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Placental MRI: Development of an MRI compatible ex vivo system for whole placenta dual perfusion

Jeffrey N Stout, PhD^{(1),*}, Shahin Rouhani, MD^{(1),(2),*}, Esra Abaci Turk, PhD⁽¹⁾, Christopher G. Ha⁽¹⁾, Jie Luo, PhD⁽¹⁾, Karen Rich, MRSO⁽³⁾ [R.T.(R)(MR)], Lawrence L. Wald, PhD⁽³⁾, Elfar Adalsteinsson, PhD^{(4),(5)}, William H. Barth Jr, MD⁽⁶⁾, P. Ellen Grant, MD, PhD^{(1),**}, Drucilla J. Roberts, MD^{(2),**}

¹Fetal-Neonatal Neuroimaging & Developmental Sciences Center, Boston Children's Hospital, Boston, MA.

²Department of Pathology, Massachusetts General Hospital, Boston, MA.

³Department of Radiology, Massachusetts General Hospital, Boston, MA, USA

⁴Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA, USA

⁵Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA, USA

⁶Maternal-Fetal Medicine, Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA, USA.

Abstract

Purpose: Placental dysfunction plays a key role in diseases that affect the fetus in utero and after birth. Aiming to develop a platform for validating in vivo placental MRI and investigations into placental physiology, we designed and built a prototype MRI-compatible perfusion chamber with an integrated MRI receive coil for high SNR ex vivo placental imaging.

Principal Results: After optimizing placenta vascular clearing and perfusion protocols, we performed contrast enhanced MR angiography and MR relaxometry on eight carefully selected placentas while they were perfused via the umbilical arteries (UAs). Additionally, two of these placentas underwent maternal perfusion via the intervillous space (IVS). Despite striving for homogenous perfusion across the whole placenta, imaging results were highly heterogeneous for both UA and IVS perfused placentas. By histology, we observed blood congestion in the villi in regions that showed low UA perfusion during MRI. In two placentas prominent chorionic arteries

Corresponding Author Info Jeffrey Stout, jeffrey.stout@childrens.harvard.edu, Boston Children's Hospital, Neonatal Neuroimaging BCH3181, 300 Longwood Ave., Boston, MA 02115.

*JNS and SR made equal contributions to this paper.

**PEG and DJR made equal contributions to this paper

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followed by adjacent veins underwent contrast enhancement in the absence of villous capillary blush. The single placenta from a pregnancy affected by IUGR had the most homogeneous villous capillary perfusion.

Major Conclusions: A dual perfusion system for ex vivo placentas compatible with MRI permitted assessment of UA and IVS placental perfusion. We observed spatial UA perfusion heterogeneity and evidence for arteriovenous shunting in placentas from normal pregnancies and deliveries, but relative villous capillary perfusion homogeneity in a single IUGR placenta. Future work will focus on system optimization, followed by physiological manipulation, and validation of in vivo placental MRI.

Graphical Abstract

Keywords

Ex vivo; placenta; MRI; pathology; angiography; relaxometry

Introduction

The placenta is an endocrine organ and the site of nutrient, oxygen, and waste exchange during fetal development. Although the placenta is vital for fetal life, it is dispensable after birth. As such, it is the only critical but temporary human organ. Placental dysfunction not only plays a key role in diseases of pregnancy such as intrauterine growth restriction and preeclampsia but also is implicated in diseases that manifest later in life in both mother and fetus [1,2]. However, currently little is known about the in utero development of placental dysfunction as abnormalities are only identified by pathological examination after birth, when the placenta is no longer functioning. This unmet need for methods to monitor placental function in vivo and in real time during pregnancy has recently received considerable attention by the research community [3]. As a result, techniques are being developed to monitor placental function in vivo. However, these techniques are still in the first phase of the development of diagnostic tests [4], that is, understanding of the technical and physiological factors that influence the range of results obtained with these techniques is needed. Ex vivo studies that aim to maintain placental function can contribute to this understanding, since the placenta is unique in its routine availability for research after delivery.

Studying the functioning placenta ex vivo offers unique opportunities to explore placental physiology in a controlled environment. Researchers have sought to overcome the challenge

of perfusing the intervillous space (IVS), and to develop suitable perfusates that maintain organ viability. Studies have demonstrated that tissue viability can be maintained for up to 12 hours [5-7]. Thus the placenta can be kept viable for hours after delivery for physiological and pharmacological experiments [8-11].

There have only been a few attempts to use the ex vivo placenta for bioimaging with most attempts focused on high resolution angiography of the fetal vessels that can only be performed after delivery since the contrast agents used are potentially toxic to the fetus in vivo, and in utero access to the fetal circulation carries some risk of fetal loss [12-16]. In recent pioneering work, MR imaging was performed during ex vivo placental perfusion via the umbilical arteries (UAs) that enabled contrast enhanced (CE) perfusion imaging [17]. There have also been efforts to bridge hemodynamics and metabolism through UA perfusion of hyperpolarized ^{13}C -pyruvate while performing MR spectroscopy and CE MRI [18].

It is important to understand how perfusion of the ex vivo placenta compares to the physiological state in vivo. Clearly after birth the placenta's normal physiological state is perturbed. Early biochemical experiments on the placenta perfused from fetoplacental arteries and the IVS often inferred the quality of perfusion by fetal to maternal seepage and overall flow rates [7,11]. Only one early study of whole placentas made direct observations of UA and IVS perfusion via x-ray angiography [6]. Recent contrast enhanced imaging work has shown heterogeneity of contrast perfusion for the fetal vasculature [17,18], and it is difficult to know if this is an effect of the explant and artificial perfusion process, or whether it reflects normal spatiotemporal heterogeneity related to the normal degradation of placental perfusion at term.

Heterogeneous perfusion is an even greater challenge when using ex vivo placental imaging to validate techniques that have been proposed to monitor placental function in vivo at earlier gestational ages [19,20]. To validate in vivo results, a whole placenta perfused from both UAs and all maternal IVSs while kept viable by an appropriate perfusate would be necessary. Crucially, the in utero perfusion state should be mimicked as closely as possible, such that ex vivo imaging experiments can explore the effect of perfusion dynamics on imaging results.

The goal of this study was to develop an ex vivo placenta perfusion system for MRI. Specifically we built an MR-compatible, dual-perfusion placental chamber with an integrated MRI receive coil so that near-physiological perfusion could be maintained during MRI. We then conducted a series of experiments to determine through both histopathology and imaging how best to mimic in vivo perfusion and visualize perfusion to both the fetal and maternal circulations. Our system will serve as a platform for future studies of placental physiology and for validation of in vivo placental MRI.

Methods

Both the chamber construction and perfusion methodology underwent iterative improvements. We describe our current state-of-the-art approach, and outline the rationale for design decisions in the results and discussion sections.

Coil and chamber construction

We modified the whole placenta dual perfusion chamber described by Maulik et al. [6] and used by Miller et al. [7] to build an MRI compatible chamber with an integrated seven channel coil array (Figure 1). The chamber consists of three compartments: the placenta compartment, the coil array compartment, and the fetal input/reservoir compartment. All compartments were 36 cm in diameter. The chamber was constructed from acrylic, and the coil housing from 3D printed material. The coil and chamber were designed to allow the coil to be isolated from the fluids and removable, yet immediately adjacent to the fetal surface of the placenta to maximize signal to noise. A multi-coil receive array was designed for 3 Tesla scanning to permit modest parallel imaging acceleration. The coil array of overlapping 10 cm loops followed conventional construction [21]. Each channel was optimized using geometric decoupling and preamplifier decoupling techniques [22].

The placenta was mounted such that the chorionic plate was placed down on a 5 cm thick, slightly concave paraffin tray with plastic toothpicks. A hole in the center of the placenta compartment and coil array compartment permits the umbilical cord to hang beneath the placenta into the lower reservoir compartment. Umbilical vein outflow drains into the reservoir compartment.

Placenta preparation for perfusion

Specimen procurement—At the Massachusetts General Hospital, placentas from all in house deliveries are received in the pathology laboratory for triage. Placentas from normal term deliveries without clinical indications for pathological examination [23] are refrigerated for up to 3 weeks pending neonatal or maternal postpartum complications necessitating pathological examination. Placentas with clinical indications undergo a full gross and histopathologic examination.

With IRB approval placentas were obtained for our study. Normal placentas were from term [24] pregnancies with no adverse clinical histories (i.e. no indications for full gross and histopathological examination of the placenta). Abnormal placentas were from term pregnancies with clinical histories of IUGR or preeclampsia with severe features. IUGR was diagnosed when the estimated fetal weight was less than the 10th percentile based on the curve published by Oken and colleagues [25]. Preeclampsia with severe features was diagnosed according to ACOG guidelines [26]. All placentas collected for this ex vivo MRI study were received within 3 hours of delivery and were complete without significant maternal floor disruption or umbilical cord injury as determined by gross examination by an experienced perinatal pathologist (DJR).

Catheterization—The placenta was immediately placed in a prewarmed normal saline bath maintained at 37° C. The umbilical cord was transected at 5 to 15 cm from its placental insertion. The UAs were dilated mechanically (by curved forceps) and cannulated with 8 Fr catheters. The catheters were gently advanced anchoring the catheters within the vessel while flushing with perfusate. The umbilical vein (UV) was catheterized with a 12 Fr catheter in a similar fashion. Monofilament non-absorbable sutures were used to secure the

catheters in place. The catheters were connected to the fetal pump with 6 m of 2.5 mm inner diameter polyvinyl tubing.

Once the placenta was placed maternal side up on the paraffin tray, 32 catheters (5 Fr) were inserted through the basal plate, evenly dispersed, 1 cm into the placental parenchyma [11]. The catheters were fed in parallel by one 2.5 inner mm diameter inflow tube that gradually stepped down to 1.6 mm inner diameter tubing segments proximal to the catheters via a series of short tube segments and “Y” tubing connectors.

Perfusion during clearing and scanning

The perfusate for clearing and for UA and UV perfusion was 37° C prewarmed phosphate-buffered saline at pH 7.4, with heparin sulfate (25000 USP units/L), nitroglycerin (200ug/L), glucose (100 mg/dL) added. A PHD Ultra dual syringe pump (Harvard Apparatus, Holliston, MA, USA) was used to provide pulsatile flow at 86 pulses/minute at a flow rate of 50-80 mL/min. Pump perfusion pressures were measured via in-line pressure gauges (Vernier Software & Technology, Beaverton, OR, USA) and were maintained at 85 and 55 mmHg for “systolic” and “diastolic” phases, respectively. During clearing, the fetal-placental blood was washed out until the venous return was clear. After clearing, perfusion continued at very low flow rates (20 mL/min) until MRI scanning, with a less than 30 min pause while transferring the placenta and perfusion system to the MRI suite.

The perfusate for IVS perfusion was 37° C prewarmed phosphate-buffered saline at pH 7.4, with glucose (100 mg/L) added. Flow to the catheters perfusing the IVS was provided by a peristaltic pump (Masterflex L/S, Cole-Palmer, Vernon Hills, IL, USA) at 85 mL/min with a pressure maintained at less than 15 mmHg as measured by an in-line pressure gauge as above.

During MR scanning the placenta was perfused through the UA catheters at 86 pulses/minute with a flow rate of 50-80 mL/min. The pump was kept outside the MR scan room with 6 m of the same tubing used for clearing to connect the pump to the placental chamber now located in the bore of the MRI through the MRI waveguide (Figure 1a). Approximately 1 m before the branch point to the UA catheters a port was installed to receive outflow from a power injector used to administer contrast during MRI scanning. At the time of contrast enhanced MR angiography (MRA) scanning, yellow India ink (10 mL per 4 L) was mixed into the perfusate reservoir. This allowed histologic ascertainment of the fetal-placental perfusion coverage. In two experiments, we switched the feed line from UA to UV input and modified the pump program to avoid rupturing the venous vasculature (50 mL/min of continuous, nonpulsatile, flow).

Maternal IVS perfusion during scanning used a similar tubing set-up to administer contrast, but this time with blue india ink (10 mL per 4 L) mixed into the perfusate reservoir. This allowed for histologic confirmation of intervillous perfusion.

MR imaging protocol

MRI took place on a 3 Tesla Skyra scanner (Siemens Healthineers, Erlangen, Germany) and consisted of a localizer, B1+ mapping performed by pre-saturation using turboFLASH [27],

multiple echo time spin echo echo planar imaging (SE-EPI) for T2 reference, multiple flip angle VIBE for T1 reference, and MRA using TWIST [28]. Protocol parameters are given in Table 1.

Contrast was used during MRA. This was either Magnevist (Bayer AG, Leverkusen, Germany) diluted to 93.8 mg of gadopentetate dimeglumine per mL, or 50-100 mg per mL of albumin conjugated gadolinium produced in house. Contrast was injected via power injector as a 4 mL bolus at 0.5 mL/s followed by an 8 mL saline flush at 0.5 mL/s.

Pathological Examination Post-Imaging

The placenta was cooled to 4 °C after imaging and, within 24 hours, the placenta underwent a full gross pathologic examination including obtaining the trimmed placental weight, measurements, and examination for lesions in the parenchyma. Sections of parenchyma and umbilical cord were obtained, directed by any gross lesions and from regions of interest (ROIs) as determined by MRI. ROIs were selected from areas of the placenta with varying degrees of villous capillary blush during MRA imaging. Spatial alignment between the gross specimen and MR imaging was achieved by manually comparing the outline from structural images to the specimen aided by two orthogonal lines of vials containing diluted Magnevist that established a fiducial grid. Alignment was also confirmed by comparing the position of prominent chorionic vessels from the MRA. Up to 8 sections were obtained per placenta. Routine formalin fixation, processing, and 5 µm sections for hematoxylin and eosin staining ensued. Pathologic slides were reviewed by an experienced perinatal pathologist (DJR) for pathology and extent of perfusion. Photomicrographs were obtained using a Olympus DP27 camera mounted on an Olympus BX41 microscope and obtained using CellSens Entry (Olympus Corp., Tokyo, Japan) and Photoshop (Adobe Inc., San Jose, CA, USA) software.

Data processing

MRA processing: The TWIST sequence generates several thousand dicoms. These were transferred offline and then combined using fslmerge (into 4D datasets). Visual inspection, merging of venous and arterial images and ROI drawing was carried out in FSLeves [29]. “Perfused” and “unperfused” regions were determined by visual inspection noting regions of villous capillary blush.

T1/T2 parameter mapping: T1 values were determined by fitting for each voxel of the multiple flip angle VIBE data using qMRLab [30,31]. The acquired B1+ map was used to account for flip angle inhomogeneity across each slice. T2 values were determined for each voxel by fitting the model $\ln(S(TE)) = A * (-TE/T_2)$ using “fit” in Matlab (The Mathworks Inc., Natick, MA, USA). For each placenta a median and interquartile range of values within a manually drawn ROI of a central coronal slice of the whole placenta were determined.

Results

Method refinement and design decisions

Anecdotally, we noticed that with high perfusion pressure in the UA (from hand perfusion or constant pressure pumping) there was histological evidence of villous damage with features

resembling chorangiosis and intravillous hemorrhage (data not shown). We did not see these “iatrogenic” pathologies when physiologic pressures and the pulsatile pump were utilized.

Correlation between pathology and MRA

Eight placentas were scanned with UA perfusion, and two of these (#7 and #8) also underwent IVS perfusion. Seven placentas were normal and placenta #6 was from an IUGR pregnancy. Details are provided in Supplemental Table 1. We sought to understand the observed UA perfusion heterogeneity, by comparing imaging and pathology. These comparisons are reported below:

Pattern of terminal villi perfusion—During the clearing procedure the placenta becomes progressively translucent as fetal blood is cleared through the UV and maternal blood is cleared via perfusate extravasation into the IVS (Figure 2a).

In all but one experiment, the pattern of tissue enhancement by MRI (Figure 3) and ink in villous capillaries by histology was heterogeneous. We observed that areas where the MRI enhancement was less appreciable there was villous vascular congestion (compare regions 1 and 2 in Figure 2c and 2d), and no ink in the terminal villous capillaries. The exception was a placenta from an IUGR pregnancy where there was unusually homogeneous enhancement (Figure 3, placenta #6). In unperfused regions, although a vascular thrombus was never observed grossly or histologically, villous vascular congestion was often observed during histological examination (e.g. Figure 2d).

Pattern of chorionic vessel perfusion—In placenta #5 and #7 chorionic plate arteries are first visualized, followed by adjacent chorionic plate veins in regions of no appreciable villous capillary enhancement (Figure 3). This is potential evidence of arteriovenous shunting.

In four experiments we sought to better visualize the chorionic plate veins by reversing the direction of the fetal circulation, and in one experiment (placenta #7) a large (~25%) region of the placenta was perfused via the UV that was previously not perfused by UA perfusion (Figure 4).

Relaxometry—Example T1 and T2 maps are shown in Figure 2. The overall mean $\pm$ standard deviation T1 and T2 values from placentas #2-8 were 2.36 ± 0.18 s and 0.181 ± 0.034 s, respectively. Relaxometry was not collected for the first placenta. Values for each placenta are given in Supplemental Table 2. Note that both T1 and T2 are lower in regions where there is less contrast enhancement during MRA, corresponding to areas where red blood cell (RBC) congestion was observed by histopathology (e.g. Figure 2, region 1 vs 2 in c,d,e,f). There is appreciably more variation in T2 than in T1 across the placenta.

In placenta #4 an intervillous thrombus (IVT) (Figure 5a) and associated infarction (Figure 5b) was found during pathological examination. In the IVT region, T1 was similar but T2 was lower than the surrounding tissue (long arrow, Figure 5c,d). In the infarcted region, both T1 and T2 were lower (short arrow, Figure 5c,d). Low T1 and T2 values were seen in

regions associated with increased RBC congestion (inferior aspect of the placenta, Figure 5c,d).

Maternal IVS perfusion

MRA demonstrated that perfusion of the IVS in our experiments was very heterogeneous. Some lobules appeared well perfused while others demonstrated no perfusion (Figure 6). The pattern of contrast enhancement in perfused lobules was a central inflow that dispersed outward toward the septae (Figure 6). The pattern of blue dye in the gross specimen after perfusion and in the prepared tissue blocks from selected regions indicated that the entirety of the IVS was perfused to some extent, but that regions of appreciable enhancement were darker blue. The histological preparation washed away the blue dye so it was not possible to observe IVS dye penetration on the microscale.

Discussion

We are developing an ex vivo placenta imaging platform that can be used to maintain placental viability and validate in vivo MRI sequences, furthering our understanding of placental physiology. We present our prototype of an MRI compatible placental perfusion chamber complete with a 7-ch MRI receive coil for parallel imaging acceleration. We developed a perfusion method that minimized iatrogenic effects while maximizing terminal villous perfusion and tested our method on eight carefully selected placentas. Our pilot data from dynamically perfused placental MRI demonstrates that ex vivo perfusion heterogeneity from both the fetal side UA and maternal side IVS is common, and is the biggest challenge to future physiological investigations using this platform. Comparing MRA and histology, we observed correlations between low villous capillary contrast enhancement and RBC congestion. In two placentas, upon UA perfusion we saw arterial followed by adjacent venous contrast enhancement, without the expected villous capillary blush, which suggests arteriovenous shunting. Relaxometry findings were consistent with MRA and histopathology, and with known physiology.

There has been a long standing interest in developing ex vivo platforms to understand placental physiology [11]. However, study of ex vivo placental MRI is more limited. Most prior MRI studies have focused on gathering information about the fetal vasculature by optimizing contrast agents for static ex vivo imaging with methods that do not preserve placental function [13,14]. Two efforts contemporaneous to our own work have explored dynamically perfusing the placenta during MRI scanning [17,18]. Both studies grappled with heterogeneous perfusion similar to what we observed in our study. One group observed a relationship between ex vivo perfusion and glucose metabolism using hyperpolarized ^{13}C imaging, which suggests that observed UA perfusion heterogeneity may be in part due to regional variability in glucose metabolism, not just vascular occlusion related to ex vivo handling of the placenta. It is difficult to quantify fluid flow through the placenta when there are tears or regions of unexplained lack of capillary perfusion. Improvements to experimental apparatus and protocols are likely necessary before meaningful flow parameters can be extracted from contrast enhanced studies.

We sought to optimize terminal villous coverage when perfusing through the UA. Careful specimen selection (without parenchymal tears) gave us confidence that UA perfusion should be homogenous. We quickly started using a pulsatile syringe pump, in order to efficiently reach the distal vessels and to minimize “iatrogenic” rupture of the perfused villous vessels. It makes sense that a more physiological approach to villous perfusion would result in better perfusion, and dye coverage increased from approximately 50% with hand injection to approximately 90% in the terminal villi utilizing the pump. As we were calibrating the pump program we had noticed rare foci of iatrogenic pathologies (chorangiosis, villous edema, and intravillous hemorrhage) by histology, which were likely due to non-physiological pumping parameters.

It remains unclear whether UA perfusion heterogeneity is caused by normal term placental physiology, placental pathology, or the insult of delivery and post-delivery degradation. Supporting the hypothesis that delivery causes an insult to normal placental function, is the observation of villi congested with RBC, but not coagulated or clotted blood. This suggests that there was at least some blood flow through these villi not long before delivery. Placental vessels are aneural, but can constrict due to hypoxia and hypoglycemia [32-35]. Although glucose was added to the perfusate, these experiments were not performed with oxygenated perfusate, so the placenta was assuredly hypoxic and vasoconstriction would be expected. However, heterogeneous constriction would not be expected suggesting another mechanism.

Supporting the hypothesis that heterogeneous UA perfusion may be a result of normal or adaptive physiology, the IUGR placenta appeared to be the most uniformly perfused of all the placentas we examined. This placenta was very small, likely with limited reserves and relative hypoxia before delivery. There is evidence that placentas from IUGR pregnancies that still have end diastolic fetoplacental flow, have more highly branched capillary beds in comparison to those with no or reversed end diastolic flow [36]. This increased terminal villous surface area, may mean that in this placenta that is too small and likely hypoxic, normal regional controls such as vasoreactivity are relaxed or impeded to improve overall exchange [32].

As we further develop this platform there are several approaches that might clarify the nature of UA perfusion heterogeneity. The wet lab space was far from the delivery suite, and therefore the normal pathological specimen handling protocols and the difficulty with catheterization prevented us from clearing the placenta with heparinized buffer until 2-3 hours after delivery. Other published methods suggest this step should be taken much sooner after delivery [18,37]. In addition our perfusate could be optimized to maintain healthy endothelium. At one extreme, we could adopt a blood replacement like that proposed to maintain a pig brain viable ex vivo [37], or we could follow more placenta specific recipes with the addition of oxygen [11]. The introduction of intravascular osmotic pressure could foreseeably increase venous pressure and thus better clear the placenta and reduce endothelial damage.

Chorionic veins were visualized in all but the first placenta. The first placenta was scanned approximately 36 hours after delivery, and we hypothesize that contrast leaked from the

arterial side of the villi into the IVS due to endothelial degradation as opposed to flowing through to the chorionic veins as we observed in every subsequent experiment.

We hypothesize that arteriovenous shunting explains the lack of villous capillary perfusion in regions of placenta #5 and #7 where there are appreciable arterial and venous phases of contrast enhancement in chorionic vessels. Arteriovenous shunts are known to cause twin-to-twin transfusion syndrome [38], but there is only limited evidence of arteriovenous shunts in singleton pregnancies [39].

The same region that lacked villous capillary contrast enhancement during UA perfusion in placenta #7, was enhanced during UV perfusion. This region was also near the hypothesized arteriovenous shunt. There was potentially an interplay between shunting and vascular resistance of individual villous trees that could lead to preferential flow to some regions and away from others explaining the difference between UA and UV perfusion. If true, when flow was reversed the pressure bias of this shunt was altered such that the villous tree was then perfused. Other explanations could include, a vasospasm that relaxed over the course of the experiment, or a blockage that was relieved via reversed flow. When combined with the results of the ex vivo perfusion study of glucose metabolism that showed regions of decreased flow were associated with decreased glucose metabolism [17,18], our findings suggest that shunting may be a mechanism to divert flow to regions of the placenta that are more functionally viable.

Relaxometry of the ex vivo placenta indicated the presence of an IVT in one experiment, but T1 and T2 values must be interpreted in light of the experimental setting where the placenta is perfused with water not blood. Water has a much longer T1 and T2 than blood. Water volume and residual blood congestion in individual voxels strongly determines the heterogeneity in T1 and T2 maps (Figure 2 and 5). In the IVT (the core of the bullseye pattern in Figure 5c), similar T1 to the surrounding tissue and lower T2 is indicative of old blood products in agreement with the histologically observed lysed RBC phase of clot development (approximately 1 week old) [40]. The lower T1 and T2 in the infarcted area is counterintuitive in the ex vivo setting given that fibrin density has been related to elevated T2 in vivo [41]. However, as previously observed, water volume fraction in the tissue appears to dominate relaxometry ex vivo. A lower water fraction, and residual deoxygenated- or met-hemoglobin, in this infarcted region would explain the lower T1 and T2 observed. A previous study that compared in vivo MRI findings with post-delivery placental pathology, observed an infarction hematoma in a placenta that had a dark rim and bright core on T2-weighted imaging [42]. In half-fourier acquisition single-shot turbo spin echo (HASTE) imaging, image contrast results from a combination of T1 and T2 effects, so assuming that the bullseye pattern results from a low T1 core and long T1 and low T2 periphery, this would be an older lesion than ours with a neutral T1 core and low T1 and low T2 periphery.

Maternal side heterogeneity is almost certainly an artifact of our experimental design. It is impossible to know how the resistance of individual spiral arteries and the vasculature feeding each spiral artery contributes to the distribution of maternal blood flow to the placenta. In vivo, it is likely affected by myometrial tone that varies over time [43].

Currently our apparatus, following past examples [7,11], uses identical catheters in parallel which means perfusate will flow to the catheters of least resistance. Obviously lobule based dual perfusion experiments will be less sensitive to this heterogeneity given the limited number of maternal catheters. However, for whole placenta ex vivo dual perfusion imaging a different experimental apparatus is needed whereby flow can be regulated across individual lobules of the placenta.

We examined eight placentas, one with significant clinical pathology, and these results already have implications for in vivo placental imaging. T2* imaging of the placenta is being developed as an in vivo indicator of placental function [19], but our results from one IUGR placenta demonstrate that among these placentas some may be functioning in an adaptively optimized hemodynamic mode, while others have failed to adapt. In other words, a large range of in vivo results may be observed for the IUGR group, that could be refined based on doppler ultrasound observations of fetoplacental vascular resistance. Our MRI results also support the concept that regional UA perfusion heterogeneity is normal, even in placentas where fetal outcomes were normal. That is, we often observe regions of abnormal relaxation, capillary perfusion or histopathology in healthy placentas which are likely due to the natural degradation of the placenta as it approaches term. These observations strongly support developing whole, as opposed to single lobule, placenta perfusion systems as test beds for physiological intervention and in vivo imaging validation.

Future work will focus on optimizing the perfusion experiment and obtaining perfusion results From placentas linked to suboptimal fetal outcomes such as IUGR. Optimization will include redesigning the maternal side perfusion system to better model the in vivo conditions of spiral artery perfusion. A crucial component missing from our perfusate is oxygen to maintain cellular respiration, and we will use an oxygenator in our future experiments. These changes should move us closer to the ultimate goal of maintaining placental functionality to study the effects of pharmacological and physiological interventions on placental imaging. As new therapies are investigated for diseases such as preeclampsia, MRI-based assessments of the placenta's vasoactive profile in vivo could prove crucial, and with improvement the platform we proposed here could be well suited to develop and validate these assessments [32,33,35]. Additionally, as we become more adept at our procedures, we hope to compare in vivo and ex vivo imaging of the same placenta with the help of software tools to coregister the images [44].

In conclusion, an ex vivo placental dual perfusion system compatible with MRI permits assessment of UA and IVS placental perfusion We built such a system capable of UA and IVS perfusion while the explant placenta is undergoing MRI and refined the placental vascular clearing and perfusion protocols. We observed spatial UA perfusion heterogeneity and evidence for arteriovenous shunting in placentas from normal pregnancies and deliveries but relative perfusion homogeneity in a single IUGR placenta. Future work will focus on system optimization, followed by physiological manipulation, and validation of in vivo placental MRI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Previously published material

Some aspects of this current work have been previously presented at conferences:

Ha, Christopher G., Esra Abaci Turk, Jie Luo, Elfar Adalsteinsson, P. Ellen Grant, Lawrence L. Wald, and Drucilla J. Roberts. "Seven Channel 3T Array Coil and MRI-Compatible Chamber Design for Ex-Vivo Placental Perfusion." In *Human Placenta Project Annual Meeting*, 13-14 Nov 2018..

Ha, Christopher G., Esra Abaci Turk, B. Muntanelli, J. Luo, W. H. Barth Jr., E. Adalsteinsson, P. E. Grant, L. L. Wald, and Drucilla J. Roberts. "An MRI Compatible Ex-Vivo Placenta Perfusion Chamber Design with 7 Channel 3T Receive Array." In *ISMRM Workshop on MRI of the Placenta*, 04-06 February 2018.

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Highlights

- Built an MRI-compatible placenta perfusion chamber for ex vivo placental imaging
- Magnetic resonance angiography and relaxometry was acquired during dynamic gadolinium perfusion
- First report of MRI during maternal intervillous space perfusion
- MRI evidence of placental arteriovenous shunting

Figure 1. MRI-compatible perfusion chamber. a) diagram of experimental equipment for MRI scanning of a dually-perfused placenta. b) 7-channel MRI coil array with each circular loop of wire (arrow) forming one channel; c) Photograph of the chamber with a catheterized placenta in place in the upper placenta compartment, the coil installed in the coil array compartment, and the lower fetal input/reservoir compartment containing perfusate effluent.

Figure 2.

An example normal placenta (placenta #3). a) After UA washout the placenta is translucent with mostly clear chorionic plate vessels. b) After imaging on gross inspection, there are areas that remain red (1) and areas that appear yellow/white (2). c) MRA maximum intensity projection during the parenchymal phase of the contrast passage showing area of lesser (1) and greater (2) enhancement. d) Histological examination of the red, less enhanced area (1) shows no yellow dye and RBC congestion throughout the villous tree, whereas the whiter, more enhanced area (2) shows dye in most of the terminal villi (arrow) and more open vasculature. e) T2 map. (f) T1 map of the placenta.

Figure 3. (movie) MRA maximum intensity projection of each placenta with green outline indicating the placental border based on structural imaging. The spatial scale is the same for all images. Temporal resolution is 0.9 s and the duration of the clip is 109 s in real time. Note the large variation in heterogeneity, with the IUGR placenta (#6) being the most homogenous and the most rapid venous phase enhancement. In two placentas (red circle), arterial and venous phase is visible in the chorionic plate vessels, but in areas of no parenchymal enhancement.

Figure 4.
(movie) Umbilical artery (UA) perfusion (left) versus umbilical vein (UV) perfusion (right) of placenta #7. Green outline reflects the extent of UV perfusion contrast enhancement. There is a large area of the terminal villi perfused by the UV and not by the UA.

Figure 5:

In placenta #4 an intervillous thrombus (#, lysed RBC) was found during histopathological examination (a). An associated infarction (*) seen as perivillous fibrin around the thrombus was also observed (b, magnified view). This intervillous thrombus and surrounding infarct corresponded to an abnormal area seen on the relaxometry maps (c, d). The long arrow points to the core of the thrombus (designated by # in a), with the short arrow pointing to the surrounding infarct (designated by *). The low T1 and T2 observed in the bottom half of this placenta correlate with villi congested by RBCs suggesting incomplete washout in that area.

Figure 6:
 (movie) 4D MRA data during IVS perfusion (gray scale) and UA perfusion (red scale) contrast injection for two placentas, visualized as overlaid data whilst contrast injections were sequential. Placenta outline is in green drawn manually from the GRE structural scan. The chorionic plate is lying in the posterior (P) most plane. Green crosshairs indicate the positions of the perpendicular slices, with the center point selected as the middle of a well perfused lobule. Temporal resolution is 1.8 s and the duration of the clip is 336 s in real time.

Table 1:**MRI Protocol Parameters**

Sequence	Resolution (mm ³)	FOV (mm)	TE (ms)	TR (ms)	FA	Total time (s)	Notes
MRF (T ₁ and T ₂)	3x3x5	390	2.7	Perlin noise pattern 12-12.81	sinusoidally varied 3-78°	9	720 TRs, continuous density spiral readout
B ₁ ⁺ mapping	6.1x6.1x5	390	2.21	15000	8	30	
VIBE (ref T ₁)	1x1x3	256	1.7	6.1	2/9/19	36	6/8 partial fourier
SE-EPI (ref T ₂)	2x2x3	256	50/150/ 250/350	5000	90	80	GRAPPA R=2, readout bandwidth = 1954 Hz/px
MRA (TWIST)	1x1x1	256	1.11	2.85	21	382	GRAPPA R=2, 6/8 partial fourier, virtual temporal resolution = 0.78 s
GRE (DSC)	1x1x3	256	4.4	2030	30	203	GRAPPA R=2, readout bandwidth = 975 Hz/px