Detailed Anatomic Microimaging of Early Stage Human Embryos

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Introduction: We are performing MRI of early and late stage human embryos as part of a MRI and EFIC microscopic database of normal and abnormal human development. Embryos of Carnegie stage (CS) 15 (~33 day) and older are sufficiently developed that tissue microimaging techniques to increase SNR and CNR such as background-free media and contrast agent enhanced fixatives (1) can be utilized. The earlier stages present a challenge due to their fragility and lack of differentiation of tissue MR characteristics, combined with the need to acquire higher resolution images on very small samples. These specimens display low internal contrast (2), and are deformable with rigid positioning or dense fluid media. The literature contains few reports of MRI at stage 12-13, and none with well resolved anatomy. We performed MR microimaging of a CS-13 (28 day) fixed embryo in aqueous media, with high contrast and differentiation of internal structures, using spin-echo (SE) imaging with a high performance gradient system at 7.0T. This approach yielded high anatomic detail in images of early embryos.

Methods: Fixed human embryos in formalin were provided by the National Museum of Health and Medicine, Washington, DC. CS-13 embryos were prepared in 5-mm tubes with a fixative layer containing the embryo supported by a layer of Fomblin (Solvay Solexis, Houston, TX). Imaging was performed on a 7.0T Bruker Biospin MRI system with 150 G/cm gradients and a 5 mm birdage coil (Bruker, Billerica,MA). 2- and 4-echo RARE and T1W gradient-echo (not shown), and 3D SE sequences (**Fig. 1**), were performed. SE parameters were, 1 echo, with 90 flipback, TR/TE=1000/20 ms, 12 NEX, FOV 9.5x4.5x4.5 mm, matrix 256x128x128, for 37x35x35 micron resolution. Images were processed in ImageJ (NIH) and Volocity (Improvision, Lexington, MA).

Results and discussion: The imaging considerations for the early embryo include less dense, less differentiated tissue and a requirement for an aqueous medium. While the use of perfluoroether media for microimaging tissue is desirable to eliminate background signal, the density and hydropobicity of perfluoroethers and silicone oils deforms embryos at this stage. Exposure of embryos to T1 contrast agents such as Magnevist prior to imaging was found to enhance the signal in the surrounding aqueous media due to diffusion, and contrast agents were not used in the study shown in **Fig. 1**. Multiecho RARE and T1W gradient echo imaging performed on the embryos produced poor differentiation of tissues and poorly distinguished embryo from the medium, while a 3D spin echo at a relatively short TR compared to water T1 and TE of 18 to 20 ms produced a high level of tissue differentiation at 35 micron resolution, with relatively low background signal. By comparison, seen in our laboratory and in the literature (3), gradient echo and fast gradient echo imaging was superior in later stage embryos treated with formalin/Gd fixative prior to imaging.

Conclusions: 3D single echo spin-echo imaging at carefully chosen echo times on a 150G/cm gradient system gave very high contrast for this stage well differentiated from the surrounding fluid signal in CS-13 human embryos. The resolution and contrast achieved allowed highly detailed anatomic structural identification including pharyngeal pouches, inflow and outflow tracts of the heart, trabeculated myocardium and rhombomeres in the hindbrain (**Fig. 1 A, B**).

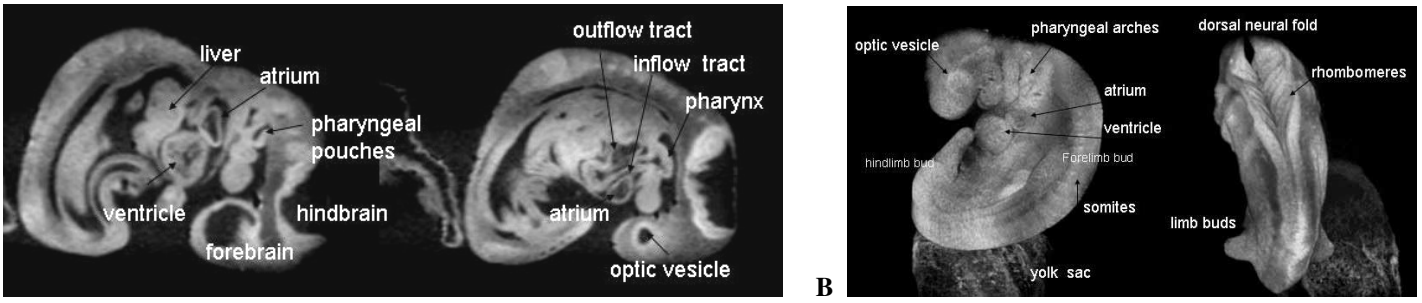

Figure 1. Carnegie stage 13 3D MRI data set with anatomic identification. A. 2D sagittal views (ImageJ); B. 3D projection (Volocity).

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