Simultaneous and rapid MR-microscopy on 32 mouse embryos *ex vivo*

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Introduction: The genetics of malformations found in humans is commonly studied in mouse models using high-throughput, phenotype-driven screens such as *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis (1,2). For example, in order to screen 100 first generation mutants per year the analysis of ~2400 embryos would be required. Importantly, an efficient identification of late developmental defects in mouse embryos such as cardiac abnormalities is difficult as the embryos are opaque. MRI has been used previously to investigate fixed mouse embryos at high spatial resolution (3,4). More recently, a multi-coil approach has been proposed in order to increase throughput (5,6). However, unlike clinical scanners, most of the experimental MR-systems are not yet equipped with multiple receive capability. Ultra-high field magnets ($B_0 \geq 10$ T) may be additionally limited in space making the multi-coil arrangement difficult. We therefore developed a single-coil approach at 11.7 T that allows us to simultaneously image up to 32 embryos at a spatial resolution < 50 µm.

Methods: Normal and genetically modified embryos were harvested at 15 days after detection of the vaginal plug and fixed in 4% paraformaldehyde at 4˚C for ~1 week. Up to 32 embryos (four embryos per layer) were embedded in 1% agarose (Seakem) containing 2 mM Gd-DTPA (Magnevist, Schering UK) in 28 mm NMR tubes. MR microscopy was performed on an MR-system that comprised an 11.7T vertical magnet with a shielded gradient system (548 mT/m, risetime 160 µs) (Magnex Scientific, Oxon, UK), a Bruker Avance console (Bruker Medical, Ettlingen, Germany) and a 28 mm quadrature driven birdcage-type resonators (Rapid Biomedical, Würzburg, Germany). Raw data were acquired using a 3D spoiled gradient echo (GE) sequence (FOV 26 × 26 × 50 mm, matrix size 608 × 608 × 1408, α=90°, TE=10 ms, TR=30 ms, NAE=4, experimental time ~12 h), zerofilled to 1024 × 1024 × 2048 points and filtered before FFT.

Results: Figure 1 shows (a) a longitudinal MR image across the multi embryo tube together with axial cross-sections through layer 1, 4, 5 and 8 (Fig. 1b-e), respectively. The pixel size is 25 × 25 × 24 µm. Sufficient contrast and resolution was obtained for all embryos to accurately identify different organs and tissues (Fig. 1c-e). We assessed the sensitivity and specificity of multi-embryo MRI in comparison to single-embryo MRI (7) using a known mouse model of *Cited2* deficiency that is characterized by adrenal agenesis, exencephaly and cardiac defects such as atrial and ventrical septal defects, outflow tract and aortic arch malformations (8). The overall sensitivity and specificity of multi-embryo imaging for cardiac malformations was 88% and 92% respectively. For bilateral adrenal agenesis, the sensitivity was 100% and specificity was 95%.

Discussion: We have established an MRI method to image up to 32 embryos simultaneously using a single volume coil. Our method used a fast GE sequence and was based on the single embryo technique reported previously (7). It represents a simpler alternative to the multi-coil approach (5,6), but achieves higher throughput and spatial resolution. This approach allows us in principle to screen 11,680 embryos per year on a single magnet in overnight runs, while leaving it free for daytime experiments.

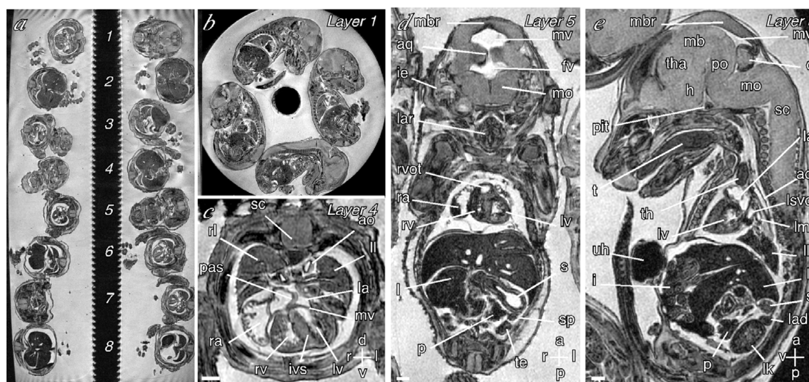

Figure 1: *(a) High-throughput high-resolution magnetic resonance microscopy on a stack of 32 embryos embedded in a single NMR tube. (b) Transverse section through the top layer (layer 1) showing the four embryos in this layer. (c-e) Transverse, coronal, and sagittal sections through individual embryos in layers 4, 5 and 8, respectively. The voxel size is 25.4 × 25.4 × 24.4 µm. Structures indicated are the spinal cord (sc), the right and left lungs, atria and ventricles (rl, ll, ra, la, rv, lv), primary atrial and interventricular septa (pas, ivs), mitral valve (mv), midbrain roof (mbr), midbrain (mb), mesencephalic vesicle (mv), thalamus (tha), hypothalamus (h), pons (po), cerebellum (c), medulla oblongata (mo), pituitary (pit), tongue (t), thymus (th), left superior vena cava and main bronchus (lsvc, lmb), aorta (ao), liver (l), stomach (s), left adrenal and kidney (lad, lk), pancreas (p), intestines (i), umbilical hernia (uh), aqueduct of Sylvius (aq), fourth ventricle (fv), inner ear (ie), larynx (lar), right ventricular outflow tract (rvot), spleen (sp), and testes (te). Scale bars = 500 µm; axes: d-dorsal; v - ventral; r - right; l - left; a- anterior, p- posterior.*

Conclusions: High-resolution MRI can be used to simultaneously image multiple specimens at high spatial resolution using a single RF coil. We are currently applying this technique in analyzing unexplained lethality in embryos generated in our and in collaborating laboratories.

References

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