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Impaired endothelial-dependent vasodilatation in old but not young female offspring of rats fed a protein restriction diet during pregnancy

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In the rat, the restriction of dietary protein during pregnancy leads to raised blood pressure and impaired endothelium-dependent vasodilatation in the male offspring (Brawley *et al.* 2003). Ageing is known to impair endothelium-dependent vasodilatation along with a number of other effects on the vasculature (Atkinson *et al.* 1994), and may be especially important in postmenopausal women, given the cardioprotective associations of oestrogen (Barrett-Connor, 1997). The present study investigated the effects of protein restriction *in utero* on vascular function in female offspring during oestrus at and post-cessation of the oestrus cycle.

Pregnant Wistar rats were fed either a control diet (C; 18% casein) or a protein-restricted diet (PR; 9% casein) throughout gestation from conception. Small mesenteric arteries (~250 μm) of female offspring at 17 weeks (n=4-6, in oestrus) and 56 weeks (n=5-6, post oestrus cycle), were dissected and mounted in the wire myograph. Segments were bathed in physiological salt solution, at 37°C and continually gassed with 95% O_2 and 5% CO_2. Following normalisation, cumulative concentration response curves were constructed to phenylephrine (PE; 10 nM-100 μM) and the endothelium-dependent vasodilator acetylcholine (ACh; 1 nM-10 μM). Data are presented as mean ± SEM and differences calculated by two-way ANOVA. Significance was accepted for $p<0.05$.

Vasoconstriction to PE did not differ between C and PR offspring at either age (p=ns), but constriction to PE did decrease with age (two-way ANOVA, $p<0.05$). Similarly, endothelial function as assessed by ACh was significantly blunted with ageing in both C and PR offspring (pEC_{50}: C, young, 7.59 ± 0.16, n=6; old, 7.06 ± 0.24, n=4; % max response: PR, young, 78.5 ± 4.2, n=5; old 26.3 ± 12.0, n=6; two-way ANOVA, $p<0.01$). The extent of the blunting with age seen in the PR group was considerably larger than that seen in the controls and unmasked a significant difference in response between the C and PR groups in the older animals (two-way ANOVA, $p<0.05$), which was not present at the 17 week timepoint (p=ns).

These data demonstrate that the vascular effects of protein restriction *in utero* seen in young female offspring are less apparent than in the male offspring (Brawley *et al.* 2003), during pregnancy (Torrens *et al.* 2003) or after the cessation of the oestrus cycle. This suggests a protective role of oestrogen in the vasculature of young female rats, which masks an underlying defect apparent in old age or during a metabolic challenge in pregnancy.

Atkinson *et al.* (1994). *Br J Pharmacol* **111**, 1184-1188.

Barrett-Conner (1997). *Circulation* **95**, 252-264.

Brawley *et al.* (2003). *Pediatr Res* **54**, 83-90

Torrens *et al.* (2003) *J Physiol* **547**, 77-84.

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C112

Tone oscillations in human placental arteries from normal and pre-eclamptic pregnancies

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The human placenta is vital for the maintenance of normal pregnancy, but mechanisms responsible for the control of vasomotor tone and blood flow within the fetoplacental circulation are poorly characterized. Agonist-induced rhythmical contractions and relaxations (oscillations) in blood vessel tone are a common feature of many vascular beds (1,2), and may allow for an acute regulation of volume flux. Alterations of oscillations may contribute to the pathophysiology of many hypertensive conditions, e.g. pre-eclampsia (PE) (3). Tone oscillations are thought to be influenced by endothelial factors and it is pertinent that PE is associated with endothelial dysfunction (1,3,4). Thus, our aim was to compare the patterns of tone oscillations in placental arteries from women with normal pregnancies and those with PE. Placental biopsies were obtained at term (≥36+6 weeks) from vaginal or Caesarean section (n=4 normal pregnancy) delivery and normalised on a wire myograph at 0.9 of $L_{5.1kPa}$ and equilibrated in physiological salt solution (37°C; 5%O_2/5%CO_2 ~7%O_2) for 20 min. Following initial exposure to a solution containing 120mM potassium to assess arterial viability, arteries were exposed to the thromboxane-mimetic U46619 ($10^{-7.5}$M) for 1 hour. Slow, large amplitude oscillations (>10% of the U46619-induced contraction) were observed in most arteries from women with normal pregnancies (n=15/18) and PE (n=11/15; defined according to international guidelines (4)). All arteries were included in subsequent analysis. Some arteries were exposed to $10^{-7.5}$M U46619 a second time in the presence or absence of the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine (L-NNA, 10^{-4}M).

Arteries from women with normal pregnancies developed oscillations with mean±SEM amplitude of 39±7% and frequency of 0.043±0.008 per minute. In the presence of L-NNA (n=6) amplitude was significantly reduced (40±12% to 18±7%, P<0.05, Friedman test), but frequency was unaltered. In arteries from women with PE, the amplitude of oscillations (not frequency) was significantly reduced (17±4%, P<0.01, ANOVA) compared to arteries from women with normal pregnancies. However, in the presence of L-NNA, although the magnitude of the U46619-induced contraction was significantly increased (10.8±1.3kPa to 13.1±1.7kPa, P<0.05, Friedman test, n=8) neither amplitude nor frequency of oscillations were altered. These data indicate that in human placental arteries (i) the patterns of tone oscillations are altered in PE and (ii) NO has a role in mediating tone oscillations in normal pregnancy and the contractile magnitude in PE. These findings may be important when considering regula-

tion of uteroplacental blood flow in normal or pathophysiological pregnancies.

Nilsson H & Aalkjaer C (2003). Mol Interv 3, 79-89.

Wareing M et al. (2002). Placenta 23, 400-409.

Pascoal IF et al. (1998). J Clin Invest 101, 464-470.

Ong SS et al. (2003). BJOG 110, 909-915.

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C113

Differential effects of hydrogen peroxide on human fetoplacental and non-pregnant uteroplacental arterial tone

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Reactive oxygen species (ROS) play a key role in the modulation of vascular tone in physiological and pathological conditions. Pre-eclampsia is associated with raised placental vascular resistance and increased superoxide (O_2^-) (1, 2). Hydrogen peroxide (H_2O_2) is a vasoactive ROS, generated spontaneously and enzymatically from (O_2^-), which may be involved in the regulation of vascular reactivity in the feto- and uteroplacental circulations in normal and complicated pregnancy. Here, we aimed to determine the effects of H_2O_2 on normal chorionic plate and myometrial arterial tone.

Normal term placentas (N=13) were collected within 30min of delivery. Myometrial biopsies (N=4) were obtained immediately following hysterectomy in pre-menopausal women with no medical complications. Chorionic plate arteries (n=20, N=5 placentas) and myometrial arteries (N=4) were dissected, mounted onto a wire myograph, and normalized at 0.9 of $L_{5.1kPa}$ (~20mmHg) and 0.9 of $L_{13.3kPa}$ (~45mmHg), respectively. The vessels were equilibrated at 37°C for 20 min and gassed at 5% O_2/5% CO_2 (chorionic plate arteries) or 5% CO_2/balance air (myometrial arteries). Preconstricted chorionic plate and myometrial arteries (U46619 thromboxane A_2 mimetic; EC_{80}) were exposed to H_2O_2 (10^{-5}M) for 10 min and responses compared to time matched controls. In separate experiments, chorionic plate arteries were exposed to H_2O_2 (10^{-4}M) for 30 min in the presence (n=6, N=3 placentas) and absence (n=16, N=8) of catalase (500U/ml; H_2O_2 scavenger) and responses compared to those of time matched controls (n=10, N=5).

H_2O_2 (10^{-5}M) induced significantly greater relaxation of preconstricted myometrial arteries than of time-matched controls (treatment vs. controls; maximum relaxation to 27±8% vs. 90±8% of U46619 EC_{80} contraction, mean+SEM, $p<0.05$ Mann-Whitney U test). In contrast, H_2O_2 (10^{-5}M) had no effect on U46619 induced tone in chorionic plate arteries. However, chorionic plate arteries exposed to H_2O_2 (10^{-4}M) constricted transiently (treatment vs. controls; peak increase in baseline tension at 3.2±0.2 mins, 3.8±0.7kPa vs. 0.5±0.4kPa, $p<0.05$, Mann-Whitney U test). The H_2O_2-induced constriction in chorionic plate arteries was completely inhibited by catalase.

Our data reveal differences in human fetoplacental and non-pregnant uteroplacental small artery responses to H_2O_2. The relaxation of myometrial arteries with 10^{-5}M H_2O_2 is consistent with observations in several systemic vascular beds and may be mediated by the endothelium (3). In contrast, the transient chorionic plate arterial constriction at 10^{-4}M H_2O_2, a pathophysiologically relevant concentration (4) is probably a direct effect on vascular smooth muscle. We suggest that in pregnancy complications such as pre-eclampsia, excess H_2O_2 could contribute to raised fetoplacental vascular resistance.

Raijmakers MT *et al.* (2005). *Curr Pharm Des* **11**, 711-734.

Sikkema JM *et al.* (2001). *Placenta* **22**, 304-308.

Thengchaisri N & Kuo L (2003). *Am J Physiol Heart Circ Physiol* **285**, H2255-2263.

Test ST & Weiss SJ (1984). *J Biol Chem* **259**, 399-405.

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C114

Tissue-level Ca^{2+} signalling in human myometrium: a possible role for ICCs

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An increase in intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) initiates contraction in all types of smooth muscle cells including uterine myocytes. On a tissue level, temporal and spatial summation of $[Ca^{2+}]_i$ transients in individual cells triggers a contraction pattern specific to a particular type of smooth muscle. The mechanisms responsible for the initiation and spread of tissue-level Ca^{2+} signals differ in different smooth muscles, involving in some cases, a specialised type of cell called interstitial cells of Cajal (ICCs). The transition of myometrium from relative quiescence throughout pregnancy to powerful contractions during labour is a very complex and precisely timed process. An increase in intercellular coupling through gap-junctions and paracrine mechanisms is thought to be important for synchronising myocyte activity at the end of pregnancy, yet the mechanism of myometrial autorhythmicity remains unclear. Recent work has established that ICC-like cells are present in both rodent and human myometrium, although they are unlikely to be the pacemakers, at least in the rat [1]. In the present study, we used laser scanning confocal microscopy to investigate the initiation and propagation of Ca^{2+} signals between uterine myocytes in their natural environment (i.e. within thin slices of intact myometrium). Myometrial biopsies were taken from the patients undergoing Caesarean section. Samples from three different patients were used in this study. Ten to fifteen slices of myometrium (200-300 μm thick) were cut using a vibroslicer (Integraslice 7550 PSDS, Campden Instruments, UK) in ice-cold physiological saline. The Ca^{2+} signalling events were recorded

from Fluo-4 loaded slices using a Zeiss LSM 510 META confocal microscope. Confocal imaging of tissue slices revealed bundles of smooth muscle cells separated by interstitial spaces. We observed synchronous and large (3.25 ± 0.35 F/F_0, n=32) rises in $[Ca^{2+}]_i$ elicited by action potentials in bundles of smooth muscle cells within the field of view (10x and 20x objective lenses). This was followed by non-propagating asynchronous Ca^{2+} transients of smaller amplitude (1.05-1.75 F/F_0, n=102), presumably due to spontaneous Ca^{2+} release from the sarcoplasmic reticulum. Immediately preceding high-amplitude $[Ca^{2+}]_i$ transients in the smooth muscle cell bundle, there were spikes of $[Ca^{2+}]_i$ originating in the interstitial space outside the bundle, and propagating towards the muscle bundle. Immunohistochemistry of fixed myometrial slices revealed vimentin-positive ICC-like cells within the interstitium and surrounding the smooth muscle cell bundles. In conclusion, our data suggest that in human myometrium, vimentin positive ICC-like cells may play a role as a pacemaker.

Duquette RA et al. (2005). Biol Reprod 72,276-283.

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SA59

Pregnancy-induced adaptation of uterine vascular structure and reactivity: patterns and pathways

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During pregnancy, dramatic increases in uterine blood flow occur via a complex and integrated process of vascular enlargement and altered reactivity. The mechanisms underlying this adaptive process, which is essential for placental perfusion and normal pregnancy outcome are poorly understood. It is well established that insufficient adaptation is associated with pregnancy complications such as preeclampsia and intrauterine growth restriction. Our laboratory has been studying the pattern and extent of arterial remodeling during pregnancy by using the rat as an experimental model. In addition to the intact animal, we also employ a model in which unilateral uterine horn ligation (under anaesthesia) eliminates implantation in one uterine horn, resulting in an animal that is "half pregnant". Although there may be some remodeling relative to nonpregnant controls, the marked differences between the implanted vs. nonimplanted horn indicate that a major part of the remodeling is due to local influences, such as increased shear stress due to the combination of a new low-resistance circulation (the placenta), and of vasodilatation of upstream vessels. Furthermore, there are localized differences in structure and reactivity between vessels that perfuse the myometrium vs. those that feed the placenta. In addition, I will present data that support the concept of venoarterial exchange as a contributing mechanism to vascular remodelling. This mechanism involves the secretion of mitogenic and/or vasoactive signals from the placenta into the venous effluent, and their transfer to the arterial side, which is facilitated by the anatomical proximity of veins and arteries in the uterine circulation of most mammals. In this regard, the potential role of VEGF and of PlGF in facilitating vessel dilation and growth will be discussed as a possible molecular pathway for gestational adaptation. Particular emphasis will be placed on VEGF-R1 (the Flt-1 receptor) in terms of signal transduction, and on the evidence that insufficient signaling due to an excess of a soluble form of this receptor (sFlt-1) is associated with preeclampsia, reduced vascular remodelling, and a significant reduction in fetal weight. The presentation will conclude with some discussion on the effects of hypertension, induced by nitric oxide inhibition, on vascular structure and reactivity, and on the beneficial fetal and vascular effects of supplementation with Sildenafil citrate (Viagra) to reinstate cGMP signalling in uterine vascular smooth muscle.

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Mechanisms of placental vasoreactivity: do structure and function match?

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The human placental circulation is crucial to providing nutrients to, and removing waste products from, the developing fetus. This vasculature comprises an intricate system of arteries *and* veins with vasoactive potential. In the villous tree, solutes are exchanged between placental capillaries and maternal blood via the syncytiotrophoblast epithelial cell layer. The venous system emanating from the capillaries transports blood, enriched in oxygen and nutrients from the maternal circulation, to the fetus. De-oxygenated blood exiting the fetus is returned by the umbilical artery, surface chorionic plate arteries and stem villi arteries to the villous capillary system for removal of waste products and re-oxygenation across the syncytiotrophoblast. Tone regulation throughout this vascular tree is an important determinant of fetal nutrient status impacting upon fetal growth and well-being. Intrauterine growth restriction (IUGR) in pregnancy, for example, is associated with impaired placental blood flow and significant infant morbidity and mortality as well as increased cardiovascular disease risk in later life. The need to understand the mechanisms of tone regulation of human placental vessels is therefore clear and makes the relative paucity of data to date somewhat surprising. Additionally, the lack of innervation of the human placenta presents an unique opportunity to consider how vascular tone may be modulated in the absence of central neuronal input. Consequently, we have embarked upon studies to investigate the structure and function of arteries and veins isolated from placentae of late term pregnant women. These have uncovered several features key to the placental vascular system that are noteworthy. Firstly, consistent with the placenta being an organ of low pressure and oxygenation, maximal agonist responsiveness of chorionic plate arteries is achieved at intralumenal pressures (or equivalent isometric stretches) and perfusate oxygenations of only ~25mmHg and ~7%, respectively (Wareing *et al.* 2006). Simultaneous measurements of $[Ca^{2+}]_i$ and tone in these intact arteries, coupled with studies of α-toxin-permeabilised vessels, illustrates that receptor-coupled agents, including endothelin-1, U44619 (a thromboxane mimetic) and sphingosin-1-phosphate (S1P), enhance tone via a prominent Ca^{2+}-sensitisation of force (Wareing *et al.* 2005; Hemmings *et al.* 2006). In the case of S1P this is almost completely ablated by the Rho-associated kinase inhibitor Y27632; thromboxane receptor stimulation, in contrast, is only partly ROK-dependent indicating the capacity to invoke multiple Ca^{2+}-sensitising signalling pathways. Electron microscopic examination of these arteries has also established unusual ultrastructural features including (i) an absence of internal elastic lamina and (ii) a paucity of smooth muscle sarcoplasmic reticulum (Sweeney *et al.* 2006). The former finding may contribute to differences in vasomotor oscillations evident in comparisons of placental and maternal arteries. The significance of the latter observation for placental vessel Ca^{2+} homeostasis remains to be established but could reflect the afore-

mentioned reliance on prominent agonist-mediated Ca^{2+}-sensitisations of tone.

Secondly, although our preliminary structural data points to chorionic plate veins having fewer smooth muscle cell layers per cross-sectional area than arteries, the maximum constrictive forces are almost comparable (Wareing *et al.* 2003; Wareing *et al.* 2006). Similar to arteries, this is accomplished at the low pressures and oxygenations approximating that anticipated *in vivo*.

Thirdly, our own evidence, and that in the literature, for a substantial agonist-mediated endothelial-dependent vessel relaxation is often elusive and certainly contentious. This is perhaps surprising given that placental endothelial cells are not separated from the underlying smooth muscle by a broad laminar structure as in maternal arteries. Vessel site is perhaps pertinent here; for example, the ability of S1P to effect NO-mediated dilatory influences may vary between chorionic plate and stem villi arteries (Hemmings *et al.* 2006).

These studies of isolated human placental blood vessels indicate that there are several structural and functional features that distinguish them from adult systemic arteries and veins. Much further work is required to firmly establish how the intracellular, and intercellular, structural specialisations may relate to the signalling intricacies that regulate tone in both arteries and veins of the human placenta. This will be aided by consideration of how the vasculature is remodelled (i) from early to late pregnancy and (ii) in situations of compromised placental blood flow such as IUGR.

Wareing M, Greenwood SL, Taggart MJ *et al.* (2003). Placenta 24, 790-796.

Wareing M, O'Hara M, Seghier F *et al.* (2005). Am J Obs Gynecol 193, 814-824.

Hemmings DG, Hudson NK, Halliday D *et al.* (2006). Biol Reprod 74, 88-94.

Wareing M, Greenwood SL & Baker PN (2006). Placenta 27, 42-48.

Sweeney M, Jones CJP, Greenwood SL *et al.* (2006). Placenta (in Press).

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SA61

Mouse models of human placental vascular disease

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Placental vascular abnormalities causing deficient placental perfusion contribute to common complications of human pregnancy including preeclampsia and intrauterine growth restriction (IUGR). Blood flow pulsatility, quantified using the Doppler Resistance index, is often elevated in the uterine and/or umbilical circulations in preeclamptic or growth restricted pregnancies. This change in hemodynamics is associated with placental vascular abnormalities which presumably elevate vascular resistance and thereby impair perfusion. However, the causes of placental vascular abnormalities in human pregnancy as well as the relationships between structural and hemodynamic changes are poorly understood. In our lab, we are using genetically-altered mice as new models to explore the etiology of abnormalities in placental vascularization and hemodynamics. We are using ultrasound biomicroscopy (30-40 MHz; Vevo770, VisualSonics, Canada) to non-invasively image and record Doppler blood velocity waveforms of isoflurane-anesthetized pregnant mice. We have monitored blood velocity in the uterine artery and intraplacental arterial canals of the mother, and in the yolk sac and umbilical circulations of the embryo both in normal pregnancy and in mouse models of intrauterine growth restriction (IUGR). Dr. Junwu Mu has shown that peak (PSV) and end-diastolic velocities (EDV) in the uterine artery increase and the uterine arterial Doppler Resistance Index (RI= (PSV-EDV)/PSV) decreases during gestation in mouse as in human pregnancy. PSV in the umbilical artery increases progressively from the day the heart starts to beat (E8.5) until term (E18.5) whereas PSV in the vitelline artery to the yolk sac increases until E13.5, and then remains stable. In the umbilical artery, EDV increases from zero to become detectable as early as E15.5 and is detectable in nearly all embryos at term. Umbilical waveforms are similar to those observed in first trimester human pregnancy. Similar to findings in human IUGR, Dr. Junwu Mu and Ms. Shathiyah Kulandavelu observed elevated resistance indices in the umbilical and uterine arteries of two mouse models of IUGR; eNOS knockouts from Jackson Laboratories, and transgenics with perinatal hepatic overexpression of IGFBP-1 created by Drs. Victor Han and Carole Watson. In collaboration with Dr. John Sled (Mouse Imaging Centre, Toronto), Ms. Monique Rennie and Ms. Kathie Whiteley are using X-ray micro-computed tomography (μCT) to image and quantify vascular branching patterns in the uteroplacental and umbilico-placental circulations following the installation of an X-ray opaque contrast agent. Preliminary results suggest this method can be used to detect normal developmental changes in placental vascularity as well as vascular defects in IUGR models. We conclude that ultrasound biomicroscopy and μCT provide viable methods for quantifying placental hemodynamics and vascularity throughout pregnancy in mice. Our early results show that there are strong parallels in placental structure and hemodynamics between mice and humans in both normal and IUGR pregnancies.

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SA62

Early development of the placental vasculature

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Blood vessels develop via two subsequent processes starting with vasculogenesis and subsequently continuing with angiogenesis. During vasculogenesis pluripotent mesenchymal cells differentiate into haemangiogenic stem cells. The latter further differentiate into angioblastic cells that give rise to endothelial precursor cells (Ribatti et al. 2002). At this stage first primitive

capillaries are formed. In subsequent stages of differentiation and formation of vessels, new blood vessels derive from already existing vessels, a process called angiogenesis (Hanahan & Folkman 1996).

Vasculogenesis and angiogenesis in the human placenta

In the very beginning of human placental development, the villous trees are composed of two layer of villous trophoblast and a core of extraembryonic mesenchyme. The process of vasculogenesis is mostly restricted to the period of villous tree formation of placental development while angiogenesis takes place throughout pregnancy until delivery.

Vascularisation inside the placenta results from local de-novo development and formation of primitive capillaries from pluripotent mesenchymal cells. Hence within the placenta there is vasculogenesis that is independent from vessel formation inside the embryo; and placental vessels do not result from sprouting of vessels from the embryo into the placenta. At day 21 post conception placental vasculogenesis starts, during the 4 somite embryo stage (Kaufmann et al. 2004; Demir et al. 2005). At this stage, the villous trees comprise primary (only trophoblastic) and secondary (with a mesenchymal core) villi. Within the mesenchymal stroma of secondary villi, haemangiogenic stem cells differentiate, which finally leads to the formation of first vessels. Thus the early placental vessels and their endothelial linings are direct mesenchymal derivatives rather than originating from embryonic blood monocytes.

Sequence of events during placental vasculogenesis and angiogenesis

The sequence of events during vasculogenesis and angiogenesis in the human placenta (Demir et al. 2004, 2005) can be summarised as follows:

(1) Vasculogenesis begins with the differentiation of haemangiogenic stem cells, followed by the formation of vascular patterns. This de novo vessel formation requires a first, so far unknown trigger to induce differentiation of the primary source, pluripotent mesenchymal cell, since already formed endothelia, pre-existing mature endothelial cells or circulating endothelial precursor cells are not available at that time.

(2) The first morphological signs of differentiating cells within vascular patterns develop in close vicinity to the villous trophoblast (Demir et al. 2004), which seems to be the major trigger of vasculogenesis (Demir et al. 2004).

(3) During ongoing villous maturation vasculogenesis continues while angiogenesis already starts. Now the Hofbauer cells are recruited to further trigger vessel formation (Demir et al. 2004). Depending on the stage of villous maturation VEGF is mostly expressed in villous cytotrophoblasts or Hofbauer cells and fibroblasts (Demir et al. 2004).

(4) Primitive vascular tubes are formed by the assembly of several haemangiogenic cells or by differentiation of a single cell to form a primitive lumen (Demir et al. 2004). During this first formation of primitive vascular tubes, differentiating cells come into direct contact with cytotrophoblasts by establishing cytoplasmic projections.

(5) With advancing formation and maturation of placental vessels, endothelial progenitor cells show increasing immunoreactivities for CD31 and CD34, while further differentiated haematopoietic cells do not show any immunoreactivity for these proteins.

(6) Further processes of developmental and differentiation continue leading to the generation of endothelial cells and also first perivascular cell populations. This development of perivascular sheets including a muscular layer to form contractile vessels is induced by growth factors and cytokines derived from the local villous environment as well as from the already established foetal circulation.

In summary, placental vasculogenesis starts with the differentiation of pluripotent mesenchymal cells into first primitive vessels. Then angiogenesis takes over leading to the formation of further primitive as well as mature vessels. Only at these later stages embryonic cells participate in placental vessel development, which starts independent from the embryo inside the human placenta.

Demir R, Kayisli UA, Seval Y, Celik-Ozenci C, Korgun ET, Demir-Weusten AY & Huppertz B (2004). Placenta 25, 560-572.

Demir R, Kayisli UA, Cayli S & Huppertz B (2005). Placenta (in press).

Hanahan D & Folkman J (1996). Cell 86, 353-364.

Kaufmann P, Mayhew TM 7 Charnock-Jones DS (2004). Placenta 25, 114-126.

Ribatti D, Vacca A, Nico B, Ria R & Dammacco F (2002). Curr Mol Med 2, 537-543.

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