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Published on: 01 Jan 2007 - Clinical Science (Portland Press)

Topics: Umbilical artery, Umbilical vein, Placenta, Fetus and Intrauterine growth restriction

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Clinical Science Immediate Publication. Published on 24 Aug 2006 as manuscript CS20060161

Angiogenic Growth Factors in Maternal and Fetal Serum in Pregnancies Complicated by Intrauterine Growth Restriction

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Supported by: Wilhelm Sander-Stiftung, Munich, Germany (No. 2000.030.001)

Key Words: Angiogenesis, vascular endothelial growth factor family, intrauterine growth restriction, maternal and umbilical serum, pregnancy, vascular endothelial growth factor, placental growth factor, sFlt-1, sKDR, fibroblast growth factor.

Short title: Maternal and fetal angiogenic factors in IUGR

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ABSTRACT:

This study was performed to compare serum concentrations of maternal and fetal angiogenic growth factors in intrauterine growth restriction (IUGR) and normal pregnancy at time of delivery. Vascular endothelial growth factor (VEGF), placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1), soluble kinase domain receptor (sKDR), and basic fibroblast growth factor (bFGF) were measured by means of ELISA in maternal peripheral vein, umbilical vein (UV) and umbilical artery (UA) serum in 15 women with IUGR and 16 controls.

In IUGR sFlt-1 was increased and PlGF and sKDR decreased both in maternal and UV serum. Additionally bFGF was increased in UV serum in IUGR patients. No significant differences in growth factor concentrations between the groups could be found in UA serum. In both groups levels of VEGF were higher and levels of sFlt-1 lower in UV and UA compared to maternal serum. PlGF levels were found to be lower in UV serum compared to maternal blood in both groups, whereas UA PlGF levels were significantly lower in control patients only.

These findings suggest an imbalance of angiogenic and anti-angiogenic factors in IUGR, with formation of an anti-angiogenic state in maternal, and to a lesser extent, umbilical venous blood. The placenta appears to play a central role through the release of sFlt-1 into maternal and umbilical blood. Umbilical arterial blood was unaffected in IUGR, indicating that the fetus does not contribute to changes in angiogenic growth factor concentrations.

INTRODUCTION:

Intrauterine growth restriction (IUGR) is considered a severe complication in pregnancy leading to increased perinatal mortality as well as morbidity [1]. Among the various causes contributing to the development of IUGR, inappropriate placentation is a primary one. Placental development can be divided into vasculogenesis in which an initial vascular network is formed. This network is then remodelled by a process referred to as angiogenesis. Additional blood vessels are generated by sprouting, branching and differential growth of the initial vessels to form a more mature system with larger and smaller vessels [2].

As angiogenesis and vascular transformation are important to normal placental development, IUGR is thought to result from impaired trophoblast invasion of the maternal spiral arteries in early pregnancy, leading to reduced uteroplacental perfusion and placental hypoxia [3]. It has been hypothesized that placental hypoxia may stimulate the release of factors by the placenta which cause widespread maternal endothelial cell damage and derangements of placental angiogenesis [4].

What causes the deficient trophoblast invasion remains unknown. There are strong indications that angiogenic growth factors related to VEGF may be implicated. From bFGF and VEGF, the study of angiogenesis has expanded to include many additional agonists, receptors and inhibitors, especially PlGF and its soluble receptor sFlt-1 [2, 5-7].

Up to now studies have focused predominantly on single angiogenic growth factors in the maternal compartment [8-15]. The purpose of this study was to investigate concentrations of angiogenic growth factors related to VEGF in maternal, placental, and fetal compartments and to compare levels in normal pregnancies and pregnancies complicated by IUGR. We hypothesized, that maternal and fetal levels of angiogenic growth factors are altered in pregnancies complicated by isolated IUGR.

METHODS:

Patients were recruited from the perinatal and labour ward of the University of Erlangen-Nuremberg. Women in whom IUGR was suspected by means of ultrasound examinations (abdominal circumference below the 5th centile) were asked to participate in the study. Postnatal IUGR was defined as birth weight below the 10th centile corrected for gestational age [16]. We finally included into the study 15 women with idiopathic IUGR with no signs of pre-existing or pregnancy induced hypertension or preeclampsia (according to the ISSHP criteria [17]) with postnatal confirmed IUGR. No other risk factors associated with IUGR were obvious. Fetuses with chromosomal or structural anomalies were excluded from the study. For controls we selected 16 healthy, normotensive pregnant women with singleton pregnancies. To avoid a possible hypoxic effect of labour on angiogenic growth factors we only selected women ultimately delivered by caesarean section without labour, women who went into labour or were successfully delivered vaginally were excluded from the final analysis. Indications for caesarean delivery were IUGR and worsening of Doppler parameters or cardiotocography recordings in the IUGR group and in the control group prior caesarean section or breech presentation. The study was approved by the ethical committee at the University of Erlangen-Nuremberg and informed written consent was obtained from all study subjects.

Maternal venous blood samples were collected from the antecubital vein prior to caesarean section. Fetal blood samples were taken from the umbilical artery and vein separately immediately after delivery of the placenta. All blood samples were centrifuged immediately after collection at 3000 rpm for 15 minutes and serum aliquoted and stored at –80° C until measurement (maximal length of storage 21 months).

Serum basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), placenta growth factor (PlGF), and soluble VEGF receptor 1 (sFlt-1) levels were measured in all three compartments [maternal, placental (umbilical vein), and fetal (umbilical artery)]. Soluble VEGF receptor 2 (sKDR) was measured only in maternal and umbilical vein serum. All factors were measured by means of quantitative sandwich immunoassay techniques. Instructions were followed as provided by the manufacturer (R&D Systems GmbH, Wiesbaden, Germany). According to the manufacturer mean minimal detectable doses are: 9 pg/ml for VEGF, 7 pg/ml for PlGF, 5.01 pg/ml for sFlt-1, 4.6 pg/ml for sKDR, and 0.22 pg/ml for bFGF. The ratio of sFlt-1/PlGF was computed using the formula introduced by Buhimschi et al. (log [sFlt-1 / PlGF x 10]) [18].

In all women proteinuria was estimated by dipstick analysis and none of the women finally included had a significant proteinuria. Blood pressure was measured multiple times throughout the pregnancy until caesarean section. For estimation of diastolic blood pressure Korotkoff V was used according to the ISSHP guidelines. Mean arterial pressure was calculated as follows: mean systolic blood pressure (mm Hg) + 2 × mean diastolic blood pressure (mm Hg)] / 3.

Pulsed Doppler ultrasonic recordings from the umbilical artery were made with a 60-Hz Filter, and absent or abnormal end-diastolic blood flow velocity was documented from a minimum of five

consecutive waveforms. Abnormal end-diastolic blood flow was defined as pulsatility index (systolic-diastolic / mean) > 95th centile. Uterine arteries were examined similarly. The mean of both uterine arteries was calculated and used for statistical analysis. During the sonographic examination the amniotic fluid was subjectively diagnosed as normal or decreased (oligohydramnios). In women with IUGR Doppler ultrasound was performed daily and in control women at the day of admission for delivery.

All analyses were conducted using the statistical software program GraphPad PRISM 4.03 for Windows (GraphPad Software, San Diego, California, USA). Test results are expressed as mean ± standard deviation. Data were tested for normal distribution. Demographic and clinical data were compared by means of independent-samples T-Test or Fisher's Exact Test if appropriate. Fetal weight and placental centiles were compared by means of Mann Whitney test. Comparison of angiogenic growth factors between both groups were done by means of independent-samples T-Test or Mann Whitney test if appropriate. Comparison of angiogenic growth factors between the compartments (maternal, umbilical vein and umbilical artery serum) were performed using one-way ANOVA followed by Bonferroni's Multiple Comparison Test, or Kruskal-Wallis Test followed by Dunn's Multiple Comparison Test if appropriate. A two-sided P value of < 0.05 was considered statistically significant.

RESULTS

Patient characteristics:

The characteristics of the study patients are shown in table I. There were no significant differences between the groups in maternal age, smoking, proteinuria, and blood pressure. Due to the study design - all women had to be delivered by caesarean section without any sign of labour, therefore more women scheduled for repeated caesarean section were included in the control group - the proportion of primigravid and women was nonsignificantly lower in the control group. Additionally the number of primiparous women was higher in the IUGR group, but did not reach statistical significance. Maternal body mass index was lower in the growth restricted group ($P = .027$) as were gestational age at delivery, fetal birth weight centile, and placental weight ($P < .0001$). No difference could be found in the fetal placental ratio (data not shown). Pulsatility indices (PI) in umbilical artery ($P < .0001$) and uterine arteries (mean of both uterine arteries, $P = .0056$) were significantly higher in women with IUGR compared to the control group.

Angiogenic growth factors:

Levels of angiogenic growth factors are shown in table II. In both the women with and without IUGR maternal VEGF serum levels were below the detection limit of the assay used. No differences between groups could be observed in maternal serum bFGF concentrations. In women with IUGR levels of PlGF ($P = .0017$) and sKDR ($P = .0071$) were significantly decreased in maternal serum whereas sFlt-1 levels were significantly increased ($P = .0086$). Similar differences could be observed for umbilical vein serum levels of PlGF ($P = .0016$), sKDR ($P = .0003$), and sFlt-1 ($P = .0183$), but there were no significant differences in umbilical artery blood. Since sFlt-1 is thought to act by binding free PlGF and VEGF was below detectable limits, we computed a ratio of sFlt-1/PlGF (log [sFlt-1 / PlGF x 10]). In IUGR this ratio was significantly increased ($P < .0001$) in maternal and umbilical vein serum (figure 1). Umbilical venous bFGF was significantly higher in IUGR ($P = .0096$) than controls.

Comparison of angiogenic growth factors between the compartments is shown in table III. In the *control group* levels of VEGF were significantly higher ($P = .001$) and sFlt-1 and PlGF significantly lower ($P = .001$) both in umbilical artery and vein, as compared to maternal blood. BFGF was found to be elevated in umbilical arterial blood as compared to umbilical venous and maternal blood ($P = .001$). There were no differences in sKDR levels between maternal and umbilical vein blood.

Within the *growth restricted group* levels of VEGF were significantly higher in umbilical artery and vein as compared to maternal serum ($P = .001$). PlGF was lower in umbilical venous blood than in maternal serum ($P = .001$). SFlt-1 was significantly lower ($P = .001$) in fetal than in maternal blood. There were no differences in sKDR and bFGF levels.

Comment:

Angiogenic growth factors play a crucial role in the normal development of the placenta. The activity of these molecules must be carefully orchestrated in order to form a functioning vascular network. We found that angiogenic growth factors of the VEGF family are altered in maternal and fetal serum from IUGR pregnancies. Furthermore we observed significant correlations between the growth factor levels and various clinical parameters indicating the severity of IUGR.

Due to the study design we could not include normotensive women at earlier gestational ages similar to those of the women with IUGR. The difference in gestational age between the groups may affect the levels of angiogenic factors observed. Nevertheless, according to the published data [10, 19-21] levels in control women could be expected to be even higher (PlGF, sKDR) or lower (sFlt-1) and thus differences even greater at gestational ages corresponding to those of women with IUGR pregnancies. Only differences in the bFGF levels might have been missed, because levels in IUGR pregnancies could actually be significantly lower compared to matched gestational age samples.

Maternal VEGF levels were below the limit of detection, which could be due to the assay used for it determines only free VEGF levels and does not reflect VEGF bound to sFlt-1 [22]. Maternal free VEGF levels were significantly lower than fetal levels, as reported by Vuorela-Vepsäläinen et al. [23].

Maternal PlGF concentrations were significantly decreased in IUGR, which has been reported by several groups [10, 12-14, 24]. Moreover our data on PlGF levels in healthy pregnant women agree with previously published levels [19]. As PlGF originates mostly from the placenta, the reduced placental size observed in IUGR can in part explain the diminished PlGF levels in our IUGR group, but as PlGF is bound by sFlt-1, the increased sFlt-1 levels found in IUGR patients compared to the control group may be mainly responsible for the diminished PlGF levels.

Significantly increased sFlt-1 levels and simultaneously reduced PlGF levels in the blood of women who deliver a growth restricted infant confirm the current hypothesis that high levels of sFlt-1 bind free PlGF, causing decreased PlGF levels. Using a sFlt-1/PlGF ratio, we found significantly higher values of sFlt-1/PlGF in IUGR pregnancies. Our results do confirm the finding of Stepan et al. [25], who also observed elevated levels of sFlt-1 levels in pregnant women with isolated IUGR. Interestingly Shibata et al. [12] as well as Wathen et al. [26] couldn't find elevated levels in normotensive women with small for gestational age babies. This controversy may be explained by the different populations studied: The mean gestational age of the SGA group was 39 weeks of pregnancy [12] or 38 weeks, respectively [26]. In our study the mean gestational age is 33 completed weeks, therefore indicating a more severe form of IUGR. Additionally Shibata defines SGA as a birth weight below the 10^{th} centile and no other information such as Doppler parameters indicating the severity of the disease are given. Wathen et al. [26] defines IUGR as a birth weight below a certain percentile (two SD values below the national average at the particular gestational age). This definition is often mixed with the term small for gestational-age (SGA) and includes not only fetuses with IUGR due to placental insufficiency, but also fetuses with a lower genetic growth potential, but which are

otherwise healthy without malnutrition or hypoxia. Although we cannot rule out that the women with IUGR in our study would have developed pregnancy induced hypertension or preeclampsia with ongoing pregnancy, we couldn't find any signs of hypertension or preeclampsia throughout pregnancy and in the postpartal period.

The significant reduction of maternal sKDR levels in women with IUGR could be explained by the fact that sKDR is derived mostly from endothelial cells [27, 28] which will be reduced in volume in the presence of shallow vasculogenesis and angiogenesis. As we could show similar maternal sKDR level alterations in preeclamptic pregnancies [29], the reduction of sKDR through reduction of endothelial cells becomes more apparent.

Unlike Hohlagschwandtner et al., who found increased levels of bFGF in hypertensive disorders in pregnancy [9], no differences in maternal bFGF levels in our IUGR study group were observed, possibly indirectly confirming the diagnosis of *isolated* IUGR.

In contrast to maternal levels, data on fetal angiogenic growth factors are sparse, and no information is available on the complete family of VEGF-related growth factors in normal pregnancy and IUGR. To our knowledge this is the first study to describe fetal serum levels of sFlt-1 and sKDR in pregnancies complicated by isolated IUGR.

PlGF umbilical vein levels were significantly reduced in IUGR. As with maternal serum, reduced PlGF could be explained by the diminished placental mass in IUGR as well as by increased sFlt-1 which binds PlGF, causing it not to be detected by the assay. The parallel increases of maternal and umbilical vein sFlt-1 and decreases in PlGF and sKDR points to a placental origin of these angiogenic factor alterations. As there was no significant change of angiogenic growth factor levels in umbilical arteries a fetal origin of the angiogenic imbalance in IUGR is unlikely.

Our observation of increased bFGF umbilical vein levels in IUGR differs from that of Hill et al. who reported a trend towards decreased bFGF levels in IUGR [21]. Since Hill et al. included patients with fetal chromosomal abnormalities, altered bFGF levels might be due to these pathologies. We hypothesize, that the increase of umbilical venous bFGF in IUGR could be caused by secretion via a non-classical pathway such as cell death [30], which appears to occur under hypoxic conditions such as IUGR [31].

In controls fetal PlGF and sFlt-1 levels from both umbilical artery and vein were significantly reduced and levels of VEGF significantly increased compared to the maternal compartment. Although in IUGR lower levels were observed, the difference for umbilical artery PlGF was not statistically significant. SKDR levels did not differ significantly between maternal and fetal compartments, nor were there differences in the sFlt-1/PlGF ratio. Flt-1 and PlGF are produced mainly in the syncytial trophoblast, whereas VEGF is produced mainly in decidual and extravillous trophoblast cells [32, 33]. PlGF and sFlt-1 should be secreted predominantly into the maternal circulation, whereas VEGF could be secreted mainly into the fetal circulation. Moreover, the substantially higher level of sFlt-1 in the

maternal compartment would remove free VEGF from the blood, causing levels of free VEGF to be extremely low.

Of note, in the control group umbilical arterial blood had greater concentrations of bFGF than maternal or umbilical venous blood. In IUGR however no significant differences were observed although umbilical arterial blood concentrations appeared to be greater than those in maternal blood.

We speculate that the fetal reaction on the angiogenic imbalances in IUGR is preferable perfusion of central organs in order to establish sufficient bFGF supply, necessary for neuronal, lung and blood vessel development [34]. Beyond a critical threshold level, bFGF could induce atherogenesis, as increased aortic wall thickness in growth restricted newborns suggests [35].

In summary, our results underline the potential of the placenta to release these angiogenic factors in maternal as well as fetal circulation supporting a placental origin of the angiogenic imbalance in IUGR. Overproduced sFlt-1 binds to VEGF and PlGF, diminishing the levels of these factors. The fetus does not seem to metabolize these factors in significant amounts or contribute significantly to the pathophysiology of the disorder.

Acknowledgements

We like to thank Mrs. Elisabeth Stiegler for the extensive laboratory work, and Dr. Richard Levine for kindly pre-reviewing this paper.

This project has been funded by the Wilhelm Sander-Stiftung, Munich, Germany (No. 2000.030.001)

References

1. Kiely, J.L., Brett, K.M., Yu, S., and Rowley, D.L. (2005) Low birth weight and intrauterine growth retardation. In: From Data to Action: CDC's Public health Surveillance for Women, Infants, and Children (Wilcox, L.S., Marks, J.S., eds.), pp. 185-202, U.S. Department for Health and Human Services, Public Health Service, Center for Disease Control and Prevention
2. Risau, W. (1997) Mechanisms of angiogenesis. Nature **386**, 671-674
3. Kingdom, J. (1998) Adriana and Luisa Castelluci Award Lecture 1997. Placental pathology in obstetrics: adaptation of failure of the villous tree? Placenta **19**, 347-351
4. Kaufmann, P., Black, S., and Huppertz, B. (2003) Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod **69**, 1-7
5. Carmeliet, P., Moons, L., Luttun, A., et al. (2001) Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med **7**, 575-583
6. Ong, S., Lash, G., and Baker, P.N. (2000) Angiogenesis and placental growth in normal and compromised pregnancies. Baillieres Best Pract Res Clin Obstet Gynaecol **14**, 969-980
7. Peters, K.G. (1998) Vascular endothelial growth factor and the angiopoietins: working together to build a better blood vessel. Circ Res **83**, 342-343
8. Helske, S., Vuorela, P., Carpen, O., Hornig, C., Weich, H., and Halmesmaki, E. (2001) Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies. Mol Hum Reprod **7**, 205-210
9. Hohlagschwandtner, M., Knofler, M., Ploner, M., Zeisler, H., Joura, E.A., and Husslein, P. (2002) Basic fibroblast growth factor and hypertensive disorders in pregnancy. Hypertens Pregnancy **21**, 235-241
10. Levine, R.J., Maynard, S.E., Qian, C., et al. (2004) Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med **350**, 672-683
11. Lyall, F., Young, A., Boswell, F., Kingdom, J.C., and Greer, I.A. (1997) Placental expression of vascular endothelial growth factor in placentae from pregnancies complicated by pre-eclampsia and intrauterine growth restriction does not support placental hypoxia at delivery. Placenta 18, 269-276
12. Shibata, E., Rajakumar, A., Powers, R.W., et al. (2005) Soluble fms-like tyrosine kinase 1 is increased in preeclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: relationship to circulating placental growth factor. J Clin Endocrinol Metab **90**; 4895-4903
13. Taylor, R.N., Grimwood, J., Taylor, R.S., McMaster, M.T., Fisher, S.J., and North, R.A. (2003) Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. Am J Obstet Gynecol **188**, 177-182

14. Torry, D.S., Wang, H.S., Wang, T.H., Caudle, M.R., and Torry, R.J. (1998) Preeclampsia is associated with reduced serum levels of placenta growth factor. Am J Obstet Gynecol **179**, 1539-1544
15. Tsatsaris, V., Goffin, F., Munaut, C., et al. (2003) Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. J Clin Endocrinol Metab **88**, 5555-5563
16. Gardosi J., Mongelli M., Wilcox M., and Chang A. (1995) An adjustable fetale weight standard. Ultrasound Obstet Gynecol **6**, 168-174 (software program www.gestation.net)
17. Roberts, J.M., Pearson, G., Cutler, J., and Lindheimer, M.D. (2003) Summary of the NHLBI Working Group on Research on Hypertension During Pregnancy. Hypertens Pregnancy **22**, 109-127
18. Buhimschi, C.S., Norwitz, E.R., Funai, E., et al. (2005) Urinary angiogenic factors cluster hypertensive disorders and identify women with severe preeclampsia. Am J Obstet Gynecol **192**, 734-741
19. R&D Systems. (2002) In Scope: Angiogenesis research reagents. February 2002, p 4
20. Molskness, T.A., Stouffer, R.L, Burry, K.A., Gorrill, M.J., Lee, D.M., and Patton, P.E. (2004) Circulating levels of free and total vascular endothelial growth factor (VEGF)-A, soluble VEGF receptors-1 and –2, and angiogenin during ovarian stimulation in non-human primates and women. Human Reproduction **19**, 822-830
21. Hill, D.J., Tevaarwerk, G.J., Arany, E., et al. (1995) Fibroblast growth factor-2 (FGF-2) is present in maternal and cord serum, and in the mother is associated with a binding protein immunologically related to the FGF receptor-1. J Clin Endocrinol Metab **80**, 1822-1831
22. Maynard, S.E., Min, J.Y., Merchan, J., et al. (2003) Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension and proteinuria in preeclampsia. J Clin Invest **111**, 649-658
23. Vuorela-Vepsalainen, P., Alfthan, H., Orpana, A., Alitalo, K., Stenman, U.H., and Halmesmaki, E. (1999) Vascular endothelial growth factor is bound in amniotic fluid and maternal serum. Hum Reprod **14**, 1346-1351
24. Bersinger, N.A., and Odegard, R.A. (2004) Second- and third-trimester serum levels of placental proteins in preeclampsia and small-for-gestational age pregnancies. Acta Obstet Gynecol Scand **83**, 37-45
25. Stepan, H., Geide, A., and Faber, R. (2004) Soluble fms-like tyrosine kinase 1. N Engl J Med **351**, 2241-2
26. Wathen, K.A., Tuutti, E., Stenman, U.H., et al. (2006) Maternal serum-soluble vascular endothelial growth factor receptor-1 in early pregnancy ending in preeclampsia or intrauterine growth retardation. J Clin Endocrinol Metab **91**, 180-4

27. Barleon, B., Hauser, S., Schöllmann, C., Weindel, K., Marme, D., Yayon, A., and Weich, H.A. (1994) Differential expression of the two VEGF receptors Flt and KDR in placenta and vascular endothelial cells. J Cell Biochem **54**, 56-66
28. Barleon, B., Siemeister, G., Martiny-Baron, G., Weindel, K., Herzog, G., and Marme, D. (1997) Vascular endothelial growth factor up-regulates its receptor fms-like tyrosine kinase 1 (FLT-1) and a soluble variant of FLT-1 in human vascular endothelial cells. Cancer Res **57**, 5421-5425
29. Schlembach D., and Beinder E. (2003) Angiogenic growth factors in preeclampsia. J Soc Gynecol Invest **10** (Suppl), 316A
30. McNeil, P.L., Muthukrishan, L., Warder, E., and D'Amore, P.A. (1989) Growth factors are released by mechanically wounded endothelial cells. J Cell Biol **109**, 811-822
31. Ferrazzi, E., Bulfamante, G., Mezzopane, R., Barbera, A., Ghidini, A., and Pardi, G. (1999) Uterine Doppler velocimetry and placental hypoxic-ischemic lesion in pregnancies with fetal intrauterine growth restriction. Placenta **20**, 389-394
32. Clark, D.E., Smith, S.K., Licence, D., Evans, A.L., and Charnock-Jones, D.S. (1998) Comparison of expression patterns for placenta growth factor, vascular endothelial growth factor (VEGF), VEGF-B and VEGF-C in the human placenta throughout gestation. J Endocrinol **159**, 459-467
33. Wulff, C., Wilson, H., Dickson, S.E., Wiegand, S.J., and Fraser, H.M. (2002) Hemochorial placentation in the primate: Expression of vascular endothelial growth factor, angiopoietins, and their receptors throughout pregnancy. Biol Reprod **66**, 802-182
34. Bikfalvi, A., Klein, S., Pintucci, G., and Rifkin, D.B. (1997) Biological roles of fibroblast growth factor-2. Endocr Rev **18**, 26-45
35. Skilton, M.R., Evans, N., Griffiths, K.A., Harmer, J.A., and Celermajer, D.S. (2005) Aortic wall thickness in newborns with intrauterine growth restriction. Lancet **365**, 1484-1486

Figure legends

Figure 1: Ratio of sFlt-1 and free PlGF in both groups (as introduced by Buhimschi et al. [18]). CTR = control group; IUGR = intrauterine growth restriction; mat = maternal; UV = umbilical vein; horizontal lines indicate mean.

Table I: Clinical characteristics of the women and infants*

Maternal Characteristic	Control (n=16)	IUGR (n=15)	P-value
Age (a)	32.75 ± 2.86	30.07 ± 5.87	NS
Body mass index (kg/m^2)	29.19 ± 3.89	25.94 ± 3.72	*P*= .027
Primigravidae [n (%)]	3 (18.75)	7 (46.67)	NS
Primiparae [n (%)]	4 (25)	9 (60)	NS
Gestational age (completed weeks)†	38	33	*P*< .0001
Smokers [n (%)]	3 (18.75)	4 (26.67)	NS
Placental weight (g)	658 ± 163	280 ± 81	*P*< .0001
Blood pressure (mm Hg)			
• systolic	125 ± 13	122 ± 17	NS
• diastolic	79 ± 8	78 ± 11	NS
• mean arterial	94 ± 9	93 ± 13	NS
Pulsatility indices			
• uterine arteries‡	0.73 ± 0.13 (n=7)	1.68 ± 0.79 (n=13)	*P*= .0056
• umbilical artery**	0.88 ± 0.12 (n=14)	1.76 ± 0.56 (n=15)	*P*< .0001
Oligohydramnios	0	12	*P*< .0001
Fetal characteristic			
Birth weight (g)	3470 ± 310	1350 ± 657	*P*< .0001
Centile	60.0 [25.0 – 96.0]	3.0 [3.0 – 9.0]	*P*< .0001
Sex (♂:♀)	11:5	5:10	NS

* Results are presented as mean ± SD or median and [range]; NS = not significant; †: median completed weeks, for statistical analysis days of gestation were used; ‡: mean of both uterine arteries; ** absent or reversed enddiastolic flow was set to value of 2.

Table II: Levels of angiogenic factors (pg/ml): Comparison of groups and relative alteration of the IUGR-group compared to the control group*

Factor		Control (n=16)	IUGR (n=15)	Relative alteration and P value
VEGF	Mat	below detection limit of 9 pg/ml		
	UV	504.57 ± 536.73	448.49 ± 504.02	NS
	UA	422.83 ± 386.10	672.63 ± 615.23	NS
PlGF	Mat	245.74 ± 217.42	48.44 ± 41.63	↓ *P*= .0017
	UV	9.32 ± 6.24	1.90 ± 3.37	↓ *P*= .0016
	UA	11.89 ± 7.58	17.45 ± 40.58	NS
sFlt-1	Mat	2199.85 ± 1824.53	4479.17 ± 2633.21	↑ *P*= .0086
	UV	121.38 ± 50.89	624.71 ± 804.08	↑ *P*= .0183
	UA	188.08 ± 108.97	546.49 ± 844.78	NS
sKDR	Mat	6367.09 ± 1766.75	4726.47 ± 1338.12	↓ *P*= .0071
	UV	6804.66 ± 1580.59	4666.07 ± 1273.55	↓ *P*= .0003
	UA	no measurement available		
bFGF	Mat	14.87 ± 15.59	12.14 ± 11.92	NS
	UV	7.95 ± 5.75	24.20 ± 22.69	↑ *P*= .0096
	UA	39.75 ± 19.87	45.04 ± 64.16	NS

* Results presented as mean ± SD; Mat = maternal; UV = umbilical vein; UA = umbilical arteries; NS = not significant; ↑/↓ = significant in-/decrease

Table III: Relative changes of angiogenic growth factors (arrows indicate changes in column B relative to column A): Comparison of compartments within the groups.

	Compared compartments		VEGF	PlGF	sFlt-1	sKDR	bFGF
	A	B					
Control	Mat	UV	↑	↓	↓	NS	NS
	Mat	UA	↑	↓	↓		↑
	UV	UA	NS	NS	NS		↑
IUGR	Mat	UV	↑	↓	↓	NS	NS
	Mat	UA	↑	(↓) NS	↓		NS
	UV	UA	NS	NS	NS		NS

Mat = maternal; UV = umbilical vein; UA = umbilical arteries; NS = not significant; ↑/↓ = significant in-/decrease ($P < .001$).

Figure 1