UNIQUE IN UTERO IDENTIFICATION OF FETUSES IN MULTI-FETAL MOUSE PREGNANCIES BY PLACENTAL BI-DIRECTIONAL ARTERIAL SPIN LABELING (BD-ASL) MRI

Reut Avni[1], Tal Raz[1], Joel Garbow[2], and Michal Neeman[1]

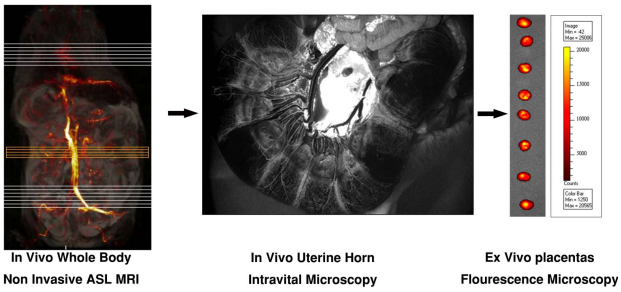
[1]*Biological Regulation, Weizmann Institute of Sceince, Rehovot, Israel,* [2]*Biomedical MR Laboratory, Mallinckrodt Institute of Radiology, Washington University, St. Louis, Missouri, United States*

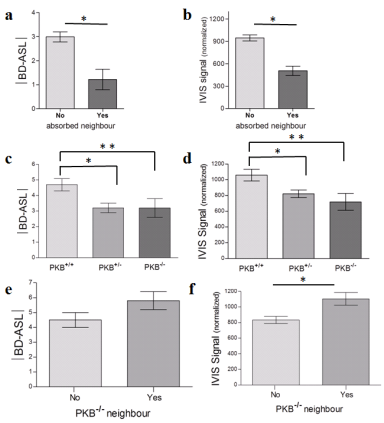
Introduction

In litter-bearing mammals, pups from the same litter must share space in their mother's uterus. This sharing of space results in pups from large litters developing in slightly different environments from one another. For example, previous studies showed that position within the uterus (relative to the ovary and the cervix) influences fetal body weight, due to differences in blood flow to the placentas in the middle versus the ends of the uterine horns. Intrauterine position, therefore, can be a source of non-genomic variability. In most studies of this phenomenon to date, the focus of attention has been based primarily on the sex of the fetuses, as hormonal steroids are able to pass between adjacent fetuses. However, local and/or systemic reciprocal or destructive effects, resulting when sibling embryos/fetuses from different genetic background are carried in the same litter or when a fetus dies naturally in mid-pregnancy, can also influence the growth of the survivors. PKBα/Akt1 is a known mediator of angiogenesis, and placentas of PKBα/Akt1 null (-/-) fetuses were shown to display decreased vascularization and significant hypotrophy. PKBα/ $Akt1^{-/-}$ mice were found to be smaller, with increased perinatal mortality and disordered fetal vasculature. The study presented here examines whether natural occurring deaths, as well as genetic manipulation to create targeted defects in placental function (PKBα/ $Akt1^{-/-}$ fetuses), affect placental perfusion and maternal blood volume in adjacent (normal) fetuses at late-gestation, as measured by *in vivo* Bi-Directional Arterial Spin Labeling (BD-ASL) MRI [1] and *ex vivo* fluorescence microscopy, respectively.

Methods

Female ICR mice and B6(C57BL/6J)/PKBα/Akt1 heterozygote (-/-) mice were analyzed using a 9.4 T MRI scanner on the last days of their pregnancies (E17.5). Blood flow along the uterine horns was measured using BD-ASL MRI, a method developed recently in our group [1]. At the end of the MRI experiment, intravital fluorescence microscopy experiments were performed following the administration of a high molecular weight fluorescent dye. After the *in vivo* imaging experiments, the placentas were removed and *ex vivo* fluorescence images were acquired to measure the blood volume inside the placentas (Figure 1).





Results

Fetuses that had a dead neighbor had lower |BD-ASL| values (1.1±0.4 vs. 3.0±0.2, p= 0.0009) and lower blood volume as measured by fluorescence imaging (536±81 vs. 946±40, p=0.0083), as compared to fetuses which had "normal neighbors" (Figure 2, b and c). Placentas of fetuses of different genetic backgrounds had different values of both BD-ASL and blood volume: |BD-ASL| values were significantly higher in WT placentas (4.7±0.4), as compared to KO (3.2±0.6) or Hetero (3.2±0.3) placentas (Figure 2d; p=0.0112). Interestingly, when WT fetuses were positioned near KO fetuses, their average fluorescence signals were significantly larger (Figure 2f) compared to WT fetuses that had only WT neighboring fetuses (1257±141 vs. 792±71; p=0.0164). |BD-ASL| values showed the same trend (Figure 2e; 5.8±0.6 vs. 4.5±0.5; p=0.07).

Conclusion

The ability to noninvasively determine fetal location along the uterine horn using BD-ASL method opens possibilities for determining and pursuing phenotypic alterations in genetic, as well as developmental, longitudinal studies. These data suggest that, in addition to steroidal hormones, there may exist additional communication mechanisms (e.g. hydrodynamic) in systems of multiple fetuses within one uterus.

References

1. Avni R, Raz T, Biton EI, Kalchenko V, Garbow JR, Neeman M. Unique In Utero Identification of Fetuses in Multifetal Mouse Pregnancies by Placental Bidirectional Arterial Spin Labeling MRI. *Magnetic Resonance in Medicine*, in press, October 2011.

Figure 1: Imaging placental perfusion: Female mice on days E17.5 of their pregnancy were analyzed using 3 imaging modalities: non-invasive BD-ASL MRI, in vivo uterine horns intravital microscopy and ex vivo analysis of the maternal blood volume in the placentas.

Figure 2: Effects of fetal death on adjacent embryos. (a) BD-ASL values and (b) fluorescence signal in placentas of fetuses located near vital fetuses, compared to those near dead fetuses in placentas of fetuses; (c) BD-ASL values and (d) fluorescence in placentas of fetuses of different genetic background; (e) BD-ASL values and (f) fluorescence signals in placentas of WT fetuses having KO neighbors, compared with WT neighbors.