# VIABILITY TESTING AND DEVELOPMENT MONITORING IN GENTOO AND ADELIE PENGUIN EGGS: A NOVEL ROLE FOR MRI

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#### Introduction

The captive raising of endangered penguin species is improved by removing eggs from parent birds for machine incubation where the environment can be strictly controlled. As in the wild, some machine incubated eggs fail to develop or die during incubation. These eggs are prone to grow bacteria and may leak or burst over neighboring developing eggs leading in turn to their death: it is therefore important to identify and remove these eggs. Candling – or shining a bright light through the eggs to identify developing blood vessels or an embryo is extraordinarily difficult in penguins eggs, and especially in Adelie eggs as the shells are so thick. This often results in dead eggs being retained with healthy developing ones. We describe 1. the MRI features of penguin egg viability throughout development using "minimal egg MRI", and 2. techniques for in ovo, in vivo anatomical imaging of anesthetized developing chicks. This has potential use as an in-vivo assay for environmental toxicity, and as a non destructive method of determining the normal anatomical appearances of penguin development for scientific and educational purposes.

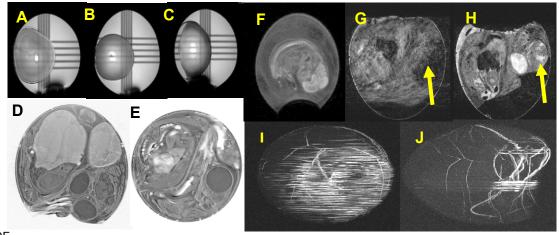
#### Methods:

27 penguin eggs were imaged in the study. Eggs were placed in a polystyrene form, with built in temperature sensor. The form was placed in a 72mm coil for 7T imaging (Bruker), or in a wrist coil (General Electric) for 3T imaging. The bore was maintained at 35'c. Later stage anatomical imaging required anesthesia to remove motion artifact. This was accomplished using 2% isoflurane in oxygen over the exposed upper half of the egg.

### Results

Ten Adelie and Gentoo eggs were imaged weekly to document the appearances of early fertility **A-C**. 4 eggs were also imaged at later stages for anatomical data **D-E**. 17 eggs were imaged diagnostically for early or late stage questionable viability. 12 were not viable or demonstrated delayed development and poor movement, and subsequently died **A&F**. Eggs that appeared non-viable were reimaged at 7 days before being removed from incubation. Isoflurane anesthesia allowed for high resolution imaging **D&E** and **G&H**, including angiograpgy **I & J**.

A: infertile egg B: early fertile 2 days: blastodisc separating yolk from shell. C: 7days: clear lucent area enlarges due to increased water. "minimal egg MRI" protocol: 7sec. gradient tri-pilot TE/TR 6/100ms. **D-E:** anatomical 31day anesthetized embryo. Anat. 3T T2W FSE-XL TE/TR 70/4000 F: Mid stage death G-H: & I-J: Mid stage embryo pre and post 2 minutes of isoflurane Anat. 7T 2D turboRARE TE/TR and I-J: 33/2500 angiography.



## **Discussion**

Eggs have a well defined though delicate anatomy which can be appreciated with MRI. This includes early characteristic changes that occur in development even before blood vessels are formed. Using MRI we were able to confirm fertility as early as 2 days. These structural changes are disrupted when the egg is opened. We could also confirm early and late stage death. MR of anesthetized chicks allowed for detailed anatomical examination including angiography, and has the potential for both generating developmental atlases and as an assay for environmental toxins without harming the developing chick.