**Author response to editor and referees**

We would like to thank the editor-in-chief and the referees for their time and constructive comments to help improve the thoroughness of this paper. According to their comments, we have made several changes to the manuscript. Most notably we have added an intra- and inter-observer analysis for flow analysis, an image scoring analysis for the ferumoxytol and PC MRA analysis, a dedicated paragraph to statistical analysis, and … , All changes from the prior submission have been marked accordingly in the revised annotated manuscript (e.g. R2.1 for Reviewer 2, comment 1).

The incorporation of new material to address reviewer suggestions left the manuscript 500+ words over the word limits.Therefore, some sections of the manuscript were trimmed to be more concise. Most notably, the analysis on cross-sectional area repeatability in the day-to-day and consecutive-day scans has been removed. This analysis is less significant than the flow repeatability, as flow should be conserved along an unbranching vessel, but area can fluctuate.

Please find our point-by-point response below.

**Response to editor-in-chief’s comments (Comments were provided in summary statement and as comments in the word document)**

**EIC.1** Methods (in regards to ferumoxytol angiogram comparison): How? By Who? Using what scale? This needs to be done by two independent observers.

*We followed the suggestion of reviewer 1 and added clarifications and additional analysis to the ferumoxytol methodology. Vessel conspicuity was scored independently by two experienced radiologists. The methods section has been appropriately updated to describe this process:*

*“To assess the relative quality of the PC MRA, the conspicuity of uteroplacental and fetal vasculature was scored in the ferumoxytol-enhanced angiogram and PC MRA for each monkey that received both scans in the same imaging session. Scoring was performed independently by two independent radiologists using the following scale: 1=not visible, 2=partially visible, 3=fully visible.”*

*“The significance of differences in image scoring between the PC MRA and ferumoxytol-enhanced angiograms for each vessel were compared using a Wilcoxon rank-sum test. Differences in inter-observer scoring for each case were assessed with paired t-tests.”*

*Scoring results were added to the Results section. On PC VIPR scoring:*

*“Radiologist scoring (right/left uterine arteries=2.8±0.6/2.9±0.4; right/left ovarian veins=2.9±0.2/2.8±0.4) confirmed these vessels were fully visible in almost all vessels. Notably absent from most 3D segmentations were the uterine veins and ovarian stem arteries, which received lower scores (right/left uterine veins=1.9±0.2/1.8±0.4; right/left ovarian arteries=1.6±0.6/1.5±0.6). These vessels were only fully observed in one rhesus macaque each. The fetal and umbilical vessels were visible in most cases (umbilical/fetal vessels=2.5±0.7/2.5±0.7).”*

*On ferumoxytol-enhanced angiogram scoring:*

*“Like the PC MRA, the ferumoxytol-enhanced angiograms had excellent visualization of the uterine arteries and ovarian veins (right/left uterine arteries=2.8±0.4/2.8±0.4; right/left ovarian veins=2.9±0.2/2.9±0.2). These angiograms displayed enhanced vessel detail and depicted smaller diameter vessels, allowing for slight improvements in visualization of the uterine veins and ovarian arteries (right/left uterine veins=2.1±0.5/2.2±0.6; right/left ovarian arteries=2.1±0.9/1.7±0.5). The average increased visibility in these vessels, however, was not statistically significant.”*

*“Assessment of inter-observer vessel scoring showed excellent agreement in scoring of the uterine arteries, ovarian veins, and fetal vasculature. Statistically significant differences in scoring were detected for the uterine veins and ovarian arteries in the PC MRA and uterine veins in the ferumoxytol-enhanced angiograms.”*

*In the Discussion section:*

*“For the vessel conspicuity scoring, one radiologist had significantly more success in identifying the uterine veins and ovarian arteries in both the PC MRA and the ferumoxytol MRA. This can be attributed to this observer’s increased experience in reading images in pregnant subjects and highlights the challenges in detecting these two vessel pairs. While this observer was able to more consistently detect the uterine veins and ovarian arteries, their conspicuity was still noticeably lower than in the corresponding arteries and veins, respectively.”*

**EIC.2** Methods: A statistical analysis paragraph is required.

*A statistical analysis paragraph has been added.*

**EIC.3** Results (in regards to description of PC MRA image): This subjective assessment needs to be mentioned in your methods section.

*Thank you for pointing out this oversight on our part. The following sentence has been added to the methods section:*

*“In addition to comparing the scores for each vessel between the two MRA techniques, the impact of gestational age on vessel conspicuity in the PC MRA was examined.”*

**EIC.4** Discussion (in regards to study limitations): Also small numbers for repeatability, and the use of an animal model.

*The following sentences have been modified from existing sentences in the limitations paragraph to address the additional constraints mentioned above:*

*“The use of a rhesus animal model for this feasibility study makes it unclear if the good image quality seen in this study will scale favorably to future human imaging studies, especially given that the mother and fetus will no longer be sedated in these cases.”*

*“In addition to these limitations, the repeatability studies had relatively small sample sizes and were not designed to completely eliminate outside influences on flow . . .*

**EIC.5** Discussion: Conclusions should be three sentences

*The conclusion has been shortened to three sentences.*

**Response to referee 3’s comments**

**R3.1** The inherent limitations of ultrasound are mentioned; any comparison between US and MRI? Complex vascular geometry is listed as a limitation of US, but also seems to be a limitation of MRI? Additionally, nonvisualization of ovarian stem arteries is described as a Doppler US limitation; however, they were unable to be assessed by MRI either. How is MRI preferable?

*US data was not acquired for this study, as we wanted to first ascertain that flow measurements could be acquired in these small vessels with PC VIPR.*

*As mentioned in the manuscript introduction, the complex vascular geometry (torturous path, patient specific anatomy, and adaptation through gestation) is certainly an issue with traditional 2D and 4D Flow PC MRI, where a limited imaging volume requires the prior acquisition of an angiogram to ensure scan planes/volumes are placed properly. We noted that PC VIPR’s large imaging volume and good quality angiograms that can inherently be derived from the data overcome these limitations. The sentences added in response to reviewer comment R4.2 further clarifies this by noting how our post-processing methods allow for accurate measurement plane placement in the presence of complex geometry.*

*A paragraph in the discussion addressing the advantages of PC VIPR relative to pre-existing MRI and US techniques has been re-written (reproduced below) to improve the description of how MRI may be preferable to US in high-risk populations, even if we are unable to probe flow in the ovarian stem arteries.*

*“We believe this technique represents an improvement over previous attempts to characterize total uteroplacental flow with US and MRI. The ability to retrospectively place measurement planes on data-derived angiograms over a large imaging volume allowed for placement of measurement planes orthogonal to the complex, tortuous, patient specific geometries of many uteroplacental vessels relatively simple. Dealing with this geometry is a major limitation with US, where challenges with properly aligning acoustic windows with these vessels may result in reduced accuracy and reproducible plan placement is more difficult for the lack of 3D vessel visualizations. As a result, clinical US examinations do not attempt to image the even more variable venous return, which PC VIPR was able to depict. In addition, using PC angiograms inherently from the data, made possible by the large FOV conferred by the radial trajectory, removed the need for a dedicated MRA acquisition, which was cited as the main limitation of previous PC MRI approaches [23,24]. The PC MRA incorporates all acquired radial projections, which would no be possible with a Cartesian acquisition because of artefacts from cardiac pulsatility. Although, like US, PC VIPR was unable to visualize the ovarian stem vessels and truly characterize total uteroplacental flow, PC VIPR may still offer enhanced diagnostic and prognostic capabilities. However, our results suggest that these flow contributions are small in the subjects imaged here. With its high isotropic spatial resolution, PC VIPR allows for more accurate characterization of velocity profiles and vessel geometry, and thus flow, as these parameters are directly measured rather than estimated from models used in US. This enhanced accuracy may prove valuable in identifying compromised flow dynamics in high-risk populations, and the ability to reproducibly assess flow by as early as late first trimester, as demonstrated here, could aid in early diagnosis. Furthermore, it is possible that when PC VIPR is scaled up for use in pregnant human subjects, the ability to probe the ovarian stem vessels may improve given the increased vessel sizes, allowing for measurements of total uteroplacental flow and subjects. Future studies will investigate whether ovarian stem vessels have larger flow contributions in some subjects, e.g. related to placentation site or vascular adaptation or if they generally contribute little to TUBF. ”*

**R3.2** Clinical applicability; specifically, what interventions can be performed based on placental flow findings? The referenced Human Placenta Project described such intervention only in hypothetical/theoretical terms. If no such intervention exists, what does this MRI acquisition add? How could information be utilized?

*Thank you for pointing this out, such statement was clearly missing. The following sentence has been added to the introduction to demonstrate how early detection of abnormal flow can lead to improved patient outcome:*

*“Prior studies, however, have established strong clinical evidence that low-dose aspirin can reduce the risk of fetal growth restring and preeclampsia in women with reduced blood flow, and intensified monitoring of these pregnancies can allow for appropriately timed delivery.”*

**R3.3** It is unclear that these results will translate well to unsedated mothers/fetuses.

*We agree that exploring this is the logical next step for this work and we have human studies already underway. Future work will explore this topic in more detail, but extensive speculation on human studies is outside of the scope of this manuscript. Our initial results show that, not surprisingly, vascular imaging of the unsedated fetus is often not feasible, but imaging of the uterine arteries and veins and placental side of the embilicial cord shows similar result as in the rhesus studies.*

**R3.4** Any safety considerations prior to attempting with human subjects? Any SAR concerns with 4D flow sequences? Any thoughts on ferumoxytol in pregnant women, if this is to be again used as a standard for comparison?

*There are no notable safety consideration prior to attempting with human subjects. All scanning sequences have already received IRB approval for humans. There are no SAR concerns with PC VIPR as it is actually a fairly low SAR sequence given the low flip angle (10°) and longer TR as a result of playing the bipolar gradients. The use of ferumoxytol for imaging in pregnant humans is currently not approved. However, we think that future approval might be possible as ferumoxytol is approved and routinely used in the clinic* *as an iron supplement to treat iron deficiencies during pregnancy. According statements have been added to the discussion.*

**Response to referee 4’s comments**

**R4.1** It would have been of interest to include characterization of the fetal arterial and venous flows to assess their variability as well.

*To avoid introducing measurement bias in fetal flow measurements by selecting the day with the best imaging quality, fetal flow analysis was always performed on the first day of imaging. Due to scan scheduling and fetal repositioning, same-day analysis is only possible on 3/10 monkeys and back-to-back day analysis is only possible on 6/10 days for which we previously analyzed fetal and umbilical flow. We don’t believe these sample sizes are large enough to draw any meaningful conclusions regardless of the results. We also do not have much more space to spare in the manuscript, which we believe is better suited for the interobserver and intraobserver analysis.*

**R4.2** Further detail regarding the flow measurement on the MIMIC Ensight software is required particularly to demonstrate how a perpendicular cross section was obtained for accurate flow measurement. Given the tortuous nature of the uterine arteries this may introduce some error.

*Thank you for this comment. The original language in the manuscript was vague, assuming prior knowledge of 4D flow post-processing on the part of the reader. We have improved the detail in the part of the Methods section describing flow measurement to now read:*

*“Segmentation of the uteroplacental vessels and fetal vessels was performed through signal thresholding and region-growing using MIMICS (Version 17.0, Materialize, Leuven, Beligum). This mask was imported into Ensight (Version 10.0, CEI Inc., Apex, NC) where 2D measurement planes could be manually oriented with respect to a 3D rendering of the segmented vessels, allowing for accurate plane placement in the presence of tortuous vasculature. Time-averaged flow was measured at the uteroplacental vessel midpoints by integrating through-plane velocity components over the segmented vessel areas.”*

**R4.3** Some assessment of interobserver and intraobserver variability of the flow measurement would be required, as well, given that this work is a validation of flow measurements. Some comment on the training of expertise of the persons doing the measurement (e.g. was a training dataset used prior to evaluation of study data).

*Thank you for this suggestion. The addition of interobserver and intraobserver variability analysis was conducted and certainly improves the thoroughness of this feasibility manuscript. The following statements have been added:*

*In the methods:*

*“Furthermore, for all animals, inter-observer and intra-observer variability in uteroplacental flow was assessed. Inter-observer comparisons were performed by comparing flow measurements from a second observer blinded to the measurement plane placements and results of the original observer. To assess intra-observer variability, the original observer repeated all uteroplacental flow measurements in each monkey. The two observers had 5 and 2 years of experience in 4D flow post-processing, respectively.”*

*In the results:*

*“The inter-observer and intra-observer repeatability analysis in the uterine arteries revealed similar measurement distributions to the same-day repeatability analysis. Inter-observer analysis showed an average measured difference in flow of 16% in the left uterine arteries and 24% in the right uterine arteries (d=0.6 mL/min; LoA=-14.1 to 13.0 mL/min; r=0.75). Intra-observer analysis showed a slightly improved relative difference of 14% in both the right and left uterine arteries (d=0 mL/min; LoA=-12.2 to 12.2 mL/min; r=0.79). The Bland-Altman analysis of all four repeatability metrics for the uterine arteries is presented in Figure 6.”*

*“Inter-observer measurements showed a relative difference of 24% in left ovarian vein flow and 12% in the right ovarian veins (d=1.6 mL/min; LoA=-24.1 to 27.3 mL/min; r=0.85). Intra-observer measurements were improved with relative differences in flow of 13% in the left ovarian vein and 8% in the right ovarian vein (d=1.6 mL/min; LoA=-21.0 to 24.3 mL/min; r=0.89). The Bland-Altman analysis of all four repeatability metrics for the ovarian veins is presented in Figure 7.”*

*In the discussion:*

*“In both the uterine arteries and ovarian veins, the repeatability studies showed good correlation and acceptable relative differences in flow measurements from multiple scans acquired on the same day, as well as inter-observer and intra-observer comparisons. Increased variability was observed between consecutive day scans.”*

**R4.4** Figure 1 is basic and can be omitted.

*Thank you for this comment, which we thoroughly discussed among the co-authors.While we understand it is not common practice to include a basic anatomy figure, we believe Figure 1 should not be omitted. Placental imaging is non-traditional and relatively new to the MRI community. As a result, many MR researchers are unfamiliar with this unique organ and vascular anatomy. Feedback on our first submission of this manuscript also showed difficulties to communicate the need for assessing total uterine blood flow, a central concept of our study design, without such a figure. We believe this figure provides ease of understanding for how we are assessing total uteroplacental flow and a frame of reference with respect to our measurement locations. In addition, most traditional anatomical drawings of this anatomy only show the arterial flow, whereas this paper also demonstrates MRI’s capability to probe venous return.*