11

Placental Angiogenesis and Fetal Growth Restriction

Victor Gourvas[1], Efterpi Dalpa[2], Nikos Vrachnis[3] and Stavros Sifakis[4]
[1]*Department of Pathology, General Hospital "G. Genimatas", Thessaloniki,*
[2]*Department of Pediatrics, General Hospital "G. Papageorgiou", Thessaloniki,*
[3]*2nd Department of Obstetrics & Gynecology, Aretaieion Hospital, University of Athens, Athens,*
[4]*Department of Obstetrics & Gynecology, University Hospital of Heraklion, Crete, Greece*

1. Introduction

1.1 Vessel growth and angiogenesis

The process that involves vessel formation and growth has been described by the terms vasculogenesis and angiogenesis, as two distinct types [Demir et al., 2007]. Both of these processes are essential for normal uteroplacental development:

a. vasculogenesis, meaning new blood vessel formation from hemangiogenic stem cells (derived from mesenchymal cells) that differentiate to hemangioblastic stem cells, essentially occurring during fetal development. Vasculogenesis consists of three major steps: i. induction of hemangioblasts and angioblasts -mediated mainly through fibroblast growth factor (FGF), ii. assembly of primordial vessels -mediated mainly by vascular endothelial growth factor/vascular endothelial growth factor receptor system (VEGF/VEGFR) iii. transition from vasculogenesis to angiogenesis [Flamme et al., 1997];
b. angiogenesis, meaning new branches from pre-existing vessels, which is occurring in the female reproductive tract during the formation of the corpus luteum, during endometrial development and during embryo implantation and placentation. Two forms of angiogenesis have been described: sprouting and non-sprouting angiogenesis (intussusception) [Folkman et al., 1992]. The process of angiogenesis has three phases: initiation, proliferation-invasion and maturation-differentiation.

The vascularisation of placental villi starts at day 21 post conception (dpc), being the result of local de novo formation of capillaries rather than protrusion of embryonic vessels into the placenta [Demir et al., 1989]. Mesenchymal cells inside the villi transform into hemangiogenic precursor cells that migrate toward the periphery. In the latter, prior to the formation of the first vessels that are observed by about 28 dpc (erythrocytes are detected by 32 dpc), mesenchymal derived macrophages (Hofbauer cells) appear. Those macrophages will express angiogenic growth factors and, as they appear early, they suggest a paracrine role in the initiation of vasculogenesis. Angiogenic growth factors are also expressed by the

maternal decidua and macrophages mediating the trohpoblast invasion [Ahmed et al., 1995]. In the meanwhile since the uterus and its contents demand an increased supply of blood during pregnancy, its vasculature undergoes three main adaptative changes: vasodilation, increased permeability, and growth and development of new vessels. So the villi mature and angiogenesis begins at around 32 dpc [Zygmunt et al., 2003].

Branching or sprouting angiogenesis (lateral ramification of pre-existing tubes) is first observed leading to the formation of a capillary network. During branching angiogenesis multistep processes are taking place including increased vascular permeability, degradation of basement membrane, increase in endothelial cell proliferation and migration, formation of endothelial cell tubes, and recruitment of pericytes to the outside of the capillary to form a stable vessel [Kaufmann et al., 2004]. From the 6th week a basal lamina begins to form around the capillaries that results to a web like arrangement of capillaries within the stroma of mesenchymal villi, and a superficially location of most of the capillaries in the immature intermediate villi (beneath the trophoblast, covering the villous surface). In the latter, from the 15th week onwards, fibrosal stromal core is being formed by the fusion of the adventicia of large central vessels, thus becoming a stem villus. In these larger villi a few central endothelial tubes (or early villous arteries and veins) have larger diameters, up to 100mm, and become surrounded by cells expressing alpha and gamma smooth muscles actins, vimentin and desmin. These contractile cells concentrate around the lumina, acquiring the full spectrum of cytoskeleton antigens. Following branching angiogenesis, non branching angiogenesis is observed from 24 weeks of gestation, due to the formation of mature intermediate villi, specialized in gas exchange. Those villi contain 1-2 long, poorly branched capillary loops, which coil and bulge through the trophoblastic surface, forming the terminal villi. These structures are the main site of diffusional gas exchange between the maternal and fetal circulations. This process includes decreased trophoblast proliferation and increased endothelial proliferation [Benirschke and Kaufmann, 1995].

As gestation increases, the terminal capillaries focally dilate and form large sinusoids, which counterbalance the effects of the long poorly branched capillaries on total fetoplacental vascular impedance. Increasing fetal blood pressure aids this dilation and fetoplacental blood flow rises throughout gestation to 40% of fetal cardiac output at term [Ahmed et al., 2000].

Pseudovasculogenesis is the process which remodelling of maternal uterine vessels occurs. Until 6 weeks post conception uterine arteries (spiral arteries) have high resistance and low capacity. After cytotrophoblast invasion they breakdown smooth muscle cells and replace maternal endothelial cells resulting to low resistance and high capacity vessels. Same process, but less extent occurs also in maternal veins. Pseudovasculogenesis is completed around 20 weeks of gestation.

1.2 Fetal growth restriction

Fetal growth restriction (FGR) is a complex condition in the field of current Obstetrics with an incidence rate of 4-7% of births and is associated with a 6- to 10-fold increased risk of perinatal morbidity and mortality [Jarvis et al., 2003]. FGR is not a disease entity with a unique pathophysiology. A variety of factors have been involved, including congenital abnormalities, drug abuse and infectious, immunological or anatomical factors. However, incomplete placentation (placental formation) is observed in most cases [Cetin et al., 2004].

Two types of FGR have been described: early onset, that the growth restriction is symmetrical and late onset that restriction of growth is asymmetrical. The first one is more severe and usually has its underline cause in a specific defect that acts from the beginning of conception such as chromosomal anomalies, infections or substance abuse. Second and more common is less severe with smaller impact on the fetus and the causes may vary. Many elements can be of greater or lesser importance in the progress of this entity. Incomplete placentation and factors controlled by hypoxia are the most common pathophysiologic mechanism.

A successful pregnancy outcome depends on the proper development of the fetoplacental vasculature in the villous core, which begins with the infiltration of cytotrophoblast in the endometrium and is completed in conjunction with the spiral arteries. It is widely accepted that shallow trophoblast invasion can lead to fetal hypoxia and impaired growth [Mayhew et al., 2004]. The proper and timely proliferation and differentiation of the villous cytotrophoblast stem cells, which are controlled by hypoxia, are crucial for adequate placentation [James et al., 2006]. Thus, the entire repertoire of hypoxia-associated growth factors is remarkably active during placental development.

1.3 Fetal growth restriction and angiogenesis

Angiogenesis is a placental factor playing an important role in the development of FGR. FGR occurs as a result of adequate vascular transformation and of terminal villous formation [Ahmed et al., 2000].

Based on the type of FGR (early or late onset) there are two models that may occur in the placental tissue. First is due to uteroplacental insufficiency and results to increased branching angiogenesis. Second and more common is due to placental failure and is accompanied by straight and unbranched capillaries, along with reduced cytotrophoblast proliferation, increased syncytial nuclei and erythrocyte congestion. All these suggest an increased rate of trophoblast proliferation. This situation has been interpreted as placental hyperoxia. Thus, fetoplacental blood flow is severely impaired and transplacental gas exchange is poor, placing the fetus at risk of hypoxia and acidosis [Macara et al., 1996].

Factors that are regulated by oxygen concentration are mainly important for the placental tissue to respond to hypoxic events. The most important known factors of this subgroup are hypoxia inducible factors (HIF).

2. Hypoxia inducible factors

Hypoxia Inducible Factors (HIF) are transcriptional factors that were discovered in mammalian cells under conditions of low oxygen, and appear to have a fundamental role in the cellular and systematic response to hypoxia, via the regulation of metabolism, cellular cycle, angiogenesis and apoptosis. HIFs are heterodimers constituted from the HIFa (1, 2 or 3) and HIFb or ARNT.

HIFa subunit is found in the cytoplasm and is transported in the nuclei in order to form HIF with the other subunit ARNT, which is expressed regularly in the nuclei, and achieve its transcriptional role. It has been found that transcriptional action of HIFa is achieved via bHLH (basic helix-loop-helix)-PAS domain in N-terminal. For this action the recruitment of

co-activators p300 and CBP is needed. While HIF-1 and HIF-2 have similar structure and action, it appears that HIF-3 presents suspensive action in the hypoxia, which has not however been confirmed [Kiichi et al., 2006].

HIFa under normoxic conditions is hydroxylated in two Prolyl domains (Pro 402 and Pro 564) by their Prolyl Hydroxylation Domains (PHD-1,2 and 3), which induces the reaction with the von Hippel-Lindau (VHL) forming a dimmer that is proteosomical degradated. It appears that VHL is downregulated in hypoxia, consequently releasing the HIFa that at the same time is upregulated. PHD utilizes O_2 as a substrate with a Km that is slightly above atmospheric concentration. The enzymatic activity is modulated by changes in O_2 concentration under physiological conditions. This regulation appears to be common for all three HIFa factors [Min et al., 2002].

Deductively, the HIFa factors are upregulated by hypoxia, while the ARNT is stably expressed and in order to they carry out their transcriptional role, they should form a heterodimmer in the nuclei. Under normal conditions, while HIFs continue to be expressed, VHL and PHD-1, 2 and 3 are upregulated in order to complete protesomical degradation of HIFs. The existing data for HIF-1a and HIF-2a show a regulating role in angiogenesis, metabolism, cellular cycle and apoptosis; for HIF-3a however there is still lack of evidence regarding his precise role [Makino et al., 2001]. On the contrary, factors PHD-1, 2 and 3 appear to play all similar roles in the degradation of HIFa subunits [Salceda et al., 1997; Maxwell et al., 1999]. Important examples of factors that are regulated by HIF-1 are VEGF, PLGF, Flt-1 and Angiopoietin-2 regarding arterial destabilization and increased vascular permeability, MMPs, Angiopoietin-1, MCP-1 and PDGF regarding migration and proliferation of endothelial cells etc. Figure 1 represents the HIF pathway during normoxic and hypoxic conditions.

2.1 Hypoxia inducible factors in placenta

It is already known that one of the most important processes during gestation is the physiologic development of feto-placental unit that begins with the cytotrophoblast invasion in the endometrium and is completed with the creation of chorionic villi [Huppertz et al., 2007]. Initially the cytotrophoblast environment can be characterized as hypoxic, but after the completion of the conjunction with the spiral vessels there is a passage to physiologic oxygenation. During this process, structural changes are required for both the epithelium of endometrium and the endothelium and walls of the spiral arteries. The wall of vessels that is found to the side of fetus is replaced from trophoblasts. Cytotrophoblasts in this process turn from an expressive pattern of molecules of epithelial specification in an expressive pattern of adherence molecules that is more of endothelium cells differentiation. Many of these molecules are regulated by HIF. The cytotrophoblast tends to proliferate in hypoxia but to differentiate in physiological oxygenation, and these data show that cytotrophoblast activity depends on the presence of oxygen.

Results from studies in animal models have shown that at the initial stages of pregnancy where PO_2 is decreased, an increased action of regulating factors exists, mainly from HIF-1, HIF-2 and HIF-3. HIF-1 comprises a basic response of a cell to the hypoxic conditions. It is constituted by two subunits, HIF-1a and HIF-1b (ARNT). HIF-1a forms a heterodimmer with the HIF-1b and HIF-1, acts on the cell nucleus and regulates the expression of many

genes that play role in angiogenesis, cellular cycle and metabolism [Semenza et al., 2000]. Similar function appears also for the HIF-2, while for HIF-3 the existing data is insufficient. The regulation of these factors is depending on the partial pressure of oxygen, a fact that makes them immediately connected with hypoxic conditions in the feto-placental circulation such as preeclampsia or FGR [Smith et al., 2001].

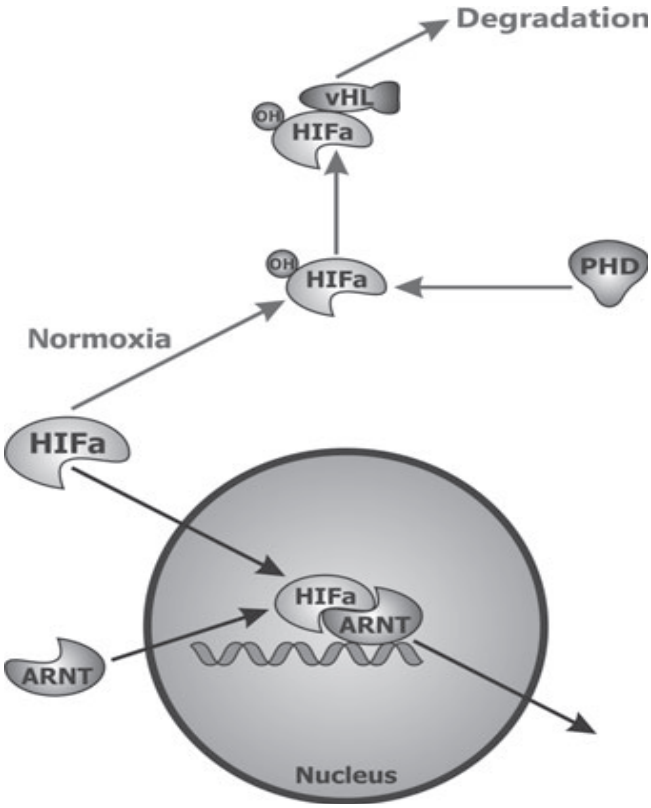


Fig. 1. Graphical representation of the HIF pathway during normoxic and hypoxic conditions. In normoxia (grey arrows) PHD hydroxylates HIF-a, which then binds to vHL, leading to its proteosomal degradation. In hypoxia (black arrows) HIF-a enters the nucleus, forms a dimer with ARNT, which then binds to DNA, leading to gene transcription activation [Gourvas et al., 2010].

Regulation of HIFs appears to relate not only with the induction/suspension of their expression, but also with their degradation. Thus, in physiologic conditions the a- subunits of HIF are degradated with a mechanism that includes factors PHD-1, 2 and 3 (prolyl hydroxylation domain) and VHL. They are responsible for the hydroxylation and degradation of HIFa, so that HIFa cannot activate ARNT in the nuclei. In a second phase one more hydroxylation can contribute in the suspension of HIF, which becomes in asn803 and suspends the interaction with co-activators p300/CBP, which is essential for the induction of transcription [Lando et al., 2002].

It is obvious that the regulation of HIF expression and function is achieved through a number of factors, such as PO_2, VHL and PHD-1, 2 and 3. Consequently, a measurement of expression only of HIF in conditions with decreased supply of oxygen may lead to false conclusions, especially by considering that their regulation is also posttranscriptional through the action of PHD and VHL. Later during gestation, when fetal-placental unit provides satisfactory quantities of O_2, HIFs are downregulated by decreased expression or degradation. In pathological situations, however, it is probable that this model is disturbed and the maintenance of decreased oxygenation affects the regulating action of these factors. Thus exists a change/imbalance in the expression of genes related with processes such as angiogenesis (VEGF, PLGF, PDGF, EPO, NOS2, FLT1 etc), metabolism (aldolase, hexokinase, pyrouvic kinase, lactic dehydrogonase etc) and cellular cycle (IGF, p21, p35srj etc). These changes may produce clinical signs and symptoms of FGR or preeclampsia or of both of them.

3. Angiogenetic growth factors in FGR

Numerous factors are thought to play a role in normal vascular adaptation to implantation. The VEGFs are specific stimulators of vascular permeability, as well as vascular endothelial cell protease production and migration, all of which are critical components of the angiogenic process [Folkman et al., 1987] Vascular endothelial growth factor also stimulate angiogenesis in a variety of in vivo and in vitro models [Klagsbrun et al., 1991]. The increased expression of VEGF-A, b-FGF, and eNOS that we have found in IUGR placentas may promote increased endothelial cell proliferation and migration and pathological angiogenesis [Kinzler et al., 2008].

VEGF, placental growth factor (PlGF), angiopoietins (Ang-1 and Ang-2) are involved not only in the regulation of vascular development and in remodeling during placentation, but also act as growth factors for driving growth and differentiation processes such as invasion. It has been hypothesized that an impairment of trophoblast invasion and a failure of spiral artery remodeling could have a role in the development of PE and FGR [Wulff et al., 2003].

Vascular endothelial growth factor-A (VEGF-A) and placental growth factor (PlGF) are probably the best-studied factors. VEGF interacts with VEGFR-1 (Flt-1) and VEGFR-2 (KDR) to promote endothelial cell proliferation, cell migration, and vascular permeability. PlGF shares biochemical and functional features with VEGF and interacts with VEGFR-1 (Flt-1). PlGF and VEGF-A have synergistic effects regarding angiogenesis, but vessels induced by PlGF are more mature and stable than vessels induced byVEGF-A [Chung et al., 2004]. PlGF is abundantly expressed in the human placenta. Both VEGF-A and PlGF may be important paracrine regulators of decidual angiogenesis and autocrine mediators of trophoblast function [Sherer et al., 2001].

A second family of growth factors, the angiopoietins, is also known for their regulating capacities regarding angiogenesis. Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) bind with equal affinity to their receptor TIE-2, but have different functions. Ang-1 maintains vessel integrity and plays a role in the later stages of vascular remodeling [Geva et al., 2000]. Ang-2 is a functional antagonist of Ang-1 and leads to loosening of cell–cell interactions and allows access to angiogenic inducers like VEGF. Coexpression of VEGF and Ang-2 induces angiogenesis, but Ang-2 results in vascular regression in the absence of angiogenic signals. Ang-1 and Ang-2 have both been detected in decidual and placental tissues [Asahara et al., 1998].

Various decidual cell types are capable of producing angiogenic factors. We recently showed the production of PlGF, KDR, Flt-1, Ang-2, and TIE-2 by endothelial cells and extravillous trophoblasts. Decidual stromal cells, glandular epithelium, and perivascular smooth muscle cells were found to produce all studied angiogenic factors [Plaisier et al., 2007]. Uterine natural killer cells are also abundantly present in first-trimester decidua and are known to produce PlGF, VEGF, Ang-1, and Ang-2.

4. Conclusion

A successful pregnancy outcome depends on the proper development of the fetoplacental vasculature in the villous core, which begins with the infiltration of cytotrophoblast in the endometrium and is completed in conjunction with the spiral arteries. It is widely accepted that shallow trophoblast invasion can lead to fetal hypoxia and impaired growth. The proper and timely proliferation and differentiation of the villous cytotrophoblast stem cells, which are controlled by hypoxia, are crucial for adequate placentation and initiation of angiogenetic pathways. Numerous factors are thought to play a role in normal vascular adaptation to implantation. There is strong evidence that abnormal levels of angiogenic and antiangiogenic growth factors could in part be responsible for the pathophysiology associated with pregnancies complicated by FGR.

5. References

Ahmed AS, Li XF, Dunk CE, Whittle MJ, Rollason T. Colocalisation of vascular endothelial growth factor and its flt-1 receptor in human placenta. Growth Factors 1995;12:235-243.

Ahmed A, Dunk C, Ahmad S, Khaliq A. Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) and soluble flt-1 by oxygen. A Review. Troph Res 2000;14:16-24.

Ahmed A, Perkins J: Angiogenesis and intrauterine growth restriction. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:981-998.

Asahara T, Chen D, Takahashi T, et al. Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. *Circ Res.* 1998;83:233-240

Benirschke K, Kaufmann P (Eds). Pathology of Human Placenta. London: Springer-Verlag 1995

Cetin I, Foidart JM, Miozzo M, et al. Fetal growth restriction: a workshop report. Placenta 2004;25:753-757.

Chung J, Song Y, Wang Y, Magness RR, Zheng J. Differential expression of vascular endothelial growth factor (VEGF), endocrine gland derived-VEGF, and VEGF receptors in human placentas from normal and preeclamptic pregnancies. *J Clin Endocrinol Metab* 2004;89:2484-2490.

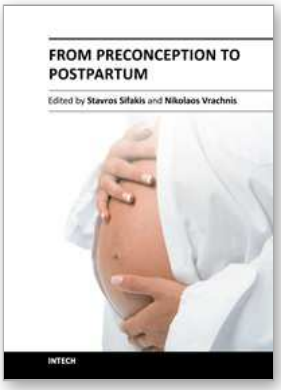
Demir R, Kaufmann P, Castellucci M, Erbengi T, Kotowski A. Fetal vasculogenesis and angiogenesis in human placental villi. Acta Anat (Basel) 1989;136:190–203

Demir R, Seval Y, Huppertz B. Vasculogenesis and angiogenesis in the early human placenta. Acta Histochem 2007;109:257-265.

Flamme I, Frolich T, Risau W. Molecular mechanisms of vasculogenesis and embryonic angiogenesis. J Cell Physiol 1997;173:206–210

Folkman J, Klagsbrun M. Angiogenic factors. Science 1987;233:442–447

Folkman J, Shing Y. Angiogenesis. J Biol Chem 1992;267:10931–10934
Geva E, Jaffe RB. Role of angiopoietins in reproductive tract angiogenesis. Obstet Gynecol Surv 2000;55:511-519.
Gourvas V, Sifakis S, Dalpa E, Soulitzis N, Koukoura O, Spandidos DA. Reduced placental prolyl hydroxylase 3 mRNA expression in pregnancies affected by fetal growth restriction. BJOG. 2010 ;117:1635-1642.
Huppertz B, Abe E, Murthi P, Nagamatsu T, Szukiewicz D, Salafia C. Placental Angiogenesis, Maternal and Fetal Vessels. A Workshop Report. Placenta 2007;28(supplement A):S94eS96
James JL, Stone PR, Chamley LW. The regulation of trophoblast differentiation by oxygen in the first trimester of pregnancy. Hum Reprod Update 2006;12:137-144.
Jarvis S, Glinianaia S, Torrioli M, et. al. Cerebral palsy and intrauterine growth in single births: European collaborative study. Lancet 2003;362:1106-1111.
Kaufmann P, Mayhew TM, Charnock-Jones DS. Aspects of human fetoplacental vasculogenesis and angiogenesis. II. Changes during normal pregnancy. Placenta 2004;25:114-126.
Hirota K, Semenza. GL Regulation of angiogenesis by hypoxia-inducible factor 1. Crit Rev Oncol Hematol 2006;59:15–26
Kinzler WL, Vintzileos AM. Fetal growth restriction. Curr Opin Obstet Gynecol 2008;2:125-131.
Klagsbrun M, D'Amore PA. Regulators of angiogenesis. Annu Rev Physiol 1991;53:217
Lando D, Peet, DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain: a hypoxic switch. Science 295: 858-861, 2002
Macara LM, Kingdom JCP, Kaufmann P, Kohen G, Hair J, More IRA, Lyall F, Greer IA. Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. Placenta 1996;17:37-48
Makino Y, Cao R, Svensson K, et al. Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. Nature 2001;414:550–554.
Maxwell PH, Wiesener MS, Chang GW, et al. The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygendependent proteolysis. Nature 1999;399:271–275.
Mayhew TM, Charnock-Jones DS, Kaufmann P. Aspects of human fetoplacental vasculogenesis and angiogenesis. III. Changes in complicated pregnancies. Placenta 2004;25:127-139.
Min JH, Yang H, Ivan M. Gertler F, Kaelin WG Jr, Pavletich NP. Structure of an HIF-1-alpha-pVHL complex: hydroxyproline recognition in signaling. Science 2002;296:1886-1889.
Plaisier M, Rodrigues S, Willems F, et al. Different degree of vascularisation and its relation to the expression of VEGF, PlGF, angiopoietins and their receptors in 1st trimester decidua. Fertil Steril 2007;88:176-187.
Salceda S, Caro J. Hypoxia-inducible factor 1a (HIF-1a) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redoxinduced changes. J Biol Chem 1997;272:22642–22647.
Semenza, G. L.HIF-1 and human disease: one highly involved factor. Genes Dev 2000;14:1983-1991.
Sherer DM, Abulafia O. Angiogenesis during implantation, and placental and early embryonic development. Placenta 2001;22:1-13
Smith SK. Angiogenesis and reproduction. BJOG 2001;108:777-783
Wulff C, Weigand M, Kreienberg R, Fraser HM. Angiogenesis during primate placentation in health and disease. Reproduction 2003;126:569–577.
Zygmunt M, Herr F, Munstedt K, et al: Angiogenesis and vasculogenesis in pregnancy. Eur J Obstet Gynecol Reprod Biol 2003;110(suppl):S10-S18.



From Preconception to Postpartum

Edited by Dr. Stavros Sifakis

ISBN 978-953-51-0353-0
Hard cover, 314 pages
Publisher InTech
Published online 23, March, 2012
Published in print edition March, 2012

Obstetrics is evolving rapidly and finds itself today at the forefront of numerous developments. Providing selected updates on contemporary issues of basic research and clinical practice, as well as dealing with preconception, pregnancy, labor and postpartum, the present book guides the reader through the tough and complex decisions in the clinical management. Furthermore, it deepens the scientific understanding in the pathogenetic mechanisms implicated in pregnancy and motivates further research by providing evidence of the current knowledge and future perspectives in this field. Written by an international panel of distinguished authors who have produced stimulating articles, the multidisciplinary readers will find this book a valuable tool in the understanding of the maternal, placental and fetal interactions which are crucial for a successful pregnancy outcome.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821