Robust Diffusion-Weighted Single-Shot MRI Can Resolve Major Mice Placental Compartments

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Introduction: The placenta is an essential and versatile organ providing life-supporting functions for the developing fetus. A placenta's wide range of physiological functions includes providing the nutrient, gases and clearance of catabolites and gases, while protecting the fetus from the maternal immune system [1-2]. Further understanding placental functions and their relation to the organization of its intervening blood vessels and cells, requires characterizing the movement of fluids within and between key compartments making up the placental structures. MRI can provide a unique window to these phenomena, particularly if assisted by Diffusion-Weighted (DW) studies [3,4]. DW MRI of these organs in mice, however, faces a number of challenges including tissue heterogeneities, continuous fetal motion, air/fat/water interfaces, maternal cardiac and breathing motions, and dealing with small structures. This explains the absence, hitherto, of literature reports on this kind of studies. Emerging spatio-temporal encoding (SPEN) methods, however, can provide a sufficiently robust imaging platform to measure these phenomena [5]; SPEN is a single-shot imaging method that can cope with the spatial, chemical and field heterogeneities that normally challenge spin-echo EPI. The aim of this study is to explore such possibility, and investigate the potential of novel diffusion-based SPEN (dSPEN) acquisition strategies to uncover diffusion-based functional aspects of mice placentas *in-vivo*. dSPEN MRI studies were thus carried out in the presence and absence of high M_w contrast agents (b-BSA-GdDTPA) to fractionate different placental compartments, at various gestation periods of pregnant mice. These *in vivo* MRI analyses, aided by histological and fluorescence microscopy validations, lead to new insights regarding *in vivo* supra- and sub-voxel morphologies, diffusion and perfusion in placentas.

Methods: MRI experiments were conducted on a 9.4 T Biospec scanner (Bruker, Germany). A total of n=12 dams and 52 placentas/fetuses in female ICR mice, were studied in these experiments; complementary optical imaging studies were done on n=6 dams, 27 placentas/fetuses. Animals were analyzed on embryonic days (E) 14.5 and 18.5 of pregnancy. Mice were anesthetized and scanned using 2D fast spin-echo (FSE; FOV = 40×40mm^2; voxel size = 0.156×0.156×1mm^3) for anatomical reference. Diffusion measurements were acquired using custom-written dSPEN acquisition and processing routines. All DW MRI experiments were acquired using a single scan segment and shared a FOV = 40x40mm^2, in plane resolution = 0.4x0.4 mm^2, 1 mm slice, and were monitored by seven b-values (0, 100, 200, 400, 600, 800, 1000 sec/mm^2) applied along three orthogonal directions to obtain the geometric mean ADCs using a $\boldsymbol{\delta}$=3.5 ms and Δ=7.7 ms. To further distinguish the placental compartments, dSPEN MRI data were collected before and after intravenous administration of b-BSA-GdDTPA, which does not cross the maternal-fetal barrier.

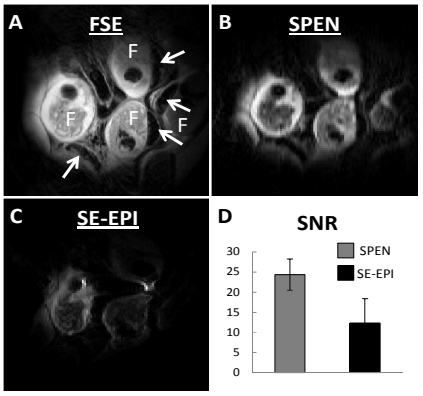


Figure 1. Images of a pregnant mouse collected on a 1 mm slice, highlighting the fetuses (F) and their placentas (white arrows). (A) 2D fast spin-echo images. (B) Single-shot 2D SPEN. (C) 2D SE-EPI. (D) Signal-to-Noise ratios of SPEN and SE-EPI averaged over the N=4 placentas for suitably chosen equal regions of noise.

Results:

Imaging Mice Placentas by SPEN and by SE-EPI: Figure 1 presents anatomical images collected on a pregnant mice at E14.5 gestation days using 2D fast spin-echo (A), single-shot 2D SPEN (B) and 2D SE-EPI (C) MRI. Both SE-EPI and SPEN uncover several fetuses, although the later does so with a higher SNR (panel D). In general, the SPEN images showed a systematically higher robustness in these placental mice studies, with little or no need for fine-tuning procedures; SE-EPI by contrast suffered from the noise and ghost artifacts known to affect its analyses of small heterogeneous regions at high fields, and failed to yield DW data for most cases. All our work therefore was based on diffusion SPEN acquisitions. Mean ADC for E18.5 mice (n=10 mice; 39 placentas) was found to be 2.9±0.6 x 10^{-3} mm2/sec, as derived using a mono-exponential fitting, and mean ADC for E14.5 mice was 2.4±0.9 x 10^{-3} mm^2/sec; (pValue=0.065 between the two gestation days).

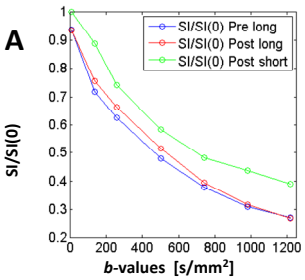
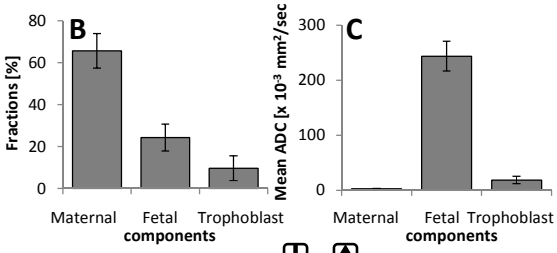


Figure 2. (A) Representative multi-exponential diffusion curves obtained before and after the administration of b-BSA-GdDTPA, at short (250 ms) and long (5000 ms) recycle times TRs. (B) Estimation of the different placental compartmental fractions, and of (C) the corresponding ADC values.



Resolving placental structures using a multi-component relaxation fitting: To differentiate maternal blood from fetal blood capillaries and trophoblast giant cells, changes in the placental signal intensity before and after administration of b-BSA-GdDTPA were analyzed for both short (250ms) and long (5000ms) TR acquisitions. An example of the multiple relaxation curve analysis based on these choices is presented in Figure 2A. A multi-fit formulation of the acquired data revealed that maternal blood constitutes 66±8%, fetal blood is 24±6%, and the TGC are 10±6% of the overall placental volume (Fig. 2B). ADCs were found to be 3.1±0.4, 244±27 and 19±7 x10^{-3} mm^2/sec for maternal, fetal and TGC compartments, respectively (Fig. 2C).

Conclusion: The use of emerging, robust single-shot imaging methods like SPEN, provides a powerful tool for investigating anatomical features even under the challenging conditions arising in *in vivo* studies of mice placentas at high fields. As shown in this work, the combination of DW SPEN and of high molecular weight contrast agents, succeeded to resolve three key placental populations and to characterize them both their fractions –which were validated by independent optical means (data not shown) – as well their apparent diffusional characteristics. The resolved features and ADCs reported here, are consistent with a dynamic scheme (Fig. 3) for the microcirculation of fluids in mice placenta, involving mainly free diffusion of the maternal blood, a strongly forced perfusion of the fetal blood in fetal microcapillaries, and a moderate fluid exchange along with endocytic uptake of the contrast media by trophoblasts lining the maternal blood pool.

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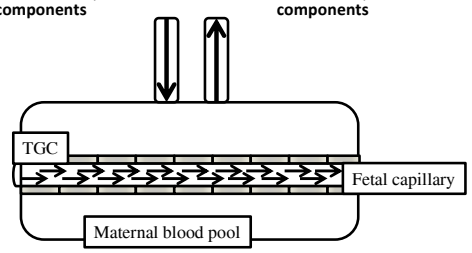

Figure 3. The movement of fluids in the placenta main compartments as visualized by diffusion MRI.