Developmental Analysis of Placental Vascularization using Dynamic Contrast- enhanced MRI

S. Sapoznik[1], V. Plaks[1], E. Berkovitz[2], R. Haffner-Krausz[2], and M. Neeman[1]

[1]Biological Regulation, Weizmann Institute of Science, Rehovot, Israel, Israel, [2]Veterinary Resources, Weizmann Institute of Science, Rehovot, Israel

Introduction:

The mammalian placenta allows respiratory gases, nutrients, and wastes to exchange between the maternal and fetal vascular systems. The importance of the placental circulation has long been recognized and is exemplified by the correlation of fetal weight with placental size, and uterine and umbilical blood flow [1]. Reduced placental blood flow can be used as a predictor of high risk pregnancy and is associated with early embryonic mortality [2], fetal growth retardation [3] and impaired neonatal survival and growth [4]. Genetically modified mice frequently exhibit growth retardation and in utero lethality which are attributed to problems in placenta function. In the current study we used macromolecular contrast-enhanced MRI for in utero assessment of placental function along pregnancy in mice. We evaluated normal placentas at different developmental stages, as well as strain specific differences. Additionally, we studied placental function during fetal resorption and during tetraploid complementation, a manipulation which allows a mouse fetus to develop supported by a placenta of a different genetic background.

Materials and Methods:

Animal model: Pregnant ICR mice were analyzed on E10.5, E13.5 and E18.5 (vaginal glug=E0.5). ICR mice carrying transferred embryos (with native or tetraploid placentas), were analyzed on E18.5 of pregnancy. Tetraploid complementation was performed as previously reported [5].

MRI experiments: Animals were placed in a 4.7T horizontal Bruker Biospec spectrometer and scanned using a whole-body rf coil. Fetal anatomy was derived from multi-slice spin echo images (TR 2000ms, TE 11 ms, 2 averages, SW 50000 Hz, matrix 128x128, FOV 5x5cm, slice thickness 1 mm, 13 slices). Placental function was derived from 3D-GE contrast enhanced images. Biotin-BSA-GdDTPA and BSA fluorescein/rhodamine were prepared as previously reported [6]. A series of images with 15, 5, 30, 50, 70 degrees flip angles were acquired to determine the precontrast R_1 (TR 10ms, TE 3.6ms, 2 averages, SW 50000 Hz, matrix 128x128x64 (zero filled to 128), FOV 5x5x5cm). For dynamic postcontrast imaging, consecutive T_1 weighted 3D-GE images (flip angle=15^0) were acquired from the time of biotin-BSA-GdDTPA tail vein injection and up to 13.5 minutes. MRI data were analyzed to derive the blood volume fraction (fBV), rate of contrast material enhancement, placental volume and fetal length.

Histology: Animals were sacrificed 3.5 or 13.5 minutes following fluorescent contrast material administration. Placentas were retrieved, fixed, embedded in paraffin and sectioned serially (4μm). Percent area of fluorescence in each field of view was calculated.

Results:

Normal placental function was characterized during different embryonic stages. Contrast enhanced MRI enabled detection of the maternal vasculature including the embryonic placentas. Analysis of the placental size showed a significant increase as pregnancy progressed. Determination of the fBV values revealed no significant change between E10.5 and E13.5. However, considerable increase was observed at E18.5. 13.5 minutes follow up of the contrast enhancement dynamics was used to derive the rate of enhancement, namely the change in contrast material concentration over time. At E10.5, a negative rate of enhancement reflected clearance of the contrast media from the circulation. This clearance was attenuated at E13.5. Towards the end of pregnancy (E18.5), the contrast material accumulated slowly in the placental vasculature, resulting in a positive rate of enhancement. A significant change was observed for all time points detected. The slow accumulation of contrast material was restricted to the maternal vasculature, and no enhancement was observed for the embryos. Thus the fetal maternal blood barrier remained intact. These data were further verified by histological analysis. In order to further explore the ability of the method to evaluate developmental changes in utero, we examined strain related differences using B6 and ICR embryo transfer experimentation. Significant differences were observed regarding fetal and placental sizes, as ICR embryos and placentas were larger than those of B6. Interestingly, no significant strain related changes were observed regarding placental fBV and rate of enhancement. Tetraploid complementation may result in an abnormal placental function. Using contrast enhanced MRI we were able to detect these abnormalities non-invasively in utero. Our results were further verified by fluorescent imaging.

Discussion:

Using macromolecular dynamic contrast enhanced MRI we were able to assess vascular parameters of placental function in normal and abnormal pregnancies, as well as to resolve strain related differences in embryo transfer experimentation. This non invasive technique may be further used for follow up of vascular deficiencies affecting placenta function in genetically modified mice.

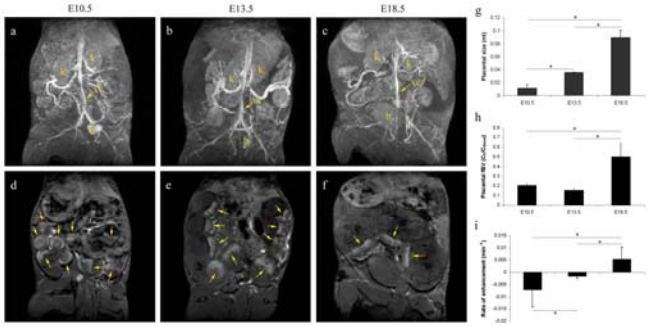

Figure 1: MRI of mouse pregnancy. (a,b,c) MIP of pregnant ICR mice, tail vein injected with biotin-BSA-GdDTPA at E10.5, E13.5, or E18.5 respectively. K-Kidney B-Bladder VC-Vena Cava. (d,e,f) Single slices of a, b and c respectively. Placentas are indicated with an arrow. (g) Placental size. (h) fBV. (i) Rate of enhancement.

References:

1. Reynolds, L. P. and Redmer, D. A. (2001). *Biol Reprod.* 64, 1033-40.
2. Bassil, S., et al. (1995). *Hum Reprod.* 10, 1497-501.
3. Harrington, K., et al. (1997). *Br J Obstet Gynaecol.* 104, 674-81.
4. Baergen, et al. (2001). *Pediatr Dev Pathol.* 4, 144-53.
5. Nagy, A., et al. (1990). *Development.* 110, 815-21.
6. Dafni, H., et al. (2003). *Magn Reson Med* 50, 904-14.