# In ovo MRI and MRS of the turkey embryo

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**Introduction:** Increasing awareness in animal protection creates a significant demand for alternatives to animal experiments. The avian embryo may replace mammalian experiments in many fields including toxicology, drug metabolism and some areas of basic biomedical research [1]. In Germany animal studies in ovo are generally exempt from legislation regarding animal testing. However only very few studies on *in ovo* MRI and MRS have been performed so far. The aim of the study was to evaluate the potential of MRI and MRS methods to study anatomical and metabolic changes of living turkey embryos *in ovo*. Immobilizations by cooling and by isoflurane anesthesia were compared concerning effects on motion artifacts and on brain metabolites. Since the avian embryo is frequently used as model for the fetal alcoholic syndrome, we exemplarily assessed the possibility to measure the time course of ethanol accumulation in the embryonic turkey brain by localized proton MRS.

**Methods:** Incubated turkey eggs underwent MR measurements at day 15 and 21 of embryonic development. T2-weighted images (2D BLADE, TR/TE = 2830/40ms, spatial resolution: 0.188-0.3 x 0.188-0.3 x 0.5-1 $mm^3$) and localized proton MR spectroscopy (PRESS, TR/TE = 2000/10 ms, VOI = 5 x 2 x 2 mm) were performed at 7T (ClinScan, Bruker BioSpin) using a circular polarized whole body RF coil (Bruker BioSpin) in transmit-received mode. For isoflurane anesthesia the eggs were positioning in a temperature controlled chamber (37±1°C) insides the magnet allowing a continuous flow of isoflurane (5%) in oxygen (flush: 4l/min over 5 min, maintenance: 1l/min) over the eggshell. To evaluate different cooling protocols eggs were either cold by ice for 10 min and 20 min or in the refrigerator (4°C) for 1h. Subsequently possible movement was assessed by continuous light inspection either at room temperature or at sustained cooling (styrofoam box). Possible influence on embryonic development were addressed by assessing the vitality as well as the embryonic weight as day 23. In one embryo (10 min ice-cooling, MRI measurement at room temperature) 250 µl of 15% Ethanol were applied via a catheter into the air chamber of the egg while continuously acquiring MRS-Datasets every 8 min.

**Results and Discussion:** After 10 minutes of isoflurane exposure a strong reduction of movement could be observed (Fig. 1a compared to 1b). However, using external exposure of the egg, it was not possible to entirely suppress movements even at 5% isoflurane over more than 2h. In contrast, 10 min of ice-cooling and MR measurement at room temperature allowed the acquisition of motion-artifact-free images over about 2.5 h. Moreover, this procedure did not affect embryo vitality or weight at day 23. With this immobilization and an in-plane resolution of 188 µm anatomical structures such as different parts of the brain, liver and kidneys were clearly visible (Fig 1c-2). Localized proton MRS of the brain revealed major metabolites as N-acetylaspartate, choline containing compounds, and glutamine/glutamate. (Fig. 2). In contrast to isoflurane anesthesia (Fig. 2, right), where significant brain lactate was detected, no lactate was seen in the cooled embryo (Fig. 2, left). Ethanol was detectable in the brain 8 min after administration via the air chamber and increased the next 88 minutes (Fig. 3)

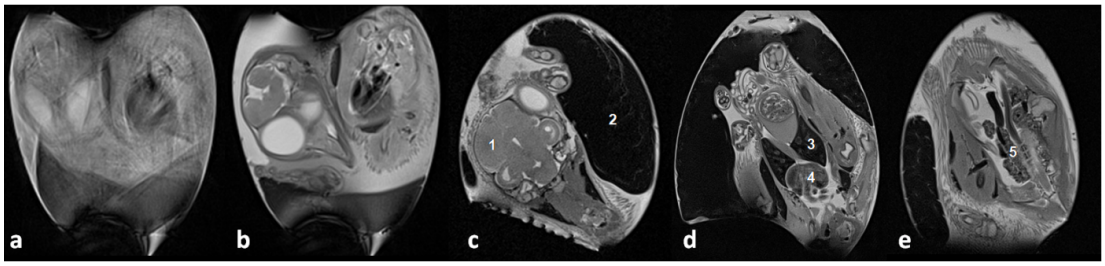


**Fig. 1** Necessity of immobilization: T2w images obtained at (a) 37°C, (b) 37°C, 10 minutes after 5% Isoflurane exposure (0.3 x 0.3 x 1 $mm^3$), (c-e) at room temperature after 10 min of ice-cooling (0.188 x 0.188 x 0.5 $mm^3$),
1 cerebellum , 2 yolk, 3 liver, 4 crop, 5 kidney

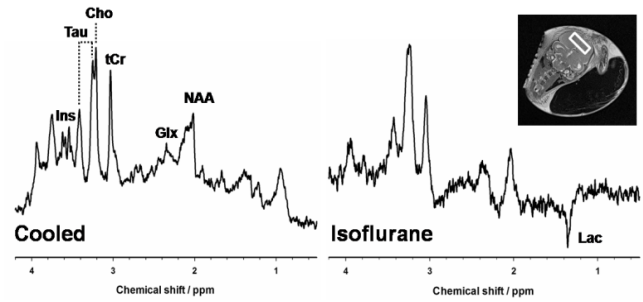


**Fig. 2** MRS obtained after 10 min of ice-cooling (left, TE = 10 ms) and with 5% isoflurane (TE = 135 ms). Compared to cooling isoflurane exposure resulted in an increase of lactate.
Cho - choline containing compounds, Ins - *myo*-inositol, Lac - lactate, Tau - taurine, tCr - total creatine, Glx - glutamine + glutamate

**Conclusion:** 10 min of ice-cooling allowed for almost motion-artifact-free MRI and MRS without any detectable impairment of viability. Moreover, as shown on the example of ethanol, localized proton magnetic resonance spectroscopy may be used in this model to obtain neurochemical profiles. Besides utilizing the advantage of MRI/MRS in avian embryonic model this study supported the use of *in ovo* models for MRI sequence and contrast agent development to reduce the number of animal experiments in mammalian used for basic research.

**References:** [1] Barile et al, JPTM, 61, 136–145 (2010)

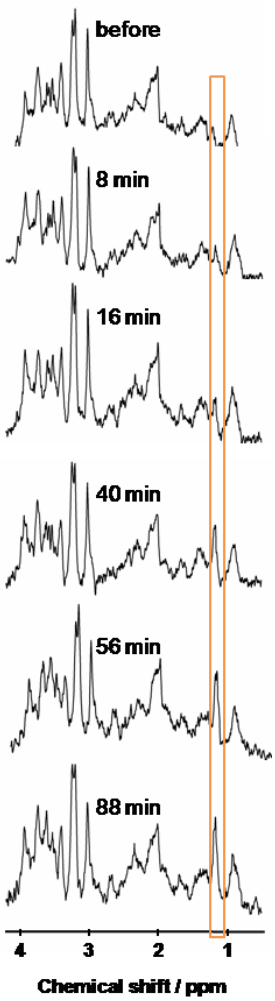


**Fig 3** Time course of ethanol uptake into the brain