


Advances in imaging feto-placental vasculature: new tools to elucidate the early life origins of health and disease

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Review

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Abstract

Optimal placental function is critical for fetal development, and therefore a crucial consideration for understanding the developmental origins of health and disease (DOHaD). The structure of the fetal side of the placental vasculature is an important determinant of fetal growth and cardiovascular development. There are several imaging modalities for assessing feto-placental structure including stereology, electron microscopy, confocal microscopy, micro-computed tomography, light-sheet microscopy, ultrasonography and magnetic resonance imaging. In this review, we present current methodologies for imaging feto-placental vasculature morphology *ex vivo* and *in vivo* in human and experimental models, their advantages and limitations and how these provide insight into placental function and fetal outcomes. These imaging approaches add important perspective to our understanding of placental biology and have potential to be new tools to elucidate a deeper understanding of DOHaD.

Introduction

The placenta ensures fetal development and maternal adaptations via regulation of nutrient and waste transfer, endocrine function, and immunological response. Placental dysfunction can result in poor fetal outcomes such as fetal growth restriction (FGR), which affects 5%–10% of pregnancies.¹ However, the ramifications of inadequate placental function extend far beyond short-term health outcomes. Epidemiological studies show that reduced placental weight and surface area are associated with low birth weight and an increased risk of hypertension, heart failure, coronary heart disease and sudden cardiac death in adult life.^{2–4} Yet despite the critical importance of the placenta, its structural and functional integrity as well as response to environmental challenges remains under-researched and poorly understood.

One of the key functions of the placenta is to transfer nutrients and waste between the mother and the fetus. Underpinning this exchange function is the architecture of the placental vasculature. The human placenta is haemochorial, with fetal (feto-placental) vasculature surrounded by trophoblast and bathed in maternal (utero-placental) blood. An elaborate feto-placental vascular tree is critical for optimal fetal growth, yet this elaboration is particularly sensitive to environmental stressors.⁵ This is exemplified by the characterisation of placentas from FGR babies: there are significant reductions in vascular volume and structural changes throughout the human feto-placental vascular tree from the chorionic plate to the capillaries.^{6–8}

Reduced placental vascular complexity has implications beyond impaired nutrient and waste exchange, and it is emerging that poor placental vascularity alters how blood flows to the developing heart and fetal vascular system. These changes in blood flow (haemodynamics) alter the mechanical forces exerted on endothelial cells, thus altering endothelial cell function and blood vessel remodelling.^{9–11} Moreover, the developing heart beats directly against the resistance of the placental vascular bed,¹² and therefore, changes in mechanical signals have potent and enduring effects on cardiac development and structure.¹³ Recent recharacterisation of mouse mutants known to be lethal in gestation revealed striking, and previously unappreciated, placental defects.¹⁴ Notably, strong correlations between placental defects (many of which involved feto-placental vascular malformation) and heart pathology or abnormal fetal vascular development were observed.¹⁴ Other genetically modified mouse experimental models (which include ablation of genes such as *PPAR γ* , *Wnt*, *Hex* and *Islet-1*) have also demonstrated the necessity of an elaborate feto-placental vasculature for optimal fetal heart development, although none have established the consequences for later cardiovascular health.^{15–25} Furthermore, severe placental insufficiency in humans results in increased loading of the right ventricle.²⁶ However, how alterations in feto-placental vascular structure associate with adult cardiovascular health remains relatively unexplored.

To comprehensively understand the emerging idea of a placenta-cardiovascular axis, and the implications for adult health, it is important for experimental studies to adequately image

feto-placental vasculature. To assist researchers with considering the best options for their research question, this review will present current methodologies and considerations for imaging of feto-placental vasculature *ex vivo* and *in vivo* in human and experimental models. The review will also provide examples of studies that have implemented the various imaging approaches and the insight they have provided into placental function and fetal outcomes.

Feto-placental vascular structure and function

Fetal blood travels to the human placenta via the umbilical arteries, which upon reaching the placenta diverge and branch into large vessels known as chorionic plate arteries.⁵ These vessels radiate across the fetal surface of the placenta, before plunging into the placenta where they branch again into multiple, elaborately branched villous trees, which arise from a stem villus in the chorionic plate.⁵ Several villous trees may occupy a lobe (also known as a cotyledon) which is distinguished by septa on the maternal side of the placenta. The finest branches of the villous trees, known as terminal villi, form grape-like structures.²⁷ The walls of these terminal villi are extremely thin, making them ideal for maternal-fetal exchange.^{28,29} By term, the terminal villi make up almost 40% of the villous volume of the placenta with the feto-placental capillary network greater than 500 km in length and 12–14 m² in surface area.³⁰ Once the fetal blood has travelled through the capillary networks within villi, fetal blood is drained through a venule at the base of each villus, and these venules will then converge into the umbilical vein which returns blood to the fetal circulation.^{30,31}

Given the inherent ethical and logistical issues of obtaining and imaging human placentas, it is imperative that experimental models are utilised to increase our understanding of placental development and function and consequences for later health outcomes. No commonly used animal model is a perfect model for human placentation, and excellent extensive reviews on comparative placentation have been published by others.^{32–34} Here, we restrict our review to focus predominantly on imaging of feto-placental vasculature on human, mouse and rat placentas as the imaging performed in other species is currently limited in comparison. Furthermore, with regard to consideration of the role of feto-placental vasculature in driving fetal cardiovascular development and subsequent health, mice and rats are particularly useful models due to ease of genetic manipulation, short lifespan and the vascular structure of the fetal side of the placenta. Like the human, the mouse and rat placenta is a discoid, haemochorial organ,^{35,36} with the rat placenta more invasive when compared to mouse.³⁵ The rodent placenta consists of three distinct regions: (i) the maternal portion (the decidua basalis and underlying myometrium), (ii) junctional zone and (iii) labyrinth zone. The junctional zone (equivalent to the human basal plate) consists of spongiotrophoblast and trophoblast giant cells, which synthesise hormones crucial for fetal development and maternal adaptations to pregnancy.^{36–38} The labyrinth zone is the site of maternal-fetal exchange and the labyrinth vasculature is argued to be analogous to a single chorionic villus in the human placenta.^{36,38–41} In rodents, arterioles from the umbilical artery divide and branch before travelling to the maternal border of the labyrinth zone where they then form a dense capillary bed.^{31,40}

Imaging feto-placental vasculature

Due to the intricate structure of feto-placental vasculature, it is imperative that imaging approaches capture the correct context

for the experimental hypothesis. Biological imaging approaches are diverse and are developing at a rapid rate to provide previously unimaginable perspectives. Analysis of feto-placental vasculature in humans and experimental models has been carried out through a range of two-dimensional (2D) and three-dimensional (3D) imaging techniques both *ex vivo* and *in vivo*. These imaging approaches include stereology, electron microscopy, confocal microscopy, micro-computed tomography (micro-CT), light-sheet microscopy, ultrasonography and magnetic resonance imaging (MRI). Each of these approaches provides benefits and limitations for understanding how feto-placental vascular structure informs placental and fetal development and thus, future health outcomes.

Stereology

Stereology enables 3D interpretation of 2D sections of tissue. In essence, random systematic sampling and measurement of tissue cross-sections can provide unbiased and quantitative data of 3D structures such as volume, surface area, length and number. Undoubtedly, stereology has been a prevalent imaging technique to characterise placental structure, particularly in the rodent. Stereology has been used to assess placental morphology in humans,^{42–47} mice,^{18,48–61} and rats.^{62–64} Via unbiased randomised sampling of 2D histological images, structures of interest can be quantitated and mathematically extrapolated to infer 3D structure. In the placenta, stereology is commonly used not only to evaluate information of placental vascular length, diameter, surface area, volume and density^{18,42–44,54,63,64} but also to estimate its theoretical diffusion capacity by measuring capillary interhaemal membrane thickness and volumes of placental components, that is, labyrinth zone, spongiotrophoblast and decidua volumes.^{53–55,59,61}

Stereology has been implemented to assess placental structure in human pregnancy complicated by FGR, pre-eclampsia and diabetes.^{42–44} FGR placentas exhibited thicker trophoblastic epithelium, a reduction in peripheral villous surface areas and a reduction in volume of peripheral villi and intervillous space.⁴³ In contrast, there were no significant differences in the volume of intervillous space and all types of villi in pre-eclampsia, when compared to normal pregnancy.^{43,44} These changes support the notion that feto-placental vascular complexity is an important determinant of fetal growth. Higgins and colleagues utilised stereological analysis to examine human placentas from diabetic pregnancies which exhibited increased terminal villous volume, capillary volume and capillary length.⁴² Interestingly, maternal gestational hyperglycaemia affects capillary, but not stromal, development, and these changes may increase specifically the surface areas for maternal-fetal exchange and potentially represent a structural mechanism which accounts for the overgrowth of fetus and abnormally high birth weight. Alternatively, hyperglycaemia elicits excessive insulin secretion which promotes abnormal vascular proliferation, causing downstream abnormalities. Overall, these findings suggest differential condition-specific effects on placental integrity: FGR (but not pre-eclampsia) leads to reduction in structural size and complexity, while gestational diabetes leads to changes in terminal structures.

The early elegant stereological assessments by Coan and colleagues established the developmental dynamics of C57BL/6J mouse placental morphology.^{59,61} Maternal blood spaces were shown to expand rapidly between E14.5 and 16.5, whereas fetal capillary development continuously increased in volume and surface area right up to late gestation.⁶¹ These findings highlight the importance for feto-placental capillary expansion to meet

increased fetal demand for nutrients in late gestation when fetal growth is rapid. These initial assessments have been extended to the comparison of different mouse strains, revealing differences in fetoplacental structure including growth of fetoplacental capillaries and interhaemal membrane thickness,⁵² an important consideration when selecting experimental models. In well-established mouse models of developmental origins of health and disease (DOHaD) such as maternal protein restriction⁵⁸ and maternal undernutrition,⁵³ stereology has demonstrated changes in placental vascular morphology. Moreover, stereological investigations of knockout-mouse models have given important insights into the dynamic nature of the placental function, especially into the co-ordination of maternal and fetal signals to in part compensate for an adverse environment *in utero*.^{48–50,57,60,62} Placental structural development in rats has also been examined by stereology and revealed greater fetoplacental complexity in comparison to mice.^{63,64} In a rat model of glucocorticoid ('stress' hormone) excess in pregnancy, the reduction in fetal weight is accompanied by markedly reduced placental weight and fetoplacental vascular volume, length and diameter, although not maternal blood spaces.⁶⁴ Similar stereological outcomes have been obtained in other mouse studies of glucocorticoid excess⁵¹ including 11 β -hydroxysteroid dehydrogenase 2 knockout (11 β -HSD2^{-/-}) mice which also exhibit reduced cardiac function.^{18,54} Interestingly, administration of pravastatin to stimulate placental vascular growth factor A and enhance fetoplacental angiogenesis in 11 β -HSD2^{-/-} fetuses also ameliorated fetal cardiac function,¹⁸ highlighting the likely direct influence of fetoplacental vascular structure on fetal organ development.^{18,54} However, none of the studies mentioned have shown a conclusive role of fetoplacental vasculature directly informing adult cardiovascular health.

Stereology is a well-established and low-cost method, albeit laborious, with the advantage of encompassing the entire placental vasculature. Stereology has also yielded important insights into placental vascular morphology in human, mouse and rat alike. However, stereology approaches necessitate dismembering the structural integrity and connectivity of placental vascular networks. Thus, this approach cannot be used to evaluate placental vascular tree topology. To overcome these limitations, high-resolution contrast-enhanced micro-CT (discussed below) may well replace stereology in years to come.

Electron microscopy

Electron microscopy is a 2D imaging technique which includes transmission electron microscopy (TEM) and scanning electron microscopy (SEM). TEM creates high-resolution and high-magnification images of fine structural characteristics by passing electron beams through tissues. However, TEM destroys the connectivity of the placental vascular networks due to the necessity of tissue-sectioning during sample preparation. SEM scans the surface of a tissue specimen and also generates a 2D image of a 3D surface via interaction of high energy electron beam with a prepared specimen. However, SEM destroys surrounding tissues due to the requirements of sample preparation and the high electron beams. In 1955, Wislocki and Dempsey implemented electron microscopy to investigate placental morphology in rats,⁶⁵ pigs⁶⁶ and humans.⁶⁷ Thereafter, electron microscopy has been used by other investigators to delineate fine detail in the placental vasculature of monkeys,⁶⁸ minks,^{69,70} horses,⁷¹ rodents^{40,52,59,72–74} and humans.^{68,75–86} Demir and colleagues have used TEM to

investigate the development of fetoplacental villous trees during the early period of ectopic human pregnancy compared to normal pregnancy.⁸⁰ A decade later, Coan and colleagues investigated the mouse placental exchange region, which included characterising cytoplasmic bridges attached to several areas of the interhaemal membrane.⁵⁹ This was the first study that combined TEM and SEM to determine ultrastructural characteristics of mouse placental development.⁵⁹ Additionally, TEM has contributed to the understanding of placental cellular mechanisms in gas exchange surrounding capillaries,^{59,85} as well as the interior of fetoplacental endothelial cells.⁸⁶ Moreover, TEM has been used to inspect the maldevelopment of the trophoblast and placental terminal villous compartment in human preterm growth-restricted⁷⁷ and pre-eclamptic pregnancies,⁸⁷ illustrating irregular trophoblast surface and elongated villi in preterm intrauterine growth restriction,⁷⁷ and excessive villous arborisation with attenuated villi in pre-eclampsia.⁸⁷

Vascular corrosion casting of fetoplacental vasculature can be visualised using SEM.^{40,73,84} Briefly, a vascular cast is generated with a polymer (i.e. Batson's casting compound) and after polymerisation, the surrounding tissue is removed with a corrosive agent to reveal the surface of the vascular cast.^{40,73,84,88,89} SEM has been utilised to reveal the intricate connectivity of fetoplacental vasculature of healthy pregnancies^{72,78,79} and complicated pregnancies such as FGR, whereby pronounced variations in placental surface were observed.^{72,75} SEM has also been used to provide anatomical comparison between humans and rodents, as well as a deeper understanding of how placental structure associates with fetal outcomes.^{40,52,72–77} For example, SEM has revealed that the human placentas of FGR pregnancies exhibit abnormalities in their terminal villi compartments, including reductions in numbers of capillary loops, branches and coiled vessels.⁷⁷ SEM has been applied to image the transition from arterioles to capillaries in the mouse fetoplacental tree and revealed that expansion of the fetoplacental vasculature in late gestation is strain dependent.⁵² Moreover, SEM revealed that chronic hypoxia during pregnancy in mice caused expansion of the fetoplacental capillary bed and thinning of the interhaemal membrane,⁷⁴ possibly as a compensatory adaptation to ensure maternal-fetal exchange.

Even though electron microscopy has obvious advantages, especially visualising the fetoplacental vasculature (particularly capillary and terminal villi) at extremely high resolutions and high magnifications, it only provides 2D images and also a very restricted field of view of a small segment over the entire vascular network. Moreover, each examination requires demanding sample preparation which destroys the connectivity of the placental vascular networks and the surrounding tissue. Other 3D imaging modalities such as confocal microscopy, light-sheet microscopy and micro-CT may overcome these limitations of electron microscopy.

Confocal microscopy

The development of 3D imaging techniques has altered the scope to study microvasculature with greater accuracy and precision. Confocal microscopy relies on point illumination; the technique utilises a confocal pinhole and a photomultiplier detector to exclude out-of-focus light surrounding the focal plane. This generates a series of 2D optical sections with enhanced contrast, precision and definition. These 2D optical sections can then be combined and reconstructed into a 3D representation which results in high-contrast and high-resolution images.⁹⁰

Confocal microscopy has been applied to human placentas to visualise the architecture of chorionic villous vascularisation in the first trimester,⁹¹ second trimester⁹² and at term.^{81,86,93,94} It has also been used to examine the spatial arrangement of the capillary bed.^{95,96} Confocal has been utilised to investigate the distribution of villous capillaries in human diabetic placentas, including the surface and volume ratios between the fetal capillaries and the villus. It has also been implemented to provide a structural basis for computational modelling of oxygen flux in human placenta.^{96,97} Recently, Sargent detailed methods of confocal microscopy in rhesus macaques placentas.⁹⁸

Although confocal microscopy can be used to visualise the architecture of chorionic villous vascularisation, a major limitation is the maximum depth of focus (~1 mm³),⁹² which means that a full-specimen coverage of the placental vasculature is currently impossible using this technique. Nonetheless, 3D confocal microscopy is still a useful approach for quantifying fine details of placental structure.

Micro-computed tomography

In recent decades, X-ray micro-CT has become a valuable and increasingly popular technique for non-destructive *ex vivo* imaging and has been used extensively to visualise placental vasculature in 3D,^{6–8,52,72,74,99–111} as well as other organs including brain,^{112–114} heart,^{112,115} lung,^{105,112,116} liver^{117–119} and kidney.^{112,115,120–123} While micro-CT scanning is clearly suitable for imaging bone, blood vessels with low-inherent contrast properties cannot be directly visualised. To overcome this issue, a radio-opaque compound, such as Microfil® (Flow Tech Inc., Carver, MA, USA), Batson's No.17 (Polysciences Inc., Warrington, PA, USA), BriteVu® (Scarlet Imaging, Murray, UT, USA) or barium sulphate, is perfused into blood vessels to heighten the contrast of the vessel lumen against surrounding tissues. This delineates the vascular structures for qualitative and quantitative analysis.^{6,7,121} In recent years, this approach has been successfully applied to examine placental vascular integrity in mice,^{52,72,74,99–106} rats^{107,108} and humans.^{6–8,109–111} Briefly, *ex vivo* micro-CT of vasculature consists of four main steps: (i) generation of vascular casts using a radio-opaque compound; (ii) *ex vivo* micro-CT scanning and 3D image reconstruction; (iii) vascular network segmentation and (iv) visualisation and characterisation of vascular network.¹⁰⁸ Importantly, the field of view using micro-CT is sufficiently large to capture the entire span of fetoplacental vasculature in rodents and humans. Moreover, casting approaches for micro-CT can be modified to distinguish arterial and venous networks within the placenta, and the non-destructive nature of the imaging means the same sample can be preserved for follow-up histological assessments.

In mice, the innovative work of Rennie and colleagues using Microfil® and micro-CT has added considerable depth of understanding in the structural changes of fetoplacental vasculature.^{52,100–102} The developmental course of mouse fetoplacental vasculature has been captured,^{52,100–102} and comparison between mouse fetoplacental arterial and venous trees has been drawn.¹⁰⁰ Again, mouse strain differences have been revealed using this technique, such as differences in the expansion of the fetoplacental arterial and capillary vasculature in late gestation.⁵² Experimental models have also been characterised using this technique. As such, endothelial nitric oxide synthase deficiency reduced fetoplacental vascular diameter and lengths in peripheral vessels.¹⁰¹ Also, maternal chronic hypoxic mice showed a significant reduction in the absolute volume of fetoplacental arterial

vasculature and the total number of vessel branches (–44% and –30%, respectively), when compared to controls.⁷⁴ Moreover, environmental pollutant exposure polycyclic aromatic hydrocarbons in C57Bl/6J mice reduced the volume and surface area of the fetoplacental arterial vasculature,⁷² as well as resulting in significantly increased fetoplacental arterial vascular tortuosity and reduced intra-placental arteriole vessels.¹⁰² These alterations can cause reduced placental blood flow, leading to ineffective maternal-fetal exchange as well as potentially impaired fetal development and outcomes. Further investigations are required however to clarify these associations, as well as the consequences for adult health.

In humans, Pratt and colleagues have evaluated different methodologies in placental preparation and imaging.¹⁰⁹ They attempted to optimise the application of contrast agent, perfusion pressure, perfusion location and perfusion vessel in tissue preparation.¹⁰⁹ As such, they found the quality of the fetoplacental cast from Microfil® more superior than barium sulphate as well as the contrast injection through umbilical artery more effective than via the umbilical vein or chorionic vessels.¹⁰⁹ Aughwane and colleagues recently investigated the degree of heterogeneity in human fetoplacental vasculature using multiscale micro-CT and histological analysis.¹¹⁰ They found that the vascular density varied throughout placentas, and the patterns of vascular branching differed across a number of normal placentas.¹¹⁰ Langheinrich and colleagues have also analysed the vascular density and surface area of human fetoplacental vasculature using micro-CT with barium sulphate perfusion.¹¹¹ Furthermore, they quantitated fetoplacental vasculature in FGR and found arterial vascular volume in FGR was significantly lower than healthy controls.⁸ Junaid and colleagues combined two techniques [corrosion casting (Batson's No.17) and micro-CT] to image the whole human placental vascular morphology in FGR.^{6,7} Notably, this study was the first to evaluate the whole human placenta. They detected an alteration of chorionic arterial branching patterns and a reduction in capillary network density in FGR placentas,⁷ as well as shorter median vessel length density in arteries but longer median vessel length density in veins.⁶

We have recently established micro-CT methodology in our laboratory to characterise mouse and rat fetoplacental vascular structures.^{107,108} The qualitative assessments of mouse and rat fetoplacental vascular structures at the end of gestation revealed striking species differences. Compared to mouse, rat fetoplacental vasculature exhibits far greater branching complexity, vascular tree span and tree depth (Fig. 1). While differences in vascular structure are readily apparent qualitatively, obtaining quantitative data has proven more challenging. Despite the excellent work of others, quantitation of these vascular trees has been limited by costly software needed for such analyses. Indeed, Aughwane and colleagues recently also highlighted these methodological issues and barriers in the analysis of human fetoplacental vasculature.¹¹⁰ To assist with this, we have recently developed an end-to-end methodology for 3D quantitative characterisation of fetoplacental arterial vasculature.¹⁰⁸ The methodology enables quantification of numerous parameters including tortuosity, vessel diameter, number and order of vessel segments.¹⁰⁸

Beyond assessing the broad expanse of the fetoplacental vascular tree, higher resolution scans with a narrower field of view can reveal the capillary structure of the placenta. We have recently assessed this in a rat placenta at the end of gestation, and the capillary tuft can be distinguished in exquisite detail (Fig. 2). Thus, micro-CT approaches may be able to replace electron microscopy

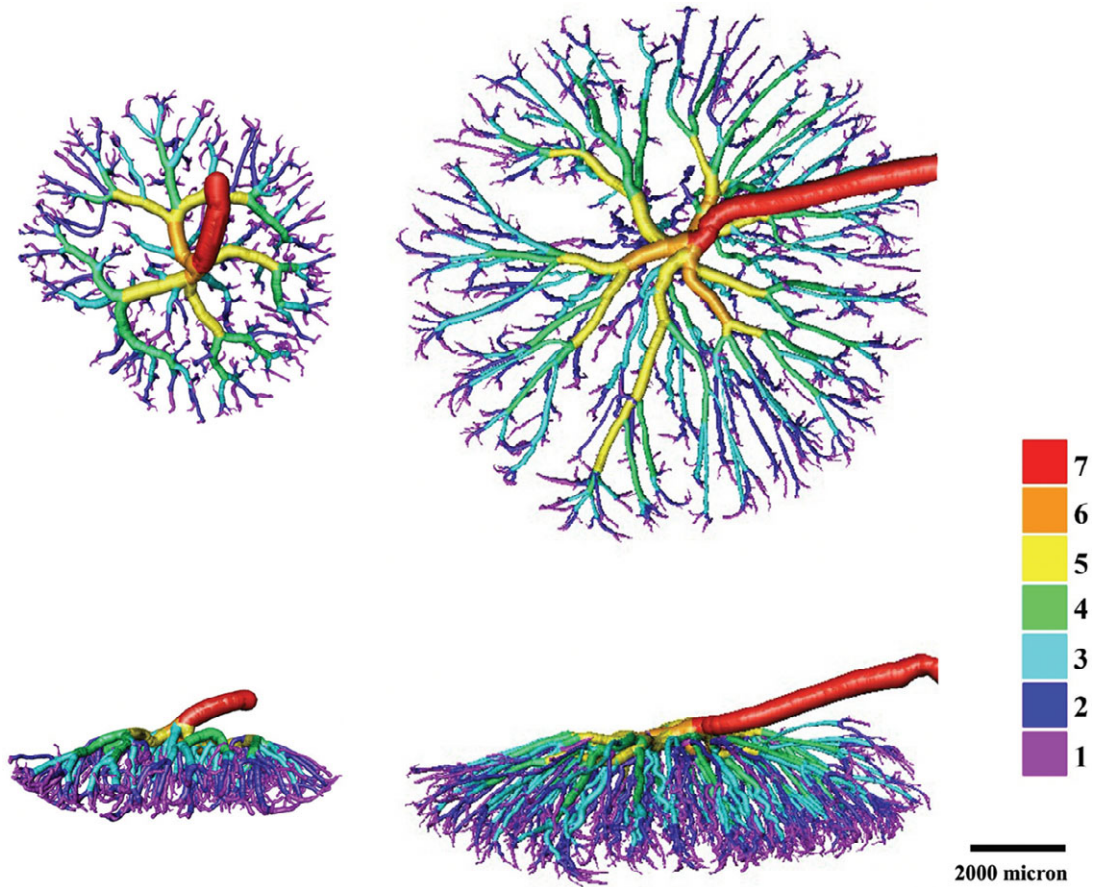


Fig. 1. Visualisation of a fetoplacental arterial network at the end of gestation in a mouse (left) and a rat (right). Colour rendering represents different levels of the fetoplacental vascular tree. Top row, superior view; bottom row, lateral view; scale bar, 2000 μm .

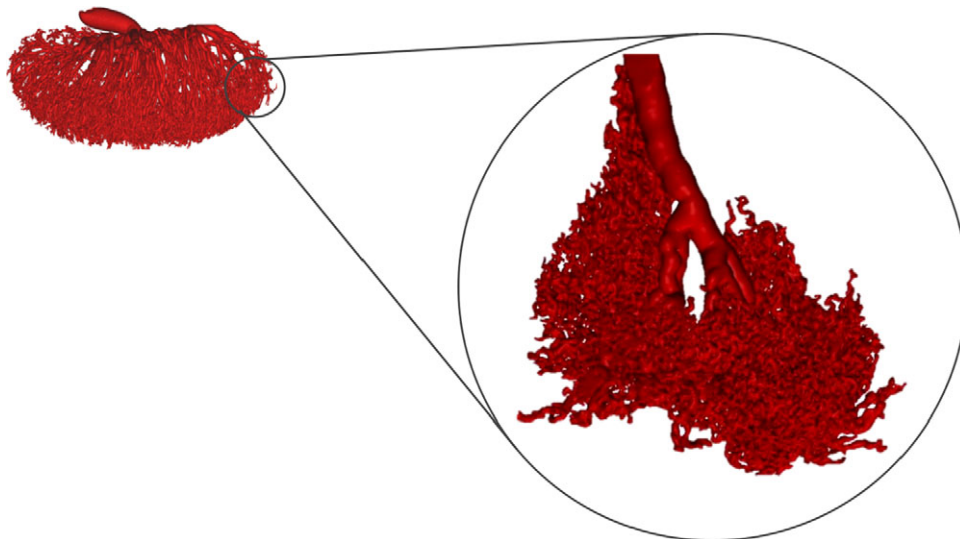


Fig. 2. High-resolution visualisation of the rat fetoplacental capillary tuft at the end of gestation.

and enable investigators a non-destructive way to image capillary networks in their experimental models. Moreover, contrast-enhanced micro-CT (work in progress in our laboratory) can provide potential novel opportunities to visualise greater detailed structures in placental vascular architecture. Iodine¹²⁴ and zirconium-substituted Keggin polyoxometalate⁹⁹ have also been used on mouse placenta and gross structures can be clearly visualised.

However, further work still needs to be developed to explore the full potential of contrast-enhanced micro-CT techniques for assessing placental vasculature.

Micro-CT is a quantitative 3D imaging technique that is advantageous for investigating 3D placental vascular networks. Evidently, the combination of the contrast agent casting technique and the micro-CT scanning can generate striking images of

feto-placental vascular geometry and connectivity in 3D. However, the methodology does involve technical challenges: the casting technique can be difficult, visualisation of data requires considerable computing power and quantitation can be challenging.

Light-sheet microscopy

Light-sheet microscopy has the potential to be a powerful tool for investigating feto-placental vasculature at a macro and micro scale, using either contrast agents or antibodies. However, to our knowledge, little work has been published on this with regard to the placenta. Light-sheet microscopes are traditionally built in-house, require significant computing power for visualisation of the images and optical clearing of the tissue (for a comprehensive review, see^{125,126}). Optical clearance can be challenging for a structure such as the mouse labyrinth zone which is dense with blood vessels. Light-sheet microscopes also traditionally have a relatively small field of view. However, there have been recent exciting developments for tissue clearance and the use of light-sheet microscopy in whole mice,¹²⁷ mouse eyes,¹²⁸ mouse hearts,¹²⁹ mouse colons,¹³⁰ mouse uterine walls,¹³¹ human brains¹³² and human fetal urogenital organs,¹³³ as well as comparison between different tissue types (i.e. bone, muscle and brain).¹³⁴ With these recent developments, there comes demands for new ways to visualise and quantitate these images. This is a rapidly emerging field and we anticipate that this method will add substantially to our understanding of placental vascular development.

Ultrasonography

None of the imaging modalities discussed thus far are able to assess the feto-placental unit in real time. Indeed, a key challenge for placental research is the ability to accurately monitor placental blood flow in real time, and in particular feto-placental blood flow *in vivo* with sufficient resolution to yield predictive and diagnostic data. Conventional 2D ultrasonography has been widely used as a primary imaging modality for the assessment of human placental structure and haemodynamics during pregnancy.^{135–137} Utero-placental vasculature can be identified towards the end of the first trimester,¹³⁸ and there have been recent striking developments in 3D ultrasonography to obtain human placental volumes and overlaying power Doppler ultrasound of the major vasculature.¹³⁹ Moreover, 3D ultrasound has been used to reveal placental volume and vascularity.^{140–144} These approaches provide not only the opportunity for a screening test for growth restriction but also novel insight into how placental vasculature informs future health outcomes.

With regard to experimental models, preclinical micro-ultrasound has provided useful insight into placental haemodynamics in models commonly used to understand early life origins of adult disease.^{18,52,101,102,145–152} For example, using preclinical high-resolution ultrasound, there is evidence for perturbed umbilical cord blood flow and velocity in 11 β -HSD2^{-/-} mice.¹⁸ However, this was ameliorated by pravastatin administration and also improved fetal cardiac function.¹⁸ Current resolution of the conventional 2D ultrasound is not adequate for evaluating 3D vascular complexity and fine branching structure. Therefore, the technique cannot investigate the entire extent to which vascular networks are altered in FGR and the downstream effects on fetal outcomes. However, colour Doppler and photoacoustics are emerging as valuable tools for understanding placental haemodynamic changes and structure in real time. At E14.5 in mouse pregnancy, the

utero-placental and feto-placental blood supply can be visualised including chorionic plate vessels and intra-placental arterioles.¹⁵³ Moreover, these assessments can be taken in conjunction with Doppler measures of fetal heart and other aspects of the fetal circulation including the aorta, inferior vena cava, ductus arteriosus, ductus venosus, inferior vena cava, carotid and pulmonary arteries.¹⁵³ Recently, photoacoustic technology has been utilised to image the human placenta in normal and pathologic pregnancy^{154,155} including maternal hypoxia and hyperoxygenation,¹⁵⁶ and pre-eclampsia.¹⁵⁷ These approaches will provide invaluable insight into how placental and fetal haemodynamic parameters in early life associate and inform adult cardiovascular outcomes.

Magnetic resonance imaging

The technology of MRI has advanced over the past few years to enable assessment of placental structure, blood flow and oxygenation. MRI is a non-invasive, *in vivo* 3D imaging modality that has been used for fetal and placental investigations and is emerging as a possible clinical tool. For instance, using MRI in obstetrics to assess human placental abnormalities^{158,159} and to predict fetal volume and birth weight.^{160–162} MRI has the advantage of capturing a large field of view, and thus full-specimen coverage, and, in the case of rodent models, multiple placentas.¹⁶³ However, in comparison to ultrasound and photoacoustics, there are significant limitations with translation of MRI studies to a clinical setting. These include machine accessibility outside of large clinical research centres, logistics for obese pregnant women undergoing an MRI and feasibility of using MRI to assess fetal oxygenation in high-risk pregnancies. The low resolution of MRI is also not suitable for detailed analysis of placental microvasculature and microcirculation.

Experimentally, MRI is used to reveal placental and fetal development across gestation. Blood oxygen level-dependent MRI (BOLD-MRI) can be used to evaluate changes in feto-placental oxygenation detecting changes in deoxyhaemoglobin concentration during a respiration challenge (i.e. T₂*). For example, BOLD-MRI has been used to measure changes in human placental oxygenation during maternal hyperoxia in normal pregnancies^{164,165} and FGR pregnancies.¹⁶⁶ Moreover, in rat models of FGR, there is a lower feto-placental response to maternal hyperoxygenation.^{167,168} Furthermore, dynamic contrast-enhanced MRI (DCE-MRI) with contrast agent intravenous injection (i.e. gadolinium chelate) has been used to quantify placental function such as blood volume, blood flow, oxygenation and perfusion rate in different placental compartments.¹⁶⁹ Recently, DCE-MRI has been used to investigate placental perfusion in hypoxic-induced FGR rodents,^{170–172} pre-eclamptic rats (induced by continuous delivery of L-nitro-arginine methyl ester to chronically inhibit nitric oxide synthase)¹⁷³ and prenatal alcohol exposure in monkeys.¹⁵¹ Placental blood flow and perfusion rate were reduced significantly in the animal models of hypoxia and ethanol exposure^{151,170,171} but not in a model of pre-eclampsia.¹⁷³ A combination of DCE-MRI and fluorescence imaging has also been used to delineate and quantify the volume of maternal and fetal compartments in mouse placenta.¹⁷⁴ Therefore, both BOLD-MRI and DCE-MRI can be used for *in vivo* 3D assessment of placental and fetal function, blood flow and oxygenation. While these approaches are highly useful in an experimental context, it remains to be seen if MRI will transition to being standard use clinically.

Future considerations and opportunities

The capacity to image the placenta, and in particular the fetoplacental vasculature, brings opportunities to reconsider models of developmental programming from a different perspective. Blood flow plays a critical role in determining heart and vascular development, yet haemodynamic placental influences have been neglected in consideration as a determinant of adult cardiovascular disease. Through imaging of established experimental models, the development of more specific models to tease out the placental-cardiac axis, and modelling approaches, new context for the early life origins of adult cardiovascular disease should emerge in the next few years. This work should also provoke possibilities of whether placental blood flow can be modified to attenuate cardiovascular disease.

There is no doubt that the various imaging approaches to visualise fetoplacental vasculature have yielded useful perspectives for placental and fetal biology. However, with the rapid advances in imaging techniques, there comes the need for computational power and technology to acquire, store, display, visualise and analyse such complex images. To advance this field, it is critical that an interdisciplinary research community is developed to collaborate not only on imaging techniques but also on software and computation resources. Alongside reaching consensus on best practice and standardisation, making open-source software available to the whole research community will be pivotal.

Though beyond the scope of this review, it is important to highlight the rapid emergence of modelling approaches in fetoplacental vasculature. These have been made possible due to the technical advances in imaging techniques and have enabled several groups to utilise either engineering or mathematical modelling approaches in order to couple structure with function.^{52,74,94,96,100–102,107,145,175–185} The *ex vivo* dual perfusion of the human placenta, *in vivo* MRI and ultrasonography (including photoacoustics) being developed in parallel will provide rich means of validation for computational models in predicting placental function, particularly oxygen transfer. This interdisciplinary placental research community is thus at a juncture of developing some important modelling tools for clinical application to help predict and manage complicated pregnancy, including FGR. Moreover, these tools will be invaluable in understanding the influence of early life environment on development, in particular the haemodynamic implications of altered placental vascular structure on fetal tissues, and how adverse health outcomes in later life may be prevented.

Conclusions

Placental function and structure in relation to DOHaD are still poorly understood, particularly in how fetoplacental vascular structure affects placental function and fetal cardiovascular development. Although multiple imaging modalities can be used to assess fetoplacental structure, each has its limitations. With the new array of imaging techniques, technology, software, computation development and modelling approaches becoming available, the field can move forward at a rapid pace. However, the speed of progress will rely on interdisciplinary and cross-research group collaborations, particularly in establishing open access ways to visualise and analyse data. Through this, the field is poised to deepen the understanding of placental development and function, and fetal health outcomes in early and adult life.

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References

- Gardosi J, Madurasinghe V, Williams M, Malik A, Francis A. Maternal and fetal risk factors for stillbirth: population based study. *BMJ*. 2013; 346, f108.
- Barker D, Osmond C, Grant S, *et al*. Maternal cotyledons at birth predict blood pressure in childhood. *Placenta*. 2013; 34(8), 672–675.
- Thornburg KL, O'Tierney PF, Louey S. Review: the placenta is a programming agent for cardiovascular disease. *Placenta*. 2010; 31, S54–S59.
- Barker DJ, Thornburg KL, Osmond C, Kajantie E, Eriksson JG. The surface area of the placenta and hypertension in the offspring in later life. *Int J Dev Biol*. 2010; 54(2–3), 525–530.
- Burton GJ, Fowden AL, Thornburg KL. Placental origins of chronic disease. *Physiol Rev*. 2016; 96(4), 1509–1565.
- Junaid TO, Bradley RS, Lewis RM, Aplin JD, Johnstone ED. Whole organ vascular casting and microCT examination of the human placental vascular tree reveals novel alterations associated with pregnancy disease. *Sci Rep*. 2017; 7(1), 4144.
- Junaid TO, Brownbill P, Chalmers N, Johnstone ED, Aplin JD. Fetoplacental vascular alterations associated with fetal growth restriction. *Placenta*. 2014; 35(10), 808–815.
- Langheinrich AC, Vormann S, Seidenstucker J, *et al*. Quantitative 3D micro-CT imaging of the human fetoplacental vasculature in intrauterine growth restriction. *Placenta*. 2008; 29(11), 937–941.
- Fisher AB, Chien S, Barakat AI, Nerem RM. Endothelial cellular response to altered shear stress. *Am J Physiol Lung Cell Mol Physiol*. 2001; 281(3), L529–L533.
- Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev*. 1995; 75(3), 519–560.
- Langille BL. Arterial remodeling: relation to hemodynamics. *Can J Physiol Pharmacol*. 1996; 74(7), 834–841.
- Rudolph AM. Hepatic and ductus venosus blood flows during fetal life. *Hepatology*. 1983; 3(2), 254–258.
- Courchaine K, Rykiel G, Rugonyi S. Influence of blood flow on cardiac development. *Prog Biophys Mol Biol*. 2018; 137, 95–110.
- Perez-Garcia V, Fineberg E, Wilson R, *et al*. Placentation defects are highly prevalent in embryonic lethal mouse mutants. *Nature*. 2018; 555(7697), 463–468.
- Maslen CL. Recent advances in placenta-heart interactions. *Front Physiol*. 2018; 9, 735.
- Crispi F, Miranda J, Gratacos E. Long-term cardiovascular consequences of fetal growth restriction: biology, clinical implications, and opportunities for prevention of adult disease. *Am J Obstet Gynecol*. 2018; 218(2S), S869–S879.
- Camm EJ, Botting KJ, Sferruzzi-Perri AN. Near to One's Heart: The Intimate Relationship Between the Placenta and Fetal Heart. *Front Physiol*. 2018; 9, 629.

18. Wyrwoll CS, Noble J, Thomson A, *et al.* Pravastatin ameliorates placental vascular defects, fetal growth, and cardiac function in a model of glucocorticoid excess. *Proc Natl Acad Sci U S A.* 2016; 113(22), 6265–6270.
19. Shen H, Cavallero S, Estrada KD, *et al.* Extracardiac control of embryonic cardiomyocyte proliferation and ventricular wall expansion. *Cardiovasc Res.* 2015; 105(3), 271–278.
20. Linask KK. The heart-placenta axis in the first month of pregnancy: induction and prevention of cardiovascular birth defects. *J Pregnancy.* 2013; 2013, 320413.
21. Gessert S, Kuhl M. The multiple phases and faces of Wnt signaling during cardiac differentiation and development. *Circ Res.* 2010; 107(2), 186–199.
22. Shaut CA, Keene DR, Sorensen LK, Li DY, Stadler HS. HOXA13 Is essential for placental vascular patterning and labyrinth endothelial specification. *PLoS Genet.* 2008; 4(5), e1000073.
23. Hemberger M, Cross JC. Genes governing placental development. *Trends Endocrinol Metab.* 2001; 12(4), 162–168.
24. Adams RH, Porras A, Alonso G, *et al.* Essential role of p38alpha MAP kinase in placental but not embryonic cardiovascular development. *Mol Cell.* 2000; 6(1), 109–116.
25. Barak Y, Nelson MC, Ong ES, *et al.* PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol Cell.* 1999; 4(4), 585–595.
26. Kiserud T, Ebbing C, Kessler J, Rasmussen S. Fetal cardiac output, distribution to the placenta and impact of placental compromise. *Ultrasound Obstet Gynecol.* 2006; 28(2), 126–136.
27. Kaufmann P, Bruns U, Leiser R, Luckhardt M, Winterhager E. The fetal vascularisation of term human placental villi. II. Intermediate and terminal villi. *Anat Embryol (Berl).* 1985; 173(2), 203–214.
28. Kaufmann P, Sen DK, Schweikhart G. Classification of human placental villi. I. Histology. *Cell Tissue Res.* 1979; 200(3), 409–423.
29. Sen DK, Kaufmann P, Schweikhart G. Classification of human placental villi. II. Morphometry. *Cell Tissue Res.* 1979; 200(3), 425–434.
30. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. *Philos Trans R Soc Lond B Biol Sci.* 2015; 370(1663), 20140066.
31. Wang Y, Zhao S. In *Vascular Biology of the Placenta*, 2010. Morgan & Claypool Life Sciences, San Rafael (CA).
32. Wooding P, Burton G. *Comparative placentation: structures, functions and evolution.* 2008; pp. 1–309. Springer, Verlag Berlin Heidelberg, Germany.
33. Furukawa S, Kuroda Y, Sugiyama A. A comparison of the histological structure of the placenta in experimental animals. *J Toxicol Pathol.* 2014; 27(1), 11–18.
34. Leiser R, Kaufmann P. Placental structure: in a comparative aspect. *Exp Clin Endocrinol.* 1994; 102(3), 122–134.
35. Vercruyse L, Caluwaerts S, Luyten C, Pijnenborg R. Interstitial trophoblast invasion in the decidua and mesometrial triangle during the last third of pregnancy in the rat. *Placenta.* 2006; 27(1), 22–33.
36. Georgiades P, Ferguson-Smith AC, Burton GJ. Comparative developmental anatomy of the murine and human definitive placentae. *Placenta.* 2002; 23(1), 3–19.
37. Matt DW, Macdonald GJ. Placental steroid production by the basal and labyrinth zones during the latter third of gestation in the rat. *Biol Reprod.* 1985; 32(4), 969–977.
38. Rossant J, Cross JC. Placental development: lessons from mouse mutants. *Nat Rev Genet.* 2001; 2(7), 538–548.
39. Soares MJ, Chakraborty D, Karim Rumi MA, Konno T, Renaud SJ. Rat placentation: an experimental model for investigating the hemochorial maternal-fetal interface. *Placenta.* 2012; 33(4), 233–243.
40. Adamson SL, Lu Y, Whiteley KJ, *et al.* Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. *Dev Biol.* 2002; 250(2), 358–373.
41. Pijnenborg R, Robertson WB, Brosens I, Dixon G. Review article: trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. *Placenta.* 1981; 2(1), 71–91.
42. Higgins M, Felle P, Mooney EE, Bannigan J, McAuliffe FM. Stereology of the placenta in type 1 and type 2 diabetes. *Placenta.* 2011; 32(8), 564–569.
43. Mayhew TM, Manwani R, Ohadike C, Wijesekera J, Baker PN. The placenta in pre-eclampsia and intrauterine growth restriction: studies on exchange surface areas, diffusion distances and villous membrane diffusive conductances. *Placenta.* 2007; 28(2–3), 233–238.
44. Mayhew TM, Ohadike C, Baker PN, Crocker IP, Mitchell C, Ong SS. Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. *Placenta.* 2003; 24(2–3), 219–226.
45. Mayhew TM. Recent applications of the new stereology have thrown fresh light on how the human placenta grows and develops its form. *J Microsc.* 1997; 186(Pt 2), 153–163.
46. Burton GJ, Reshetnikova OS, Milovanov AP, Teleshova OV. Stereological evaluation of vascular adaptations in human placental villi to differing forms of hypoxic stress. *Placenta.* 1996; 17(1), 49–55.
47. Jackson MR, Mayhew TM, Boyd PA. Quantitative description of the elaboration and maturation of villi from 10 weeks of gestation to term. *Placenta.* 1992; 13(4), 357–370.
48. Nuzzo AM, Camm EJ, Sferruzzi-Perri AN, *et al.* Placental adaptation to early-onset hypoxic pregnancy and mitochondria-targeted antioxidant therapy in a rodent model. *Am J Pathol.* 2018; 188(12), 2704–2716.
49. Higgins JS, Vaughan OR, Fernandez de Liger E, Fowden AL, Sferruzzi-Perri AN. Placental phenotype and resource allocation to fetal growth are modified by the timing and degree of hypoxia during mouse pregnancy. *J Physiol.* 2016; 594(5), 1341–1356.
50. Sferruzzi-Perri AN, Lopez-Tello J, Fowden AL, Constanica M. Maternal and fetal genomes interplay through phosphoinositol 3-kinase(PI3K)-p110alpha signaling to modify placental resource allocation. *Proc Natl Acad Sci U S A.* 2016; 113(40), 11255–11260.
51. Vaughan OR, Sferruzzi-Perri AN, Coan PM, Fowden AL. Adaptations in placental phenotype depend on route and timing of maternal dexamethasone administration in mice. *Biol Reprod.* 2013; 89(4), 80.
52. Rennie MY, Detmar J, Whiteley KJ, Jurisicova A, Adamson SL, Sled JG. Expansion of the fetoplacental vasculature in late gestation is strain dependent in mice. *Am J Physiol Heart Circ Physiol.* 2012; 302(6), H1261–H1273.
53. Coan PM, Vaughan OR, Sekita Y, *et al.* Adaptations in placental phenotype support fetal growth during undernutrition of pregnant mice. *J Physiol.* 2010; 588(Pt 3), 527–538.
54. Wyrwoll CS, Seckl JR, Holmes MC. Altered placental function of 11beta-hydroxysteroid dehydrogenase 2 knockout mice. *Endocrinology.* 2009; 150(3), 1287–1293.
55. Coan PM, Angiolini E, Sandovici I, Burton GJ, Constanica M, Fowden AL. Adaptations in placental nutrient transfer capacity to meet fetal growth demands depend on placental size in mice. *J Physiol.* 2008; 586(18), 4567–4576.
56. Veras MM, Damaceno-Rodrigues NR, Caldini EG, *et al.* Particulate urban air pollution affects the functional morphology of mouse placenta. *Biol Reprod.* 2008; 79(3), 578–584.
57. Coan PM, Fowden AL, Constanica M, Ferguson-Smith AC, Burton GJ, Sibley CP. Disproportional effects of Igf2 knockout on placental morphology and diffusional exchange characteristics in the mouse. *J Physiol.* 2008; 586(20), 5023–5032.
58. Rutland CS, Latunde-Dada AO, Thorpe A, Plant R, Langley-Evans S, Leach L. Effect of gestational nutrition on vascular integrity in the murine placenta. *Placenta.* 2007; 28(7), 734–742.
59. Coan PM, Ferguson-Smith AC, Burton GJ. Ultrastructural changes in the interhaemal membrane and junctional zone of the murine chorioallantoic placenta across gestation. *J Anat.* 2005; 207(6), 783–796.
60. Sibley CP, Coan PM, Ferguson-Smith AC, *et al.* Placental-specific insulin-like growth factor 2 (Igf2) regulates the diffusional exchange characteristics of the mouse placenta. *Proc Natl Acad Sci U S A.* 2004; 101(21), 8204–8208.
61. Coan PM, Ferguson-Smith AC, Burton GJ. Developmental dynamics of the definitive mouse placenta assessed by stereology. *Biol Reprod.* 2004; 70(6), 1806–1813.

62. Napso T, Hung YP, Davidge ST, Care AS, Sferruzzi-Perri AN. Advanced maternal age compromises fetal growth and induces sex-specific changes in placental phenotype in rats. *Sci Rep*. 2019; 9(1), 16916.
63. Serman L, Zunic I, Vrsaljko N, *et al*. Structural changes in the rat placenta during the last third of gestation discovered by stereology. *Bosn J Basic Med Sci*. 2015; 15(1), 21–25.
64. Hewitt DP, Mark PJ, Waddell BJ. Glucocorticoids prevent the normal increase in placental vascular endothelial growth factor expression and placental vascularity during late pregnancy in the rat. *Endocrinology*. 2006; 147(12), 5568–5574.
65. Wislocki GB, Dempsey EW. Electron microscopy of the placenta of the rat. *Anat Rec*. 1955; 123(1), 33–63.
66. Dempsey EW, Wislocki GB, Amoroso EC. Electron microscopy of the pig's placenta, with especial reference to the cell membranes of the endometrium and chorion. *Am J Anat*. 1955; 96(1), 65–101.
67. Wislocki GB, Dempsey EW. Electron microscopy of the human placenta. *Anat Rec*. 1955; 123(2), 133–167.
68. Lee MM, Yeh MN. Fetal circulation of the placenta: a comparative study of human and baboon placenta by scanning electron microscopy of vascular casts. *Placenta*. 1983; 4 Spec No, 515–526.
69. Krebs C, Winther H, Dantzer V, Leiser R. Vascular interrelationships of near-term mink placenta: light microscopy combined with scanning electron microscopy of corrosion casts. *Microsc Res Tech*. 1997; 38(1–2), 125–136.
70. Pfarrer C, Winther H, Leiser R, Dantzer V. The development of the endotheliochorial mink placenta: light microscopy and scanning electron microscopical morphometry of maternal vascular casts. *Anat Embryol (Berl)*. 1999; 199(1), 63–74.
71. Macdonald AA, Chavatte P, Fowden AL. Scanning electron microscopy of the microcotyledonary placenta of the horse (*Equus caballus*) in the latter half of gestation. *Placenta*. 2000; 21(5–6), 565–574.
72. Detmar J, Rennie MY, Whiteley KJ, *et al*. Fetal growth restriction triggered by polycyclic aromatic hydrocarbons is associated with altered placental vasculature and AhR-dependent changes in cell death. *Am J Physiol Endocrinol Metab*. 2008; 295(2), E519–530.
73. Whiteley KJ, Pfarrer CD, Adamson SL. Vascular corrosion casting of the uteroplacental and fetoplacental vasculature in mice. *Methods Mol Med*. 2006; 121, 371–392.
74. Cahill LS, Rennie MY, Hoggarth J, *et al*. Feto- and utero-placental vascular adaptations to chronic maternal hypoxia in the mouse. *J Physiol*. 2018; 596(15), 3285–3297.
75. Bobinski R, Pieliesz A, Waksmanska W, *et al*. Surface structure changes of pathological placenta tissue observed using scanning electron microscopy - a pilot study. *Acta Biochim Pol*. 2017; 64(3), 533–535.
76. Baykal C, Sargon MF, Esinler I, Onderoglu S, Onderoglu L. Placental microcirculation of intrauterine growth retarded fetuses: scanning electron microscopy of placental vascular casts. *Arch Gynecol Obstet*. 2004; 270(2), 99–103.
77. Krebs C, Macara LM, Leiser R, Bowman AW, Greer IA, Kingdom JC. Intrauterine growth restriction with absent end-diastolic flow velocity in the umbilical artery is associated with maldevelopment of the placental terminal villous tree. *Am J Obstet Gynecol*. 1996; 175(6), 1534–1542.
78. Burton GJ. Scanning electron microscopy of intervillous connections in the mature human placenta. *J Anat*. 1986; 147, 245–254.
79. Burton GJ. The fine structure of the human placental villus as revealed by scanning electron microscopy. *Scanning Microsc*. 1987; 1(4), 1811–1828.
80. Demir R, Demir N, Ustunel I, Erbenli T, Trak I, Kaufmann P. The fine structure of normal and ectopic (tubal) human placental villi as revealed by scanning and transmission electron microscopy. *Zentralbl Pathol*. 1995; 140(6), 427–442.
81. Palaiologou E, Etter O, Goggin P, *et al*. Human placental villi contain stromal macrovesicles associated with networks of stellate cells. *J Anat*. 2020; 236(1), 132–141.
82. Lee MM, Yeh MN. Fetal microcirculation of abnormal human placenta. I. Scanning electron microscopy of placental vascular casts from small for gestational age fetus. *Am J Obstet Gynecol*. 1986; 154(5), 1133–1139.
83. Lee MM, Yeh MN. Fetal microcirculation of abnormal human placenta. II. Scanning electron microscopy of placental vascular casts from fetus with severe erythroblastosis fetalis. *Am J Obstet Gynecol*. 1986; 154(5), 1139–1146.
84. Leiser R, Krebs C, Ebert B, Dantzer V. Placental vascular corrosion cast studies: a comparison between ruminants and humans. *Microsc Res Tech*. 1997; 38(1–2), 76–87.
85. Sooranna SR, Oteng-Ntim E, Meah R, Ryder TA, Bajoria R. Characterization of human placental explants: morphological, biochemical and physiological studies using first and third trimester placenta. *Hum Reprod*. 1999; 14(2), 536–541.
86. Cronqvist T, Tannetta D, Morgelin M, *et al*. Syncytiotrophoblast derived extracellular vesicles transfer functional placental miRNAs to primary human endothelial cells. *Sci Rep*. 2017; 7(1), 4558.
87. Abdel Salam GA, Alam OA, Ahmed UF, Al-Sherbeny MF. Light and electron microscopic study of placenta in pre-eclampsia: a trial to define underlying changes and its clinical impact. *Tanta Medical Journal*. 2015; 43(4), 134–145.
88. Sricharoenvej S, Tongpob Y, Lanlua P, Piyawinijwong S, Roongruangchai J, Phoungpetchara I. Renal microvascular changes in streptozotocin-induced, long-termed diabetic rat. *J Med Assoc Thai*. 2007; 90(12), 2677–2682.
89. Bamroongwong S, Somana R, Rojananeungnit S, Chunhabundit P, Rattanachaiakunsopon P. Scanning electron microscopic study of the splenic vascular casts in common tree shrew (*Tupaia glis*). *Anat Embryol (Berl)*. 1991; 184(3), 301–304.
90. Liu YC, Chiang AS. High-resolution confocal imaging and three-dimensional rendering. *Methods*. 2003; 30(1), 86–93.
91. Lisman BA, van den Hoff MJ, Boer K, Bleker OP, van Groningen K, Exalto N. The architecture of first trimester chorionic villous vascularization: a confocal laser scanning microscopical study. *Hum Reprod*. 2007; 22(8), 2254–2260.
92. Merz G, Schwenk V, Shah RG, Necaie P, Salafia CM. Clarification and 3-D visualization of immunolabeled human placenta villi. *Placenta*. 2017; 53, 36–39.
93. Plitman Mayo R, Abbas Y, Charnock-Jones DS, Burton GJ, Marom G. Three-dimensional morphological analysis of placental terminal villi. *Interface Focus*. 2019; 9(5), 20190037.
94. Perazzolo S, Lewis RM, Sengers BG. Modelling the effect of intervillous flow on solute transfer based on 3D imaging of the human placental microstructure. *Placenta*. 2017; 60, 21–27.
95. Jirkovska M, Janacek J, Kalab J, Kubinova L. Three-dimensional arrangement of the capillary bed and its relationship to microrheology in the terminal villi of normal term placenta. *Placenta*. 2008; 29(10), 892–897.
96. Plitman Mayo R, Charnock-Jones DS, Burton GJ, Oyen ML. Three-dimensional modeling of human placental terminal villi. *Placenta*. 2016; 43, 54–60.
97. Jirkovska M, Kucera T, Kalab J, *et al*. The branching pattern of villous capillaries and structural changes of placental terminal villi in type 1 diabetes mellitus. *Placenta*. 2012; 33(5), 343–351.
98. Sargent JA, Roberts V, Gaffney JE, Frias AE. Clarification and confocal imaging of the nonhuman primate placental micro-anatomy. *Biotechniques*. 2019; 66(2), 79–84.
99. De Clercq K, Persoons E, Napso T, *et al*. High-resolution contrast-enhanced microCT reveals the true three-dimensional morphology of the murine placenta. *Proc Natl Acad Sci U S A*. 2019; 116(28), 13927–13936.
100. Rennie MY, Cahill LS, Adamson SL, Sled JG. Arterio-venous fetoplacental vascular geometry and hemodynamics in the mouse placenta. *Placenta*. 2017; 58, 46–51.
101. Rennie MY, Rahman A, Whiteley KJ, Sled JG, Adamson SL. Site-specific increases in utero- and fetoplacental arterial vascular resistance in

- eNOS-deficient mice due to impaired arterial enlargement. *Biol Reprod.* 2015; 92(2), 48.
102. Rennie MY, Detmar J, Whiteley KJ, *et al.* Vessel tortuosity and reduced vascularization in the fetoplacental arterial tree after maternal exposure to polycyclic aromatic hydrocarbons. *Am J Physiol Heart Circ Physiol.* 2011; 300(2), H675–H684.
 103. Mohammadi H, Papp E, Cahill L, *et al.* HIV antiretroviral exposure in pregnancy induces detrimental placenta vascular changes that are rescued by progesterone supplementation. *Sci Rep.* 2018; 8(1), 6552.
 104. Chi L, Ahmed A, Roy AR, *et al.* G9a controls placental vascular maturation by activating the Notch Pathway. *Development.* 2017; 144(11), 1976–1987.
 105. Yang J, Yu LX, Rennie MY, Sled JG, Henkelman RM. Comparative structural and hemodynamic analysis of vascular trees. *Am J Physiol Heart Circ Physiol.* 2010; 298(4), H1249–H1259.
 106. Conroy AL, Silver KL, Zhong K, *et al.* Complement activation and the resulting placental vascular insufficiency drives fetal growth restriction associated with placental malaria. *Cell Host Microbe.* 2013; 13(2), 215–226.
 107. Bappoo N, Kelsey LJ, Parker L, *et al.* Viscosity and haemodynamics in a late gestation rat fetoplacental arterial network. *Biomech Model Mechanobiol.* 2017; 16(4), 1361–1372.
 108. Tongpob Y, Xia S, Wyrwoll C, Mehnert A. Quantitative characterization of rodent fetoplacental vasculature morphology in micro-computed tomography images. *Comput Methods Programs Biomed.* 2019; 179, 104984.
 109. Pratt R, Hutchinson JC, Melbourne A, *et al.* Imaging the human placental microcirculation with micro-focus computed tomography: optimisation of tissue preparation and image acquisition. *Placenta.* 2017; 60, 36–39.
 110. Aughwane R, Schaaf C, Hutchinson JC, *et al.* Micro-CT and histological investigation of the spatial pattern of fetoplacental vascular density. *Placenta.* 2019; 88, 36–43.
 111. Langheinrich AC, Wienhard J, Vormann S, Hau B, Bohle RM, Zygmunt M. Analysis of the fetal placental vascular tree by X-ray micro-computed tomography. *Placenta.* 2004; 25(1), 95–100.
 112. Vasquez SX, Gao F, Su F, *et al.* Optimization of microCT imaging and blood vessel diameter quantitation of preclinical specimen vasculature with radiopaque polymer injection medium. *PLoS One.* 2011; 6(4), e19099.
 113. Ghanavati S, Yu LX, Lerch JP, Sled JG. A perfusion procedure for imaging of the mouse cerebral vasculature by X-ray micro-CT. *J Neurosci Methods.* 2014; 221, 70–77.
 114. Chugh BP, Lerch JP, Yu LX, *et al.* Measurement of cerebral blood volume in mouse brain regions using micro-computed tomography. *Neuroimage.* 2009; 47(4), 1312–1318.
 115. Jorgensen SM, Demirkaya O, Ritman EL. Three-dimensional imaging of vasculature and parenchyma in intact rodent organs with X-ray micro-CT. *Am J Physiol.* 1998; 275(3), H1103–H1114.
 116. Kampschulte M, Schneider CR, Litzlbauer HD, *et al.* Quantitative 3D micro-CT imaging of human lung tissue. *Rofo.* 2013; 185(9), 869–876.
 117. Op Den Buijs J, Bajzer Z, Ritman EL. Branching morphology of the rat hepatic portal vein tree: a micro-CT study. *Ann Biomed Eng.* 2006; 34(9), 1420–1428.
 118. Ananda S, Marsden V, Vekemans K, *et al.* The visualization of hepatic vasculature by X-ray micro-computed tomography. *J Electron Microscop.* (Tokyo). 2006; 55(3), 151–155.
 119. Kline TL, Zamir M, Ritman EL. Relating function to branching geometry: a micro-CT study of the hepatic artery, portal vein, and biliary tree. *Cells Tissues Organs.* 2011; 194(5), 431–442.
 120. Nordsletten DA, Blackett S, Bentley MD, Ritman EL, Smith NP. Structural morphology of renal vasculature. *Am J Physiol Heart Circ Physiol.* 2006; 291(1), H296–H309.
 121. Bentley MD, Ortiz MC, Ritman EL, Romero JC. The use of microcomputed tomography to study microvasculature in small rodents. *Am J Physiol Regul Integr Comp Physiol.* 2002; 282(5), R1267–1279.
 122. Garcia-Sanz A, Rodriguez-Barbero A, Bentley MD, Ritman EL, Romero JC. Three-dimensional microcomputed tomography of renal vasculature in rats. *Hypertension.* 1998; 31(1 Pt 2), 440–444.
 123. Marxen M, Thornton MM, Chiarot CB, *et al.* MicroCT scanner performance and considerations for vascular specimen imaging. *Med Phys.* 2004; 31(2), 305–313.
 124. Zhou YQ, Cahill LS, Wong MD, Seed M, Macgowan CK, Sled JG. Assessment of flow distribution in the mouse fetal circulation at late gestation by high-frequency Doppler ultrasound. *Physiol Genomics.* 2014; 46(16), 602–614.
 125. Power RM, Huisken J. A guide to light-sheet fluorescence microscopy for multiscale imaging. *Nat Methods.* 2017; 14(4), 360–373.
 126. Reynaud EG, Peychl J, Huisken J, Tomancak P. Guide to light-sheet microscopy for adventurous biologists. *Nat Methods.* 2015; 12(1), 30–34.
 127. Cai R, Pan C, Ghasemigharagoz A, *et al.* Panoptic imaging of transparent mice reveals whole-body neuronal projections and skull-meninges connections. *Nat Neurosci.* 2019; 22(2), 317–327.
 128. Henning Y, Osadnik C, Malkemper EP. EyeCi: optical clearing and imaging of immunolabeled mouse eyes using light-sheet fluorescence microscopy. *Exp Eye Res.* 2019; 180, 137–145.
 129. Ding Y, Bailey Z, Messerschmidt V, *et al.* Light-sheet fluorescence microscopy for the study of the murine heart. *J Vis Exp.* 2018; 139, e57769.
 130. Li T, Hui H, Hu C, Ma H, Yang X, Tian J. Multiscale imaging of colitis in mice using confocal laser endomicroscopy, light-sheet fluorescence microscopy, and magnetic resonance imaging. *J Biomed Opt.* 2019; 24(1), 1–8.
 131. Kagami K, Shinmyo Y, Ono M, Kawasaki H, Fujiwara H. Three-dimensional visualization of intrauterine conceptus through the uterine wall by tissue clearing method. *Sci Rep.* 2017; 7(1), 5964.
 132. Mano T, Albanese A, Dodt HU, *et al.* Whole-brain analysis of cells and circuits by tissue clearing and light-sheet microscopy. *J Neurosci.* 2018; 38(4), 9330–9337.
 133. Isaacson D, McCreedy D, Calvert M, *et al.* Imaging the developing human external and internal urogenital organs with light sheet fluorescence microscopy. *Differentiation.* 2019; 111, 12–21.
 134. Buytaert J, Goyens J, De Greef D, Aerts P, Dirckx J. Volume shrinkage of bone, brain and muscle tissue in sample preparation for micro-CT and light sheet fluorescence microscopy (LSFM). *Microsc Microanal.* 2014; 20(4), 1208–1217.
 135. Abramowicz JS, Sheiner E. Ultrasound of the placenta: a systematic approach. Part I: imaging. *Placenta.* 2008; 29(3), 225–240.
 136. Abramowicz JS, Sheiner E. Ultrasound of the placenta: a systematic approach. Part II: functional assessment (Doppler). *Placenta.* 2008; 29(11), 921–929.
 137. Kingdom JC, Audette MC, Hobson SR, Windrim RC, Morgen E. A placenta clinic approach to the diagnosis and management of fetal growth restriction. *Am J Obstet Gynecol.* 2018; 218(2S), S803–S817.
 138. Collins SL, Birks JS, Stevenson GN, Papageorghiou AT, Noble JA, Impey L. Measurement of spiral artery jets: general principles and differences observed in small-for-gestational-age pregnancies. *Ultrasound Obstet Gynecol.* 2012; 40(2), 171–178.
 139. Mathewlynn S, Collins SL. Volume and vascularity: using ultrasound to unlock the secrets of the first trimester placenta. *Placenta.* 2019; 84, 32–36.
 140. Mulcahy C, Mone F, McParland P, *et al.* The impact of aspirin on ultrasound markers of uteroplacental flow in low-risk pregnancy: secondary analysis of a multicenter RCT. *Am J Perinatol.* 2019; 36(8), 855–863.
 141. Stevenson GN, Noble JA, Welsh AW, Impey L, Collins SL. Automated visualization and quantification of spiral artery blood flow entering the first-trimester placenta, using 3-D power Doppler ultrasound. *Ultrasound Med Biol.* 2018; 44(3), 522–531.
 142. Costa J, Rice H, Cardwell C, Hunter A, Ong S. An assessment of vascularity and flow intensity of the placenta in normal pregnancy and pre-eclampsia using three-dimensional ultrasound. *J Matern Fetal Neonatal Med.* 2010; 23(8), 894–899.

143. Hata T, Tanaka H, Noguchi J, Hata K. Three-dimensional ultrasound evaluation of the placenta. *Placenta*. 2011; 32(2), 105–115.
144. Looney P, Stevenson GN, Nicolaides KH, *et al*. Fully automated, real-time 3D ultrasound segmentation to estimate first trimester placental volume using deep learning. *JCI Insight*. 2018; 3(11), e120178.
145. Lin M, Mauroy B, James JL, Tawhai MH, Clark AR. A multiscale model of placental oxygen exchange: the effect of villous tree structure on exchange efficiency. *J Theor Biol*. 2016; 408, 1–12.
146. Yu Y, Yang O, Fazli L, Rennie PS, Gleave ME, Dong X. Progesterone receptor expression during prostate cancer progression suggests a role of this receptor in stromal cell differentiation. *Prostate*. 2015; 75(10), 1043–1050.
147. Morris RK, Malin G, Robson SC, Kleijnen J, Zamora J, Khan KS. Fetal umbilical artery Doppler to predict compromise of fetal/neonatal wellbeing in a high-risk population: systematic review and bivariate meta-analysis. *Ultrasound Obstet Gynecol*. 2011; 37(2), 135–142.
148. Salati JA, Roberts VHJ, Schabel MC, *et al*. Maternal high-fat diet reversal improves placental hemodynamics in a nonhuman primate model of diet-induced obesity. *Int J Obes (Lond)*. 2019; 43(4), 906–916.
149. Rahman A, Zhou YQ, Yee Y, *et al*. Ultrasound detection of altered placental vascular morphology based on hemodynamic pulse wave reflection. *Am J Physiol Heart Circ Physiol*. 2017; 312(5), H1021–H1029.
150. Hernandez-Andrade E, Ahn H, Szalai G, *et al*. Evaluation of utero-placental and fetal hemodynamic parameters throughout gestation in pregnant mice using high-frequency ultrasound. *Ultrasound Med Biol*. 2014; 40(2), 351–360.
151. Lo JO, Schabel MC, Roberts VH, *et al*. First trimester alcohol exposure alters placental perfusion and fetal oxygen availability affecting fetal growth and development in a non-human primate model. *Am J Obstet Gynecol*. 2017; 216(3), e301–e302, e308.
152. Lopez-Tello J, Barbero A, Gonzalez-Bulnes A, *et al*. Characterization of early changes in fetoplacental hemodynamics in a diet-induced rabbit model of IUGR. *J Dev Orig Health Dis*. 2015; 6(5), 454–461.
153. Foster FS, Hossack J, Adamson SL. Micro-ultrasound for preclinical imaging. *Interface Focus*. 2011; 1(4), 576–601.
154. Maneas E, Aughwane R, Huynh N, *et al*. Photoacoustic imaging of the human placental vasculature. *J Biophotonics*. 2019; e201900167. doi: [10.1002/jbio.201900167](https://doi.org/10.1002/jbio.201900167).
155. Yamaleyeva LM, Brosnihan KB, Smith LM, Sun Y. Preclinical ultrasound-guided photoacoustic imaging of the placenta in normal and pathologic pregnancy. *Mol Imaging*. 2018; 17, 1536012118802721.
156. Arthuis CJ, Novell A, Raes F, *et al*. Real-time monitoring of placental oxygenation during maternal hypoxia and hyperoxygenation using photoacoustic imaging. *PLoS One*. 2017; 12(1), e0169850.
157. Lawrence DJ, Huda K, Bayer CL. Longitudinal characterization of local perfusion of the rat placenta using contrast-enhanced ultrasound imaging. *Interface Focus*. 2019; 9(5), 20190024.
158. Otake Y, Kanazawa H, Takahashi H, Matsubara S, Sugimoto H. Magnetic resonance imaging of the human placental cotyledon: proposal of a novel cotyledon appearance score. *Eur J Obstet Gynecol Reprod Biol*. 2019; 232, 82–86.
159. Allen BC, Leyendecker JR. Placental evaluation with magnetic resonance. *Radiol Clin North Am*. 2013; 51(6), 955–966.
160. Damodaram M, Story L, Eixarch E, *et al*. Placental MRI in intrauterine fetal growth restriction. *Placenta*. 2010; 31(6), 491–498.
161. Sinding M, Peters DA, Frokjaer JB, *et al*. Prediction of low birth weight: comparison of placental T2* estimated by MRI and uterine artery pulsatility index. *Placenta*. 2017; 49, 48–54.
162. Damodaram MS, Story L, Eixarch E, *et al*. Foetal volumetry using magnetic resonance imaging in intrauterine growth restriction. *Early Hum Dev*. 2012; 88 (Suppl 1), S35–S40.
163. Avni R, Neeman M, Garbow JR. Functional MRI of the placenta—from rodents to humans. *Placenta*. 2015; 36(6), 615–622.
164. Sorensen A, Peters D, Frund E, Lingman G, Christiansen O, Ulbjerg N. Changes in human placental oxygenation during maternal hyperoxia estimated by blood oxygen level-dependent magnetic resonance imaging (BOLD MRI). *Ultrasound Obstet Gynecol*. 2013; 42(3), 310–314.
165. Sorensen A, Sinding M, Peters DA, *et al*. Placental oxygen transport estimated by the hyperoxic placental BOLD MRI response. *Physiol Rep*. 2015; 3(10), e12582.
166. Ingram E, Morris D, Naish J, Myers J, Johnstone E. MR imaging measurements of altered placental oxygenation in pregnancies complicated by fetal growth restriction. *Radiology*. 2017; 285(3), 953–960.
167. Aimot-Macron S, Salomon LJ, Deloison B, *et al*. In vivo MRI assessment of placental and foetal oxygenation changes in a rat model of growth restriction using blood oxygen level-dependent (BOLD) magnetic resonance imaging. *Eur Radiol*. 2013; 23(5), 1335–1342.
168. Chalouhi GE, Alison M, Deloison B, *et al*. Fetoplacental oxygenation in an intrauterine growth restriction rat model by using blood oxygen level-dependent MR imaging at 4.7 T. *Radiology*. 2013; 269(1), 122–129.
169. Kording F, Forkert ND, Sedlacik J, *et al*. Automatic differentiation of placental perfusion compartments by time-to-peak analysis in mice. *Placenta*. 2015; 36(3), 255–261.
170. Alison M, Quibel T, Balvay D, *et al*. Measurement of placental perfusion by dynamic contrast-enhanced MRI at 4.7 T. *Invest Radiol*. 2013; 48(7), 535–542.
171. Tomlinson TM, Garbow JR, Anderson JR, Engelbach JA, Nelson DM, Sadovsky Y. Magnetic resonance imaging of hypoxic injury to the murine placenta. *Am J Physiol Regul Integr Comp Physiol*. 2010; 298(2), R312–319.
172. Deloison B, Siauve N, Aimot S, *et al*. SPIO-enhanced magnetic resonance imaging study of placental perfusion in a rat model of intrauterine growth restriction. *BJOG*. 2012; 119(5), 626–633.
173. Lemery Magnin M, Fitoussi V, Siauve N, *et al*. Assessment of placental perfusion in the preeclampsia L-NAME rat model with high-field dynamic contrast-enhanced MRI. *Fetal Diagn Ther*. 2018; 44(4), 277–284.
174. Solomon E, Avni R, Hadas R, *et al*. Major mouse placental compartments revealed by diffusion-weighted MRI, contrast-enhanced MRI, and fluorescence imaging. *Proc Natl Acad Sci U S A*. 2014; 111(28), 10353–10358.
175. Erlich A, Nye GA, Brownbill P, Jensen OE, Chernyavsky IL. Quantifying the impact of tissue metabolism on solute transport in fetoplacental microvascular networks. *Interface Focus*. 2019; 9(5), 20190021.
176. Erlich A, Pearce P, Mayo RP, Jensen OE, Chernyavsky IL. Physical and geometric determinants of transport in fetoplacental microvascular networks. *Sci Adv*. 2019; 5(4), eaav6326.
177. Lofthouse EM, Perazzolo S, Brooks S, *et al*. Phenylalanine transfer across the isolated perfused human placenta: an experimental and modeling investigation. *Am J Physiol Regul Integr Comp Physiol*. 2016; 310(9), R828–836.
178. Pearce P, Brownbill P, Janacek J, *et al*. Image-based modeling of blood flow and oxygen transfer in fetoplacental capillaries. *PLoS One*. 2016; 11(10), e0165369.
179. Plitman Mayo R, Olsthoorn J, Charnock-Jones DS, Burton GJ, Oyen ML. Computational modeling of the structure-function relationship in human placental villi. *J Biomech*. 2016; 49(16), 3780–3787.
180. Widdows KL, Panitchob N, Crocker IP, *et al*. Integration of computational modeling with membrane transport studies reveals new insights into amino acid exchange transport mechanisms. *FASEB J*. 2015; 29(6), 2583–2594.
181. Panitchob N, Widdows KL, Crocker IP, *et al*. Computational modelling of placental amino acid transfer as an integrated system. *Biochim Biophys Acta*. 2016; 1858(7 Pt A), 1451–1461.
182. Panitchob N, Widdows KL, Crocker IP, *et al*. Computational modelling of amino acid exchange and facilitated transport in placental membrane vesicles. *J Theor Biol*. 2015; 365, 352–364.
183. Gill JS, Salafia CM, Grebenkov D, Vvedensky DD. Modeling oxygen transport in human placental terminal villi. *J Theor Biol*. 2011; 291, 33–41.
184. Lecarpentier E, Bhatt M, Bertin GI, *et al*. Computational fluid dynamic simulations of maternal circulation: wall shear stress in the human placenta and its biological implications. *PLoS One*. 2016; 11(1), e0147262.
185. Clark AR, Lin M, Tawhai M, Saghian R, James JL. Multiscale modelling of the fetoplacental vasculature. *Interface Focus*. 2015; 5(2), 20140078.