Monitoring chick development in ovo using a 7T MRI System

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INTRODUCTION:

Several approaches have been developed to study the dynamics of chick embryo development and growth *in ovo* but most require the embryos to be sacrificed at different stages of incubation to allow body size and individual organs to be measured. An alterative approach is to make repeated observations on the same embryo using non-invasive techniques such as MR imaging. MRI has significant advantages over other less invasive systems in that the number of embryos needed to attain statistical significance is significantly reduced, and repeated observations can be made on the same chick throughout the incubation process and therefore related to the final phenotype. However chick movement has previously been shown to adversely affect image quality after about the 10th day of incubation [1, 2].

The aim of this study was to test the feasibility of using a state-of-the-art ultra high field (7 Tesla) MRI system to continuously monitor chick growth *in ovo* throughout the incubation period (21 days). In an attempt to minimise motion artefact, we decided to try cooling the eggs for 1 hour at 4°C in a refrigerator prior to imaging once motion artefacts became problematic. Another aim was to see if repeatedly cooling and imaging incubating eggs was detrimental to chick survival, growth and hatching success.

METHODS:

Eggs and treatments: Two experiments were carried out. In the first experiment three groups of 10 broiler breeder eggs were set in a Brinsea 40 digital tabletop incubator. Group A eggs were used as a control and left in the incubator for the full duration of the experiment (21 days); Group B eggs were removed from the incubator on days 12, 15, 17, 18, 19, and 20 of incubation, cooled for 1 hr in a refrigerator set at 4°C then returned to the incubator; Group C eggs were cooled as for Group B on days 12,15,17,18, 19, and 20, but were then individually imaged for 25 minutes using a 7 T MRI system before being returned to the incubator. In the second experiment 5 eggs were imaged from day 0 through to hatching (day 21) and only cooled when image quality became adversely affected by chick movement (>day 8)

***Imaging and image analysis*:** T2-weighted imaging was carried out using an SE TR/TE 6424/56msec, 195 µm in-plane resolution, 0.5 mm slice thickness, 0.2 mm inter-slice distance. 60 slices were required to cover the entire egg, with a total acquisition time of 25 minutes per egg. In the first experiment, measurements of the volume of the yolk sac, heart, liver and brain were carried out using the Bruker ParaVision software.

RESULTS:

Prior to day 8 it was not necessary to cool the eggs in order to obtain excellent image quality. At day 0, the blastodisc, latebra , yolk and albumen could be distinguished. Yolk compartmentalisation and the presence of the sub-embryonic fluid were apparent from day 3. The embryo itself was recognisable from day 6. Examples of T2 MRI multi-slice scans obtained from days 12, 17, and 20 of incubation are shown in Figure 1. The average size (volume) of 3 different organs viz. the heart, liver and brain at 12, 15, 17, 18, 19,and 20 days of incubation are presented in Figure 2. This figure also shows published data relating to the weight of these individual organs [3]. A similar pattern of growth was obtained using both techniques. The survivability and hatching success of the chick embryos was not adversely affected by either the cooling treatment in isolation (Group B Experiment 1) or when the eggs were cooled then imaged for 25 minutes. With just one exception (group B) all the experimental eggs hatched within 4-6 hours of the controls.

CONCLUSION:

MRI systems can be used to non-invasively monitor chick development *in ovo* throughout the incubation period provided the eggs are cooled for 4°C prior to imaging from the 8th day of incubation. Chick development and growth are not adversely affected by these treatments.

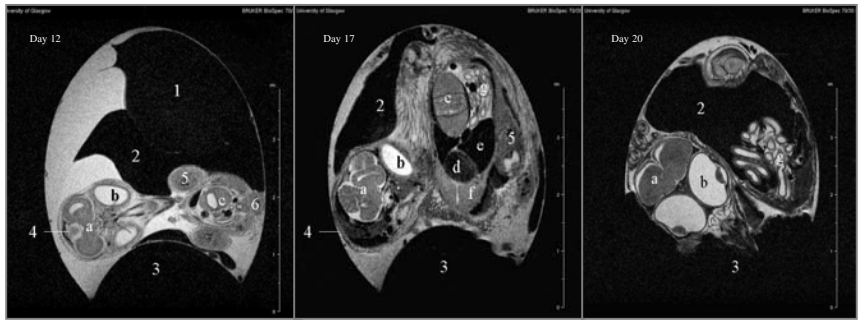

Figure 1: *Representative examples of T2 MRI multi-slice scans of a chick in ovo at 12 17, and 20 days of incubation. albumen(1); yolk(2); airsac(3); head(4); limb (5); rump(6); brain(a); eye(b); gizzard(c); heart(d); liver(e); pectoral muscles(f); intestine(g).*
The complete scans can be viewed at: ww.gla.ac.uk/7tmr/chickegg.htm

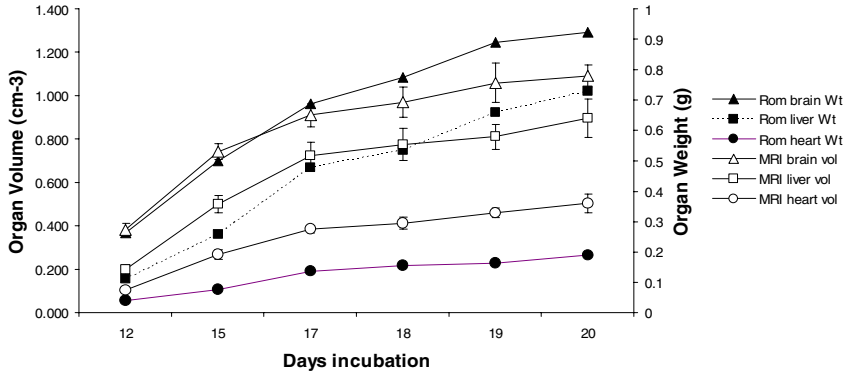

Figure 2: *Mean +/- standard deviation for heart, liver and brain volume measured on the same embryo at days 12, 15, 17, 18, 19 and 20 days incubation (n=5) compared with organ weights measured invasively (cited from Romanoff, 1960).*

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