Magnetic resonance microscopy of mouse embryos in utero

Hogers, B.; Gross, D.; Lehmann, V.; Groot, H.J.M. de; Gittenberger-De Groot, A.C.; Poelmann, R.E.; Zick, K.

Magnetic Resonance Microscopy of Mouse Embryos In Utero

BIANCA HOGERS,[1] DIETER GROSS,[2] VOLKER LEHMANN,[2] KLAUS ZICK,[2] HUUB J.M. DE GROOT,[3] ADRIANA C. GITTENBERGER-DE GROOT,[1] AND ROBERT E. POELMANN[1]*

[1]Department of Anatomy, Leiden University Medical Center, Leiden, The Netherlands
[2]Bruker Analytic GmbH, Rheinstetten, Germany
[3]Leiden Institute of Chemistry, Leiden University, Leiden, The Netherlands

ABSTRACT

Magnetic resonance microscopy (MRM) was used to study mouse embryonic development in utero. MRM is a non-invasive imaging technique to study normal and abnormal embryonic development. To overcome image blurring as a result of embryonic movement, fast imaging sequences were used (less than 1 min scanning time). Clear morphologic proton images were obtained by diffusion spin echo and by rapid acquisition with relaxation enhancement (RARE), revealing living mouse embryos with great anatomical detail. In addition, functional information about embryonic blood flow could be obtained, in the absence of a contrast agent. This was achieved by combining two imaging sequences, RARE and very fast gradient echo. We expect that MRM will soon become a feasible method to study longitudinally both normal and abnormal (transgenic) mouse development. Anat Rec 260:373–377, 2000.

Key words: heart development; magnetic resonance imaging; blood flow; transgenic mice

In this new era of transgenic mouse models, the need to evaluate the effects of gene manipulation during embryonic development is growing. Traditional embryological studies are invasive, embryos are sacrificed and processed to reveal specific information like morphology, histology, antigen distribution, gene expression patterns, or physiological parameters. Each objective requires a specific method that excludes the use of one specimen for several questions, whereas for temporal information each method has to be repeated on other specimens at subsequent stages of development. Due to the non-invasive character of magnetic resonance microscopy (MRM) this technique offers the possibility to study, over an extended period of time, subsequent stages of normal development in a single embryo in utero. In addition, due to its excellent resolution, MRM offers the possibility to follow the onset and course of a malformation during development. MRM has proven to be a powerful tool for fixed embryos (Smith et al., 1996; Huang et al., 1998) and living chicken embryos in ovo (Bone et al., 1986; Effman et al., 1988). Recently, Smith and coworkers (Smith et al., 1998) managed to visualize and follow living rat embryos in utero in a 2.0T MR microscope by a 3D projection encoding technique, with a total scanning time of 27 min. The objective of the present study is to visualize living embryos inside a mother mouse. The size of a mouse is approximately one fifth of that of a rat, and imaging of the embryo in utero requires very high resolution in combination with excellent contrast. To achieve this, we used moderately high magnetic fields of 7.0T and explored various fast imaging sequences. Fast imaging is necessary to avoid artifacts from embryonic movements that cannot be controlled by the researcher.

MATERIALS AND METHODS

Animal Support

CPB-S mice were mated overnight and checked for vaginal plugs the next morning. Mice carrying embryos of 13, 16, or 17 days of development were used. The mother

Grant sponsor: Leiden University (grant-in-aid).

*Correspondence to: Prof. Dr. R.E. Poelmann, Dept. Anatomy, Leiden University Medical Center, PO Box 9602, 2300 RC Leiden, The Netherlands. E-mail: R.E.Poelmann@lumc.nl

Received 13 June 2000; Accepted 6 September 2000

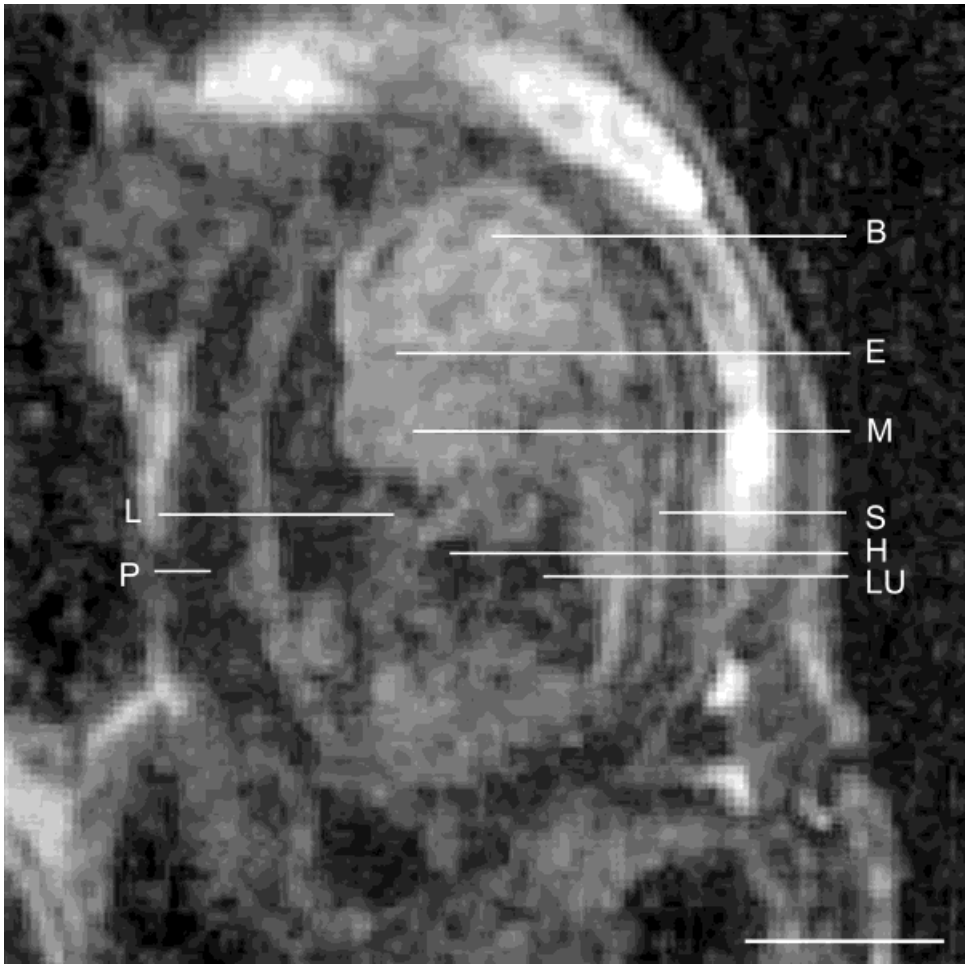

Fig. 1. Image of a mouse embryo of 16 days of development obtained with the Spin Echo diffusion method. The selected slice represents a longitudinal section of the embryo, with the nose pointing to the left. Solid tissue, like the brain (B) is bright, whereas fluid-filled structures, like the eye (E), and mouth (M) are darker grey. Regions with flowing fluid (blood, placenta (P), liver, heart (H) and amnion) are black. L, limb; LU, lung; S, spine. Scale bar = 4 mm.

mouse was connected to an inhalation anesthesia unit (N_2O/O_2/isoflurane). The anesthetized mouse was mounted into an in vivo probe with an internal diameter of 38 mm. Cardiac and respiratory motion were monitored via an ECG and a fiber-optic sensor method (Wilson et al., 1993), whereas gating of these signals was achieved by a Physiogard SM 785 NMR trigger unit (Bruker), equipped with a light-diode transmitter/detector system. The body temperature was kept constant by tubes filled with circulating warm water. The mouse was secured with adhesive tape (Leukosilk), and inserted head-up into the resonator. The experiments were performed in accordance with the *Guide for the Care and use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No. 85-23, revised 1985).

MRI

Embryos were imaged at 7.0T using an AVANCE MRM set-up (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany). We used a vertical 300 MHz, 150 mm bore magnet, equipped with a 89 mm bore shim system with an inner diameter of 72 mm. An in vivo probe (Mini 0.5 probe) was used in combination with a gradient system of 20 G/cm. The ParaVision software (Bruker) was used for data acquisition and processing on a Silicon Graphics O2 workstation with the Irix 6.5.3 operating system.

In Vivo Embryonic Imaging

Before each measurement the magnetic field homogeneity was optimized by shimming. Each measurement session began with a multislice orthogonal gradient echo sequence for position determination and selection of the desired region for subsequent analyses.

Diffusion spin echo was used for a first set of experiments to obtain anatomical clear images of living mouse embryos of 14 days of pregnancy. With a field of view (FOV) of 40 mm and a matrix size (MTX) of 256 by 256, we obtained a resolution (R) of 156 μm. The repetition time (TR) for pulse sequences was 2000 msec, and the echo time (TE) was 30.3 msec, with two averages (NEX) for each phase encoding step, resulting in a total acquisition time (T) of 17 min and 4 sec.

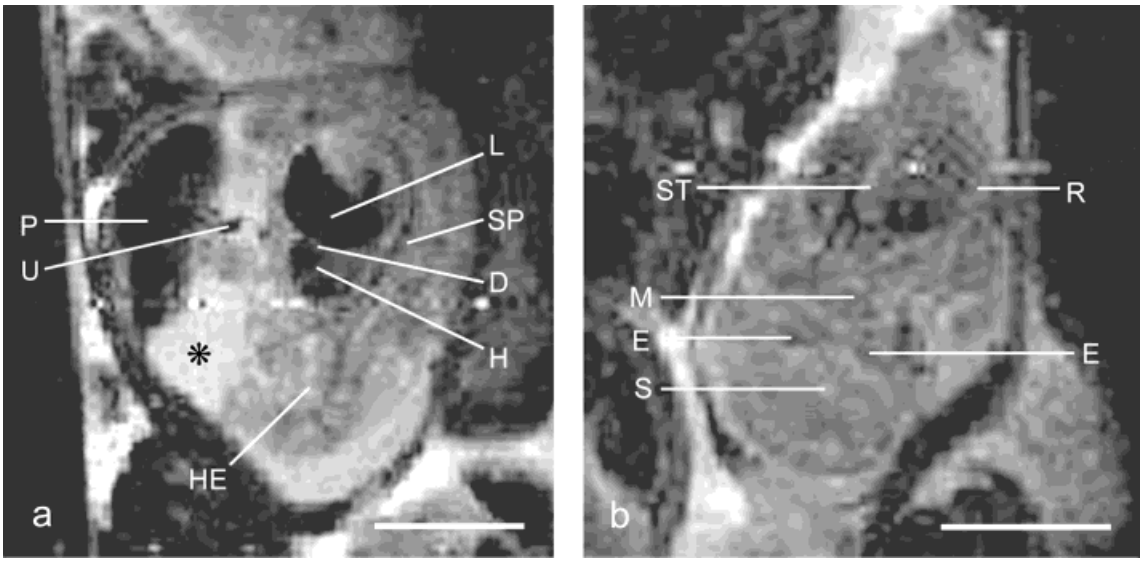

Fig. 2. In utero images of mouse embryos of 17 days development. Rapid acquisition with relaxation enhancement method. **a:** Embryo is positioned with its head (HE) down, the spine (SP) is at the right hand side. RARE results in a contrast-rich image with details like the heart (H), diaphragm (D), liver (L), and placenta (P). In contrast to the SE diffusion method the amniotic fluid (*) is now white. U, umbilical cord. **b:** Another embryo from the same litter. Position and structures similar to (a), but this is a frontal section. Visible are the skull (S), eyes (E), mouth (M), individual ribs (R), and sternum (ST). Scale bar = 4 mm.

To reduce the total acquisition time, we explored an imaging method known as the multi-slice RARE method (Henning et al., 1986). This method employs a single excitation step followed by the collection of multiple phase encoded echoes. For this purpose, mice of 13 and 17 days of pregnancy were imaged. With a FOV of 50 mm (16 slices) and a MTX of 256, 256, 256, we obtained a R of 195 μm. The TR was 3910 msec for the 13 days embryos, and 5000 msec for the 17-days embryos, whereas the TE was 11 msec. We used a RARE factor of 16, with four averages for each phase encoding for the youngest embryos and two averages for the older embryos, resulting in a T of 2 min and 10 sec for the Day 13 embryos and a T of 5 min and 31 sec for the Day 17 embryos.

To simultaneously obtain functional information we combined two imaging methods, RARE and very fast gradient echo (VFGE). Therefore, we selected as reference proton image one slice from the RARE data set. Subsequently, gradient echo with short TR (30 msec) and TE (4.3 msec) and a 90° pulse angle was performed on the same slice, with a T of 30 sec.

RESULTS

Diffusion spin echo is an adequate method for the detection of abdominal structures of the mother mouse, including uterine tissue, and of embryos with brain vesicles, eyes, thorax region, and extremities (Fig. 1). Solid tissues, like the brain, were depicted bright, whereas fluid-filled structures, like the eye and mouth were darker grey. Regions that contain flowing fluid, like blood in the placenta, liver and heart, and even amniotic fluid in the amniotic cavity, were black. Although diffusion spin echo resulted in clear anatomical images, the total scanning time of 17 min was considered as unfavorably long.

The RARE sequence was the ultimate method for embryonic contrast. This method resulted in high-contrast T2 weighted proton images of the embryos and the placenta. As this is a very fast method, it is worthwhile to increase the resolution at the expense of the time by increasing the number of averages per slice. This improves the signal-to-noise ratio within a reasonable amount of time. From the 17-days pregnant mouse a detailed image of the placenta with the umbilical vessels within the umbilical cord, the head of the embryo with brain vesicles, heart, liver, extremities, spine, ribs, and even the dorsal aorta and the carotid artery was obtained (Fig. 2). In contrast to the SE diffusion method the amniotic fluid is depicted white with RARE. We were able to obtain similar results in a 13-days pregnant mouse, resulting in a clear image of an embryo with head, eyes, extremities, and heart (Fig. 3).

Finally, we succeeded in demonstrating intraembryonic blood flow in a 13-day pregnant mouse without the use of contrast agent. We used VFGE on one slice selected from the RARE data set (Fig. 4a). The resulting image was representative of fluid flow perpendicular to the image plane (Fig. 4b). With postprocessing, color was added to the image and superimposed on the RARE image. In this way, it was possible to visualize blood flow through the heart and dorsal aorta of the embryo, and also through the placenta and uterine arteries (Fig. 4b). This method is very sensitive as it also detected amniotic fluid motion inside the amniotic cavity.

DISCUSSION

The MRI sequences presented here allow for contrast-rich images of living mouse embryos in utero. To visualize heart development in very small objects, i.e., embryos from approximately 3 mm to 2 cm, the highest possible resolution is necessary. Imaging at 7.0 Tesla was adequate to discern the heart as a separate structure from 13 days of development onwards, but we expect that higher

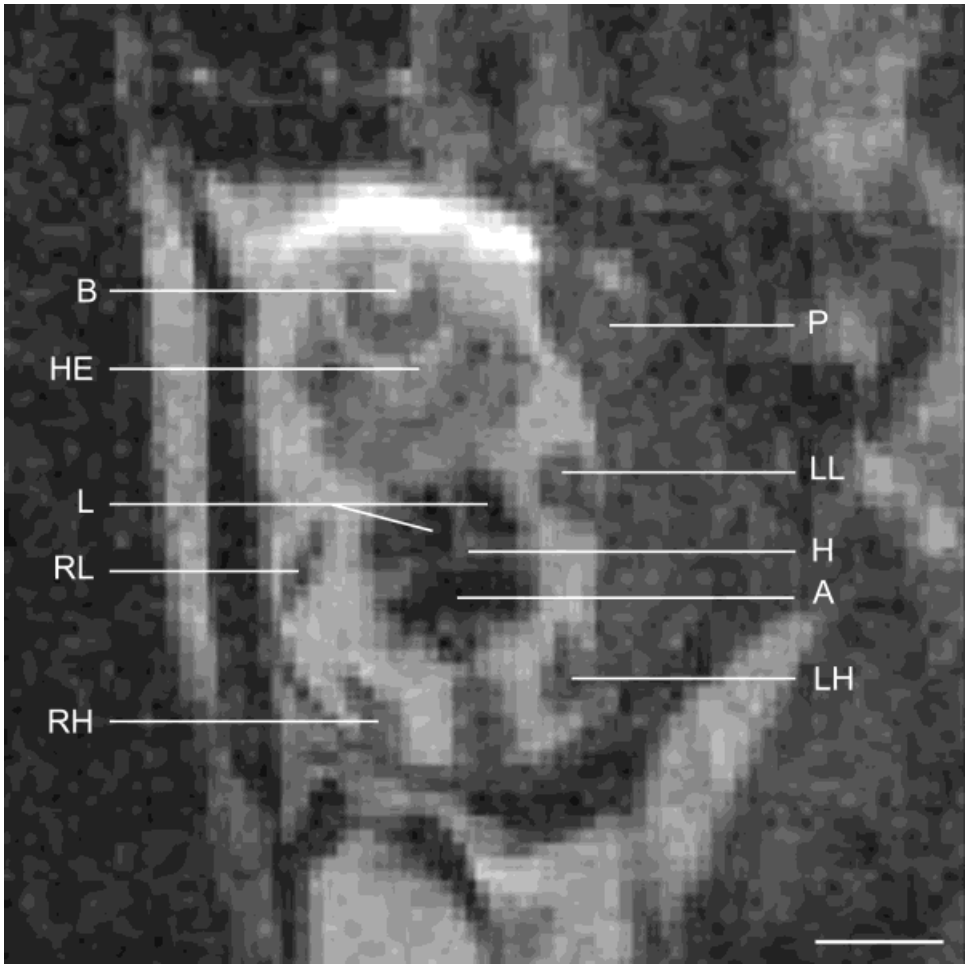

Fig. 3. Image of a mouse embryo of 13 days of development, obtained with RARE. This is a mid-frontal section with the head of the embryo upwards. The semilunar shape of the placenta (P) is visible at the right hand side. In the head (HE) the neural tissue of the brain (B) is visible. Furthermore, the forelimbs (RL, LL) and hindlimbs (RH, LH) are seen. Within the body we can discern the lungs (L), the heart (H), the diaphragm and the abdomen (A). Scale bar = 2 mm.

magnetic fields are necessary to obtain more cardiac anatomical details. Imaging of fixed embryos at 17.6 Tesla (unpublished results) improved the image quality by a factor 2.5. We are planning to repeat these in utero experiments at 9.4 and 17.6 Tesla and expect to visualize the heart in more detail and at earlier stages of development.

By combining the RARE method for obtaining a contrast-rich image of an embryo with fast gradient echo, we were able to obtain functional information in addition to the morphological image. The fast repetition time combined with a 90° excitation pulse caused a saturation of the magnetization from the static spins in the selected slice. As a consequence, the image intensity of the static tissue disappeared completely, but blood flowing into the selected imaging plane replaces the saturated magnetization, that results in a net enhancement of the signal of the flowing blood above background levels. By postprocessing this was converted into a color scale and superimposed on the RARE image. This method is very sensitive as it also clearly demonstrated amniotic fluid motion. By choosing different planes at the level of the embryonic heart, the direction and velocity of blood flow can be determined. In case of abnormal heart development, as is pertinent in many transgenic mouse models, this method might reveal functional cardiac abnormalities well before anatomic defects can be detected (due to low resolution).

Another advantage of these fast imaging sequences is that all embryos can be examined individually in a reasonable time. After scanning the complete abdominal region of the mother, very small fields of view can be selected for the individual embryos resulting in higher spatial resolution. Imaging 10 embryos, each with a resolution of 40–50 μm, takes no longer than 15–20 min, that is shorter than the total scanning time of 27 min used by Smith et al. (1998) for the imaging of 4 to 8 embryos with a resolution of 195 μm.

In conclusion, fast imaging sequences result in respectable morphological images of living mouse embryos in utero, at this moment, whereas development of MRM technology in the near future will improve the quality and time of acquisition.

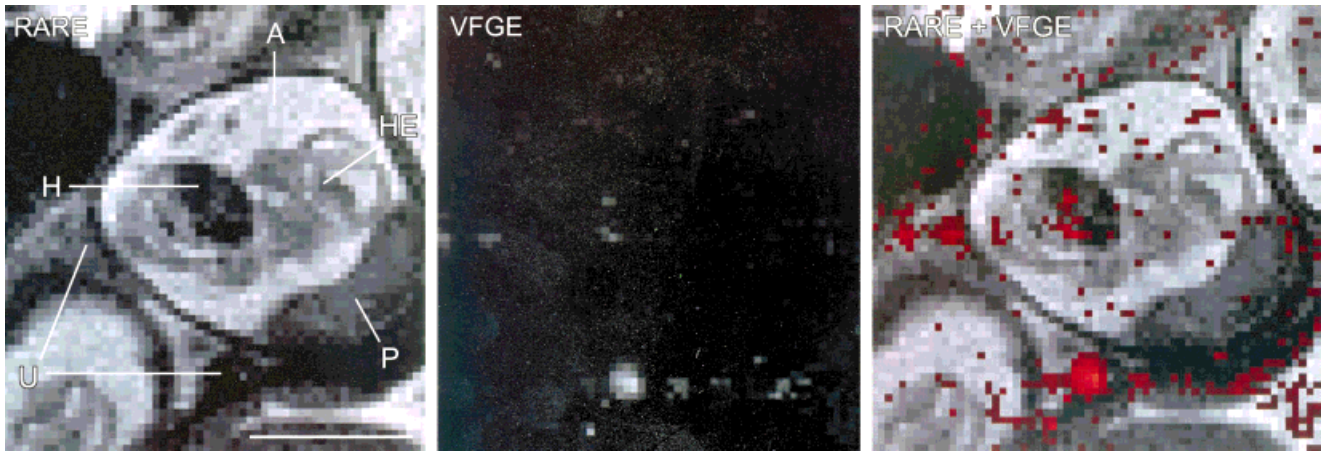

Fig. 4. Mouse embryo at 13 days of development. **a:** Contrast-rich image obtained with RARE. This is a frontal section (dorsally) with the head (HE) of the embryo to the right. **b:** Detection of flow perpendicular to the image plane, obtained with very fast gradient echo. Postprocessing of (b) resulted in a red colored image that was superimposed on (a). **c:** Combining (a) and (b) demonstrates blood flow in a 13-days mouse embryo in utero. High flow as in the uterine arteries and through the heart of the embryo results in a brighter spot. A, amniotic fluid; H, heart; HE, head region; P, placenta; U, uterine arteries. Scale bar = 4 mm.

ACKNOWLEDGMENTS

We thank C. Erkelens for his technical advice and Jan Lens for photographical assistance. This research was supported by a Grant-in-aid from the Leiden University.

LITERATURE CITED

Bone SN, Johnson GA, Thompson MB. 1986. Three-dimensional magnetic resonance microscopy of the developing chick embryo. Invest Radiol 21:782–787.

Effman EL, Johnson GA, Smith BR, Talbott GA, Cofer G. 1988. Magnetic resonance microscopy of chick embryos in ovo. Teratology 38: 59–65

Henning J, Nauerth A, Friedburg H. 1986. RARE imaging: a fast imaging method for clinical MR. Magn Reson Med 3:823–833.

Huang GY, Wessels A, Smith BR, Linask KK, Ewart JL, Lo CW. 1998. Alteration in connexin 43 gap junction gene dosage impairs conotruncal heart development. Dev Biol 198:32–44.

Smith BR, Linney E, Huff DS, Johnson GA. 1996. Magnetic resonance microscopy of embryos. Comput Med Imaging Graph 20:483–490.

Smith BR, Shattuck MD, Hedlund LW, Johnson GA. 1998. Time-course imaging of rat embryos in utero with magnetic resonance microscopy. Magn Reson Med 39:673–677.

Wilson SJ, Brereton IM, Hockings P, Roffmann W, Doddrell DM. 1993. Respiratory triggered imaging with an optical displacement sensor. Magn Reson Imaging 11:1027–1032.