# AN IN-OVO LONGITUDINAL μ-MAGNETIC RESONANCE IMAGING STUDY OF QUAIL EGGS AND EMBRYONIC DEVELOPMENT

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**Introduction:** Avian embryos are important models for understanding embryonic development. The research benefits from the fact that the egg is a viable unit containing the essential components for embryonic development, needing only oxygen, humidity and heat. The majority of the research in this area is engaged in investigating the development of the embryo. In comparison, the role of the extra- and non-embryonic components within the egg has attracted less attention, although they are essential for embryonic development. The extra-embryonic tissues (e.g. yolk sac, allantois and amnion) are temporary living appendages of the embryo participating in fundamental metabolic processes such as respiration, nutrition and excretion. The non-embryonic components of the egg (e.g. yolk, albumen and shell) provide nutrients and also physical and microbial protection for the growing embryo. In this paper, we present the results from longitudinal MRI study of a quail egg imaged at 24 hour intervals from 8 days, the three dimensional (3D) changes in the extra- and non-embryonic components as well as the embryo are quantified.

**Method:** Fertilized Japanese quail (Coturnix japonica) eggs were obtained from Rosedean, Cambs. The eggs were arranged vertically in the humidified incubator, at 38 $^{0}$C, with air sac uppermost. Prior to MR imaging, an egg was removed and cooled; at the end of the experiment the egg was returned to the incubator. Eggs were imaged with a Bruker Avance FT NMR spectrometer and a wide bore 7.1 T magnet resonating at 300.15 MHz for $^{1}$H. A birdcage rf resonator with an internal diameter of 30 mm was used. All acquisitions were made at 19$^{o}$C. 128 x 128 x 128 RARE-8 MRI experiments were performed with TE=20 ms and TE= 500ms. The field of view was 30 mm and voxel dimensions were 234 μm. MRI data were Fourier transformed and visualised using Amira imaging software, regions of interest segmented and data reconstructed to produce 3D rendered surfaces.

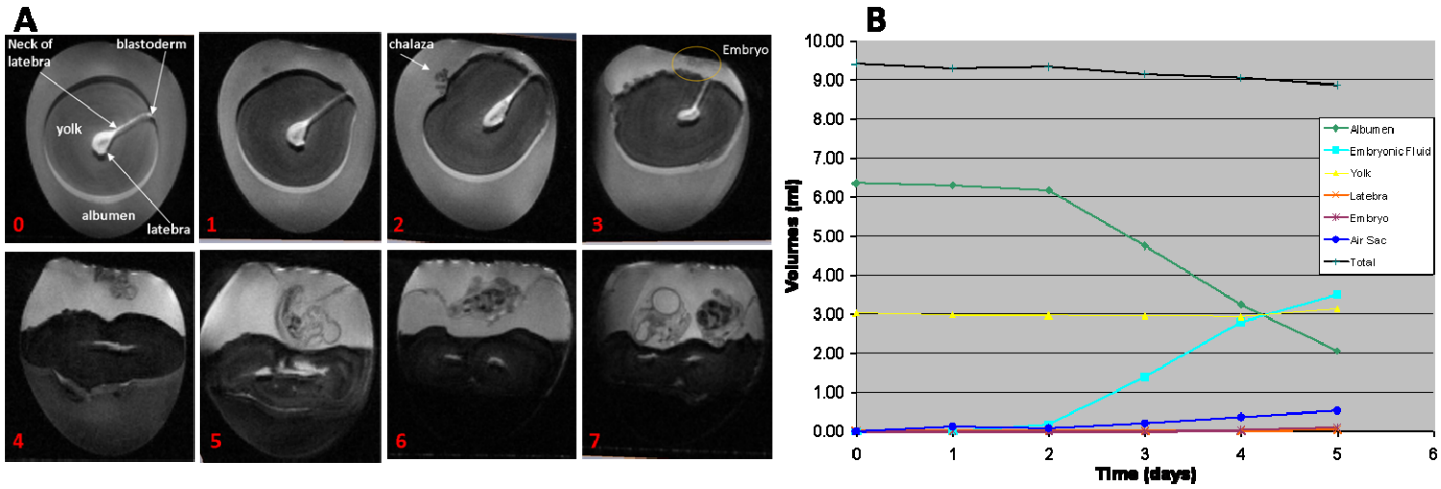


**Figure 1 μMRI of quail egg:** (A) 2D images from 3D data sets of the same egg at Incubation Day 0, 1, 2, 3, 4, 5, 6 and 7 (days in red); (B) Volume of the albumen, embryonic fluid, yolk, latebra, air sac and embryo plotted against Incubation Day.

**Results and Discussion:** The results from a longitudinal μ-MRI study of a quail egg (Figure 1) provides an informative 3D snap-shot of the changes that occur in the internal structure of the egg during embryonic development. At Day 0 the yolk is spherical, lies in the centre of the egg surrounded by the albumen. The latebra, neck of the latebra and chalaza are all visible in the image. The vitelline membrane surrounding the yolk starts to rupture after Day 1. The in-ovo quail embryo becomes visible in the MR images on Day 3. By Day 4 the vitelline membrane is completely ruptured, the yolk is distributed in a layer across the centre of the egg separating the lower, denser, lower image intensity aqueous albumen layer from the upper, less dense, higher image intensity aqueous embryonic fluid. 3D surface reconstruction of the embryo, yolk, albumen, embryonic fluid, latebra and air sac are determined and their respective volumes calculated. The volumetric changes over time are shown in the graph in Figure 1B.

**Conclusion:** The growth of a quail embryo was visualised in-ovo by 3D MRI during the first 8 days of embryonic development. The volumetric changes in the embryo, albumen, embryonic fluid, yolk, latebra and air sac were quantified during the longitudinal study and presented graphically.