PERFUSION OF MULTIPLE EMBRYOS IN MOUSE PREGNANCY - VISUALIZATION AND CHARACTERIZATION USING ASL MRI

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Introduction

A critical adaptive change necessary to support the growing fetus during pregnancy is the development of a functional placenta, together with an appropriate maternal blood supply. The vasculature of the maternal uterus changes dramatically during pregnancy, with a sharp increase in both uterine and umbilical blood flow. Our understanding of the dramatic changes in uterine vasculature is limited by our ability to examine these remodeling processes *in vivo*. Mice offer an important model for the development of novel imaging tools that would help advance clinical imaging of pregnancy. The mouse uterus is considered to be duplex, having two entry points for blood: blood vessels from the ovarian artery feed the upper portion of each uterine horn, whereas the uterine artery supplies blood at the lower end. Thus, blood reaches central embryos from both directions. The aim of this study is to develop a non-invasive MRI tool to characterize the pattern of blood flow in the (crowded) uterine horns in mice. Results of the MRI studies were validated by intravital microscopy studies of blood flow in the mouse uterus and *ex vivo* fluorescence microscopy studies of mouse placentas.

Methods

Female ICR mice were analyzed in a 9.4 T MRI scanner on the last days of their pregnancy (E17.5-E18.5). Blood flow along the uterine horns was measured using Arterial Spin Labeling (ASL) MRI. The complex spatial distribution of arterial blood vessels in the uterine horn in pregnant mice required designing a new set of control/label saturation pulses. Two sequential images were collected for each set of embryos located at the same axial plane: one with cardiac tagging of ovarian arterial water and a second with tagging of arterial water in the uterine artery. This dual tagging strategy allows distinction between embryos located closer to the heart and those embryos located near the bottom of the uterine horn. Changes in signal intensities of the images represent water exchange between the blood and the embryo/placenta. At the end of the MRI experiment, intravital fluorescence microscopy experiments were performed following the administration of a high molecular fluorescent dye. Following the *in vivo* imaging experiments, the placentas were removed and *ex vivo* fluorescence images of the placentas were acquired.

Results

The normalized difference between the two acquired images produced saturation transfer maps, which were color- coded to generate maps of directionality dependent blood flow. Histogram plots of the saturation transfer maps were generated for each placenta and the peak of the histogram was plotted as a function of its embryo position along the uterine horn. A correlation was found between the average ASL values and the positions of the embryos along the uterine horn: For embryos positioned at the lower end, closer to the cervix, a negative ASL signal was observed, suggesting that in these embryos, the maternal blood flow in the placenta is mainly through the lower end of the uterine artery. For embryos in the middle of the uterus, the ASL signal increased gradually, whereas in the upper embryos, closer to the ovary, the ASL signal was mainly positive, suggesting that in the upper portion of the uterine horn, the majority of blood is supplied through the ovarian branch (Figure). The fluorescence microscopy data validated the notion that blood enters the uterine horn from both directions and, in addition, suggested that there is an asymmetric arterial blood supply to the uterine horn with the uterine artery being more dominant than the ovarian one.

Conclusion

This study demonstrates that ASL methodology is able to measure the entire blood input to both the placenta and the embryo, and is sensitive to the embryo location along the uterine horn, thus enabling the non-invasive visualization of the vascular remodeling process that exists during pregnancy in a mouse uterus. To help provide insight into serious prenatal conditions such as fetal growth restriction, preeclampsia, and fetal death *in utero*, conditions characterized by abnormal placental function, this new methodology can be applied to the characterization of genetically modified animal models having targeted defects in placental perfusion.

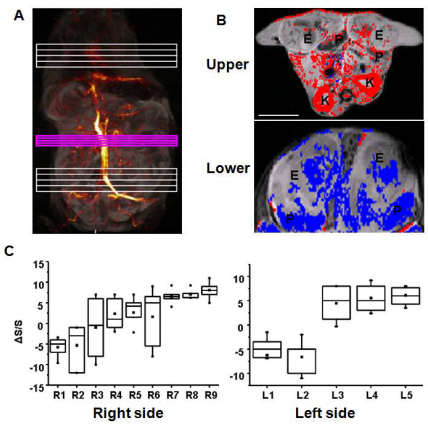

Figure (a) 3D Maximum Intensity Projection (MIP) angiography overlaid on top of a coronal anatomical image showing the major blood vessels; (b) Saturation transfer maps at two different locations within the pregnant mouse. Red represents pixels showing positive change in the saturation transfer map and blue represents negative pixels. Upper embryos are those closer to the ovarian artery, and lower embryos are closer to the uterine artery; (c) Plot of ΔS/S as function of embryo position along the uterine horn. 1 represents the embryo closest to the cervix and 5/ 9 are the embryos closest to the ovarian end. Data are expressed as box plots showing median value, upper and lower quartiles, range, and outliers of the ΔS/S values for each placenta for every imaging slice it in which it appeared.

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