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Dear Editor,

Please accept our review article for peer review. We have had previous communication with Sally Howells who gave approval for the peer review process to happen for our paper. The article should be considered for publication in the Special Edition of *The Journal of Physiology* devoted to Perinatal Physiology in 2018, collated to celebrate the work of Prof Parer.

Our paper considers early advances in placental oxygen transfer physiology. It primarily focuses on new and emerging technologies to unravel this mechanistic "black box" behind transfer efficacy across the placental barrier in health and disease, by explaining the scope for evidence based modelling of the structural and haemodynamic factors governing this transfer. We explain how *in silico* modelling tools will enhance obstetric practice in the future, aiding the diagnosis of pregnancies at risk from failed placental oxygen transfer, enabling closer clinical management of problem pregnancies.

Kind regards

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Review: Human placental oxygenation in late gestation: experimental and theoretical approaches

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Abstract

The placenta is crucial for life. It is an ephemeral but complex organ acting as the barrier interface between maternal and fetal circulations, providing exchange of gases, nutrients, hormones, waste products and immunoglobulins. Many gaps exist in our understanding of the detailed placental structure and function, particularly in relation to oxygen handling and transfer in healthy and pathological states *in utero*.

Measurements to understand oxygen transfer *in vivo* in the human are limited, with no general agreement on the most appropriate methods. An invasive method for measuring partial pressure of oxygen in the intervillous space through needle electrode insertion at the time of Caesarean sections has been reported. This allows for direct measurements *in vivo* whilst maintaining near normal placental conditions, however there are practical and ethical implications in using this method for determination of placental oxygenation. Furthermore, oxygen levels are likely to be highly heterogeneous within the placenta.

Emerging non-invasive techniques, such as MRI, and *ex vivo* research are capable of enhancing and improving current imaging methodology for placental villous structure and increase the precision of oxygen measurement within placental compartments. These techniques, in combination with mathematical modelling have stimulated novel cross-disciplinary approaches that could advance our understanding of placental oxygenation and its metabolism in normal and pathological pregnancies, improving clinical treatment options and ultimately outcomes for the patient.

Key Words

placenta, oxygen, perfusion, MRI, intervillious space, FGR, modelling

Introduction

The placenta is vital for fetal growth and development, adapting its physiology, architecture and signalling throughout gestation to meet changing demands. Despite this, there remain many unanswered questions in our detailed understanding of placental structure, function and transfer efficacy in both normal and diseased states *in utero*.

It is an ephemeral but complex organ acting as the interface between mother and fetus, providing a hub for exchange. One of the key functions of the placenta is to mediate transfer of oxygen to the fetus. However, there is poor consensus on the oxygenation of placental compartments; most notably oxygen gradients within the intervillous space (IVS) of the maternal circulation, and how the partial pressure of this gas (PO_2) differs between normal pregnancies and those complicated by placental diseases. A full understanding of spatio-temporal oxygenation and associated placental villous architecture in healthy and diseased states, aiding mathematical model development on transplacental oxygen transfer, will ultimately be useful to obstetricians trying to understand and treat placental disease. In this review, we will present current views on human placenta structure and function with respect to oxygen transfer. This will include discussions on the strengths and weaknesses of the current methods used to measure placental oxygenation both *in vivo* and *ex vivo*. It will also summarise reported oxygen levels within the placenta-fetal unit, with an emphasis on dysregulated materno-fetal oxygen transfer in pregnancy pathologies.

Placental structure and function

The human placenta is a discoid haemomonochorial dually-perfused organ, which in a healthy term pregnancy has a mean mass of 650g and a surface area for exchange of $13m^2$ (Mayhew *et al.*, 2007). It contains 25% (80ml) of the total fetal blood volume (Luckhardt *et al.*, 1996). Fetal blood flow from the two umbilical arteries is forced through two elaborately branched networks across the chorionic plate, before delving into the placental mass, where they branch again entering approximately fifty placental villous trees. Several villous trees might occupy a single lobule, which is the semi-compartmentalised structure defined by septa as seen from basal plate aspect. Villous trees are elaborately branched, commencing with stem villi (Leiser *et al.*, 1985). Stem

villi divide extensively to form intermediate villi, with the mature type branched-off to form the terminal villi (Kaufmann *et al.*, 1985). In later pregnancy, having arrived to the villous capillaries via an arteriolar microcirculatory system, the fetoplacental capillary blood enriched with oxygen and nutrients is forced into the venular systems of the mature intermediate and stem villi; and then into veins on the chorionic plate, subsequently traveling to the fetus via a single umbilical vein. Architectural events unfold in the developing placenta throughout gestation to arrive at the position of mature villous trees capable of servicing the ever-increasing demands of the fetus for oxygen. See e.g. (Huppertz, 2008; Wang & Zhao, 2010), for the detailed anatomy and physiology of the human placenta and its developmental aspects.

During the third trimester, terminal villi formation increases exponentially (Risau & Rubanyi, 2000). From 24-26 weeks of gestation, branching angiogenesis ensues, leading to capillary outgrowths and the maturation of intermediate villi. Capillary loops are hypothesised to dilate and remodel laterally under transmural hydrostatic pressure between the fetal and maternal placental circulations (Burton *et al.*, 1996). Furthermore, fetal and maternal blood are brought into close proximity at specialised adaptive capillary structures known as vasculosyncytial membranes. These represent a thinning of the combined fetoplacental endothelium and syncytilaised trophoblast, with extensive lateral displacement of single-celled trophoblasts and membrane associated organelles (Castellucci *et al.*, 1990). This structural adaptation confines the endo-epithelial placental barrier to a diffusion distance of 2-3μm, an important facet of Fick's Law of Diffusion, appertaining more particularly to the efficiency of transfer of hydrophilic molecules (Sibley *et al.*, 1998), but also in-part to fast diffusing gases like oxygen. The pathway of blood between the placenta and fetus with compartmentalised reported oxygen ranges in late pregnancy has been summarised for purpose of this review in Figure 1.

Although the placenta is involved in essential functions to maintain fetal health, little is known about how human placenta transfer *in vivo* relates to fetal oxygen acquisition in the human. The placenta is dynamic, with the capability of adapting to possible net reductions in maternal blood flow, ensuring there is an adequate supply of oxygen to the fetus (Wilkening & Meschia, 1983). *In vivo* work using animal models, most notably

the sheep, shows a normal tolerance to reduced maternal-side placental blood flow, before placental metabolic demands out-compete fetal demands for oxygen provision from the maternal circulation (Gu *et al.*, 1985). The work of Gu, who collaborated with Prof Julian Parer whilst he was at the University of California, showed that a reduction in uterine artery blood flow by up to 30% had no effect on fetal oxygenation levels (Gu *et al.*, 1985). Whilst the anatomy of the sheep placenta is substantially different to the human placenta, broader concepts such as hypoxic fetoplacental vasoconstriction and the possible role of oxygen-sensitive voltage-gated potassium channels in this process, as found in humans, might provide a cross species mechanism by which fetomaternal blood flow matching could arise (Byrne *et al.*, 1997; Hampl *et al.*, 2002; Kiernan *et al.*, 2010).

Modern *in vivo* imaging, *ex vivo* placental spatial oxygen mapping technology and mathematical modelling are now available to investigate these early observations and aim to unravel the intricacies of how fetal oxygen acquisition is regulated by placental structure and function in health and disease. This multidisciplinary approach has shown the impact of sinusoidal capillaries on placental function (Pearce *et al.*, 2016; Plitman Mayo *et al.*, 2016b) where mathematical modelling indicates the existence of an optimal capillary dilation size that maximises oxygen uptake. A fuller understanding of the remaining aspects of the placental oxygen transfer in health and disease now seems possible.

Oxygen metabolism and levels within the human placenta

In the placenta, gases diffuse due to partial pressure gradients which are maintained by maternal and fetal blood flow. As diffusion of gasses across the placenta is rapid, placental gas transfer is flow limited (Meschia *et al.*, 1967). Oxygen transfer depends on a partial pressure gradient being present between the maternal blood in the intervillious space (IVS) and the fetal blood in the fetoplacental capillaries and is enhanced by the Bohr-Haldane effect – as maternal blood takes up fetal carbon dioxide and becomes acidotic, oxygen release to the fetus is favoured. Simultaneously fetal blood takes up oxygen while decreasing its storage capacity for carbon dioxide and releasing it into the maternal circulation (Pinnock, 2002). Additionally, there is higher affinity of the fetal haemoglobin for oxygen, containing two alpha and two gamma sub-units, compared to

maternal haemoglobin, which has two alpha and two beta sub-units. When considering oxygen levels within the placenta, the differing compartments need to be viewed separately. The normal range for oxygenation of adult blood is between 75 and 100 mmHg. Although there may be a slight reduction in this during pregnancy, it is not expected to differ greatly. IVS soluble oxygenation values are expected to be reduced by the metabolic demand of placental tissue and the transfer to the fetal circulation. It is known that 40% of the total oxygen consumption occurs in the syncytiotrophoblast layer (Carter, 2000). However, where there is failure of spiral arteries to transform to a wider aperture, this potentially leads to altered hemodynamics, with a reduced net IVS flow, more localised blood flow patterns, and disparate skewed oxygen gradients around placental villi (Burton *et al.*, 2009). This in turn, ultimately leads to placental dysfunction and disease.

Poor placental oxygenation – a trigger for placental dysfunction

The placenta is developed to maximise the transfer of gases and nutrients to aid the growth of the fetus. When this fails, a wide range of maternal and fetal complications can occur, one of the most common complications being fetal growth restriction (FGR) where the fetus fails to reach its genetic growth potential. Over half of neonatal deaths worldwide are associated with low birth weight (UNICEF, 2004). Surviving FGR neonates face developmental problems and an increased risk of cardiovascular diseases in later life.

There are many causes of FGR involving both maternal and placental factors (Sharma *et al.*, 2016). One key factor is a reduction in oxygen transfer to the baby. Placentas measured from FGR babies are on average 24% smaller in weight than normal pregnancies (Heinonen *et al.*, 2001). In the absence of genetic abnormalities and underlying maternal conditions, this suggests a reduced functional capacity of the placenta. One hypothesised aetiology is a reduced placental surface area for gas exchange, coupled with dysregulated placental blood flow, leading to sub-optimal oxygen transfer from maternal to fetal circulations (Yu, 1992), an alternative is that dysregulated placental morphology might also reduce the oxygen transfer across the placenta. Non-placental aetiologies relate to maternal lifestyle factors which include smoking, living at high altitude and heart or lung disease, all of which depress the PO_2

in the maternal circulation, diminishing placental oxygen transfer (Sharma *et al.*, 2016). In such low oxygen environments, as found in some cases of FGR, it should be borne in mind that placental metabolism might shift to a high glucose and low oxygen consumption mode, which could have bearing on relative oxygen transfer rates to the fetus. This indirect evidence comes from an analogous study of high altitude pregnancies, referring to a reduced maternal oxygen supply to the placenta (Zamudio *et al.*, 2010).

Conversely, Kingdom *et al* proposed processes whereby changing oxygen levels can alter the structure of capillarisation within the terminal villous tree (Kingdom *et al.*, 2000). Potentially this can compound an already compromised disturbance in PO_2, further reducing oxygenation of the fetal circulation. The hypothesis remains to be tested that compromised villous tree architecture coupled with existing reduced oxygen levels in the maternal circulation exceed a critical threshold leading to FGR.

A comprehensive study including mathematical modelling of complex placental architecture, coupled with *ex vivo* physiological perfusion experimentation and *in vivo* MRI will provide further answers. This may then permit an interrogation of transplacental transfer efficacy of oxygen, providing translational tools for obstetricians in their diagnosis and management of FGR associated with oxygen transfer deficiency.

Current understanding of placental oxygen levels

As discussed previously, the *in vivo* measurement of placental oxygen has proved difficult and has only been recorded in a handful of studies. Schaaps *et al* published the most recent cross-sectional study (Schaaps *et al.*, 2005) using IVS blood sampling which was achieved through insertion of a 21 gauge needle through the chorionic plate, with blood being collected once baby was delivered, but before the placenta was expelled. (Schaaps *et al.*, 2005). This had previously been done in 1960 Quilligan *et al.* (1960) and again in 1996, who both recorded lower average values (Fujikura & Yoshida, 1996). The Schaaps paper also published a PO_2 ratio between the uterine vein and the IVS of 1.5 (Schaaps *et al.*, 2005). Using this ratio, values of 50mmHg in the uterine vein (Sibley *et al.*, 2002) would lead to an approximate IVS value of 33mmHg. With near infrared spectroscopy (Kakogawa *et al.*, 2010), predicted an IVS value of

30mmHg. Although providing the best-available estimate of IVS PO_2, caution must be applied to the uterine vein-IVS ratio extrapolator. The ratio first appears counter-intuitive, since uterine vein blood occurs downstream of the IVS. However, arterio-venous placental shunting and preferential IVS flow pathways evading PO_2 measurement may lead to higher than expected uterine vein oxygen values. Ideally, further ubiquitous IVS real-time data must be sought before relying solely on this reported ratio.

The few studies recording IVS oxygenation on term placentas show a value of ≈ 36 mmHg. This is much lower than the PO_2 of peripheral maternal arterial blood, which does not drop below 100 mmHg throughout gestation (Templeton & Kelman, 1976), potentially indicating transfer loss and a highly metabolic cellular layer of the IVS.

Compartmentalised *in vivo* values of soluble human placental oxygenation are given in Figure 1 and corresponding published values are summarised in Table 1. A small reduction in the oxygen levels between the IVS and the umbilical vein is evident with an average IVS oxygen recording of 30 mmHg and a further reduction in values between the umbilical vein and arteries (22 mmHg). However there is much greater variation in the recorded values in both measures potentially due to different experimental methods. In particular, there are differences in practice regarding clamping of the umbilical cord after delivery. As shown in Table 1, other studies measuring both venous and arterial values from the same cord recorded similar reductions in the arterial values (Nicolaides *et al.*, 1989; Link *et al.*, 2007). The venous oxygen level recorded by Schaaps *et al* is comparatively lower than other recorded values and lower than the average value by 10 mmHg which is possibly due to measurement of unclamped cords influencing the oxygen value (Schaaps *et al.*, 2005). It is expected that cord clamping will yield results closer to peripartum PO_2 levels, due to cord samples being compartmentalised away from the highly metabolic placental tissue.

Measuring and modelling oxygen transfer function in the human placenta

Assessing function through *in vivo* electrodes

A reported method for analysing placental oxygen status of the IVS is through the insertion of a needle into the placental tissue during routine Caesarean sections

(Quilligan *et al.*, 1960; Fujikura & Yoshida, 1996) (Table 1). Although this allows for direct measurement *in vivo* whilst still under normal conditions, there is a sampling efficiency problem, due to the limited number of IVS PO_2 measurements that can realistically be taken during surgery in such a large tissue. The heterogeneity of PO_2 levels within the IVS and the potential for contamination of IVS samples from disruption of the fetal capillaries are also major problems with this early method. However, there have been recent moves towards *in vivo* techniques to measure oxygen more ubiquitously in the human placenta.

Assessing function through magnetic resonance imaging

Magnetic resonance imaging (MRI) techniques have demonstrated the ability to measure non-compartmentalised changes in oxygen levels within defined placental spatial parameters. One such technique, Blood-Oxygen-Level-Dependent (BOLD) MRI (Figure 2A), can effectively measure changes in placental oxygen saturation following a maternal oxygen challenge. Deoxyhaemoglobin acts as an endogenous contrast agent, due to the differing magnetic properties of both haemoglobin and deoxyhaemoglobin. Changes in oxygen saturation, and therefore deoxyhaemoglobin levels, alter the local magnetic field susceptibility, thus affecting transverse relaxation times and BOLD signal. The first human placental BOLD MRI study (Sorensen *et al.*, 2013), described results from eight women with uncomplicated singleton pregnancies at 28-36 weeks' gestation. An increased BOLD signal was detected in areas proximal to the chorionic plate of the placenta. However, the potential application of BOLD in placental pathology is uncertain with conflicting data concerning FGR pregnancies in early comparative studies (Ingram *et al.*, 2017). The interpretations of BOLD signal changes is complex due to its relation to haemoglobin concentration and potential oxygen-related changes in local perfusion. The signal is also potentially affected by undetected uterine activity and there is a tendency for BOLD signal intensity to be correlated more closely with fetal haemoglobin oxygen saturation than with maternal haemoglobin oxygen saturation. This may be due to the relative hypoxic condition of the normal fetus, which results in a significant BOLD signal change, with changes in oxygen concentration, operating along the exponential phase of the sigmoidal fetal haemoglobin oxygen association curve.

In addition to BOLD MRI, effective changes in tissue PO_2 have been determined using a complementary technique: Oxygen-Enhanced (OE) MRI. In OE MRI, changes in longitudinal relaxation rates (R1) occur due to an increase in the paramagnetic dissolved oxygen content in the tissue with maternal hyperoxia. An increase in R1 following maternal hyperoxia, reflecting an increase in PO_2, was first demonstrated in the placenta in 2013 (Huen *et al.*, 2013). Increases in R1 following hyperoxia diminish with gestational age, which is thought to be a consequence of rapid materno-fetal O_2 transfer and utilisation. Additionally, in pregnancies affected by FGR, R1 changes are significantly lower presumably demonstrating a relative placental hypoxia as more of the dissolved oxygen is bound to deoxyhaemoglobin (Ingram et al 2017). The benefit of these techniques are their non-invasive nature, however they are limited in availability and expensive (Sorensen *et al.*, 2013). Essentially these techniques provide measures of relative change in tissue oxygen status. However, these techniques cannot provide absolute PO_2 or saturation values without further phantom validation.

Understanding placental blood flow rates is also important in deciphering placental oxygen transfer efficacy. Within *in vivo* imaging capability, several options might be available to the researcher in appreciating flow: dynamic contrast enhanced imaging (DCE) (Marcos *et al.*, 1997), arterial spin labelling (ASL) (Gowland *et al.*, 1998) and phase contrast imaging (Jansz *et al.*, 2010). DCE MRI provides spatial images of villous capillary (fetal) and IVS (maternal) flow. Substances such as Omniscan^{TM}; (a gadolinium chelate) are unstable and therefore are potentially toxic when used *in vivo* and have not been characterised for vascular leakage and signal stability. They therefore are only suited for *ex vivo* perfusions or acute animal experiments. These validations are essential in proving that acquired flow signals are truly compartmentalised. The future research agenda in placenta MR imaging is optimization of acquisition techniques and combining MR imaging approaches, such as OE with ASL to fully characterise the placenta.

In ASL, blood is intrinsically labelled, thus avoiding the concerns of exogenous contrast agents, ASL has been used *in vivo* to determine placental flow however to date this has been performed on a placental region-of-interest (ROI) which incorporates both materno-placental and feto-placental compartments. (Shao *et al.*, 2017). ASL quantifies

flow per gram of tissue mass, however the technique is hampered by poor signal-to-noise ratio and the few studies that have been performed demonstrate considerable variation in derived normal values.

Whilst functional MRI (fMRI) may be of benefit through the measurement of placental perfusion and oxygen status, its use in the placenta is still limited by a lack of data and there are no accepted MRI-based definition of normal/abnormal placental tissue flow rates (Avni *et al.*, 2015). Again, this could be due to differences in cost and availability but also through a lack of consensus on protocols, and poor image quality due to the challenges of correcting for maternal and fetal motion. However, these MRI techniques could be exploited *ex vivo*, through phantom perfusion investigations, utilising the human dual placental perfusion model to quantify flow and validate *in vivo* perfusion measures, improving our understanding of the imaging response as a proxy to tissue oxygenation.

Assessing function through *ex vivo* placental perfusion

There is a limited capability to manipulate *in vivo* physiological variables during human pregnancy. In this stance, the utilisation of *ex vivo* physiological research techniques is now coming to the fore. *Ex vivo* dual perfusion of the human placenta is now a widely used system for investigating a range of pharmacological and physiological functions including drug transfer whilst maintaining placental structure and an approximate *in vivo* state (Figure 2B). Perfusion has advantages over cell culture, tissue slices and explant studies due to the maintenance of villous architecture and relative IVS volume density (Brownbill *et al.*, 2018). Vascularised fetoplacental and IVS perfusate flows are key features of the model, in which the placental tissue maintains a higher metabolic level than in other human placental models (Hauguel *et al.*, 1983). This technique involves isolating a whole placental cotyledon from a freshly delivered placenta. The fetal side is cannulated on both arterial and venous sides and either near-anoxic blood or physiological buffer is pumped through the villous microcirculation (Schneider & Huch, 1985). The maternal side is also supplied with blood or physiological buffer at normoxic or superoxic PO_2 levels. Flow rates of perfusate are similar to, but less than, *in vivo* conditions (fetal side, 6ml/min) to reduce the overall resistance encountered during the experiment. Placental blood flow on the fetal side is calculated to be approximately 0.35

mL / min / g *in vivo* at term, based on the placental receiving 40% of fetal left ventricular output, being 480 mL/min at term (Rudolf, 1975). This compares to *ex vivo* fetal-side flow of 0.17 mL/min based on a perfused tissue mass of 35g being perfused at 6 mL/min (Desforges *et al.*, 2017). Once perfusion is established, a number of experiments can be undertaken that are not possible *in vivo*. Examples include increasing or decreasing the flow rate of either fetal or maternal perfusate, introducing drugs such as vasodilators/constrictors, or multiple sampling to monitor perfusate gases. There are disadvantages however, there is a high preparation failure rate; it is reasonably expensive and time consuming to run an experiment; and only one lobule from each placenta is usually suitable intact for perfusion, preventing parallel control investigations.

To simplify our understanding of placental oxygen transfer, a new adapted version of this model is being trialled by our laboratory. This involves scaling down the established maternal-side multi cannula dual perfusion model, so that the IVS irrigation volume is limited, employing just one maternal cannula, delivering normoxic perfusate and measuring oxygen gradients within the IVS (Figure 2B). Unlike *in vivo* oxygen sampling, extensive IVS oxygen sampling under steady-state experimental conditions is possible by means of an oxygen-sensitive needle optode, inserted through the decidual plate at set X-Y-Z planes controlled with a micro-manipulator (Figure 2B). With further experimentation the placental metabolic component of IVS oxygen consumption can be elucidated. IVS oxygen gradient data can be acquired and interrogated for metabolism and transfer, and it may be possible to discover how variable perfusate flow rates and fetoplacental vasoactive endocrine agents affect fetal-side oxygen acquisition, gaining an understanding of how the associated underlying villous architecture enhances or constrains oxygen transfer across the placental barrier.

Post-perfused human placental tissue can be successfully imaged using a wholemount confocal and lightsheet microscopy. These three-dimensional approaches confer advantages over traditional two-dimensional techniques, such as transmission-electron and phase-contrast microscopy (Figure 3A), and allow the surface of the villi and the fetal vascular system to be differentially labelled (Figure 3B & C). However these higher-resolution approaches are only able to image smaller regions of tissue. MicroCT

allows visualisation of larger regions of placental villi placenta but at different scales (Figure 3D). Imaging of the fetoplacental vascular system can be enhanced by perfusing contrast agents into the fetal circulation to image the arterial and venous circulation (Junaid *et al.*, 2017). From this, information on vessel branching patterns, interbranching length and capillary loop dilations are useful in predicting the placenta's ability to optimally transfer oxygen; a portion of the placenta often inaccessible by other means (Junaid *et al.*, 2017). However, while microCT can image large regions of tissue, when doing this its ability to image the microcirculation is limited. Micro CT imaging of fetoplacental capillaries is possible but in smaller pieces of tissue.

Integrating structure and function relationships through modelling

The increased availability of 3D imaging approaches including confocal microscopy and microCT have allowed for a recent increase in the efforts to build multiscale computational models that go hand-in-hand with refined experimental models (Pearce *et al.*, 2016; Plitman Mayo *et al.*, 2016a; Perazzolo *et al.*, 2017; Roth *et al.*, 2017) (Figure 4). In tissues with complex structures such as the human placenta, computational modelling has allowed the full structure to be visualised and analysed allowing assessment of structure-function relationships (Clark *et al.*, 2015; Plitman Mayo *et al.*, 2016a) (Figure 4A). Mathematical models have also been created to explain properties of the placenta that would not be possible to understand using *in vivo* measurements alone (Chernyavsky *et al.*, 2011; Serov *et al.*, 2015; Pearce *et al.*, 2016) (Figure 4B, C & D). However, there is open challenge of effective extraction of structural information from imaging data as well as of identifying key parameter values necessary for mathematical modelling. Once validated, theoretical models could provide a bridge between *in vivo* and *ex vivo* or *in vitro* approaches to characterise placental structure and oxygenation in normal and pathological pregnancies (Lecarpentier *et al.*, 2016).

Conclusion

The physiology of placental oxygen transfer is crucial for optimal fetal development and survival. Gross placental structure is well characterised and newer techniques, such as MRI, micro CT and advanced microscopy scanning techniques are affording greater detail. However, the function of the placenta remains poorly understood. Key

measurements of oxygen and carbon dioxide levels in a normal human placenta remain elusive, due to logistical and ethic complications with experimenting on *in vivo* human placenta, which are compounded by slow advances of *ex vivo, in vitro* and *in silico* work.

This lack of fundamental understanding has led to slow progress in terms of treating pathological states such as in cases of fetal growth restriction. It is our theory that impaired placental oxygen transfer and metabolism (Schneider, 2015) may well be a key factor in many cases of FGR, however whether this is due to abnormal structure or abnormal function is currently unknown. It is only with robust measures and consensus in experimental design that we can develop an integrated understanding of structure function relationships within the placenta. This in turn will provide a basis for developing therapeutic interventions for the treatment of the placental in fetal diseases *in vivo.*

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Additional Information

All authors approved the final version of the manuscript and all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Competing Interests

The authors declare no conflicts of interest

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682 **Table 1** In vivo IVS and ex vivo umbilical artery and vein PO_2 values in term normal
683 human placentas

	PO2 values, mmHg (# of Samples)	**Type of measurement**	**Time of measurement**	**Reference**
Pre-partum IVS	34 (n=4)	18 Gauge needle	Before placental shedding	(Quilligan *et al.*, 1960)
	30 (n=12)	21 Gauge needle	Before placental shedding	(Fujikura & Yoshida, 1996)
Post-partum IVS	49 (n=12)	18 Gauge needle	N/A	(Haruta *et al.*, 1986)
	33 (n=6)	Uterine vein analysis	Post placental shedding	(Sibley *et al.*, 2002)
	29 (n=9)	21 Gauge needle		(Schaaps *et al.*, 2005)
	30 (n=15)	Uterine vein analysis		(Kakogawa *et al.*, 2010)
Range, mmHg	**29 – 49**			
Weighted mean ± SD, mmHg	**34 ± 9**			
Umbilical artery	28 (n=53)	Cordocentesis	Pre caesarean section	(Nicolaides *et al.*, 1989)
	18 (n=681)	N/A	Post placental shedding	(Dudenhausen *et al.*, 1997)
	21 (n=60)	21 Gauge needle		(Daniel *et al.*, 1998)
	30 (n=18)	Blood Gas analyser		(Ochiai *et al.*, 1999)
	16 (n=1281)	N/A		(Arikan *et al.*, 2000)
	19 (n=46)	N/A		(Link *et al.*, 2007)
	26 (n=60)	N/A		(Fardiazar *et al.*, 2013)
	23 (n=46)	N/A		(Di Tommaso *et al.*, 2014)
Range, mmHg	**16 - 30**			
Weighted mean ± SD, mmHg	**18 ± 4**			
Umbilical vein	35 (n=14)		Pre	(Pardi *et al.*, 1987)

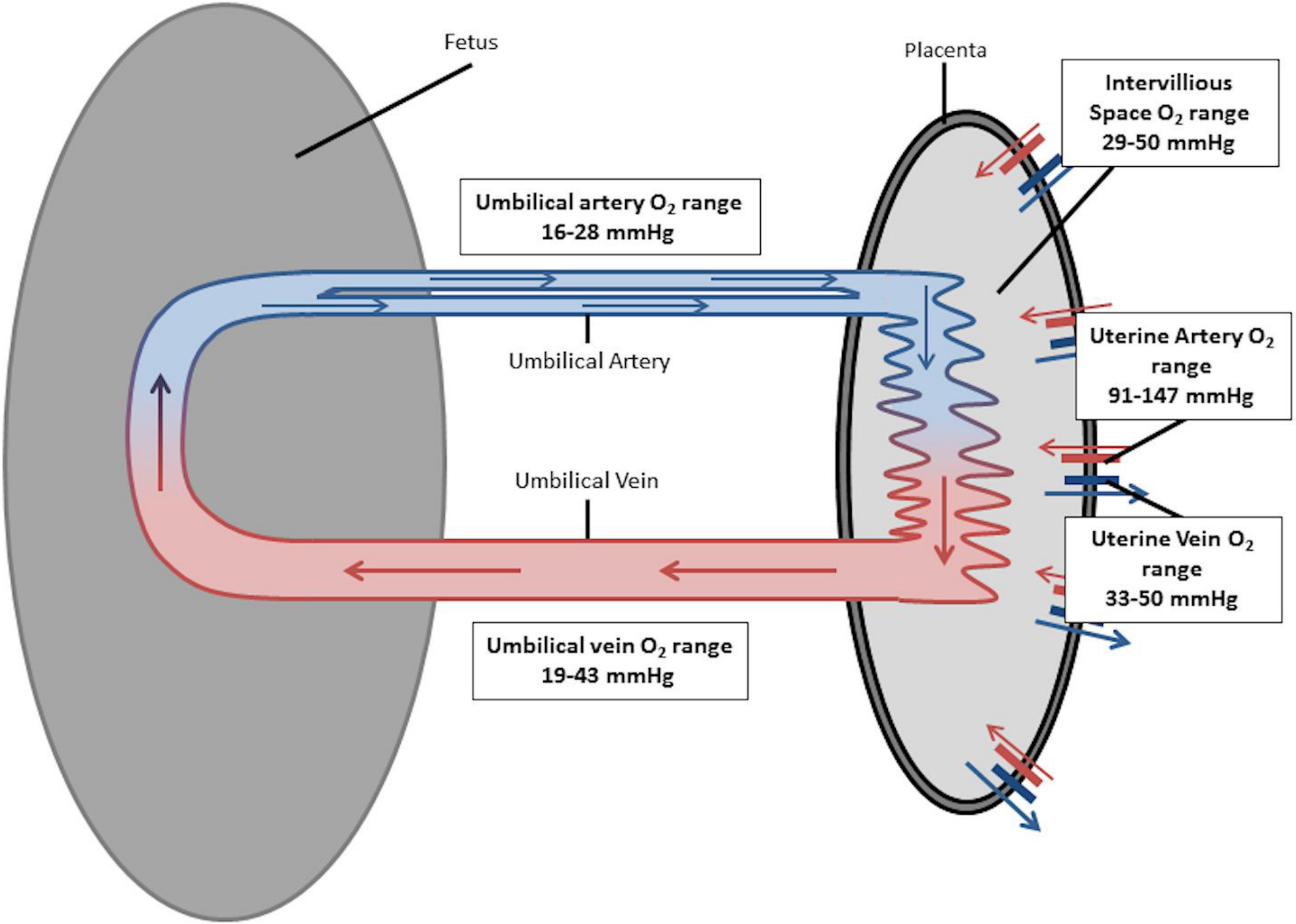
	43 (n=143)	Cordocentesis	caesarean section	(Nicolaides *et al.*, 1989)
	31 (n=60)	21 Gauge needle	Post placental shedding	(Daniel *et al.*, 1998)
	18 (n=18)	Blood Gas analyser		(Ochiai *et al.*, 1999)
	19 (n=9)	21 Gauge needle		(Schaaps *et al.*, 2005)
	25 (n=46)	N/A		(Link *et al.*, 2007)
	29 (n=300)	N/A		(Bernardez-Zapata & Moreno-Rey, 2014)
	27 (n=46)	N/A		(Di Tommaso *et al.*, 2014)
Range, mmHg	**19 – 43**			
Weighted mean ± SD, mmHg	**32 ± 8**			
Uterine Artery	97 (n=50)	Electrode	Post placental shedding	(Blechner *et al.*, 1968)
	147 (n=18)	Blood Gas analyser		(Ochiai *et al.*, 1999)
	91 (n=168)			(Postigo *et al.*, 2009)
Range, mmHg	**91 – 147**			
Weighted mean ± SD, mmHg	**97 ± 22**			
Uterine Vein	33	Electrode	Post placental shedding	(Stave, 1970)
	50 (n=6)	Blood Gas analyser		(Sibley *et al.*, 2002)
	46 (n=10)	21 Gauge needle	Before placental shedding	(Fujikura & Yoshida, 1996)
Range, mmHg	**33-50**			

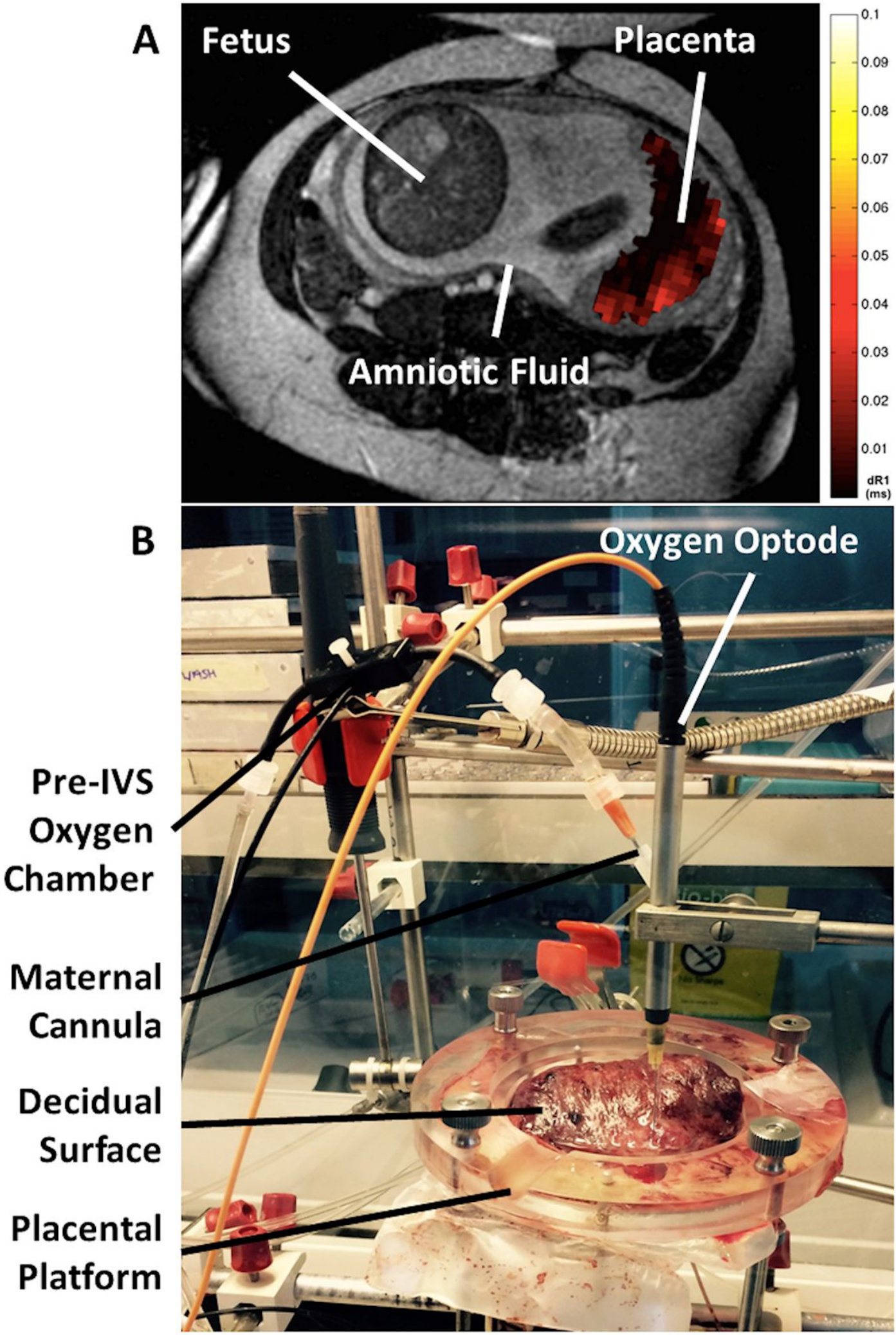
Figure 1 Simplified schematic of the maternal and fetal placental circulations, showing the major compartments and published attributed *in vivo* oxygen values (See Table 1).

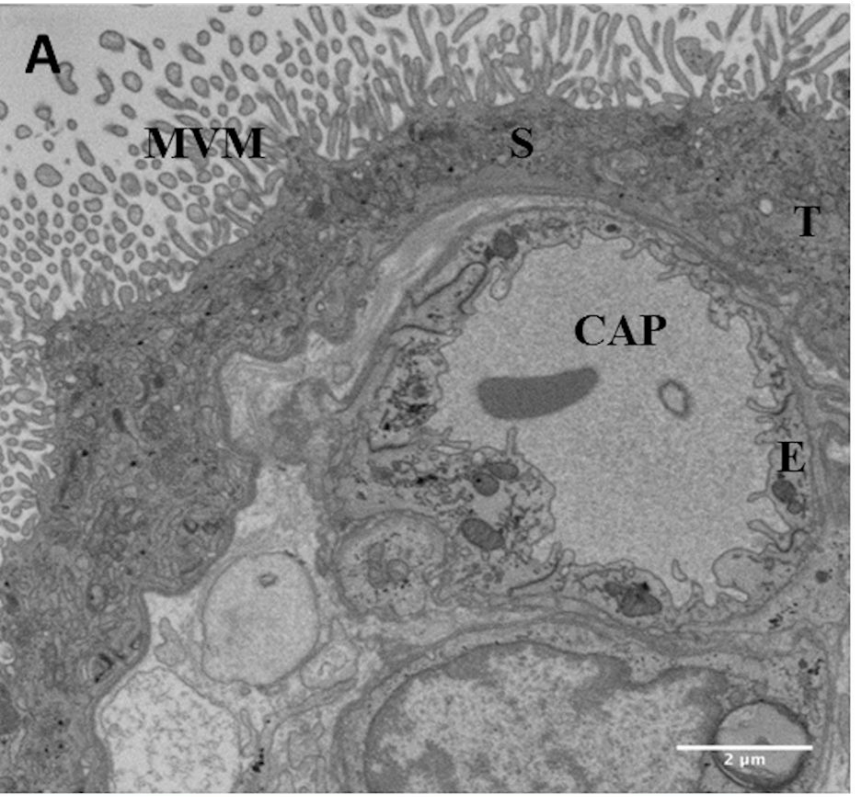
Figure 2 Measuring oxygen distribution in the human placenta. A) Demonstrating a normal placenta imaged using oxygen-enhanced (OE) MRI techniques. This shows an axial T2-weighted structural MR image through maternal abdomen demonstrating the uterine cavity, fetus (abdominal cross-section) and placenta with a superimposed dR1 map showing the placental region of interest (ROI). B) Soluble oxygen measurement of the IVS in the *ex vivo* dually perfused placental lobule via a flow- through cell (black box – top-left) and via an optode needle inserted into the placental tissue

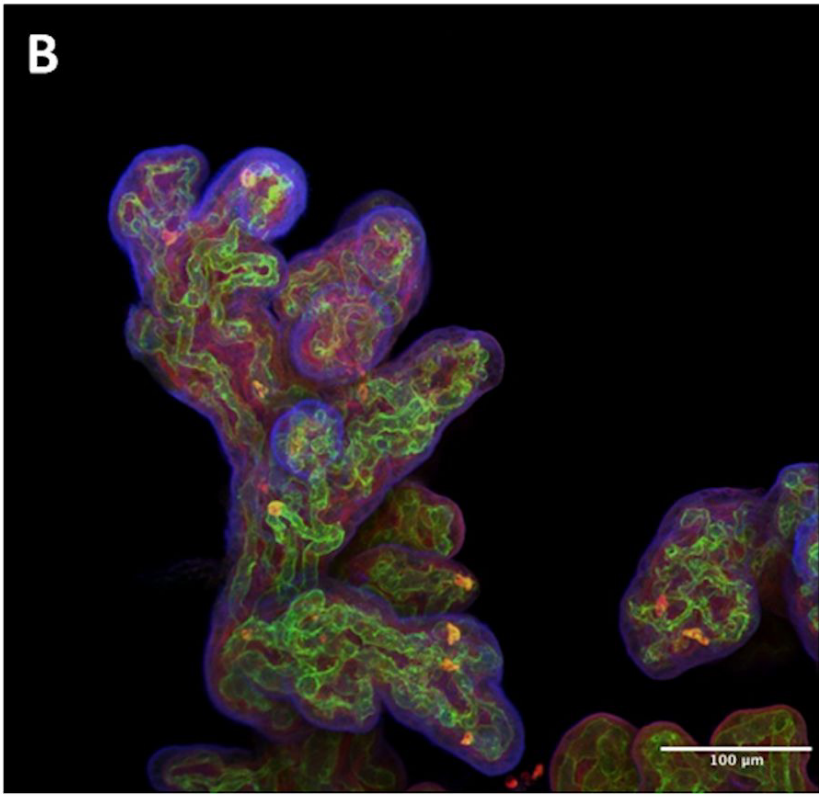
Figure 3 A) A transmission electron micrograph of terminal villi showing microvillous membrane (MVM), an underlying capillary (CAP), a syncytiotrophoblast (S), trophoblast (T) and endothelium (E). B) Projection of an imaged stack (wholemount confocal microscopy); stained with lectin fitce-AAL from the endothelium (green), rhodamine-psa for the stroma (red) and biotin-dsl for the trophoblast (violet); the dsl was detected with streptavidin 680; imaging was on a Leica Sp5 confocal microscope, presented as an imaged stack. C) Villous microcirculation of a term normal placenta perfused with a UEA lectin linked to biotin and detected with streptavidin 800. D) microCT image of a vascular corrosion cast of a term placenta, infused through the umbilical artery with Batson's resin, which was then set and underwent tissue corroded steps for several days in 20% (w/v) potassium hydroxide.

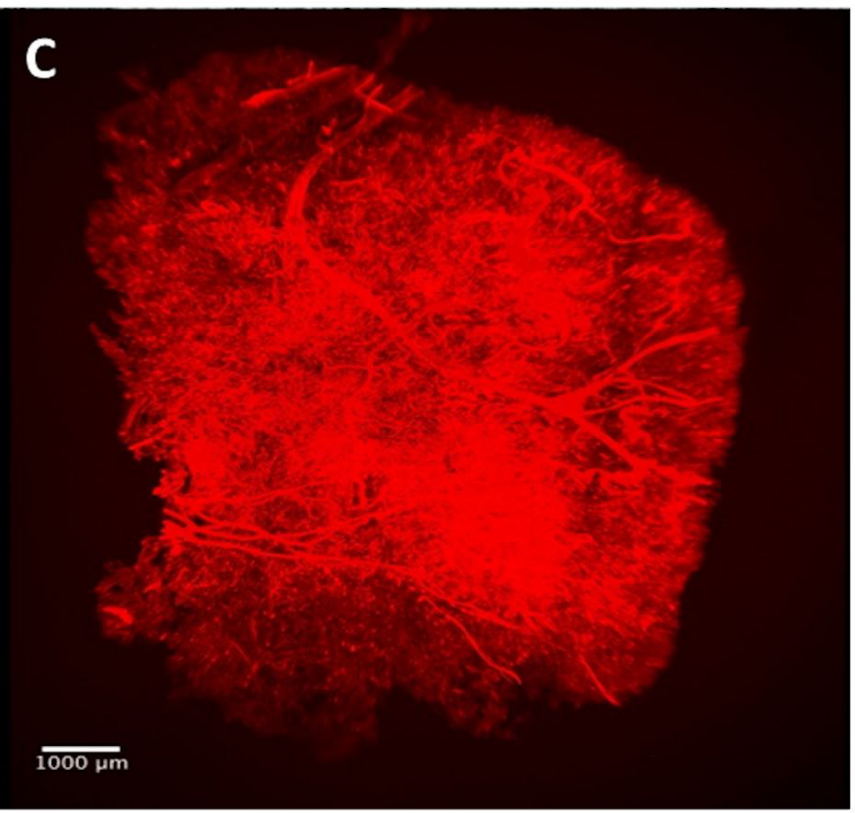
Figure 4 Mathematical modelling of human placental perfusion and oxygenation at different scales. A) variability of perfusion in a feto-placental vascular network (colour scale shows pressure for chorionic vessels and relative capillary flow for terminal capillaries, plotted as spheres) (Clark *et al.*, 2015); B) distribution of a passive solute in the intervillous space of a single placental lobule (Chernyavsky *et al.*, 2010); C) oxygen flux distribution over the capillary and syncytiotrophoblast surfaces of a single terminal villus (Pearce *et al.*, 2016); D) microscopic flow in the intervillous space(Perazzolo *et al.*, 2017). Images are reproduced with permission, subject to the respective copyrights.

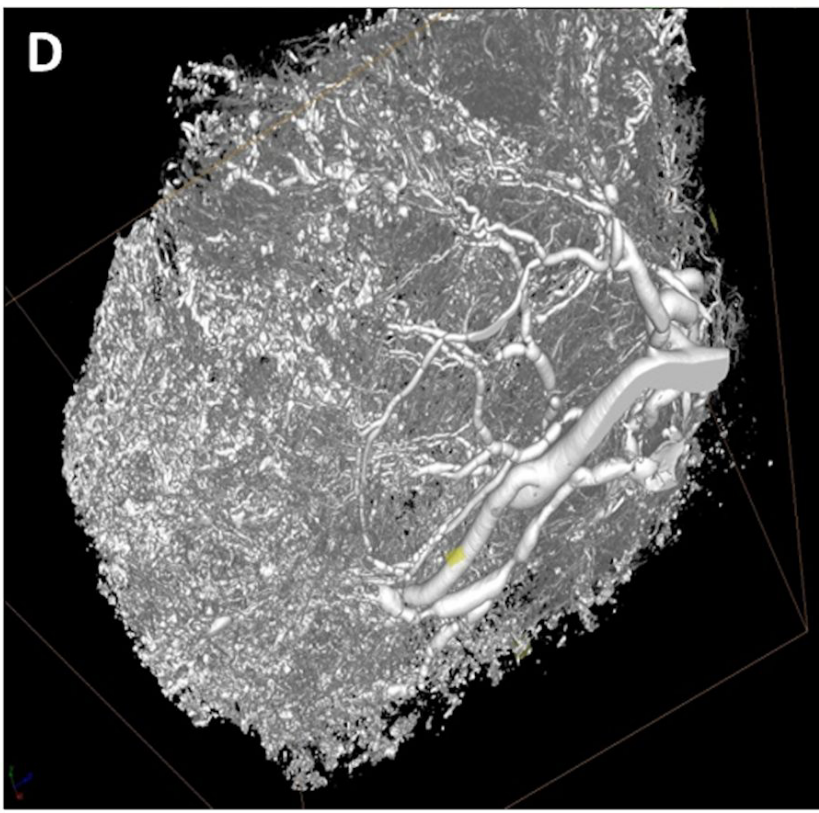

Fetus
Placenta
Intervillious Space O_2 range 29-50 mmHg
Umbilical artery O_2 range 16-28 mmHg
Umbilical Artery
Uterine Artery O_2 range 91-147 mmHg
Umbilical Vein
Uterine Vein O_2 range 33-50 mmHg
Umbilical vein O_2 range 19-43 mmHg

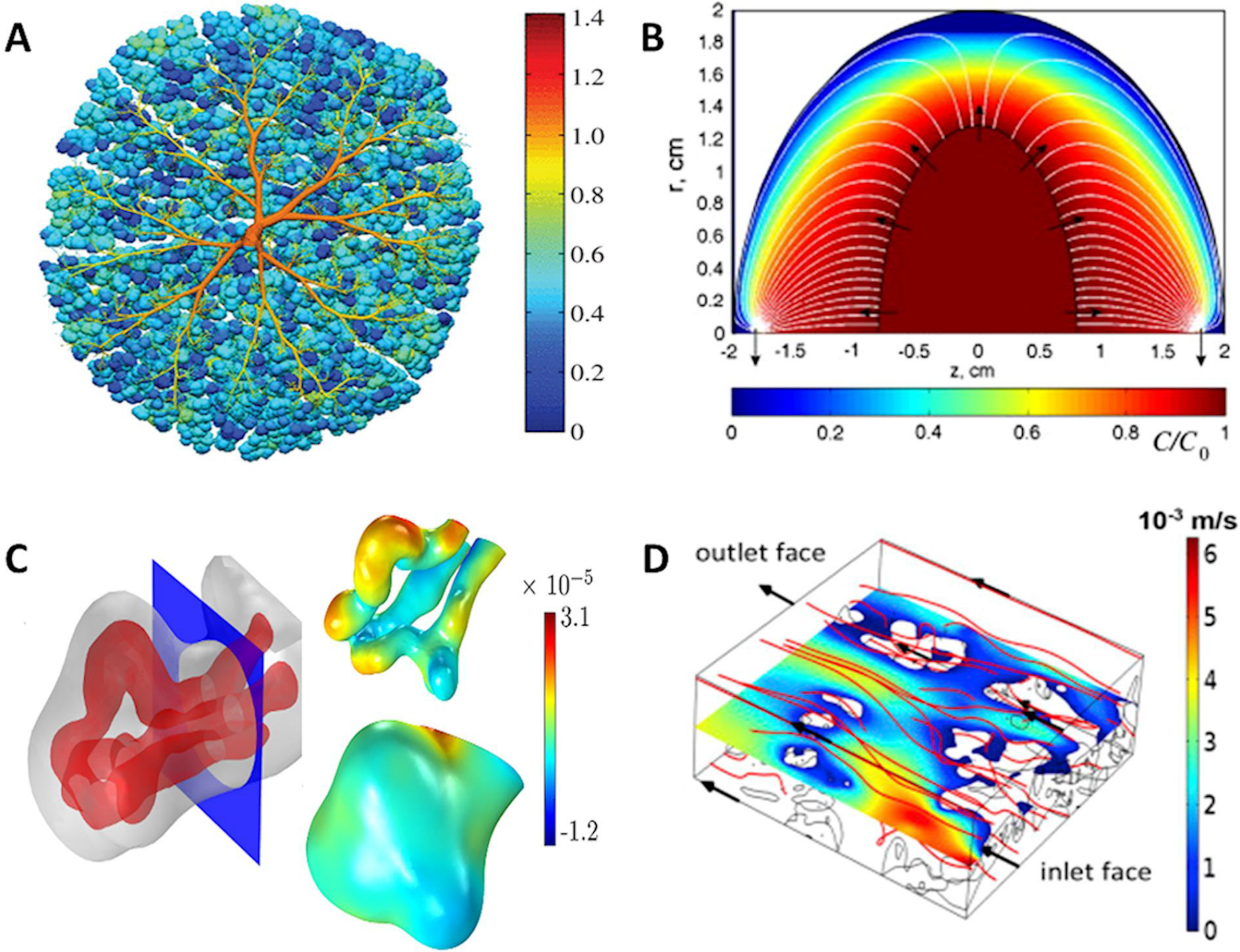

A
Fetus
Placenta
Amniotic Fluid
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
dR1 (ms)
B
Oxygen Optode
Pre-IVS Oxygen Chamber
Maternal Cannula
Decidual Surface
Placental Platform

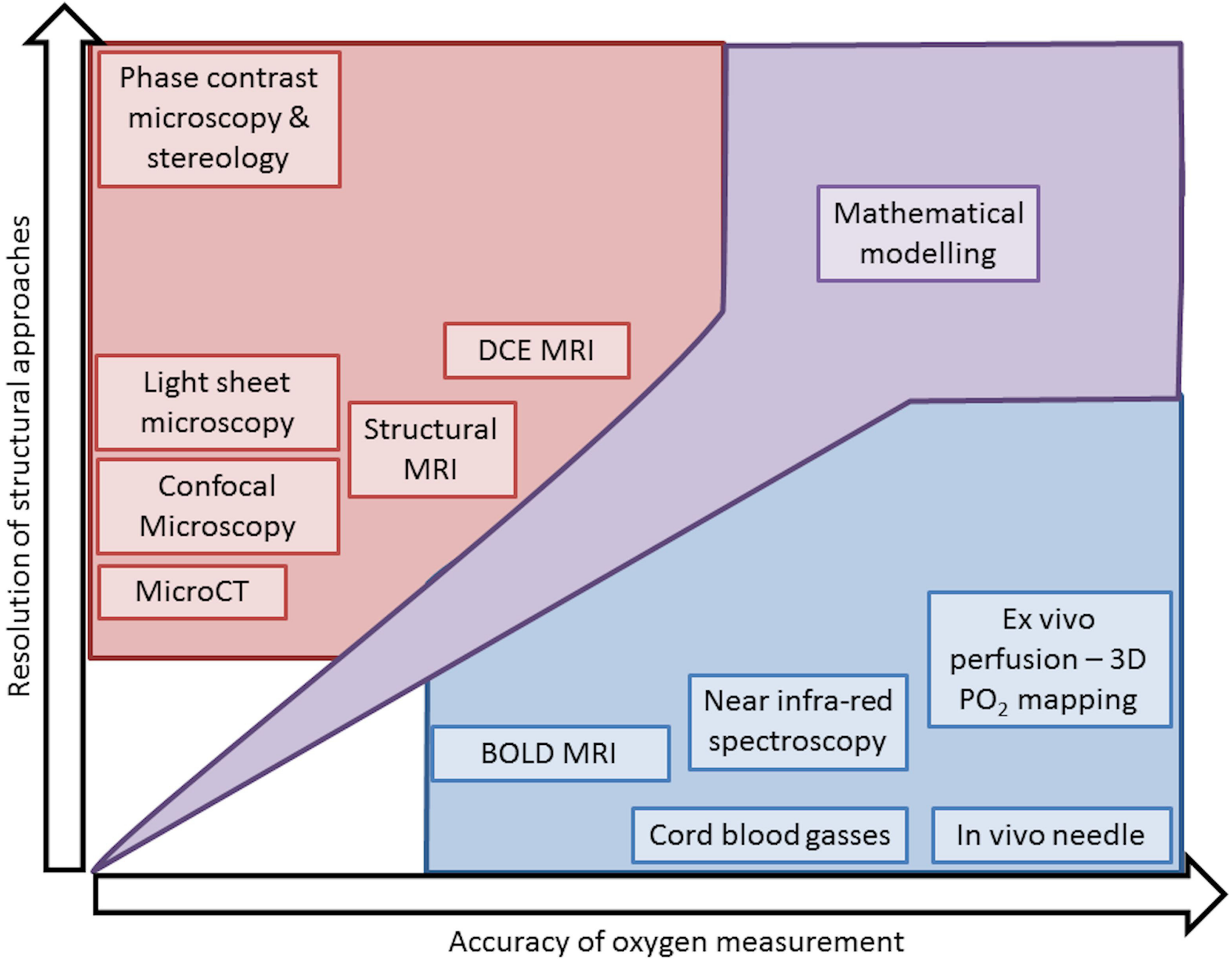

A
MVM
S
T
CAP
E
2 µm


B
100 µm


C
1000 µm


D


A
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
B
r, cm
z, cm
C/C_0
C
× 10^{-5}
3.1
-1.2
D
outlet face
inlet face
10^{-3} m/s


Phase contrast microscopy & stereology
Mathematical modelling
DCE MRI
Light sheet microscopy
Structural MRI
Confocal Microscopy
MicroCT
Ex vivo perfusion – 3D PO_2 mapping
Near infra-red spectroscopy
BOLD MRI
Cord blood gasses
In vivo needle
Resolution of structural approaches
Accuracy of oxygen measurement