# Retinal Folding in the Term Rabbit Fetus - Developmental Abnormality or Fixation Artefact?

**J. Halliday[1], J. French[2], M. Scott[1], C. Liess[1], J. Waterton[1], and J. Stewart[2]**

[1]Imaging & Antibodies, Translational Sciences, AstraZeneca, Macclesfield, Cheshire, United Kingdom, [2]Global Safety Assessment, AstraZeneca, Macclesfield, Cheshire, United Kingdom

**INTRODUCTION:** Prior to administration of candidate medicines to women of child bearing potential, studies must be performed in pregnant animals to assess the effects on embryofetal development. Rabbits are routinely used in studies of this kind, in which pregnant rabbits are dosed with candidate drugs during the organogenesis phase of gestation. Fetuses are removed at the end of the pregnancy and examined for developmental defects. One such defect is the presence of retinal folds.

The retina is a multi-layered structure, which lines the inside of the back portion of the eyeball. Folding or buckling of the retina can result in various degrees of vision loss. In the human this is caused by conditions such as retinopathy of prematurity, Norrie's disease or Familial Exudative Virtreoretinopathy (FEVR).

Retinal folds have been recorded in term rabbit fetuses in both slight and severe forms. Whilst the severe form is rare, the slight form has been recorded at unexpectedly high incidences in this laboratory and others, and has been observed in control group fetuses as well as those gestationally exposed to test compound. The high incidence suggests this is either a common developmental variation in the rabbit or that it may be artefactual.

Currently, the fetuses are decapitated and the heads are fixed in Bouin's fluid for a minimum of 2 weeks. Coronal sections are prepared using a freehand blade technique, and the eye lens and vitreous removed to allow the retinal surface to be examined. It has been suggested that the folds form during the period of fixation and are therefore artefactual in origin but there is no experimental proof of this. Obviously the correct assignation of these findings is essential in the risk assessment for any candidate drug regarding its likelihood of causing fetal harm. The current draft version of the IFTS (International Federation of Teratology Societies) international glossary of terms notes that "retinal folds may be due to processing artefact". [1]

**AIM:** This study used MRI in conjunction with freehand blade dissection and aims to provide definitive evidence as to whether the slight retinal folds in rabbit fetuses are developmental or artefactual in origin.

**METHODS:** Pregnant New Zealand white rabbits were killed on Day 29 post-coitum by IV injection of sodium pentobarbital. Live fetuses present were removed and killed using an injection of sodium pentobarbital. Fetuses were examined externally and decapitated. A total of 24 fetuses from 4 litters were examined. Heads were then imaged prior to fixation.

All imaging was performed on a Varian Inova 9.4T horizontal bore scanner equipped with 40G/cm gradients and a 25mm diameter transit/receive surface coil. T1-weighted 3D gradient echo images (TR = 13.9msec, TE = 5.9msec, flip angle = 40°, 128 x 128 x 64 matrix, 24 averages) of the eyes were acquired with an isotropic resolution of 210µm.

After MRI examination, the heads were fixed for a minimum of 2 weeks in Bouin's fluid (picric acid solution 15 parts, 40% formaldehyde solution 5 parts, glacial acetic acid 1 part). Post fixation a further MR Image of each head was acquired using identical parameters to those used pre-fixation with exception of the flip angle, which was set to 60° in order to obtain optimum tissue contrast. Coronal head sections were prepared using a freehand razor blade technique[2] to allow examination of the retinal surface following removal of the lens and vitreous using a low power (x8) stereomicroscope.

Corresponding MRI scans of the fresh and fixed heads were compared with each other, as well as with the head sections, for the occurrence of retinal folds. The difference between the incidence before fixing and the incidence after fixing was assessed using a test for the equality of two proportions. The number of fetuses displaying the folding after fixing was compared to the pre-fixing incidence using Fisher's exact test. Each test was performed at the one-sided 5% level.

**RESULTS:** MR Images of the fresh and fixed fetuses can be seen in figures 1A and B respectively. A photograph of the dissected eye is shown in figure 1C. Figures 1B and C show that the MRI technique used had sufficient resolution to allow detection of retinal folds and good correlation was achieved using both techniques in the fixed fetuses. No retinal folding could be detected using MRI in any of the fresh fetuses. Bilateral folding was observed in 14 fetuses post-fixation, with unilateral folding observed in a further 5 fetuses ($p<0.001$ and $p<0.025$ respectively, as compared to pre-fixation). Further investigations have examined the effects of an alternative fixation process.[3] Fetal heads which were fixed first in Davidson's fixative (IMS 3 parts, Formaldehyde 2 parts, Glacial Acetic Acid 1 part, water 3 parts) for 2 weeks followed by 1 week in Bouin's fluid showed a far lower incidence of retinal folding than that seen when heads were fixed in Bouin's fluid alone.

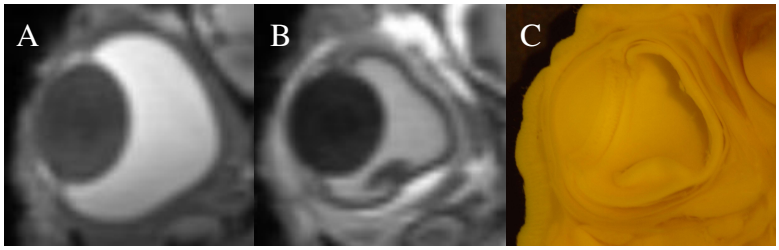


Figure 1. MRI images of fresh (A) and fixed (B) eyes from the same fetus. A photographic image of the dissected eye is also included (C) and shows good correlation with the post-fixation MRI image

**CONCLUSION:** There is strong evidence from this study that the rabbit retinal folds are artefactual in origin, and occur as a result of fixation in Bouin's fluid. Studies of candidate drugs which report folds in fixed retinas should now be interpreted accordingly.

**REFERENCES:** 1. Addition to: Wise LD et al. Terminology of developmental abnormalities in common laboratory (version 1). Teratology. 55, 249-292. 1997. Addition in preparation. 2. Van Julsingha EB, Bennett CB. A dissecting procedure for the detection of abnormalities in the rabbit fetal head. Methods in Prenatal Toxicology. Ed. Neubert D Georg Thieme, Stuttgart. 1977. 3. Latendresse JR et al. Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's Fluid. Toxicologic Pathology. 30(4) 524-533. 2002.