISA. Non-parametric statistics and logistic regression analysis were applied.

RESULTS—1) Patients with a fetal death had a significantly lower median plasma concentration of sVEGFR-2 than normal pregnant women ($p<0.001$). The median plasma concentration of sVEGFR-2 in patients with unexplained fetal death and in those with preeclampsia/abruption, but not that of those with congenital anomalies, was lower than that of normal pregnant women ($p=0.006$, $p<0.001$and $p=0.2$ respectively); 2) the association between plasma sVEGFR-2 concentrations and preterm unexplained fetal death remained significant after adjusting for potential confounders (OR 3.2; 95% CI 1.4–7.3 per each quartile decrease in plasma sVEGFR-2

Address correspondence to: Roberto Romero, MD, Perinatology Research Branch, NICHD, NIH, DHHS, Wayne State University/ Hutzel Women's Hospital, 3990 John R, Box 4, Detroit, MI 48201, USA, Telephone (313) 993-2700, Fax: (313) 993-2694, prbchiefstaff@med.wayne.edu.

Presented as a poster presentation at the 30th annual meeting of the Society for Maternal Fetal Medicine February 1–6, 2010, Hilton Chicago, IL

concentrations); 3) each subgroup of fetal death had a higher median amniotic fluid concentration of sVEGFR-2 than the control group ($p<0.001$ for each); 4) the association between amniotic fluid sVEGFR-2 concentrations and preterm unexplained fetal death remained significant after adjusting for potential confounders (OR 15.6; 95% CI 1.5–164.2 per each quartile increase in amniotic fluid sVEGFR-2 concentrations); and 5) among women with fetal death, there was no relationship between maternal plasma and amniotic fluid concentrations of sVEGFR-2 (Spearman Rho: 0.02; $p=0.9$).

CONCLUSION—Pregnancies with a fetal death, at the time of diagnosis, are characterized by a decrease in the maternal plasma concentration of sVEGFR-2, but an increase in the amniotic fluid concentration of this protein. While a decrease in sVEGFR-2 concentration in maternal circulation depends upon the clinical circumstances of fetal death, an increase in sVEGFR-2 concentration in amniotic fluid seems to be a common feature of fetal death. It remains to be determined if the perturbation in sVEGFR-2 concentrations in maternal and fetal compartments observed herein preceded the death of a fetus.

Keywords

Amniotic fluid; angiogenesis; angiogenic factor; congenital anomaly; intrauterine demise; sKDR; maternal plasma; preeclampsia; stillbirth; unexplained fetal death; sVEGFR-2

INTRODUCTION

The rate of fetal death decreased substantially from the 1950s through the 1980s. However, this rate has remained relatively stable for the past 20 years [1–3]. In the United States, there are approximately 26,000 stillbirths annually, a rate of 6.4 /1000 births which accounts for almost half of all perinatal deaths [4]. Despite the magnitude of the problem, relatively little attention has been focused on fetal death. A better understanding of the causes of stillbirth may lead to the development of measures to prevent this pregnancy complication.

Since successful pregnancy requires a balance between angiogenic and anti-angiogenic processes [5,6], examining these factors in fetal death may help to decipher its mechanisms of disease. Angiogenesis, a process by which new vessels are formed from pre-existing vasculature, is regulated by several growth factors, cytokines and their receptors. Among them, VEGF-signaling represents a critical step in both physiologic and pathologic angiogenesis [7,8]. VEGF is essential for fetal development since the loss of only one copy of the VEGF gene leads to embryonic lethality [5,6]. VEGF exerts its biological effects through two high-affinity tyrosine kinase receptors: VEGFR-1 and VEGFR-2. While VEGFR-1 is considered a ‘decoy’ receptor, VEGFR-2 is the major mediator of the mitogenic, angiogenic, permeability enhancing, and endothelial survival properties of VEGF [7,8]. VEGFR-2 knock-out mice die due to a lack of blood vessel formation, indicating that this receptor is also essential for the development of vascular systems in the embryo.[9] Both VEGF receptors are present in two forms; the membranous and the soluble forms. An excess of the soluble form of VEGFR-1 in maternal blood has been implicated in the pathophysiology of several obstetrical syndromes including preeclampsia,[10–27] fetal growth restriction [28–30], placental abruption [31], “mirror syndrome”[32], twin-to-twin transfusion syndrome[33], and fetal death.[34]

The soluble form of VEGFR-2 has been detected in human plasma.[35] Under experimental conditions, this protein can bind to VEGF [35] and its recombinant form has anti-angiogenic activity [36,37]. The role of sVEGFR-2 in human disease is unclear. However, several studies have evaluated its potential as a surrogate biomarker for tumor progression in malignant melanoma [38], myelodysplastic syndrome [39] and acute leukemia [40,41]. In

non-malignant conditions, plasma sVEGFR-2 concentration is lower in patients with systemic lupus erythematosus [42], dengue hemorrhagic fever [43], and pregnant women with acute pyelonephritis [44] compared to controls. Recent studies have also indicated that patients with preeclampsia, both prior to and at the time of clinical diagnosis, have lower plasma concentrations of sVEGFR-2 than normal pregnant women [21,45–47]. It is possible that patients with fetal death might also demonstrate perturbation of sVEGFR-2 concentration in maternal circulation.

Placenta condition media was found to have angiogenic properties as determined in a bioassay (chick chorioallantoic membrane) [48]. Subsequently, VEGF and sVEGFR-1 were identified in human amniotic fluid [49–52], and amniotic fluid concentrations of sVEGFR-1 were reported to be higher in patients with preeclampsia than in controls [51,52]. Both VEGF and its receptors (VEGFR-1 and VEGFR-2) are also expressed in the human amnion [53–55]. We hypothesize that successful pregnancies require coordination between the fetus, the placenta and the mother, and that this synchronization may be altered in cases of fetal death. The purpose of this study was to determine if fetal death is associated with changes in the concentrations of sVEGFR-2 in maternal plasma and amniotic fluid.

PATIENTS AND METHODS

Study design

A cross-sectional study was conducted by searching our clinical database and bank of biologic samples. This study included only singleton pregnancies. Maternal plasma was evaluated in 59 patients with fetal death and 134 normal pregnant women. Thirty-six patients with fetal death underwent amniocentesis. Since amniocenteses could not be performed in normal pregnant women without clinical indication, the control group for comparisons of amniotic fluid results included 1) patients with preterm labor (PTL) who delivered at term without intra-amniotic infection/inflammation (n=122); and 2) women at term not in labor without intra-amniotic infection (n=38). Women in the control group who delivered small-for-gestational age (SGA) neonates were excluded.

Clinical definition

Fetal death was defined as death of the fetus after 20 week of gestation diagnosed by ultrasound examination. This group was sub-classified according to clinical circumstances into: 1) unexplained fetal death (n=36); 2) fetal death with preeclampsia and/or placental abruption (n=15); and 3) fetal death with a known chromosomal abnormality and/or major malformation (n=8). Fetuses in the latter group included those with trisomy 21 (n=3), trisomy 13 (n=1), non-immune hydrops fetalis (n=3), and a cardiovascular defect with single umbilical artery (n=1). Preeclampsia was defined as hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on at least two occasions, 4 hours to 1 week apart) and proteinuria (≥300 milligrams in a 24-hour urine collection or at least one dipstick measurement ≥2+) diagnosed after 20 weeks of gestation [30]. Placental abruption was diagnosed based on clinical presentation (vaginal bleeding and abdominal pain) and the presence of a retroplacental blood clot, not associated with vasa previa, placenta previa, or uterine rupture [31].

Normal pregnant women were enrolled from either a labor-delivery unit (in cases of scheduled cesarean section) or an antenatal clinic, and followed until delivery. The inclusion criteria for the normal pregnancy group included: 1) no medical, obstetrical or surgical complications; 2) not in labor; and 3) delivery of a normal term (≥37 weeks) infant whose birth weight was between the 10^{th} and 90^{th} percentile for gestational age. PTL was defined by the presence of regular uterine contractions occurring at a frequency of at least 2 every 10

minutes and cervical change < 37 completed weeks of gestation. Intra-amniotic inflammation was defined as an amniotic fluid interleukin-6 concentration ≥2.6 ng/mL [56]. Intra-amniotic infection was defined as a positive amniotic fluid culture for micro-organisms. The diagnosis of SGA was based on an ultrasonographic estimated fetal weight and confirmed by a birthweight below the 10th percentile for gestational age, according to the reference range proposed by Alexander et al [57] or Gonzalez et al [58] depending on ethnicity of the patients.

The collection and utilization of maternal plasma and amniotic fluid samples was approved by the Human Investigation Committees of the Sotero del Rio Hospital, Santiago, Chile (a major affiliate of the Catholic University of Santiago), the Institutional Review Boards of Wayne State University, (Detroit, MI) and the IRB of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD/NIH/DHHS). Many of these samples have been used in previous studies.

Sample collection and sVEGFR-2 immunoassay

Maternal blood samples were obtained by venipuncture and collected into tubes containing EDTA. Amniotic fluid samples were obtained by transabdominal amniocentesis performed for genetic indications, evaluation of the microbial status of the amniotic cavity and/or assessment of fetal lung maturity in patients approaching term. Samples of amniotic fluid were transported to the laboratory in a sterile capped syringe and cultured for aerobic/anaerobic bacteria and genital mycoplasmas. Samples were centrifuged and stored at −70°C. Maternal plasma and amniotic fluid concentrations of sVEGFR-2 were determined by sensitive and specific immunoassays obtained from R&D Systems (Minneapolis, MN). Briefly, the immunoassay utilized the quantitative sandwich technique and their concentrations were determined by interpolation from the standard curves. The inter- and intra-assay coefficients of variation obtained were 2% and 4%, respectively. The sensitivity was 0.019 ng/ml.

Statistical analysis

Kolmogorov Smirnov and Shapiro-Wilk were used to test for normal distribution of the data. Kruskal Wallis with post-hoc Mann-Whitney U test was utilized to determine the differences of the median among and between groups. Contingency tables, Chi-square and Fischer's Exact test were employed for comparisons of proportions. Logistic regression (backward step-wise) was applied to determine the association between the presence of fetal death and plasma/amniotic fluid concentrations of sVEGFR-2 while adjusting for potential confounders. Analysis was conducted with SPSS V.12 (SPSS Inc., Chicago, IL). A p value of <0.05 was considered significant.

RESULTS

Maternal plasma concentrations of sVEGFR-2 in women with fetal death

Demographic and clinical characteristics of women with fetal death and those with a normal pregnancy are displayed in Table I. The normal pregnancy group had a higher median gestational age at blood sampling than the fetal death group (p<0.001). There was no significant difference in the distribution of ethnicity and nulliparity. The fetal death group had a higher rate of smokers than those with a normal pregnancy (p=0.04). The median plasma concentration of sVEGFR-2 was significantly lower in women with a fetal death than that in normal pregnant women (p<0.001; Figure 1).

Table II describes the demographic and clinical characteristics according to the subgroups of fetal death. The majority of fetal deaths were categorized as unexplained (61%). The median

plasma concentration of sVEGFR-2 in the unexplained fetal death group was lower than that in the normal pregnancy group (p=0.006; Figure 2), but higher than that in the fetal death with preeclampsia and/or placental abruption group (p=0.001). There was no significant difference in the median plasma sVEGFR-2 concentration between women with fetal death with chromosomal and/or congenital anomalies and normal pregnant women (p=0.2). The association between plasma sVEGFR-2 concentrations and fetal death overall, unexplained fetal death and fetal death with preeclampsia and/or placental abruption remained significant after adjusting for gestational age at blood sampling and other potential confounders (see Table III). There was no significant relationship between maternal plasma concentrations of sVEGFR-2 and neonatal birthweight (p=0.9).

To examine whether changes in maternal plasma sVEGFR-2 concentration in unexplained fetal death varies with the gestational age at which fetal death was diagnosed, patients with unexplained fetal death and those with normal pregnancy were stratified into those who had blood sampling performed before and after 37 weeks of gestation. There were no significant differences in the median gestational age at blood collection between patients with an unexplained fetal death at < 37 weeks and normal pregnant women at preterm gestation or between patients with fetal death at term gestation and normal pregnant women at term [preterm fetal death (n=27): median 28.6 weeks, range 20–36 weeks vs. preterm normal pregnancy (n=64): median 30.7 weeks, range 20–36 weeks; p= 0.2 and term fetal death (n=9): median 38.7 weeks, range 37–40 weeks vs. term normal pregnancy (n=70): median 39 weeks, range 37–41 weeks; p=0.8]. Unexplained fetal death in preterm, but not in term gestations, had a significantly lower maternal median plasma sVEGFR-2 concentration than normal pregnant women (p<0.001 and p=0.7 respectively; Figures 3 and 4). There was no significant difference in the median plasma concentrations of sVEGFR-2 between women with an unexplained preterm fetal death with and without SGA [SGA (n=13): median 9.4 ng/ml, range 6.5–12.6 ng/ml vs. without SGA (n=14): median 8.8 ng/ml, range 5.9–13.6 ng/ml; p=0.5]

To examine the association between plasma sVEGFR-2 concentrations and the presence of a preterm unexplained fetal death, plasma sVEGFR-2 concentrations among preterm patients were stratified into quartiles (Q). As plasma concentrations of sVEGFR-2 decreased from the 4th quartile (Q4), the rate of fetal death increased [Q4: 0% (0/27); Q3: 26.3% (5/19), Q2: 38% (8/21) and Q1: 58% (14/24); Chi-square for trend p<0.001]. The association between plasma sVEGFR-2 concentrations and preterm unexplained fetal death remained significant after adjusting for gestational age at blood sampling (weeks), maternal age (years), African-American ethnicity, smoking, nulliparity, duration of sample storage (years) and the presence of SGA (OR 3.2; 95% CI 1.4–7.3 per each quartile decrease in plasma sVEGFR-2 concentrations).

Amniotic fluid concentrations of sVEGFR-2 in women with fetal death

Clinical characteristics of the study population included for the evaluation of amniotic fluid sVEGFR-2 concentrations are displayed in Table IV. The control group was comprised of 122 women with PTL who delivered at term and 38 women at term without labor. Overall, the concentrations of sVEGFR-2 were lower in amniotic fluid than those in maternal plasma (median 0.6 ng/ml vs. 10.6 ng/ml in the control group and median 2.6 ng/ml vs. 8.8 ng/ml in the fetal death group). Among patients with fetal death, there was no relationship between maternal plasma and amniotic fluid concentrations of sVEGFR-2 (Spearman Rho: 0.02; p=0.9).

In contrast to the results in maternal plasma, the median amniotic fluid sVEGFR-2 concentration in women with fetal death was significantly higher than that of women in the control group (p<0.001; Figure 5). Among women with fetal death, there were no significant

relationships between amniotic fluid concentrations of sVEGFR-2 and amniotic fluid concentrations of glucose, white blood cell counts or red blood cell counts ($p>0.05$ for each). In contrast, amniotic fluid concentrations of sVEGFR-2 were correlated inversely with neonatal birthweight (Spearman's Rho −0.5; $p=0.004$).

When the fetal death group was subdivided according to clinical categories, women with unexplained fetal death, those with fetal death with preeclampsia and/or placental abruption and those with fetal death and major chromosomal/ congenital anomalies had a significantly higher median amniotic fluid concentration of sVEGFR-2 than women in the control group ($p<0.001$, $p=0.03$ and $p=0.005$ respectively; Figure 6). The association between amniotic fluid concentrations of sVEGFR-2 and the presence of fetal death in each subgroup remained significant after adjusting for gestational age at amniocentesis (weeks) and other potential confounders (Table V).

To examine whether changes in amniotic fluid sVEGFR-2 concentration in unexplained fetal death varies with gestational age at which fetal death was diagnosed, patients with unexplained fetal death were stratified into those who had amniocentesis performed before and after 37 weeks of gestation. The clinical characteristics of women with PTL who delivered at term, women at term without labor and those with unexplained fetal death at preterm and term gestation are displayed in Table VI. Women with a preterm unexplained fetal death had a lower median gestational age at amniocentesis and a higher rate of SGA neonates than women with PTL who delivered at term ($p<0.001$ for both). Unexplained preterm fetal death, but not term fetal death, had a significantly higher median amniotic fluid sVEGFR-2 concentration than women in the control groups ($p<0.001$ and $p=0.2$ respectively; Figure 7 and 8). There was no significant difference in the median amniotic fluid concentrations of sVEGFR-2 between women with an unexplained preterm fetal death with SGA and those without SGA [SGA (n=11): median 2.6 ng/ml, range 0.9–8.1 ng/ml vs. without SGA (n=9): median 2.4 ng/ml, range 0.4–5.7 ng/ml; $p=0.8$)

To examine the association between amniotic fluid sVEGFR-2 concentrations and the presence of unexplained preterm fetal death, amniotic fluid sVEGFR-2 concentrations were stratified into quartiles. As amniotic fluid concentrations of sVEGFR-2 increased from the 1st quartile, the prevalence of fetal death increased [Q1: 0% (0/32); Q2: 2% (1/43), Q3: 9% (3/34) and Q4: 48% (16/33); Chi-square for trend $p<0.001$]. The association between amniotic fluid sVEGFR-2 concentrations and unexplained preterm fetal death remained significant after adjusting for gestational age at amniocentesis (weeks), maternal age (years), African American ethnicity, smoking, nulliparity, duration of sample storage (years) and the presence of SGA (OR 15.6; 95% CI 1.5–164.2 per each quartile increase in amniotic fluid sVEGFR-2 concentrations).

DISCUSSION

Principal findings

1) Women with a fetal death had a lower median plasma concentration of sVEGFR-2 than normal pregnant women, whereas the median amniotic fluid concentration of sVEGFR-2 was higher in patients with a fetal death than that of controls; 2) Unexplained preterm fetal death and fetal death with preeclampsia and/or placental abruption, but not fetal death with chromosomal or major congenital anomalies, were associated with lower plasma sVEGFR-2 concentrations than normal pregnant women; and 3) Unexplained preterm fetal death, fetal death with preeclampsia and/or placental abruption, and fetal death with chromosomal or major congenital anomalies were all associated with higher amniotic fluid concentrations of sVEGFR-2 than the control group.

Fetal death is associated with decreased maternal plasma concentrations of sVEGFR-2

The finding that fetal death was associated with lower maternal plasma sVEGFR-2 concentrations than normal pregnancy is novel and consistent with two previous reports of increased maternal plasma sVEGFR-1 concentrations in pregnant women with fetal death compared to normal pregnancy [34,59]. A decrease in plasma sVEGFR-2 concentrations has also been observed in patients with preeclampsia and pregnancies with small for gestational age fetuses[21] as well as in pregnant women with systemic inflammation (acute pyelonephritis) [44]. Since all of these obstetrical syndromes are associated with various degrees of endothelial cell dysfunction [60–67], plasma sVEGFR-2 concentration might be a surrogate marker of endothelial cell function in the maternal circulation. Given that VEGF signaling through the membranous isoform of VEGFR-2 is essential for endothelial cell function/survival and that the soluble form of VEGFR-2 can bind to VEGF and inhibit VEGF function, it is possible that a decrease in plasma sVEGFR-2 concentrations may be a physiologic response designed to increase availability of free VEGF. Alternatively, plasma sVEGFR-2 concentration may simply be a marker of unbound VEGF. Consistent with this hypothesis, a recent study demonstrated that VEGF could stimulate a disintegrin and metalloproteinase (ADAM)-17, and this protein, in turn, cleaves the extra-cellular portion of VEGFR-2 from endothelial cells [68]. This mechanism of protein ectodomain shedding by transmembrane proteases has recently emerged as a key posttranslational regulator of several growth factor receptors involved in angiogenesis [69–71]. Although sVEGFR-2 concentrations in cell lysates of human colonic microvascular endothelial cells are reduced in response to hypoxia [72,73], it is unlikely that the decreased plasma sVEGFR-2 concentrations in women with fetal death results from a response of vascular endothelial cells against local hypoxic insults.

Unexplained fetal death at preterm gestation is associated with decreased maternal plasma concentrations of sVEGFR-2

The findings that unexplained fetal death at preterm, but not at term, was associated with a decrease in maternal plasma sVEGFR-2 concentration is also consistent with previous studies that observed a higher magnitude of angiogenic imbalance in complicated pregnancies (e.g., preeclampsia and pregnancies with SGA fetuses and abnormal Doppler velocimetry) at preterm than at term gestations [16,17,28,30]. Moreover, abnormalities of uterine artery Doppler velocimetry at 22–24 weeks are considered a better predictor of subsequent fetal death due to placental causes than other unexplained stillbirths [74]. These observations support the view that unexplained preterm fetal death, similar to preeclampsia, is associated with decreased uteroplacental perfusion and the presence of an anti-angiogenic state in maternal circulation.

A solid body of evidence supports an association between reduced uteroplacental perfusion, fetal growth restriction, and fetal death [75,76]. Absence of physiologic transformation of the spiral arteries has been reported in patients with second trimester spontaneous abortion [76]. Moreover, 40% of unexplained fetal deaths are SGA [75], the rate of which is consistent with the observation in the current study [44 % (16/36)]. Of note, similar to the changes of plasma sVEGFR-1 concentrations in unexplained fetal deaths [34], there was no significant difference in the median plasma sVEGFR-2 concentration in patients with unexplained fetal death between those with and without SGA neonates. We have proposed that an elevation of sVEGFR-1 may be a protective mechanism of the fetoplacental unit [34]. Some cases of fetal death, in which there is no elevation of plasma VEGFR-1 concentrations in response to decreased utero-placental perfusion (e.g., in cases of abnormal uterine artery Doppler velocimetry), may represent failure of this mechanism. It is unclear whether or not this is the case for sVEGFR-2. However, a previous study indicates that there was no significant difference in the mean plasma sVEGFR-2 concentration among patients

with SGA whether or not they had an increased impedance to blood flow in the uterine artery (as measured by uterine artery Doppler velocimetry) [21]. Moreover, there was no relationship between plasma sVEGFR-1 and sVEGFR-2 concentrations in maternal blood of women with unexplained fetal deaths (Chaiworapongsa T, et al., unpublished observation).

Fetal death is associated with increased amniotic fluid concentrations of sVEGFR-2

The current study is the first to report the detection of sVEGFR-2 in human amniotic fluid. Amniotic fluid concentrations of sVEGFR-2 in the control group were generally much lower than that in the maternal plasma of normal pregnant women. In contrast with the findings in maternal plasma, fetal death was associated with an increase in amniotic fluid concentrations of sVEGFR-2. The findings that there was no significant relationship between amniotic fluid concentrations of sVEGFR-2 and amniotic fluid concentrations of glucose, white blood cell counts or red blood cell counts suggest that the increase of amniotic fluid sVEGFR-2 concentrations in fetal death is unlikely to be a consequence of intra-amniotic infection/inflammation or traumatic amniocenteses.

VEGF and sVEGFR-1 are present in human amniotic fluid, and thus, it is likely that sVEGFR-2 is also involved in the regulation of VEGF activity in the amniotic cavity. Since there are no blood vessels in the human amnion, the function of VEGF in the amniotic cavity might be related to the growth and development of the fetus [77] or the regulation of amniotic fluid volume and its content [55]. Interestingly, amniotic fluid concentrations of VEGF in the midtrimester of pregnancy with subsequent fetal death have been reported to be lower than those of normal pregnancy [49]. However, another study found that immunoreactive VEGF in amniotic fluid of normal pregnant women could not be detected by ELISA, since VEGF was bound to other unidentified high molecular weight proteins [78]. The authors concluded that the soluble form of VEGFR-1 is unlikely to represent one of these binding proteins since the determination of PlGF (which also can bind to sVEGFR-1) by ELISA was not affected [78]. These observations do not preclude sVEGFR-1 and/or sVEGFR-2 as potential candidates for VEGF binding proteins, since they could form heterodimers and have a higher molecular weight than their original soluble forms [78,79]. Therefore, it is possible that the increased amniotic fluid concentrations of sVEGFR-2 observed in patients with fetal death could bind to VEGF, leading to the decreased availability of unbound VEGF, a critical angiogenic protein for fetal growth and development [5,6,77], in the amniotic cavity. The observations that amniotic fluid concentrations of sVEGFR-2 in patients with fetal death were correlated inversely with neonatal birthweight support this view.

The findings that all clinical circumstances of fetal death in the current study were associated with increased amniotic fluid concentrations of sVEGFR-2 suggest that the changes in sVEGFR-2 concentrations in amniotic fluid observed herein might be either a terminal event of impending fetal death or a fetal postmortem phenomenon.

Strength and limitation of the study

This study is the first to examine the changes of sVEGFR-2 concentration in patients with fetal death in both maternal plasma and amniotic cavity simultaneously. Moreover, we have described a perturbation of sVEGFR-2 concentrations in women with fetal death according to the clinical circumstances related to fetal death and to the gestational age at which the fetal death was diagnosed. The findings that there was a dose-response relationship between maternal plasma/amniotic fluid concentrations of sVEGFR-2 and fetal death strengthen the relationship between the changes of this protein in maternal/fetal compartments and fetal death.

Three potential limitations of this study should be mentioned. First, since plasma sVEGFR-2 concentrations change with gestational age, the delta values or multiple of medians (deviation from the median concentrations for gestational age) may be more interpretable than absolute values in reflecting differences in plasma sVEGFR-2 concentrations from normal pregnant women. However, the maternal plasma sVEGFR-2 concentrations were not normally distributed even after logarithmic transformation of the data and the best regression equation of plasma sVEGFR-2 as a function of gestational age could explain only 22% of the variance of plasma sVEGFR-2 concentrations in normal pregnant women. These observations led us to apply multivariable logistic regression analysis, which does not require the data to be normally distributed, to determine the association between plasma/amniotic fluid concentrations of sVEGFR-2 and the presence of fetal death while adjusting for gestational age at sampling and other potential confounders. Second, due to the cross-sectional nature of the study, the temporal relationship between the changes in plasma/amniotic fluid concentrations of sVEGFR-2 and the death of a fetus could not be determined. Finally, due to the small sample size of women with fetal death and chromosomal/congenital anomalies, the changes of sVEGFR-2 concentrations in this group should be interpreted with caution.

We conclude that pregnancies with a fetal death at the time of diagnosis are characterized by a lower maternal plasma concentration of sVEGFR-2, but a higher amniotic fluid concentration of this protein than those of the control group. While a decrease in sVEGFR-2 concentrations in the maternal circulation depends upon the clinical circumstances of fetal death, the increase in sVEGFR-2 concentration in amniotic fluid seems to be a common feature of fetal demise. However, whether these changes precede or follow fetal death remains to be determined.

Acknowledgments

This research was supported by the Perinatology Research Branch, Division of Intramural Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, DHHS.

Reference List

1. Silver RM. Fetal death. Obstet Gynecol. 2007; 109:153–167. [PubMed: 17197601]
2. Fretts RC. Etiology and prevention of stillbirth. Am J Obstet Gynecol. 2005; 193:1923–1935. [PubMed: 16325593]
3. Smith GC, Fretts RC. Stillbirth. Lancet. 2007; 370:1715–1725. [PubMed: 18022035]
4. Silver RM, Varner MW, Reddy U, Goldenberg R, Pinar H, Conway D, Bukowski R, Carpenter M, Hogue C, Willinger M, Dudley D, Saade G, Stoll B. Work-up of stillbirth: a review of the evidence. Am J Obstet Gynecol. 2007; 196:433–444. [PubMed: 17466694]
5. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature. 1996; 380:439–442. [PubMed: 8602242]
6. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 1996; 380:435–439. [PubMed: 8602241]
7. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat.Med. 2003; 9:669–676. [PubMed: 12778165]
8. Shibuya M. Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. BMB.Rep. 2008; 41:278–286. [PubMed: 18452647]
9. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature. 1995; 376:62–66. [PubMed: 7596435]

10. Torry DS, Wang HS, Wang TH, Caudle MR, Torry RJ. Preeclampsia is associated with reduced serum levels of placenta growth factor. Am J Obstet Gynecol. 1998; 179:1539–1544. [PubMed: 9855593]
11. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. Am J Obstet Gynecol. 2003; 188:177–182. [PubMed: 12548214]
12. Reuvekamp A, Velsing-Aarts FV, Poulina IE, Capello JJ, Duits AJ. Selective deficit of angiogenic growth factors characterises pregnancies complicated by pre-eclampsia. Br J Obstet Gynaecol. 1999; 106:1019–1022. [PubMed: 10519425]
13. Koga K, Osuga Y, Yoshino O, Hirota Y, Ruimeng X, Hirata T, Takeda S, Yano T, Tsutsumi O, Taketani Y. Elevated serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) levels in women with preeclampsia. J Clin.Endocrinol.Metab. 2003; 88:2348–2351. [PubMed: 12727995]
14. Tsatsaris V, Goffin F, Munaut C, Brichant JF, Pignon MR, Noel A, Schaaps JP, Cabrol D, Frankenne F, Foidart JM. Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. J Clin.Endocrinol.Metab. 2003; 88:5555–5563. [PubMed: 14602804]
15. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin.Invest. 2003; 111:649–658. [PubMed: 12618519]
16. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. N.Engl.J Med. 2004; 350:672–683. [PubMed: 14764923]
17. Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Mee KY, Goncalves LF, Gomez R, Edwin S. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. Am J Obstet Gynecol. 2004; 190:1541–1547. [PubMed: 15284729]
18. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat.Med. 2006; 12:642–649. [PubMed: 16751767]
19. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N.Engl.J Med. 2006; 355:992–1005. [PubMed: 16957146]
20. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, Kusanovic JP, Gotsch F, Erez O, Mazaki-Tovi S, Gomez R, Edwin S, Chaiworapongsa T, Levine RJ, Karumanchi SA. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern.Fetal Neonatal Med. 2008; 21:9–23. [PubMed: 18175241]
21. Chaiworapongsa T, Romero R, Gotsch F, Espinoza J, Nien JK, Goncalves L, Edwin S, Kim YM, Erez O, Kusanovic JP, Pineles BL, Papp Z, Hassan S. Low maternal concentrations of soluble vascular endothelial growth factor receptor-2 in preeclampsia and small for gestational age. J Matern.Fetal Neonatal Med. 2008; 21:41–52. [PubMed: 18175243]
22. Erez O, Romero R, Espinoza J, Fu W, Todem D, Kusanovic JP, Gotsch F, Edwin S, Nien JK, Chaiworapongsa T, Mittal P, Mazaki-Tovi S, Than NG, Gomez R, Hassan SS. The change in concentrations of angiogenic and anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and small-for-gestational age. J Matern.Fetal Neonatal Med. 2008; 21:279–287. [PubMed: 18446652]
23. Vatten LJ, Eskild A, Nilsen TI, Jeansson S, Jenum PA, Staff AC. Changes in circulating level of angiogenic factors from the first to second trimester as predictors of preeclampsia. Am J Obstet Gynecol. 2007; 196:239-6. [PubMed: 17346536]

24. Robinson CJ, Johnson DD, Chang EY, Armstrong DM, Wang W. Evaluation of placenta growth factor and soluble Fms-like tyrosine kinase 1 receptor levels in mild and severe preeclampsia. Am J Obstet Gynecol. 2006; 195:255–259. [PubMed: 16813756]
25. Wikstrom AK, Larsson A, Eriksson UJ, Nash P, Norden-Lindeberg S, Olovsson M. Placental growth factor and soluble FMS-like tyrosine kinase-1 in early-onset and late-onset preeclampsia. Obstet Gynecol. 2007; 109:1368–1374. [PubMed: 17540809]
26. Unal ER, Robinson CJ, Johnson DD, Chang EY. Second-trimester angiogenic factors as biomarkers for future-onset preeclampsia. Am J Obstet Gynecol. 2007; 197:211–214. [PubMed: 17689655]
27. Park CW, Park JS, Shim SS, Jun JK, Yoon BH, Romero R. An elevated maternal plasma, but not amniotic fluid, soluble fms-like tyrosine kinase-1 (sFlt-1) at the time of mid-trimester genetic amniocentesis is a risk factor for preeclampsia. Am J Obstet Gynecol. 2005; 193:984–989. [PubMed: 16157098]
28. Crispi F, Llurba E, Dominguez C, Martin-Gallan P, Cabero L, Gratacos E. Predictive value of angiogenic factors and uterine artery Doppler for early- versus late-onset pre-eclampsia and intrauterine growth restriction. Ultrasound Obstet Gynecol. 2008; 31:303–309. [PubMed: 18058842]
29. Savvidou MD, Noori M, Anderson JM, Hingorani AD, Nicolaides KH. Maternal endothelial function and serum concentrations of placental growth factor and soluble endoglin in women with abnormal placentation. Ultrasound Obstet Gynecol. 2008; 32:871–876. [PubMed: 18814185]
30. Chaiworapongsa T, Espinoza J, Gotsch F, Kim YM, Kim GJ, Goncalves LF, Edwin S, Kusanovic JP, Erez O, Than NG, Hassan SS, Romero R. The maternal plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated in SGA and the magnitude of the increase relates to Doppler abnormalities in the maternal and fetal circulation. J Matern.Fetal Neonatal Med. 2008; 21:25–40. [PubMed: 18175242]
31. Signore C, Mills JL, Qian C, Yu K, Lam C, Epstein FH, Karumanchi SA, Levine RJ. Circulating angiogenic factors and placental abruption. Obstet Gynecol. 2006; 108:338–344. [PubMed: 16880304]
32. Espinoza J, Romero R, Nien JK, Kusanovic JP, Richani K, Gomez R, Kim CJ, Mittal P, Gotsh F, Erez O, Chaiworapongsa T, Hassan S. A role of the anti-angiogenic factor sVEGFR-1 in the 'mirror syndrome' (Ballantyne's syndrome). J Matern.Fetal Neonatal Med. 2006; 19:607–613. [PubMed: 17118734]
33. Kusanovic JP, Romero R, Espinoza J, Nien JK, Kim CJ, Mittal P, Edwin S, Erez O, Gotsch F, Mazaki-Tovi S, Than NG, Soto E, Camacho N, Gomez R, Quintero R, Hassan SS. Twin-to-twin transfusion syndrome: an antiangiogenic state? Am J Obstet Gynecol. 2008; 198:382–388. [PubMed: 18395032]
34. Espinoza J, Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Nien JK, Kusanovic JP, Erez O, Bujold E, Goncalves LF, Gomez R, Edwin S. Unexplained fetal death: another anti-angiogenic state. J Matern.Fetal Neonatal Med. 2007; 20:495–507. [PubMed: 17674262]
35. Ebos JM, Bocci G, Man S, Thorpe PE, Hicklin DJ, Zhou D, Jia X, Kerbel RS. A naturally occurring soluble form of vascular endothelial growth factor receptor 2 detected in mouse and human plasma. Mol.Cancer Res. 2004; 2:315–326. [PubMed: 15235107]
36. Lin P, Sankar S, Shan S, Dewhirst MW, Polverini PJ, Quinn TQ, Peters KG. Inhibition of tumor growth by targeting tumor endothelium using a soluble vascular endothelial growth factor receptor. Cell Growth Differ. 1998; 9:49–58. [PubMed: 9438388]
37. McLeod DS, Taomoto M, Cao J, Zhu Z, Witte L, Lutty GA. Localization of VEGF receptor-2 (KDR/Flk-1) and effects of blocking it in oxygen-induced retinopathy. Invest Ophthalmol.Vis.Sci. 2002; 43:474–482. [PubMed: 11818393]
38. Tas F, Duranyildiz D, Oguz H, Camlica H, Yasasever V, Topuz E. Circulating serum levels of angiogenic factors and vascular endothelial growth factor receptors 1 and 2 in melanoma patients. Melanoma Res. 2006; 16:405–411. [PubMed: 17013089]
39. Cortelezzi A, Fracchiolla NS, Mazzeo LM, Silvestris I, Pomati M, Somalvico F, Bertolini F, Mancuso P, Pruneri GC, Gianelli U, Pasquini MC, Cortiana M, Deliliers GL. Endothelial precursors and mature endothelial cells are increased in the peripheral blood of myelodysplastic syndromes. Leuk.Lymphoma. 2005; 46:1345–1351. [PubMed: 16109613]

40. Aref S, El SM, Goda T, Fouda M, Al AH, Abdalla D. Soluble VEGF/sFLt1 ratio is an independent predictor of AML patient out come. Hematology. 2005; 10:131–134. [PubMed: 16019458]
41. Faderl S, Do KA, Johnson MM, Keating M, O'brien S, Jilani I, Ferrajoli A, Ravandi-Kashani F, Aguilar C, Dey A, Thomas DA, Giles FJ, Kantarjian HM, Albitar M. Angiogenic factors may have a different prognostic role in adult acute lymphoblastic leukemia. Blood. 2005; 106:4303–4307. [PubMed: 16123221]
42. Robak E, Sysa-Jedrzejewska A, Robak T. Vascular endothelial growth factor and its soluble receptors VEGFR-1 and VEGFR-2 in the serum of patients with systemic lupus erythematosus. Mediators.Inflamm. 2003; 12:293–298. [PubMed: 14760936]
43. Srikiatkhachorn A, Ajariyakhajorn C, Endy TP, Kalayanarooj S, Libraty DH, Green S, Ennis FA, Rothman AL. Virus-induced decline in soluble vascular endothelial growth receptor 2 is associated with plasma leakage in dengue hemorrhagic Fever. J Virol. 2007; 81:1592–1600. [PubMed: 17151115]
44. Chaiworapongsa T, Romero R, Gotsch F, Kusanovic JP, Mittal P, Kim SK, Erez O, Vaisbuch E, Mazaki-Tovi S, Kim CJ, Dong Z, Yeo L, Hassan SS. Acute pyelonephritis during pregnancy changes the balance of angiogenic and anti-angiogenic factors in maternal plasma. J Matern.Fetal Neonatal Med. 2009:1–12.
45. Chaiworapongsa T, Romero R, Tarca A, Kusanovic JP, Gotsch F, Mittal P, Erez O, Vaisbuch E, Mazaki-Tovi S, Dong Z, Kim C, Kim S, Yeo L, Hassan S. A decrase in Maternal Plasma Concentrations of sVEGFR-2 precedes the clinical diagnosis of preeclampsia. Reproductive Science. 2009; 16:264 A.
46. Kim SY, Park SY, Kim JW, KIm YM, Yang JH, KIm MY, Ahn HK, Shin JS, Kim JO, Ryu HM. Circulating endothelial progenitor cells, plasma VEGF, VEGFR-1 and VEGFR-2 levels in preeclampsia. Am J Obstet Gynecol. 2005; 193:S74.
47. Wallner W, Sengenberger R, Strick R, Strissel PL, Meurer B, Beckmann MW, Schlembach D. Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. Clin.Sci.(Lond). 2007; 112:51–57. [PubMed: 16928195]
48. Burgos H. Angiogenic and growth factors in human amnio-chorion and placenta. Eur.J Clin.Invest. 1983; 13:289–296. [PubMed: 6193964]
49. Tranquilli AL, Giannubilo SR, Bezzeccheri V, Ciavattini A, Scagnoli C, Mazzanti L. Amniotic levels of nitric oxide and vascular endothelial growth factor in pregnancy with subsequent intrauterine fetal death. Eur.J Obstet Gynecol Reprod.Biol. 2004; 114:162–165. [PubMed: 15140509]
50. Park CW, Park JS, Shim SS, Jun JK, Yoon BH, Romero R. An elevated maternal plasma, but not amniotic fluid, soluble fms-like tyrosine kinase-1 (sFlt-1) at the time of mid-trimester genetic amniocentesis is a risk factor for preeclampsia. Am J Obstet Gynecol. 2005; 193:984–989. [PubMed: 16157098]
51. Vuorela P, Helske S, Hornig C, Alitalo K, Weich H, Halmesmaki E. Amniotic fluid--soluble vascular endothelial growth factor receptor-1 in preeclampsia. Obstet Gynecol. 2000; 95:353–357. [PubMed: 10711543]
52. Staff AC, Braekke K, Harsem NK, Lyberg T, Holthe MR. Circulating concentrations of sFlt1 (soluble fms-like tyrosine kinase 1) in fetal and maternal serum during pre-eclampsia. Eur.J Obstet Gynecol Reprod.Biol. 2005; 122:33–39. [PubMed: 15935542]
53. Daneshmand SS, Cheung CY, Brace RA. Regulation of amniotic fluid volume by intramembranous absorption in sheep: role of passive permeability and vascular endothelial growth factor. Am J Obstet Gynecol. 2003; 188:786–793. [PubMed: 12634658]
54. Astern J, Gowin-rown J, Kendal-wright C. The expression of vascular endothelial growth factor and its receptors in primary human amniotic epithelial celss (AEC). Reproductive Sciences. 2009; 16:116 A.
55. Cheung CY. Vascular endothelial growth factor activation of intramembranous absorption: a critical pathway for amniotic fluid volume regulation. J Soc Gynecol Investig. 2004; 11:63–74.
56. Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, Jun JK. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Obstet Gynecol. 2001; 185:1130–1136. [PubMed: 11717646]

57. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet Gynecol. 1996; 87:163–168. [PubMed: 8559516]
58. Gonzalez RP, Gomez RM, Castro RS, Nien JK, Merino PO, Etchegaray AB, Carstens MR, Medina LH, Viviani PG, Rojas IT. A national birth weight distribution curve according to gestational age in Chile from 1993 to 2000. Rev.Med Chil. 2004; 132:1155–1165. [PubMed: 15631202]
59. Rana S, Rauh-Hain A, Karumanchi A, Thadhani R. First-trimester serum angiogenic factors and the risk of intrauterine fetal demise. Am J Obstet Gynecol. 2007; 197:S12.
60. Roberts JM. Endothelial dysfunction in preeclampsia. Semin.Reprod.Endocrinol. 1998; 16:5–15. [PubMed: 9654603]
61. Johnson MR, Anim-Nyame N, Johnson P, Sooranna SR, Steer PJ. Does endothelial cell activation occur with intrauterine growth restriction? BJOG. 2002; 109:836–839. [PubMed: 12135223]
62. Chaiworapongsa T, Romero R, Yoshimatsu J, Espinoza J, Kim YM, Park K, Kalache K, Edwin S, Bujold E, Gomez R. Soluble adhesion molecule profile in normal pregnancy and pre-eclampsia. J Matern.Fetal Neonatal Med. 2002; 12:19–27. [PubMed: 12422905]
63. Roberts JM, Von Versen-Hoeynck F. Maternal fetal/placental interactions and abnormal pregnancy outcomes. Hypertension. 2007; 49:15–16. [PubMed: 17116760]
64. Czabanka M, Peter C, Martin E, Walther A. Microcirculatory endothelial dysfunction during endotoxemia--insights into pathophysiology, pathologic mechanisms and clinical relevance. Curr.Vasc.Pharmacol. 2007; 5:266–275. [PubMed: 17979793]
65. Quinton AE, Cook CM, Peek MJ. The relationship between cigarette smoking, endothelial function and intrauterine growth restriction in human pregnancy. BJOG. 2008; 115:780–784. [PubMed: 18355365]
66. Schouten M, Wiersinga WJ, Levi M, van der PT. Inflammation, endothelium, and coagulation in sepsis. J Leukoc.Biol. 2008; 83:536–545. [PubMed: 18032692]
67. Kanagalingam MG, Nelson SM, Freeman DJ, Ferrell WR, Cherry L, Lowe GD, Greer IA, Sattar N. Vascular dysfunction and alteration of novel and classic cardiovascular risk factors in mothers of growth restricted offspring. Atherosclerosis. 2009; 205:244–250. [PubMed: 19022445]
68. Swendeman S, Mendelson K, Weskamp G, Horiuchi K, Deutsch U, Scherle P, Hooper A, Rafii S, Blobel CP. VEGF-A stimulates ADAM17-dependent shedding of VEGFR2 and crosstalk between VEGFR2 and ERK signaling. Circ.Res. 2008; 103:916–918. [PubMed: 18818406]
69. Garton KJ, Gough PJ, Raines EW. Emerging roles for ectodomain shedding in the regulation of inflammatory responses. J Leukoc.Biol. 2006; 79:1105–1116. [PubMed: 16565325]
70. Higashiyama S, Nanba D. ADAM-mediated ectodomain shedding of HB-EGF in receptor cross-talk. Biochim.Biophys.Acta. 2005; 1751:110–117. [PubMed: 16054021]
71. Bauvois B. Transmembrane proteases in cell growth and invasion: new contributors to angiogenesis? Oncogene. 2004; 23:317–329. [PubMed: 14724562]
72. Jayasinghe C, Simiantonaki N, Michel-Schmidt R, Kirkpatrick CJ. Hypoxia-induced reduction of sVEGFR-2 levels in human colonic microvascular endothelial cells in vitro: Comparative study with HUVEC. Int.J Mol.Med. 2009; 23:49–55. [PubMed: 19082506]
73. Jayasinghe C, Simiantonaki N, Michel-Schmidt R, Kirkpatrick CJ. Comparative study of human colonic tumor-derived endothelial cells (HCTEC) and normal colonic microvascular endothelial cells (HCMEC): Hypoxia-induced sVEGFR-1 and sVEGFR-2 levels. Oncol.Rep. 2009; 21:933–939. [PubMed: 19287991]
74. Smith GC, Yu CK, Papageorghiou AT, Cacho AM, Nicolaides KH. Maternal uterine artery Doppler flow velocimetry and the risk of stillbirth. Obstet Gynecol. 2007; 109:144–151. [PubMed: 17197600]
75. Gardosi J, Mul T, Mongelli M, Fagan D. Analysis of birthweight and gestational age in antepartum stillbirths. Br.J Obstet Gynaecol. 1998; 105:524–530. [PubMed: 9637122]
76. Khong TY, Liddell HS, Robertson WB. Defective haemochorial placentation as a cause of miscarriage: a preliminary study. Br.J Obstet Gynaecol. 1987; 94:649–655. [PubMed: 3620413]
77. Cheung CY. Vascular endothelial growth factor: possible role in fetal development and placental function. J Soc Gynecol Investig. 1997; 4:169–177.

78. Vuorela-Vepsalainen P, Alfthan H, Orpana A, Alitalo K, Stenman UH, Halmesmaki E. Vascular endothelial growth factor is bound in amniotic fluid and maternal serum. Hum.Reprod. 1999; 14:1346–1351. [PubMed: 10325292]

79. Kendall RL, Wang G, Thomas KA. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. Biochem.Biophys.Res.Commun. 1996; 226:324–328. [PubMed: 8806634]

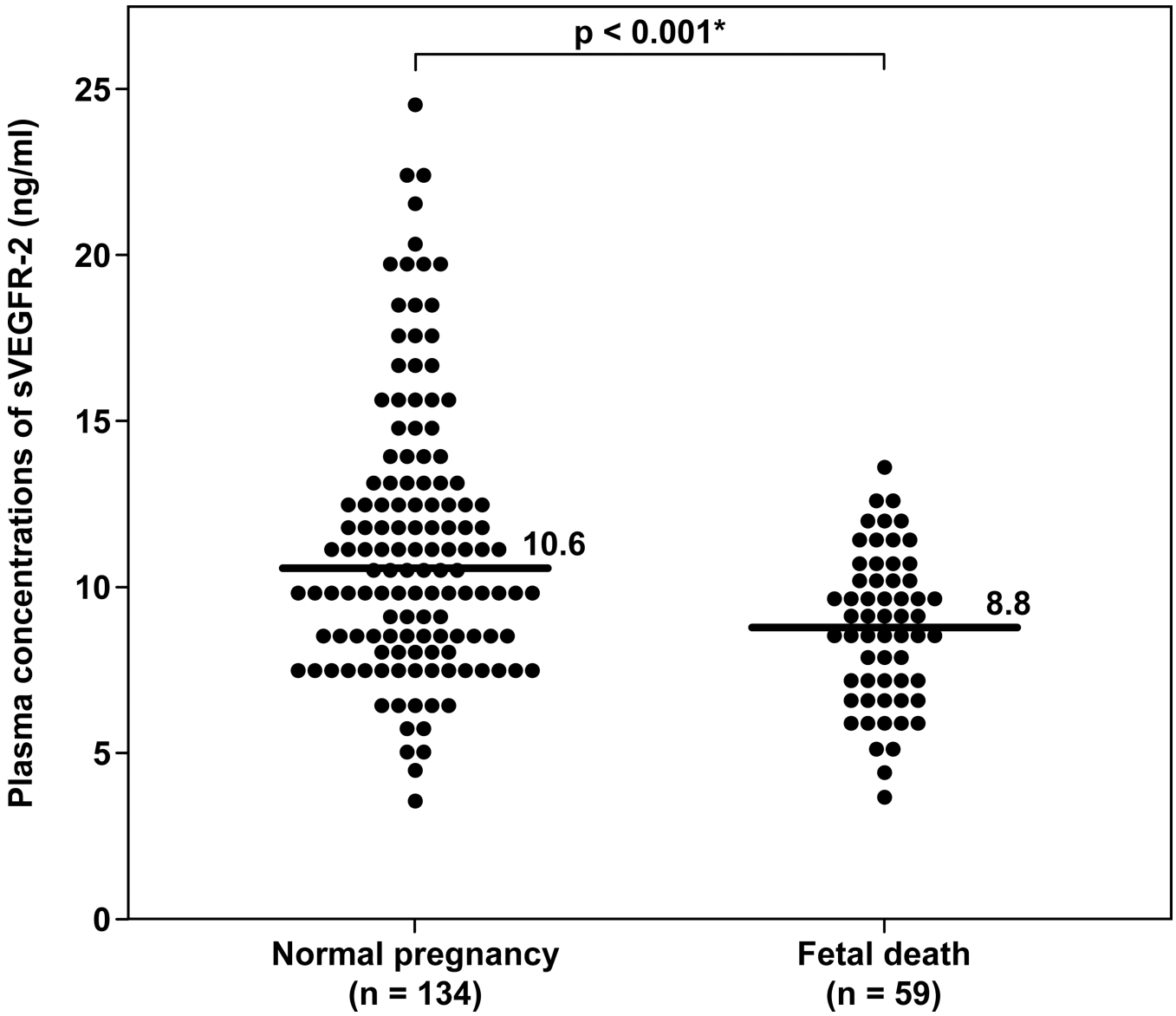

Figure 1.

Plasma concentrations of sVEGFR-2 in normal pregnant women and patients with fetal death. Women with fetal death had a significantly lower median plasma concentration of sVEGFR-2 than normal pregnant women (median 8.8 ng/ml, range 3.4–13.6 ng/ml vs. median 10.6 ng/ml, range 3.4–24.7 ng/ml; respectively; p<0.001).* $p<0.05$

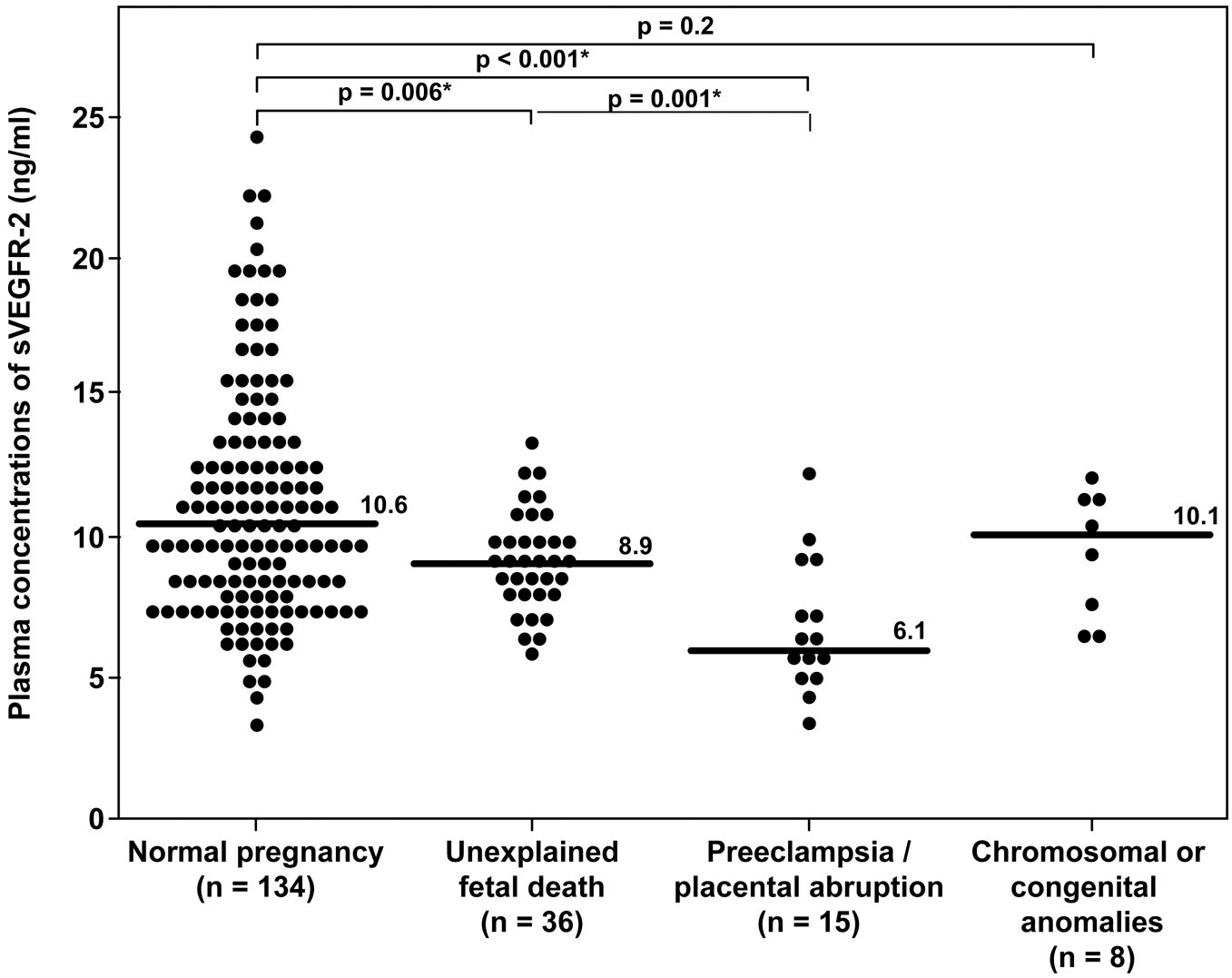

Figure 2.

Plasma concentrations of sVEGFR-2 in normal pregnant women, unexplained fetal death, fetal death with preeclampsia and/or placental abruption, and fetal death with chromosomal or congenital anomalies. The median plasma concentration of sVEGFR-2 in unexplained fetal death was lower than that of the normal pregnancy group (median 8.9 ng/ml, range 5.9–13.6 ng/ml vs. median 10.6 ng/ml, range 3.4–24.7 ng/ml; respectively; p=0.006), but higher than that of fetal death with preeclampsia and/or placental abruption (median 6.1 ng/ml, range 3.4–12.4 ng/ml; p=0.001). There was no significant difference in the median plasma sVEGFR-2 concentration between women with a fetal death with major chromosomal and/or congenital anomalies (median 10.1 ng/ml, range 6.4–12.1 ng/ml) and those with a normal pregnancy (median 10.6 ng/ml, range 3.4–24.7 ng/ml; p=0.2). * $p<0.05$

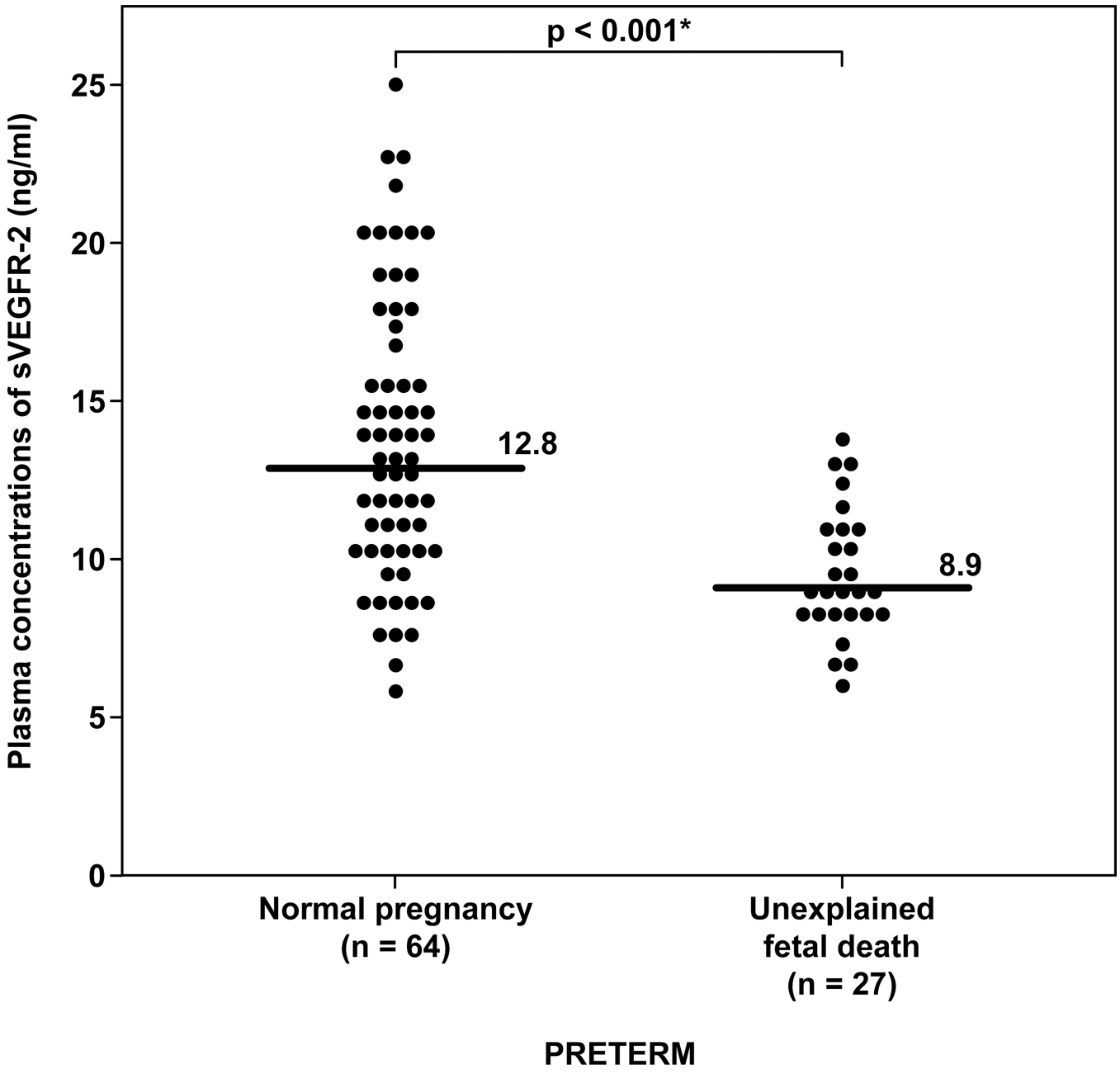

Figure 3.

Plasma concentrations of sVEGFR-2 in normal pregnant women at preterm gestation and unexplained preterm fetal death. Unexplained preterm fetal death had a significantly lower median plasma sVEGFR-2 concentration than normal pregnant women at preterm gestation (median 8.9 ng/ml, range 5.9–13.6 ng/ml vs. median 12.8 ng/ml, range 5.9–24.7 ng/ml; respectively; p<0.001). * $p<0.05$

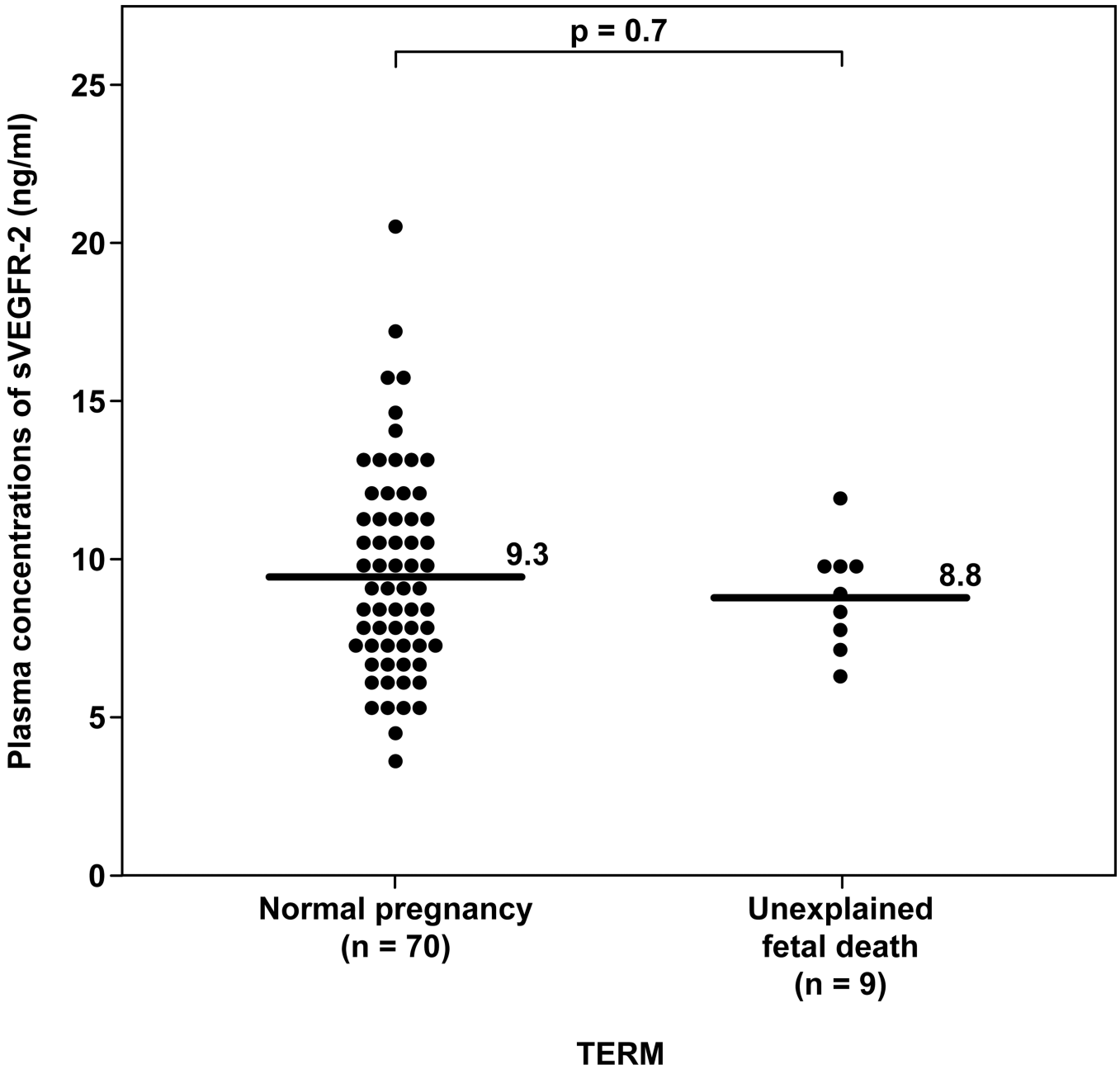

Figure 4.

Plasma concentrations of sVEGFR-2 in normal pregnant women at term gestation and unexplained term fetal death. There was no significant difference in the median plasma concentration of sVEGFR-2 between patients with unexplained fetal death at term and women at term without labor (median 8.8 ng/ml, range 6.5–11.6 ng/ml vs. median 9.3 ng/ml, range 3.4–20.2 ng/ml; respectively; p=0.7).

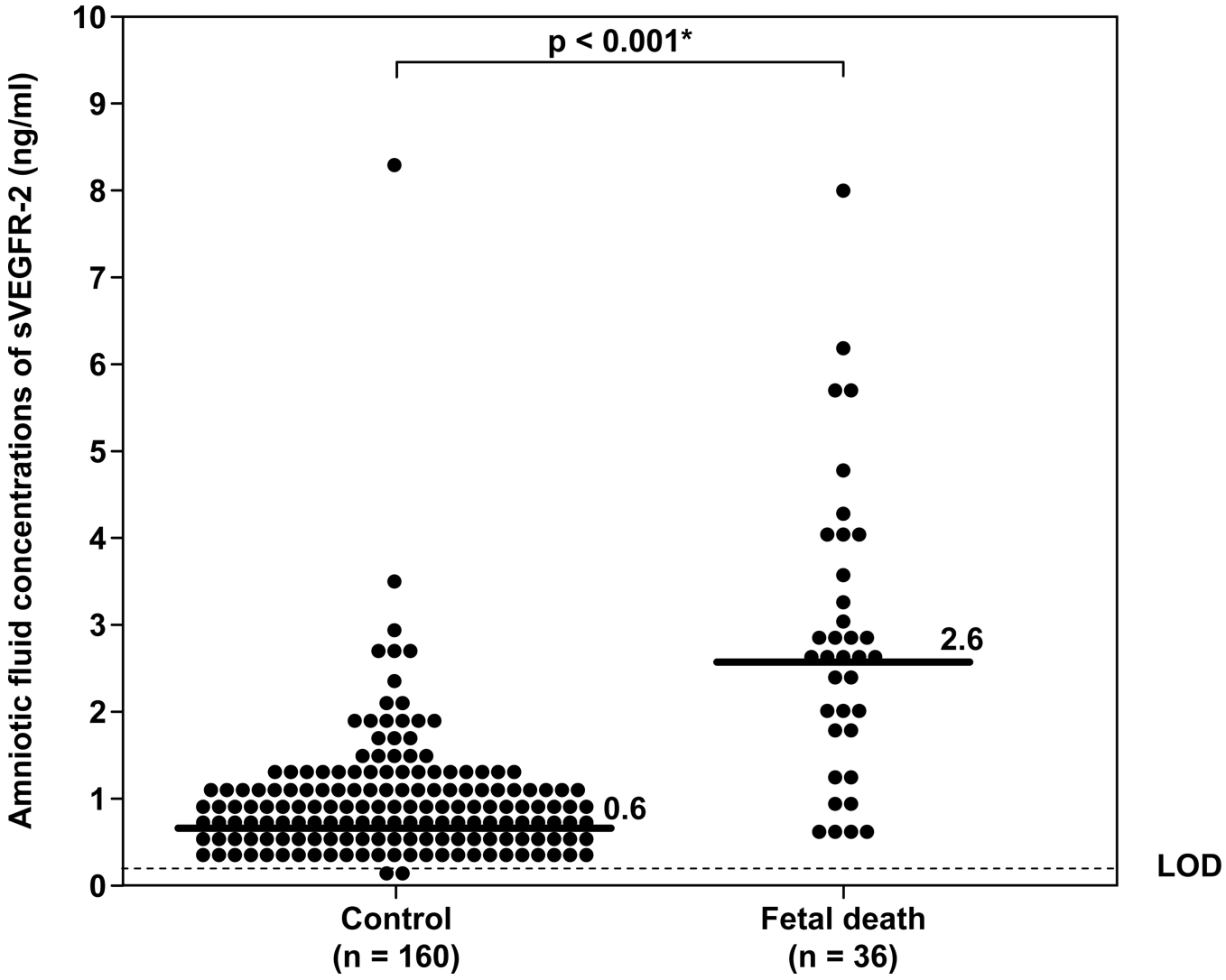

Figure 5.

Amniotic fluid concentrations of sVEGFR-2 in patients with fetal death and women in the control group. The median amniotic fluid concentration of sVEGFR-2 was significantly higher in women with a fetal death than that of women in the control group (median 2.6 ng/ml, range 0.3–8.1 ng/ml vs. median 0.6 ng/ml, range 0–9.3 ng/ml; respectively; p<0.001). Two patients in the control group had amniotic fluid concentrations of sVEGFR-2 below the limit of detection (LOD). * p<0.05

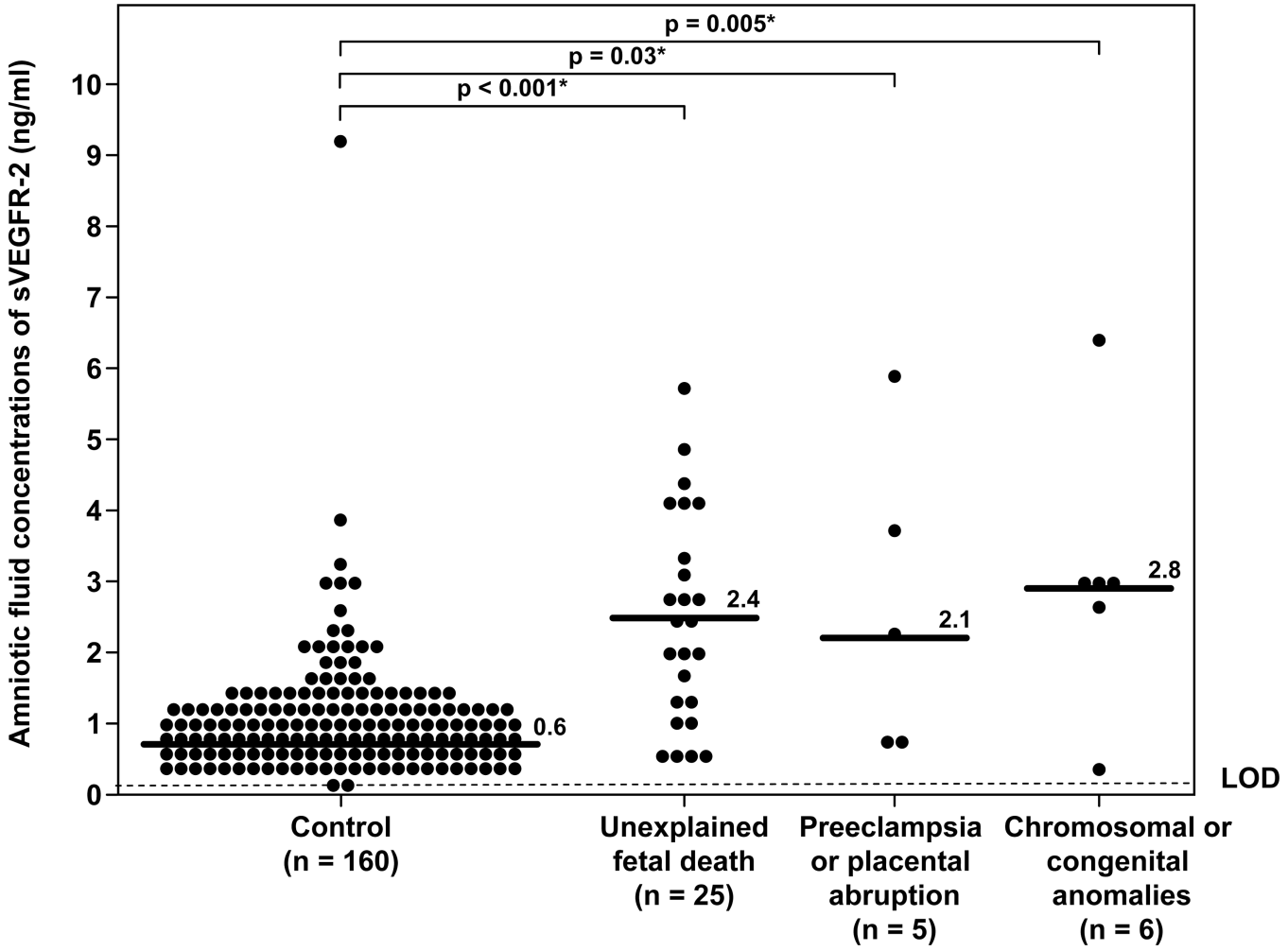

Figure 6.

Amniotic fluid concentrations of sVEGFR-2 in the control group, unexplained fetal death, fetal death with preeclampsia and/or placental abruption, and fetal death with chromosomal or congenital anomalies. Women with unexplained fetal death (median 2.4 ng/ml, range 0.4–8.1 ng/ml), those with fetal death with preeclampsia and/or placental abruption (median 2.1 ng/ml, range 0.5–5.8 ng/ml), and those with fetal death and major chromosomal/congenital anomalies (median 2.8 ng/ml, range 0.3–6.3 ng/ml) had a significantly higher median amniotic fluid concentration of sVEGFR-2 than women in the control group (median 0.6 ng/ml, range 0–9.3 ng/ml; p<0.001, p=0.03 and p=0.005; respectively; see Figure 6). Two patients in the control group had amniotic fluid concentrations of sVEGFR-2 below the limit of detection (LOD). * p<0.05

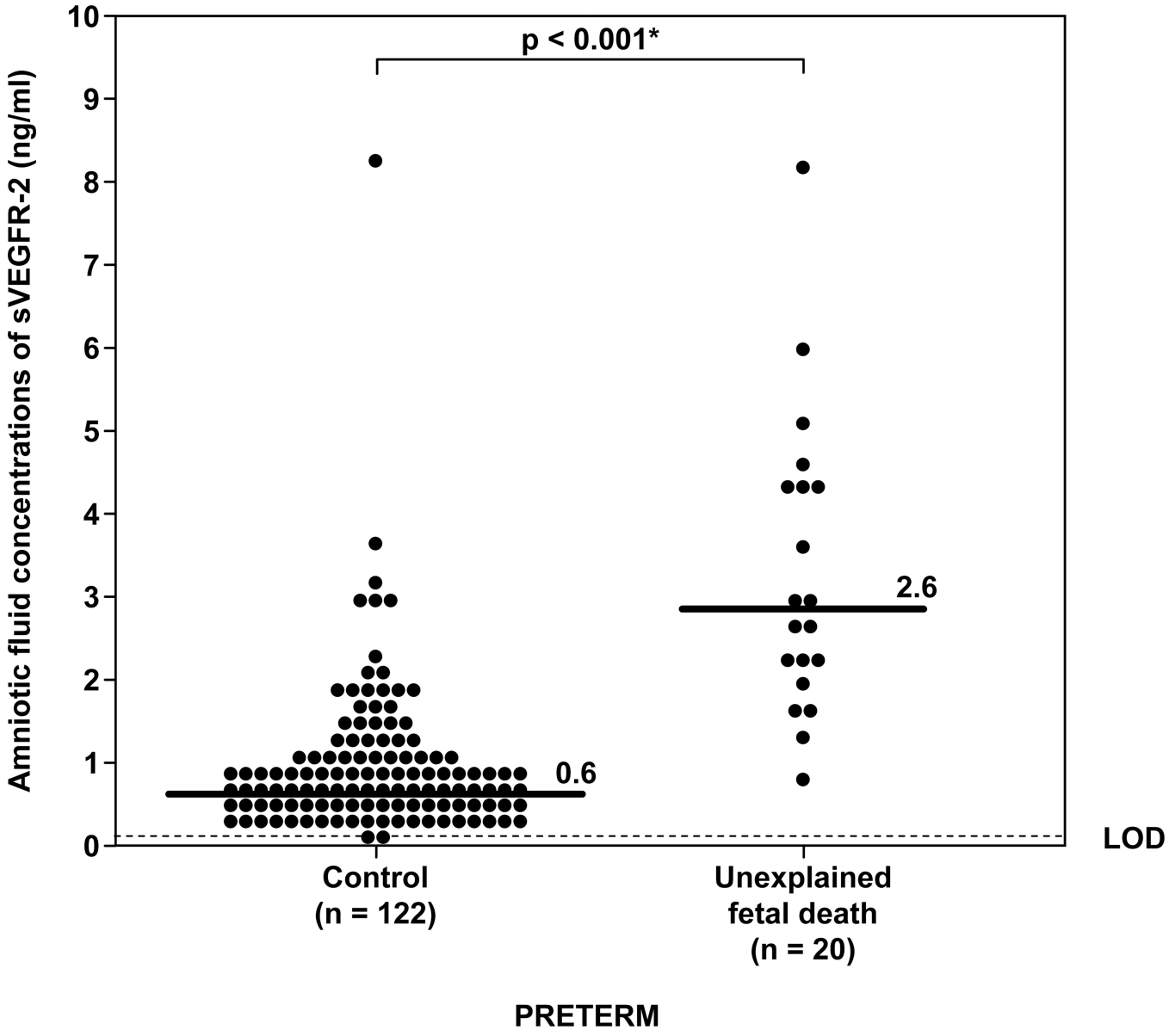

Figure 7.

Amniotic fluid concentrations of sVEGFR-2 of women in the control group and those with unexplained fetal death at preterm gestation. Unexplained preterm fetal death had a significantly higher median amniotic fluid sVEGFR-2 concentration than the control group (median 2.6 ng/ml, range 0.4–8.1 ng/ml vs. median 0.6 ng/ml, range 0–3.7 ng/ml; respectively; p<0.001). Two patients in the control group had amniotic fluid concentrations of sVEGFR-2 below the limit of detection (LOD). * p<0.05

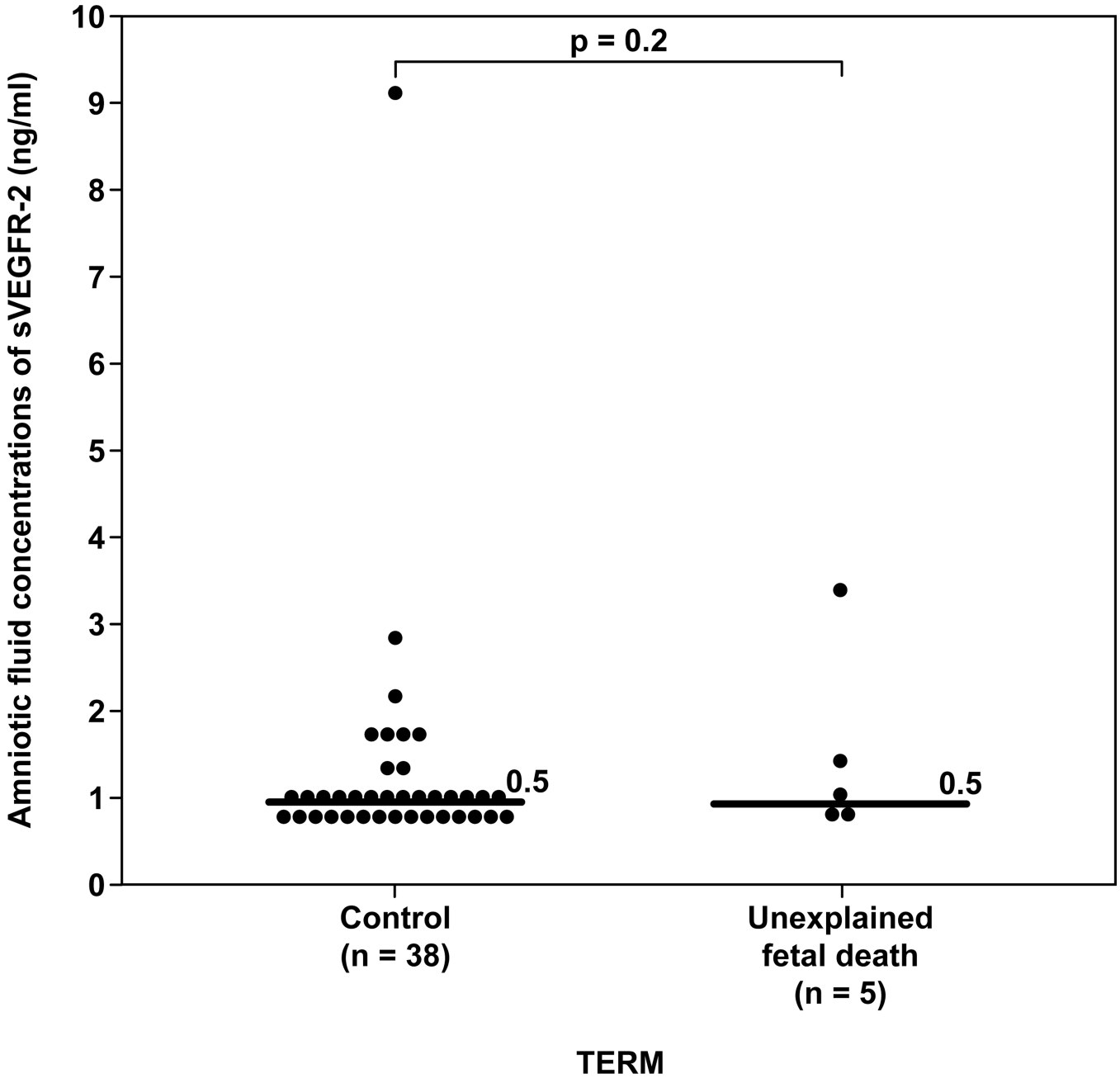

Figure 8.

Amniotic fluid concentrations of sVEGFR-2 in the control group and those with unexplained fetal death at term gestation. There was no significant difference in the median amniotic fluid concentration of sVEGFR2 in patients with unexplained fetal death at term and women at term without labor (median 0.5 ng/ml, range 0.4–3.0 ng/ml vs. median 0.5 ng/ml, range 0.1–9.3 ng/ml; respectively; p=0.2).

Table I

Demographic and clinical characteristics of normal pregnant women and women with fetal death (for plasma evaluation)

	Normal pregnancy n = 134	Fetal death n=59	p
Age (years)	25 (17–40)	25 (17–41)	0.8
Nulliparity	37 (28%)	23 (39%)	0.1
Ethnicity			
African American	106 (79%)	51 (86%)	0.6
Caucasian	15 (11%)	4 (7%)	
Hispanic	7 (5%)	3 (5%)	
Others	6 (5%)	1 (2%)	
Smoking	23 (17%)[a]	18 (31%)	0.04*
GA at blood sampling (weeks)	37.6 (20–41)	31.0 (20.1–40.6)	<0.001*
GA at delivery (weeks)	39.3 (37–42)	31.0 (20.6–40.7)	<0.001*
Birthweight (grams)	3,345 (2,610–4,080)	1,400 (140–5,755)	<0.001*
Birthweight <10th percentile	0	27 (46%)	<0.001*
Birthweight <5th percentile	0	20 (34%)	<0.001*

Value expressed as median (range) or number (percent)
GA: gestational age

[a]: n=133;

*: p<0.05

Table II

Demographic and clinical characteristics of normal pregnant women and subgroups of fetal death (for plasma evaluation)

	Normal pregnancy n = 134	Unexplained fetal death n = 36	p	Fetal death with preeclampsia/ Abruption n=15	p	Fetal death with chromosomal/congenital anomalies n = 8	p
Age (years)	25 (17–40)	25 (18–41)	0.8	23 (17–37)	0.7	27 (17–40)	0.4
Nulliparity	37 (28%)	11 (30.6%)	0.7	7 (46.7%)	0.1	5 (62.5%)	0.04*
Ethnicity							
African American	106 (79%)	33 (91%)	0.2	13 (87%)	0.7	5 (63%)	0.5
Caucasian	15 (11%)	0		2 (13%)		2 (25%)	
Hispanic	7 (5%)	2 (6%)		0		1 (12%)	
Others	6 (5%)	1 (3%)		0		0	
Smoking	23 (17%)^{a}	12 (33%)	0.04*	4 (26.7%)	0.4	2 (25%)	0.6
GA at blood sampling (weeks)	37.6 (20–41)	31.0 (20.1–40.6)	0.002*	31.0 (20.1–40.0)	0.08	23.7 (22.0–34.6)	0.001*
GA at delivery (weeks)	39.3 (37–42)	31.0 (20.6–40.7)	<0.001*	31.1 (20.6–40.0)	<0.001*	25.4 (22.1–34.7)	<0.001*
Birthweight (grams)	3,345 (2,610–4,080)	1,328 (140–5,755)	<0.001*	1,780 (387–3,620)	<0.001*	590 (180–1,980)	<0.001*
Birthweight <10th percentile	0	16 (44.4%)	<0.001*	7 (46.7%)	<0.001*	4 (50%)	<0.001*
Birthweight <5th percentile	0	11 (30.6%)	<0.001*	6 (40%)	<0.001*	3 (37.5%)	<0.001*

All p value compared to normal pregnancy
Value expressed as median (range) or number (percent)
GA: gestational age;

^{a}: n=133;

*: p<0.05

Table III

Odds ratio for the associations between the presence of fetal death and plasma concentrations of sVEGFR-2 (per each ng/ml increase)

Model	Dependent variables (vs. normal pregnancy)	Odds ratio	95% CI
1	Fetal death	0.7	0.6–0.8
2	Unexplained fetal death	0.8	0.6–0.9
3	Fetal death with preeclampsia/abruption	0.5	0.3–0.8
4	Fetal death with chromosomal or congenital anomalies	0.6	0.3–1.3

Adjusting for gestational age at blood sampling (weeks), maternal age (years), African American ethnicity, smoking, nulliparity, duration of sample storage (years) and the presence of SGA

Table IV

Clinical characteristics of the study population (for amniotic fluid evaluation)

	Control n = 160	**Fetal death n=36**	**p**
Age (years)	23 (14–42)	26 (17–41)	0.8
GA at amniocentesis (weeks)	32.9 (20–42)	30.1 (20.3–40.6)	<0.001 *
GA at delivery (weeks)	38.7 (37–43)	30.3 (20.6–40.7)	<0.001 *
Birthweight (grams)	3,255 (2,630–4,750)	1,328 (140–5,755)	<0.001 *
Birthweight <10th percentile	0	18 (50%)	<0.001 *

Value expressed as median (range) or number (percent)
GA: gestational age

*: $p<0.05$

Table V

Odds ratio for the associations between the presence of fetal death and amniotic fluid concentrations of sVEGFR-2 (per each ng/ml increase)

Model	Dependent variables (vs. control)	Odds ratio	95% CI
1	Fetal death	5.5	2.8–10.9
2	Unexplained fetal death	6.7	3.1–14.9
3	Fetal death with preeclampsia/abruption	4.9	1.7–14.1
4	Fetal death with chromosomal or congenital anomalies	13.9	1.5–132.2

Adjusting for gestational age at amniocentesis (weeks), smoking, nulliparity and duration of sample storage (years)

Table VI

Demographic and clinical characteristics of women with PTL who delivered at term, women at term no labor and those with unexplained fetal death at preterm and term gestation (for amniotic fluid evaluation)

	PTL who delivered at term n = 122	Preterm unexplained fetal death n=20	p	Women at term without labor n = 38	unexplained fetal death at term n=5	p$^{\beta}$
Age (years)	22 (14–42)	27 (18–41)	0.8	27 (14–40)	24 (18–30)	0.6
GA at amniocentesis (weeks)	32.0 (20.3–36)	29.4 (20.4–36.9)	<0.001 *	38.8 (37.0–42.0)	39.4 (37.6–40.6)	0.3
GA at delivery (weeks)	39.4 (37.6–40.7)	29.4 (20.6–36.7)	<0.001 *	38.8 (37.0–42.0)	39.4 (37.6–40.7)	0.3
Birthweight (grams)	3,250 (2,630–4,750)	992 (140–2,370)	<0.001 *	3,260 (2,840–4,530)	3,487 (2,450–5,755)	0.8
Birthweight <10th percentile	0	11 (55%)	<0.001 *	0	1 (20%)	0.1

Value expressed as median (range) or number (percent)
GA: gestational age; PTL: preterm labor with intact membranes

β: compared between women at term no labor and unexplained fetal death at term

*: p<0.05

J Matern Fetal Neonatal Med. Author manuscript; available in PMC 2012 August 26.