REPRODUCTION
REVIEW

Non-invasive evaluation of placental blood flow: lessons from animal models

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Abstract

In human obstetrics, placental vascularisation impairment is frequent as well as linked to severe pathological events (preeclampsia and intrauterine growth restriction), and there is a need for reliable methods allowing non-invasive evaluation of placental blood flow. Uteroplacental vascularisation is complex, and animal models are essential for the technical development and safety assessment of these imaging tools for human clinical use; however, these techniques can also be applied in the veterinary context. This paper reviews how ultrasound-based imaging methods such as 2D and 3D Doppler can provide valuable insight for the exploration of placental blood flow both in humans and animals and how new approaches such as the use of ultrasound contrast agents or ultrafast Doppler may allow to discriminate between maternal (non-pulsatile) and foetal (pulsatile) blood flow in the placenta. Finally, functional magnetic resonance imaging could also be used to evaluate placental blood flow, as indicated by studies in animal models, but its safety in human pregnancy still requires to be confirmed.

Reproduction (2017) **153** R85–R96

Introduction

Placental vascularisation impairment occurs in more than 15% of pregnancies in humans, leading to placental insufficiency, including potentially severe disease such as preeclampsia (PE, 4–7%) or intrauterine growth restriction (IUGR, 10%). These pathologies account for 20–30% of prenatal and perinatal morbidity and mortality in humans and are also associated with increased risk of developing a metabolic syndrome at adulthood (Barker *et al*. 2002). Precocious screening for the diagnosis of women at risk of developing placental insufficiency is therefore of uppermost importance but remains unsatisfactory. There is, thus, a crucial health interest to develop reliable methods for non-invasive evaluation of placental blood flow in the human obstetrics context.

Uteroplacental vascularisation in humans and animals

Uteroplacental vascularisation is very complex (Jaffe *et al*. 1997, Charnock-Jones *et al*. 2004, Mayhew *et al*. 2004). In humans, the placenta is discoid, haemochorial and villous. The afferent vascularisation originates predominantly from the uterine arteries. The spiral arteries, located in the myometrium, are remodelled by extravillous trophoblastic cells shortly after implantation. These cells replace the endothelial lining of the arteries and form trophoblastic plugs, preventing maternal blood from entering the intervillous space throughout the first trimester (Jaffe *et al*. 1997). In one study, colour Doppler imaging demonstrated blood flow within the uteroplacental circulation in pregnancies starting from 7 weeks of gestation (Jaffe 2001). Whether the detected flow in the early placenta results only from foetal circulation remains unknown to date. The plugs gradually dissolve to let maternal blood enter the intervillous space at the end of the first trimester. Concomitantly, the spiral arteries dilate and lose their vasoconstrictive characteristics. Moreover, a dense anastomotical vascular network (arteriovenous shunt) develops in the myometrium located under the placenta, as demonstrated using 3D colour Doppler techniques (Schaaps & Tsatsaris 2001). Simultaneously, on the foetal side, the chorionic villous tree expands, linked to the foetus through the umbilical cord (Fig. 1).

Placental vascularisation can be evaluated indirectly by the assessment of uterine or umbilical artery blood flow or by directly imaging the placenta, as described in this review (Fig. 2). For obvious ethical reasons, the testing of functional imaging techniques is limited in

ISSN 1470–1626 (paper) 1741–7899 (online)
DOI: 10.1530/REP-16-0428
Online version via www.reproduction-online.org

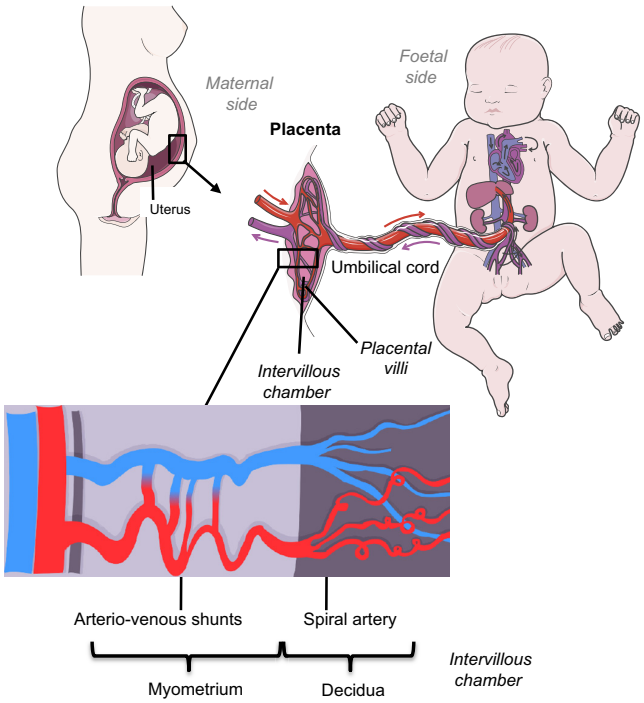


Figure 1 Utero–foeto–placental vascularisation in human. The human placenta is haemochorial and of discoid shape. Placental villi are directly in contact with maternal blood into the intervillous chamber. An arterio-venous shunt is present into the myometrium under the placenta. Adapted from Burton *et al.* (2009).

pregnant women. As a consequence, animal models are needed. Nevertheless, there is no such placentation as human placentation and intimate knowledge of the anatomic and physiological characteristics for each species is needed (Chavatte-Palmer & Tarrade 2016).

Despite their resemblance to humans, the use of non-human primates (NHP) is greatly limited owing to important ethical questions and high cost. Because of their haemochorial placenta of discoid shape, close to that of humans, rodents and lagomorphs are naturally favoured as models. Rodents are relatively cheap, easy to handle and offer a wide range of genetic tools. Rabbits are larger with a placental structure closer to that of humans than rodents. Nevertheless, in both rodents and rabbits, the exchange zone is of labyrinthine structure (mesh-like), in contrast to the villous structure of the human placenta. Although ruminant placentation is very different (cotyledonary placentation), sheep are often used for obstetrical research due to their long gestation (5 months) and large size, allowing the use of medical imaging equipment. Pigs, horses and carnivores are generally excluded for biomedical purposes due to their very different placental structures compared to humans, although information generated in these species when used may also be relevant to infer general principles in placental imaging (Fig. 3). Finally, blood flow to the placenta varies according to species' size, placental morphology and gestational age (Table 1).

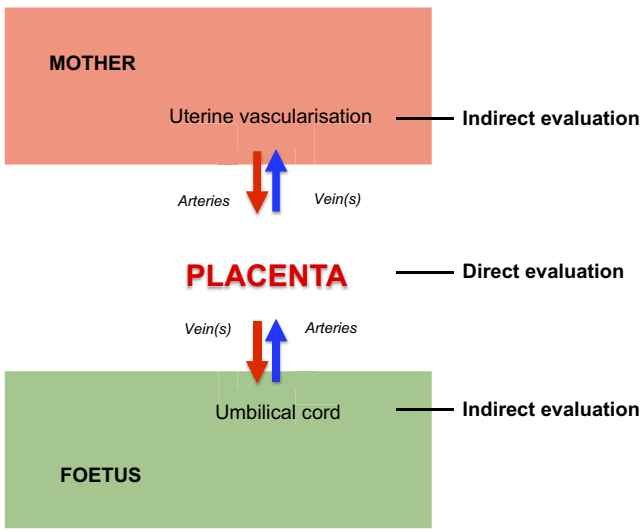


Figure 2 General scheme of placental vascularisation evaluation.

Non-human primates

Among NHPs, large primates could be considered as the gold standard because of their important similarities to humans such as maternal blood circulation in the intervillous space and spiral artery remodelling. The baboon is particularly interesting because uteroplacental ischaemia provoked by uterine artery ligation in pregnancy in this species leads to signs of PE (Makris *et al.* 2007). In rhesus macaques, it is important to notice that the placenta possesses two lobes (vs a single lobe in humans) (Roberts *et al.* 2012).

Rodents and lagomorphs

In mice, definitive placentation only occurs at midterm of a very short pregnancy (18–19 days). Trophoblastic invasion of maternal arteries is shallow and limited to proximal decidua, thus reducing this species' use for investigating arterial remodelling (Carter 2007). In contrast, the rat is considered an appropriate model for studying the mechanisms of decidualisation as well as subsequent remodelling of the uterine tissues (Fonseca *et al.* 2012). Although there is no known equivalent of PE in animals, many transgenic, surgical or pharmaceutical mouse and rat models have been developed, which mimic some aspects of PE (Clark 2014).

In rabbits (31–32 days gestation), long spiral arteries perfuse both myometrium and placenta (Duncan & Lewis 1969). Before reaching the labyrinth, maternal blood is released in arterial sinuses located in the decidual part of the placenta, slowing down blood flow to promote transplacental exchange in the placental labyrinth. Blood from the labyrinth is collected by an extensive decidual venous system (Carter *et al.* 1971).

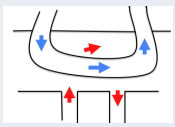
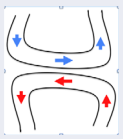
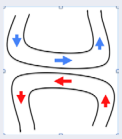
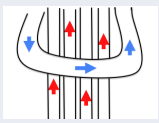
	NHP	Rabbit	Mouse / rat	Sheep / cow
Major source of uterine vascularisation	Uterine artery principally	Anastomotical network with uterine, ovarian, vaginal artery Arcade formed of uterine and ovarian arteries	Anastomotical network with uterine, ovarian, vaginal artery Arcade formed of uterine and ovarian arteries	Uterine artery principally
Placental vascular interrelations	Multivillous 	Counter current 	Counter current 	Cross current 
Umbilical cord	1 vein + 2 arteries	1 vein + 2 arteries	1 vein + 2 arteries	2 vein + 2 arteries

Figure 3 Comparative inter-species utero–foeto–placental vascularisation (Barone 1978, Evain-Brion 2010).

Sheep

In sheep, placental angiogenesis begins as early as day 18 (Reynolds & Redmer 1995, Bairagi *et al.* 2016). Each placental unit (placentome, $N = 70–100$) consists of a maternal caruncle with close interdigitation with the foetal cotyledon. Maternal caruncular vascularity increases gradually throughout gestation, whereas foetal cotyledonary vascularity expands slowly until midterm and then intensively during the last third of gestation. Maternal caruncular tissues are characterised near term by large capillaries, reducing blood flow velocity and thus promoting transplacental exchange. Placentation in goats and cattle is also cotyledonary and shares many similarities with that of sheep.

Given the complexity of placental vascularisation, it is impossible to mimic placental hypoperfusion in animals as in humans perfectly. Placental perfusion can be disturbed, however, using many different approaches. For example, in NHP, a decrease in the volume of placental blood flow associated to placental infarctions was reported in overnourished pregnant Japanese macaques (Frias *et al.* 2011). In rodents, a decrease in maternal uteroplacental blood flow has been obtained by feeding rat dams with a low-sodium diet over the last week of gestation, which was also associated with foetal IUGR (Bibeau *et al.* 2016). Inducing elevated maternal testosterone concentrations (Gopalakrishnan *et al.* 2016) or bilateral uterine artery ligation in the rat (Nusken *et al.* 2008) also reduces placental perfusion. In rabbits, placental hypoperfusion was obtained by the inhibition of nitric oxide synthase using NG-nitro-L-arginine methylester (L-NAME) (Lecarpentier *et al.* 2012, Tarrade *et al.* 2014) or by selective ligature of uteroplacental vessels

Table 1 Mean uteroplacental blood flow (UBF) measured in model animal species, before and during pregnancy.

	UBF non pregnant (NP) or beginning of pregnancy (day)	**References**	**UBF end of pregnancy** (day)	**References**
Rabbit	3–5 mL/min (NP to day 10)	Nesbitt *et al.* (2005)	18–30 mL/min (day 28)	Carter *et al.* (1971), Nesbitt *et al.* (2005), McArdle *et al.* (2009)
Mouse	NA		100–180 mL/min/100 g of placental tissue (day 16)	Salomon *et al.* (2005, 2006), Taillieu *et al.* (2006)
			0.35 mL/min (day 17.5)	Kulandavelu *et al.* (2013)
Rat	0.5 mL/min	Lashley *et al.* (2015)	159 mL/min/100 g of placental tissue	Deloison *et al.* (2012)
			2.1 mL/min (day 20)	Lashley *et al.* (2015)
Sheep	180 mL/min (day 30)	Reynolds and Redmer (1995)	1500 mL/min (day 145)	Reynolds and Redmer (1995)
Macaque	<3 mL/min/g of tissue	Keator *et al.* (2011)		
Human	60 mL/min (1st trimester)		800 mL/min (3rd trimester); 176 mL/100 g/min	Gowland *et al.* (1998)

NA, not available.

(40–50%) (Eixarch *et al*. 2011). In sheep, modulation of placental perfusion was obtained by altering maternal diet (Wallace *et al*. 2008, Redmer *et al*. 2009, Ma *et al*. 2010), by surgical removal of uterine caruncles prior to pregnancy (Robinson *et al*. 1979) or by inducing maternal hyperthermia (Bell *et al*. 1989).

In the following paragraphs, the current approaches for non-invasive evaluation of placental blood flow and how animal models contribute to technical development for human clinical use are described.

Techniques used to evaluate placental blood flow *in vivo*

Because it is widely available, low-cost and innocuous, Doppler is the first-line technique for the evaluation of uteroplacental blood flow. The Doppler technology is based on the analysis of the change in frequency or intensity of ultrasound waves when they are reflected by a moving target such as erythrocytes. It is used to study blood flow in arteries and veins and to evidence modifications due to artery stiffening or reduction in blood vessel volume. Alternatively, magnetic resonance imaging (MRI) with or without injection of ferro-magnetic contrast agents may also be used to explore placental blood flow, whereas methods based on X-ray imaging are generally banned due to the possible damage to the growing foetus (Fig. 4).

Indirect evaluation of placental blood flow using 2D Doppler

Placental blood flow can be assessed with Doppler ultrasound using maternal (uterine artery; UtA) and foetal circulation (umbilical artery; UmA) as an indirect estimate of placental function (Gudmundsson *et al*. 2009). Two Doppler modes are used: colour Doppler (CD) is based on the colour-coding of blood flow according to velocity, and pulse wave Doppler (PWD) is based on the spectral analysis of the Doppler signal. CD enables blood flow imaging over an entire region of interest without quantification. PWD allows the quantification of flow characteristics through the measurement of peak or mean flow velocity or resistance and pulsatility indices (RI and PI, respectively). In practice, PI is the most extensively used. Those modes are displayed in real time at frame rates that are usually quite low (approximately a few hertz) (Tanter & Fink 2014).

The utero–foeto–placental blood flow is linked to the gross morphology (size and shape) of the placenta (Salavati *et al*. 2016). In clinics, UtA Doppler flow (mean PI of the left and right arteries) provides a proxy measurement for the degree of vascular remodelling. Moreover, when the foetal villous vasculature of the placenta is damaged, vascular resistance within the placenta increases, leading to increased UmA RI (Llurba *et al*. 2013). The flow velocity waveform of UtA evolves physiologically from a high resistance prior to

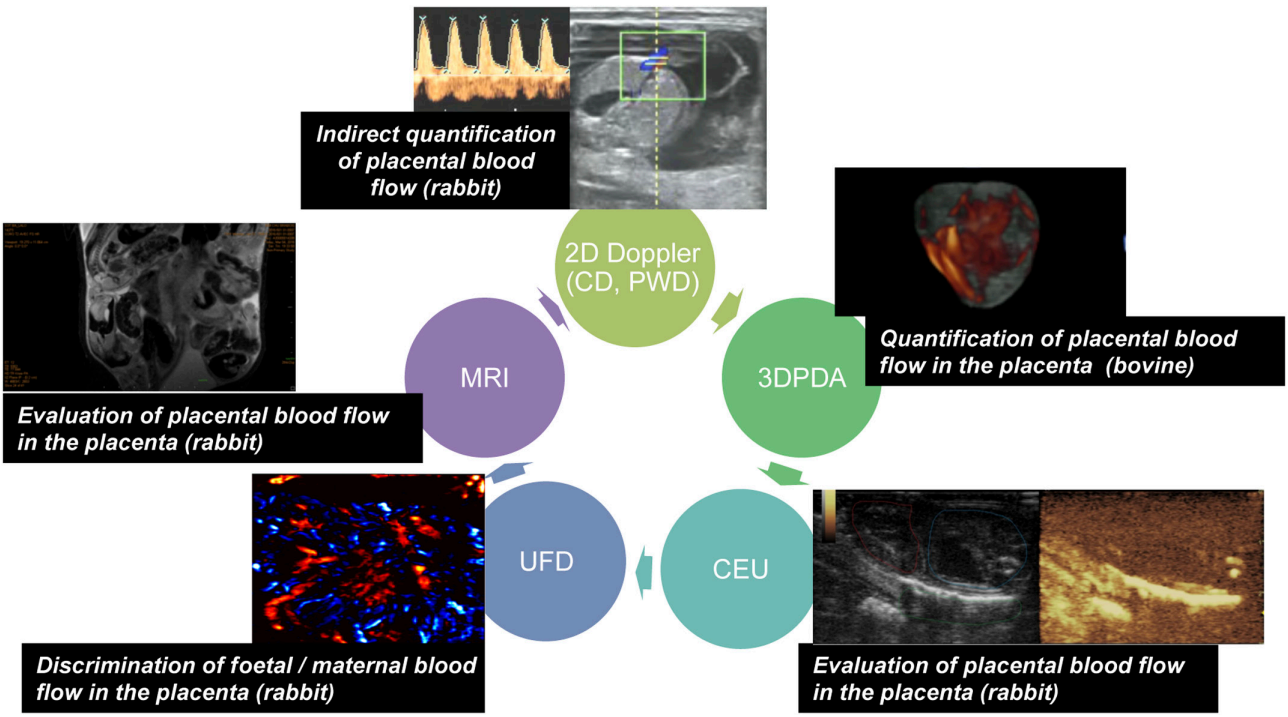


Figure 4 Current approaches for non-invasive evaluation of placental blood flow. The 5 most important methods are 2D Doppler with colour Doppler (CD) and pulse-wave Doppler (PWD), 3D power Doppler angiography (3DPDA), contrast-enhanced ultrasound (CEU), ultrafast Doppler (UFD) and magnetic resonance imaging (MRI). Examples of the obtained image are given in various model species.

pregnancy to a low resistance flow pattern in the first half of pregnancy (invasion of the maternal vessels by the trophoblast). Persistence of high resistance patterns in the UtA at mid-gestation have been associated with increased risk of obstetric complications. The flow velocity waveform of UmA is normally that of low resistance in the last trimester of pregnancy, reflecting the villous vascular tree development, whereas a high resistance pattern indicates abnormal growth of the tertiary villi. In a prospective cohort study of 2120 women, both UtA and UmA Doppler flow velocity waveforms were associated with different aspects of the size and shape of the placenta after birth, both in relation with foetal growth rate (Savasan *et al*. 2014). In humans, however, there is no evidence that the use of UmA Doppler or a combination of UmA and UtA Doppler in low-risk or unselected populations benefits either mother or baby (Alfirevic *et al*. 2015). Therefore, UtA and UmA Doppler measurements are not used in routine examinations, but can be used at any time when a pathological condition is suspected, although their interest for predicting PE remains controversial (Savasan *et al*. 2014, Zhu *et al*. 2015).

This approach has been successfully used in numerous animal species, as a basis for preclinical studies or to monitor high-risk pregnancies in veterinary medicine. In rabbits (Polisca *et al*. 2010), bitches and queens (Nautrup 1998, Di Salvo *et al*. 2006, Scotti *et al*. 2008, Giannico *et al*. 2014), normal ranges of UtA and UmA blood flow parameters have been detailed throughout pregnancy, as well in mice using high-frequency ultrasound (Hernandez-Andrade *et al*. 2014). In both rabbits and mice, they were also used to compare functional haemodynamic changes of the placenta in pathophysiologic conditions (Venditti *et al*. 2013, Lopez-Tello *et al*. 2015, Valentino *et al*. 2016). In any case, differences in blood flow could vary depending on foetal number (UtA) and foetal position in the uterine horn (UmA), limiting the use of these multicotous species as models for humans. In cattle and horses, UtA blood flow can be monitored with transrectal Doppler ultrasound and appears valuable for monitoring high-risk pregnancies (Panarace *et al*. 2006, Klewitz *et al*. 2015). In these species, the reliability of the Doppler exam of foetal and placental blood flow, however, depends on the operator's technical skills, on foetal and maternal movements and on the presence of gas in the gut. Moreover, the stress induced by the examination procedure may impair haemodynamic data.

Three-dimensional power Doppler angiography (3DPDA)

Placental perfusion can be directly investigated with three-dimensional power Doppler angiography (3DPDA). 3DPDA is a combination of three-dimensional ultrasound (3DUS) and power Doppler (PD) techniques. Using 3DUS, a region of interest (ROI) is scanned and a 3D reconstruction obtained either by manual drawing or automatically. With the PD mode, movements are detected through the comparison of pixels in successive B-images, resulting in colour cartography of global amplitude of erythrocyte movement. PD is well adapted to quantify vascularisation in the placenta because its sensibility to slow flows and small vessels is high. 3DUS and PD mode can be associated: acquired volumes or a virtual biopsy of these volumes can be analysed quantitatively using VOCAL software (virtual organ computer analysis, General Electrics Healthcare). The perfusion within the ROI is not defined in terms of mL/min or vascular resistance indexes, but using three specific indices. The vascularisation index (VI) is the ratio of colour/grey voxels and estimates the percentage of blood vessels within the ROI, the flow index (FI) represents the mean power Doppler signal intensity value and represents placental blood flow and the vascularisation flow index (VFI = VI × FI/100) represents tissue perfusion. FI and VFI are expressed as absolute values between 0 and 100. The correlation between perfusion to the uteroplacental unit and quantitative 3DPD indices was confirmed in a sheep model (Morel *et al*. 2010). Because of the heterogeneity of intraplacental vascularisation (Tuuli *et al*. 2011), manual drawing of the whole organ provides more reproducible results than the automatic drawing mode, although it is more time consuming (Cabezas López *et al*. 2016). Due to the size and shape variation, however, the complete placenta cannot always be scanned. Nevertheless, in women, a large volume of both placenta and myometrium can be examined (Morel *et al*. 2011). The resolution is about 0.3 mm. Qualitatively, it is possible in the 3rd trimester to visualise branches of principal placental vessels down to tertiary villous vessels. Quantitatively, changes in intraplacental blood flow seem to appear precociously compared to increased UmA RI observed using 2D Doppler (Matijevic & Johnston 1999). 3D power Doppler indices were studied in normal pregnancies, and reference values are available for physiological situations (De Paula *et al*. 2009, Pomorski *et al*. 2014). One drawback is that foetal blood flow cannot be differentiated from maternal blood flow.

In humans, 3DPDA has been extensively tested in the 2nd trimester. Findings suggest that it has the potential to identify women at risk for subsequent development of PE in the 2nd (Neto & Ramos 2016) and in 3rd trimester (Guiot *et al*. 2008, Duan *et al*. 2016). Current research, however, focuses on its early prognostic value in the hope that 3DPDA may constitute a new tool for 1st trimester intraplacental flow analysis (Tonni *et al*. 2015, Cabezas López *et al*. 2016). Indeed, at this stage, the complete vascular tree can be assessed, theoretically providing a very accurate analysis of placental vascularisation. In a prospective study between 10 and 13 weeks

of pregnancy, using the sphere biopsy mode, it was shown that 1st trimester patients who later develop PE have lower 3DPDA indices than healthy controls, suggesting 3DPDA can help predict PE (Dar *et al*. 2010). Technically, the quantification of the vascularisation is more representative of real perfusion when the largest portion of the uteroplacental unit is included within the volume of interest (Morel *et al*. 2011).

Animal models have been used to explore this new approach. Using experimental reduction of placental perfusion with L-NAME in rabbits, quantitative 3DPDA indices were demonstrated to be sensitive enough to detect placental vascular deficiency (Lecarpentier *et al*. 2012) as confirmed subsequently by *ex vivo* stereological approaches (Tarrade *et al*. 2014, Valentino *et al*. 2016).

This relatively new approach enables the direct evaluation of the organ of interest and is safe during pregnancy, as contrast agents or exposure to radiation is not needed. Its potential for early screening for PE needs to be confirmed. So far, however, GE equipment and VOCAL software are needed for clinical use of 3DPDA, and the indices provided by the software are not a universal method of quantification (in contrast to blood flow quantification in mL/min/g of placenta, for example). Moreover, the exploration field of transducers become limited with increased placental size as pregnancy advances. Physical characteristics of the patient (due to attenuation of ultrasounds with increasing depth) and machine settings (power Doppler gain, pulse repetition frequency and wall motion filter) may also affect 3DPDA indices and limit comparison between patients. Finally, as any real-time ultrasound technique, this method remains operator dependent.

Contrast-enhanced ultrasound (CEU)

Because of the difference of density between tissue and gas, ultrasounds are entirely reflected by gaseous volumes. This physical characteristic has been used to develop the gas-filled, lipid-encapsulated micro-bubbles as injectable ultrasound contrast agents. Contrast-enhanced ultrasound (CEU) enhances the grey scale or colour Doppler mode signal, thus enabling the visualisation of vascularisation down to the microvascular perfusion. The mean diameter of micro-bubbles ranges from 2 to 8 µm, less than that of a red blood cell but sufficiently large to be trapped within the vascular space (Roberts *et al*. 2016). Thus, this technique could discriminate foetal and maternal circulatory systems by imaging the intervillous space (IVS) alone and could be used to diagnose the abnormalities of placental blood flow (Abramowicz 1997).

Because of non-elucidated safety issues, however, ultrasound contrast agents are not currently used in on-going human pregnancies. In one very recent study, CEU was used in macaques and in human pregnancy immediately before scheduled first-trimester termination. Examination of placentas by electron microscopy showed no evidence of ultrastructure damage to microvilli syncytiotrophoblast and indicated that the use of CEU does not results in placental structural damage and may offer a safe clinical tool (Roberts *et al*. 2016). Studies in macaques and rats also suggest that CEU is safe (Keator *et al*. 2011, Roberts *et al*. 2016), without significant effects either on foetal circulation (Schmiedl *et al*. 1998) or on foetal weight (Arthuis *et al*. 2013). One study, however, suggests that it may affect placenta. Indeed, in pregnant rats submitted to CEU in combination with Evans blue perfusion, the amount of Evans blue in the placenta was much higher when CEU was used compared to ultrasound only or contrast agent only, suggesting that ultrasound combined with micro-bubbles can induce cavitation, affecting the permeability of blood vessels and inducing Evans blue leakage (Hua *et al*. 2009).

In humans, CEU was used to analyse changes in blood flow after injection of vasoactive products in the *ex vivo*-perfused term human placenta, in association with colour Doppler imaging and spectral analysis (Abramowicz *et al*. 1999). Clinically, when placental perfusion was visualised at 11 weeks of gestation immediately before scheduled first-trimester pregnancy termination, CEU demonstrated that there are regional differences in intervillous space perfusion within an individual placenta (Roberts *et al*. 2016).

CEU has been used in NHP and mice for fundamental and/or biomedical purposes. Maternal circulation in the placenta was visualised during the last third of pregnancy in macaques (Schmiedl *et al*. 1998). More recently, a study using rhesus monkeys showed that CEU used with grey scale harmonic imaging (enabling detection of blood flow without requiring the use of Doppler methods) allowed the visualisation of maternal blood flow in the IVS only, thus separating maternal from foetal blood flow (Ragavendra & Tarantal 2001). A study in cynomolgus monkeys showed the interest of contrast agents to visualise and quantify intervillous blood flow during early pregnancy (Simpson *et al*. 1998). Technically, ultrasound exams were performed trans-abdominally with a 7 MHz linear array probe, and the flow of blood into and out of the intervillous compartment was readily assessed and quantified using pulsed and colour Doppler. A pulsed-wave Doppler gate was placed in the intervillous compartment, and the waveforms obtained from the IVS were expressed quantitatively using PI (Simpson *et al*. 1998).

Others have used time-intensity curves and software indices to quantify blood in the IVS in humans undergoing second-trimester pregnancy termination (Poret-Bazin *et al*. 2013). In these cases, the bolus mode (immediately after an injection of a bolus of contrast agent) or the destruction-replenishment method can be used. In the latter, after the micro-bubbles are homogeneously distributed through the placenta, high

power pulses are emitted that burst bubbles locally. The replenishment of the contrast is subsequently recorded and analysed using a multi-pulse contrast-specific algorithm derived from modelling time-intensity data acquired after acoustic destruction of micro-bubbles, providing highly accurate estimates of microvascular blood volume and velocity. The combination of the two approaches provides spatial and quantitative information on vascular perfusion in the region of interest (Arthuis *et al*. 2013). Using Japanese macaques, CEU imaging after a micro-bubble burst demonstrated that the rate of perfusion increases with advancing gestation in the 2nd trimester of pregnancy (Roberts *et al*. 2016). In macaques during early pregnancy, CEU and Doppler were combined to calculate blood flow in a manually identified ROI and subsequently quantify volume (mL/100 mL), flow rate (s-1) and blood flow (mL/min/g) (Keator *et al*. 2011). Finally, in rats, CEU has been used for quantitative analysis of placental perfusion through the visualisation of the central arterial canal (Zhou *et al*. 2013) and to compare uteroplacental perfusion between controls and a rat model of PE (Yan *et al*. 2014).

Ultrafast Doppler (UFD)

A technologically disruptive solution to perform blood flow imaging and quantification has been recently proposed under the terminology ultrafast Doppler (UFD). In conventional ultrasound Doppler methods, due to a relatively low frame rate, a balance must be found between spatial resolution (full quantitative Doppler information is obtained on a limited sample volume with PWD) and temporal resolution (an averaged Doppler velocity and/or power estimation is obtained on a large region of interest with CD). By increasing the frame rate by ×100, UFD offers the possibility to analyse the flow with a high spatio-temporal resolution and enables the acquisition of hundreds of samples simultaneously at several locations over a large field of view, thus providing new information about the flow behaviour (Tanter & Fink 2014). The whole ultrasonic information required to assess the two-dimensional behaviour of the flow is acquired, and the PWD information was processed for each pixel within the wide two-dimensional field. Thus, with its very high frame rates applied to a wide field of view, UFD relies on both advantages of CD and PWD in a single mode, i.e. respectively imaging and quantification (Udesen *et al*. 2008, Bercoff *et al*. 2011).

In a recent preliminary study carried out on pregnant rabbits ($n=4$), both imaging and quantification capacity of UFD were used to map placental blood flow (Osmanski *et al*. 2015) (Fig. 5). It is important to note that, within the labyrinth, blood flow characterised as foetal was not negligible compared to blood flow characterised as maternal.

Magnetic resonance imaging (MRI) of the placenta

Magnetic resonance imaging (MRI) is a technique of choice for the anatomical visualisation of soft tissues. It was applied to the placenta first for the diagnosis of placenta *accreta* (when the invasive trophoblast reaches the myometrium) and provides beneficial information in case of posterior placental implantation or of maternal obesity when ultrasounds cannot reach deeply to the placenta. Functional aspects such as vascularisation can be assessed by functional MRI (fMRI) techniques.

MRI is based on the physical phenomenon called nuclear magnetic resonance. Magnetic properties of an atom's nucleus such as its spin are linked to its number of protons and neutrons, allowing it to react to an external magnetic field, absorbing and emitting energy

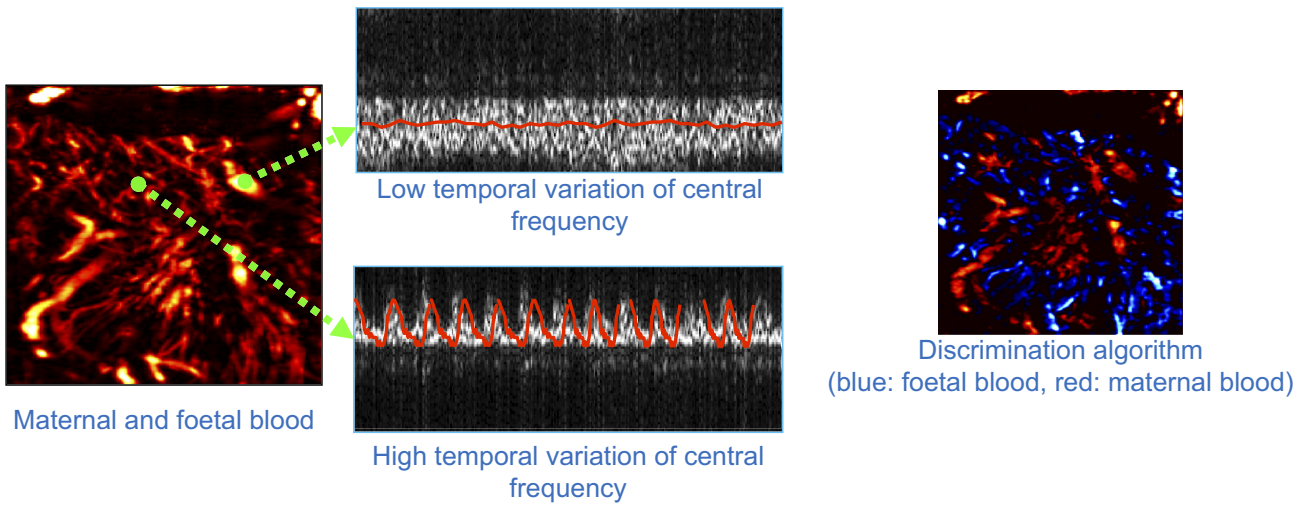


Figure 5 Placental blood flow mapping with discrimination of foetal and maternal circulation using ultrafast Doppler (UFD) in the rabbit model (Osmanski *et al*. 2015). With UFD, the pulsatility of each placental vessel is analysed. Discrimination between maternal and foetal blood flows is performed using specific algorithms.

as it comes back to their equilibrium state (relaxation). The nuclear resonance of Hydrogen (H) atoms in water trapped in biological tissues is used for MRI. During a MRI exam, the body is first submitted to a high constant magnetic field, leading to the alignment (polarisation) of the magnetic nuclear spins. Then, another variable magnetic field is applied, perpendicular to the first one, causing a shift of the alignment of nuclear spins. According to their composition, tissues react differently to these magnetic fields and will behave differently in terms of energy emission. The capture of the tissue's response is converted to image. Different sequences of magnetic fields can be applied, and T1 relaxation times (longitudinal relaxation time of water protons after a pulse of radiofrequency) and T2 relaxation times (transverse relaxation time of water protons after a pulse of radiofrequency) are studied. The precision of the images increases with the intensity of the magnetic field, which is measured in Tesla (T).

Paramagnetic compounds such as gadolinium are often used to enhance tissue contrast in areas that are difficult to visualise. In particular, dynamic contrast enhancement (DCE) MRI is used to explore and quantify tissue perfusion. It requires the acquisition of serial images, before, during and after the administration of the contrast agent, which allows a complete description of the contrast kinetics within tissues (Fig. 6). Thus, each sequence, with different contrasts, provides different information, allowing the imaging of the different tissues.

Animals are used to develop the techniques and to assess its safety. MRI is so far not yet applied in veterinary medicine in the field of reproduction and development, probably because of its cost and complexity (Dekan *et al*. 2012). Experimental studies in rats (Mevissen *et al*. 1994) and retrospectives studies in humans (Tocchio *et al*. 2015) are in favour of probable safety of MRI exams during pregnancy, although MRI uses large static and changing magnetic fields. Nevertheless, precautionary principles are currently applied, avoiding MRI exams during pregnancy unless needed under life-threatening conditions (Kanal *et al*. 2013) as well as gadolinium-based contrast agents, which are known to cross the placenta (Muhler *et al*. 2011).

In any case, this technique is giving promising insight into placental function *in vivo*. In mice and rats, DCE MRI was used to quantify placental perfusion during the last third of pregnancy (Table 1) (Salomon *et al*. 2005, 2006, Taillieu *et al*. 2006, Tomlinson *et al*. 2010, Deloison *et al*. 2012). Alternative contrast agents that do not cross the placenta are emerging and are currently tested in rat.

Alternative approaches to the use of contrast agents include the use of arterial spin labelling (ASL) MRI that measures tissue perfusion using the protons of water within arterial blood as endogenous markers. Using this method, values of normal placental perfusion have been assessed in human pregnancies (176 ± 91 mL/min/100 g of placental tissue) (Francis *et al*. 1998, Gowland *et al*. 1998, Avni *et al*. 2015).

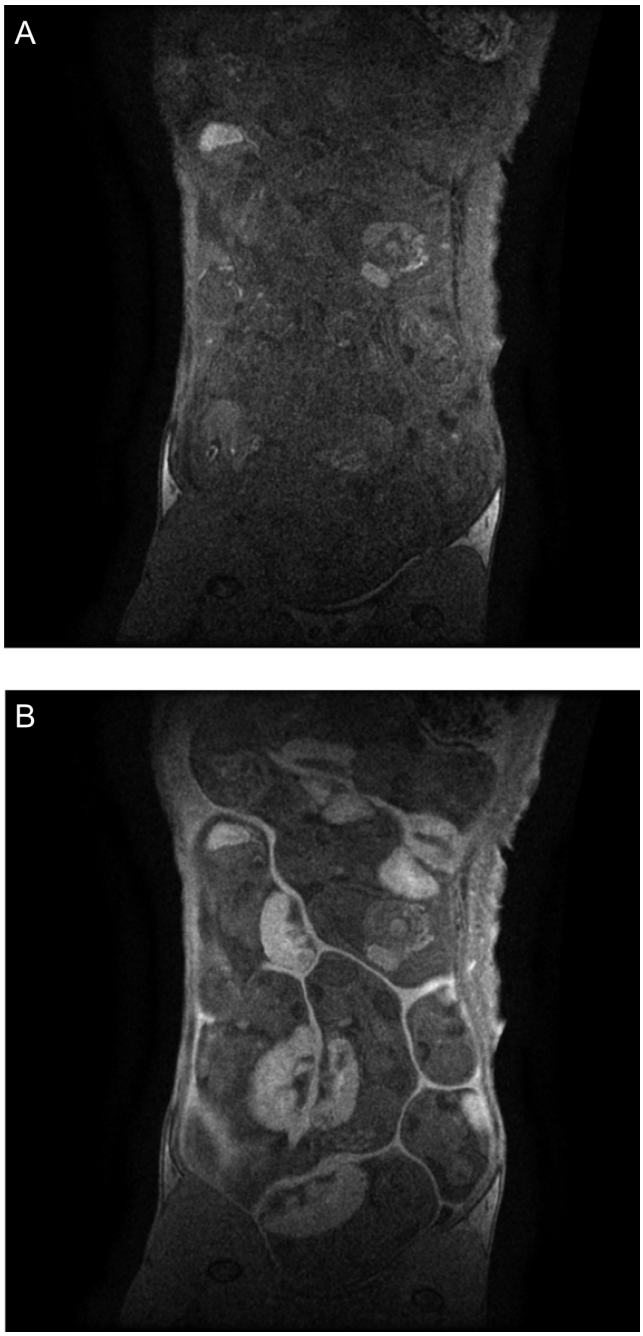


Figure 6 Evaluation of placental blood flow with magnetic resonance imaging (MRI) in the rabbit model before (A) and after (B) injection of contrast product.

Finally, the blood oxygen level-dependent (BOLD) MRI also uses blood as endogenous marker. This technique is based on haemoglobin, which possesses different magnetic properties depending on its deoxygenated/oxygenated state. BOLD MRI thus provides information on tissue oxygenation, which can complement information on tissue perfusion

(Aimot-Macron *et al*. 2013, Chalouhi *et al*. 2013, Sinding *et al*. 2016).

Altogether, MRI provides a large field of view, multiplanar and volume capabilities, an improved soft tissue contrast as compared to ultrasound, with acceptable spatial and temporal resolutions of high-contrast images. Data are independent from the magnet and can be compared between places and machines. The limits of the technique are that it is expensive and complex and not widely available. Moreover, the necessity for the patient to remain motionless and the very load noise generated during the examination requires that animals are anaesthetised, which may modify haemodynamics. Finally, it is important to note that the spatial resolution obtained with MRI is much lower than the resolution obtained with superficial ultrasound probes. Future explorations may combine ultrasound and MRI approaches, although so far, these combinations are worthy for anatomical exploration but do not provide new functional insight (Salomon *et al*. 2013).

Conclusion

In conclusion, recent developments both in Doppler ultrasound and magnetic resonance-based techniques are giving new insight into the evaluation of placental vascularisation. Before their application in the clinical context, their evaluation on animal models is required. Because of their small size and despite the availability of micro-Doppler equipment, studies in mice are technically limited. The use of larger animals such as NHP, rabbits or ruminants remains a valuable alternative.

Declaration of interest

The authors of this paper declare that they have no conflict of interest. Authors are members of European COST action BM1308 SALAAM on Sharing Advances in Large Animal Models.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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Received 8 August 2016
First decision 13 October 2016
Revised manuscript received 3 November 2016
Accepted 14 November 2016