Introduction
Interventions for ameliorating fetal brain injury due to hypoxia-ischemia (H-I) are urgently needed. There are life-long consequences of cerebral palsy (CP), mental retardation, and learning disabilities of fetal H-I. Advancement in this area is stymied from the lack of sufficient diagnostic tools for brain injury to the fetus in utero. We utilized a clinically relevant fetal animal model recently developed by us (Derrick, 2004) which exhibits a CP phenotype in newborn rabbit pups following fetal H-I, to examine the feasibility of developing prognostic biomarkers of later brain injury.

Hypothesis
1) Diffusion weighted imaging (DWI) and specifically, the apparent diffusion coefficient (ADC) can identify brain regions of selective vulnerability. 2) Depth of ADC drop and recovery time after acute H-I is proportional to the degree of fetal brain injury and thus, postnatal outcome.

Method
Survival H-I. In vivo global H-I of fetuses was induced in timed pregnant New Zealand white rabbits at 79% gestation (E25, n=4) as described previously (Derrick, 2004). Briefly, a balloon catheter was introduced via the femoral artery into the descending aorta. Imaging was performed on 3 T GE clinical scanner using extremity coil. Body temperature was maintained with a heated water blanket wrapped around the dam’s abdomen. Single shot fast spin echo (SSFSE) images were taken for anatomical reference with slice thickness 4 mm and matrix 256x192, field of view 16 cm, covering all fetuses. 25-32 axial slices were placed to obtain series of DWI images with b=0,700 s/mm² and 4 averages. Baseline readings were obtained for 10 min. With the dam in the magnet, the aortic catheter was inflated for 40 min, DWI of fetuses obtained during H-I; then, the balloon was deflated and DWI obtained for another 20 min. After the imaging session, catheter was removed, femoral artery repaired and the dam was allowed to recover. DWI was performed 3 more times at 4, 24, and 72 hours after H-I. ADC was calculated from the entire brain. On E31, 1 day before term, kits were delivered by caesarian section and underwent neurobehavioral assessment to determine presence of dystonia and motor deficits.

Regional Changes After Acute H-I. To investigate regional differences during H-I with higher spatial resolution, we performed DWI on fetuses at E25 (n=2) and E29 (n=2) with a surface coil. After anesthesia and arterial catheter insertion in the dam, as described above, a portion of uterus containing a head of a fetus was exposed through 4 cm incision in the abdominal wall. The fetal head with the intact uterine wall was positioned between 2 plastic restrainers and a 25 mm surface coil. The dam was then placed in a cradle inside a 4.7 T Bruker scanner and imaged for at least 20 min before, during 40min H-I period, and at least 20 min during reperfusion. Diffusion weighted spin-echo images were 1.5 mm slice thickness, FOV 2 cm, matrix 128x64, 8 slices, 2 averages, TR/TE 2000/21 ms. Each image without diffusion weighting was followed by 3 images with b=860 mm²/s. ADC values were calculated for the ROIs, placed on olfactory bulbs, frontal and posterior cortex, basal ganglia, hippocampus, thalamus, corpus callosum, using then extirpated after DWI and processed.

Results
Individual fetuses and their uterine position were identified and followed between sessions (Fig.1, arrow points to brain). ADC of each fetal brain was determined (Fig. 2 – ADC map). ADC slowly decreases after onset of H-I, falls to ~85% of baseline value at nadir, and takes 30-40 min to return to the baseline after reperfusion (Fig. 3). There was a second fall of ADC at 48 hours, but this may represent normal maturational development.

The investigation of different brain regions revealed that after onset of H-I, ADC values of E29 kits experienced more abrupt and profound decrease at about 20 min of H-I (Fig. 4 -blue), compared to that of E25 kits, where ADC decrease was steady and shallow (Fig 4 - green). Recovery of cerebral cortex at E29 was slower than at E25, and failed to occur in basal ganglia even 40 min after cessation of H-I. In general, the fall in ADC was consistently larger and the recovery slower in thalamus and basal ganglia compared to that of the cerebral cortex.

Conclusions
The ADC response to fetal hypoxia-ischemia is more gradual and the drop is less compared to the response to stroke in postnatal animals. Different fetuses respond differently to the same H-I and it is possible that the fetuses with the most changes in ADC are the worst kits on neurobehavioral examination. Basal ganglia and thalamus may be more vulnerable to H-I as they show more severe injury on DWI.

References

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