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Two is not necessarily better than one: a double lens in a pre-metamorphic adult axolotl (*Ambystoma mexicanum*)

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Abstract

An 8-year-old female pre-metamorphic axolotl (*Ambystoma mexicanum*) was examined for a suspected anterior lens luxation. Slit-lamp biomicroscopy revealed two lens-like structures in the anterior chamber of the right eye (OD), each with cataractous change. Ultrasound biomicroscopy and optical coherence tomography (OCT) were performed without sedation, and revealed small lenticular structures each with distinct nuclei and cortices. Although a distinct connection of the two lenticular structures could not be definitively ruled out, the structures appeared separate. Each of the lenticular structures was closely associated with its respective iris leaflet. This report demonstrates application of advanced imaging for diagnostic use in axolotl ophthalmology, showing that imaging of the lens can be performed without sedation, topical anesthetic, nor contact gel with high diagnostic quality. Although two distinct lenses were diagnosed with no historical evidence of trauma, the small sizes of each lenticular structure, with no detectable connection between them, is suggestive of a possible regenerative abnormality. This report opens discussion for the regenerative capabilities of the pre-metamorphic adult axolotl and possible implementations of their use in regenerative medicine research for development of future therapies.

Keywords

amphibian; salamander; ophthalmology; optical coherence tomography; ultrasound biomicroscopy; Mexican axolotl

Introduction

Amphibians consist of three Orders: the Gymnophiona (caecillians), the Urodela (salamanders, newts, axolotl), and the Anura (frogs and toads). Most amphibians undergo metamorphosis (paedomorphism) when transitioning from a larval stage in a fully aquatic

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environment, which is highlighted by the continued development of certain structures (e.g. legs, lungs), and resorption of others (e.g. gills and tails).[1] Compared to anurans (frogs), most urodeles are considered neotenic (pre-metamorphic) species in that they retain some of their larval physical characteristics as adults by not undergoing complete metamorphosis. A common neotenic example among the Urodela is the retention of their tails throughout life.

Metamorphosis is mediated by thyroid hormone and in some species can be induced by stimulation of the pituitary thyroid axis or triggered by specific environmental cues.[1,2] Both anurans and urodeles are capable of regenerating the tissue at different stages of development.[3,4] Interestingly, compared to anurans in which regenerative capabilities are halted at the pre-metamorphic stage, some urodeles can regenerate body parts, including limbs, tails, heart ventricles, and even parts of the eye including the lens and retina, with and without known internal or environmental triggers, at any life stage.[2,5,6] However, it has been historically considered that the adult axolotl has been considered different from other urodeles in that it is unable to regenerate ocular tissues beyond 14 days post-hatching.[3,5]

Here, we present a case of two lenticular structures in the same eye of a pre-metamorphic adult Mexican axolotl (*Ambystoma mexicanum*). This species is a critically endangered aquatic salamander native to only two freshwater lakes in Mexico. Although metamorphosis can be induced in axolotls, they do not undergo metamorphosis under normal conditions in their native habitat, and instead retain larval characteristics as neotenic adults. Given that they have become quite popular in the pet trade in recent years, and have been reported to live up to 15 years in captivity (with an average of approximately 6 to 8 years), developing a better understanding of their ocular anatomy, development, and pathologies is important for their proper care.[7] This report is not only the first known example of a vertebrate having multiple complete ocular lenses, but also provides opportunity to open discussion of the regenerative capability of ocular tissue and what it could mean for the future of clinical medicine.

Case Report

An 8-year-old female pre-metamorphic axolotl was presented to the Purdue University Ophthalmology Service for a suspected anterior lens luxation. The owner, a veterinarian, reported a grossly visible abnormality in the right eye (OD) that was initially noticed 10 days prior to presentation. The patient had no known history of ocular abnormalities. No visual deficits were noted in her environment prior to the abnormality being detected. However, immediately following, the owner reported a right visual field deficit evidenced as a difficulty detecting food on the ipsilateral side.

Ophthalmic examination demonstrated no appreciable discomfort or discharge. Slit lamp biomicroscopic examination (SL-17, Kowa Company Ltd., Tokyo, Japan) revealed no corneal abnormalities. In the anterior chamber OD, there were two similarly sized spherical lenses or lobes of a single lens, but whether they were attached or separate was unable to be determined. One was positioned anterior and dorsal and the other posterior and ventral with iridal contact causing slight dyscoria. The dorsal-most lens/lobe had an immature nuclear cataract and the ventral-most lens/lobe had an immature cortical cataract with brunescence.

Indirect ophthalmoscopy (binocular headset by HEINE Optotechnik GmbH & Co, Germany; 90 D indirect lens by VOLK Optical, Ohio, USA) revealed a pigmented anangiotic fundus in both eyes (OU) that appeared green when viewed through the ocular lenses (Figure 1A,B). The remainder of the ophthalmic examination OD was within normal limits, and no abnormal findings were noted OS.

Ultrasound biomicroscopy (50 MHz linear B-Scan Probe, Aviso, Quantel Medical, France) was performed out of water without sedation, topical anesthesia, nor stand-off gel to avoid toxicity by absorption through the skin. Two spherical structures were visible protruding into the anterior chamber OD, each of a smaller size approximately 50% that of the normal lens OS (Figure 1C,D). Resolution was not high enough to determine relatedness of the two structures. The dorsal lenticular structure OD had a hyperechoic nucleus surrounded by a hypoechoic cortex, corresponding to the nuclear cataract observed during slit lamp exam. The ventral lenticular structure OD had a hyperechoic cortex also consistent with a cortical cataract. The lens of the left eye (OS) was large, protruding into the anterior chamber and occupying approximately half of the vitreous chamber. It imaged normally.

Optical coherence tomography (OCT; Spectralis OCT System HRA+OCT, Anterior Segment Module, Heidelberg Engineering, Germany) was performed similarly, and supported the findings on clinical examination and ultrasound biomicroscopy but provided higher resolution (Figure 1E,F). Distinct nuclei and cortices were present in both lenticular structures OD, and no definitive connection between the structures was detected. Each lenticular structure appeared closely associated with its corresponding iris leaflet. A single lenticular structure, also with distinct nuclear and cortical layers, was found OS when imaged for comparison (Figure 1F).

No treatment was elected by the owner. Following the visit, there was no progression of visual disturbance reported by the owner, and no additional ophthalmic signs. After 6 months, the patient was lost to follow-up.

Discussion

There are differences in the timing of lens regeneration between species groups.[8] Despite similar induction enzymes in embryonic development of anurans and urodeles, lens induction and regeneration have been documented beyond an embryonic state, even into an adult life stage, in some species.[8] For example, in anurans, lens regeneration has been demonstrated up until metamorphosis, compared to some species of urodeles, where lens regeneration has been documented at later stages of development.[3,4] It has been shown that Axolotls have lenticular regenerative capabilities up to 14 days post-hatching, after stage 44 of development.[3] Additionally, following lensectomy, a well-differentiated lens was identifiable within one to two days.[3] However, the present report suggests that pre-metamorphic adult axolotls may also have lens regeneration capabilities years after hatching.

The process of lens regeneration also differs greatly between species groups. Lens regeneration in anurans, such as African clawed frogs (*Xenopus laevis)*, is dependent on diffusible factors from the neural retina to induce this differentiation from the corneal epithelium.[9,10] A previous study reported lens regeneration in an adult *Xenopus laevis* after removal of the lens.[11] The researchers believe this occurred from remaining lens epithelial cells after the lentectomy, making this more of a repair mechanism than a regenerative process; however, no tracing of these cells was performed to support this theory.[11] Contrarily, urodele lens regeneration can occur after removal of the lens at any life stage in some species, and the newt can regenerate the lens from pigmented epithelial cells of the dorsal, but not the ventral, iris.[5,6] In the larval axolotl, however, immunohistochemical analysis demonstrated new lenticular tissues at both the dorsal and ventral iris leaflets in one case, and a second case developed new lenticular tissue at the ventral iris leaflet.[3] Although no evidence of this has been documented in adult animals, these findings correlate with the clinical findings in the present case in that the two lenticular structures were closely associated to their respective iris leaflets (Figure 1E).

This clinical case report presents a few considerations. First, we report that advanced imaging can be performed on the awake axolotl without topical anesthetics to acquire diagnostically adequate images. Ultrasound biomicroscopy and OCT served to provide better clinical assessment of the microanatomy of the axolotl eye. Although OCT has been utilized in lens regenerative research in newts,[12] this is the first report of OCT in a clinically abnormal urodele and supports advanced imaging as a feasible and applicable diagnostic tool in small exotic animals. Additionally, lens regeneration may be a possible adaptation of the pre-metamorphic adult axolotl with lens injury or removal/loss. Although this case was not conclusive on the origin of the double lens structure in the anterior chamber, it strongly suggests that it may be an example of lenticular regeneration in response to injury. Attempts to identify underlying stem cell populations in the iris and cornea of amphibians that could explain these regenerative capabilities have been unsuccessful, suggesting that regeneration of tissues occurs from existing differentiated cell populations responding to induction enzymes.[9] With further investigation of the mechanism of lens regeneration in response to injury, potential utilization of similar mechanisms to regenerate lens cells *in vitro* could provide insight into enzymatic and gene therapy for organ regeneration in humans.[9]

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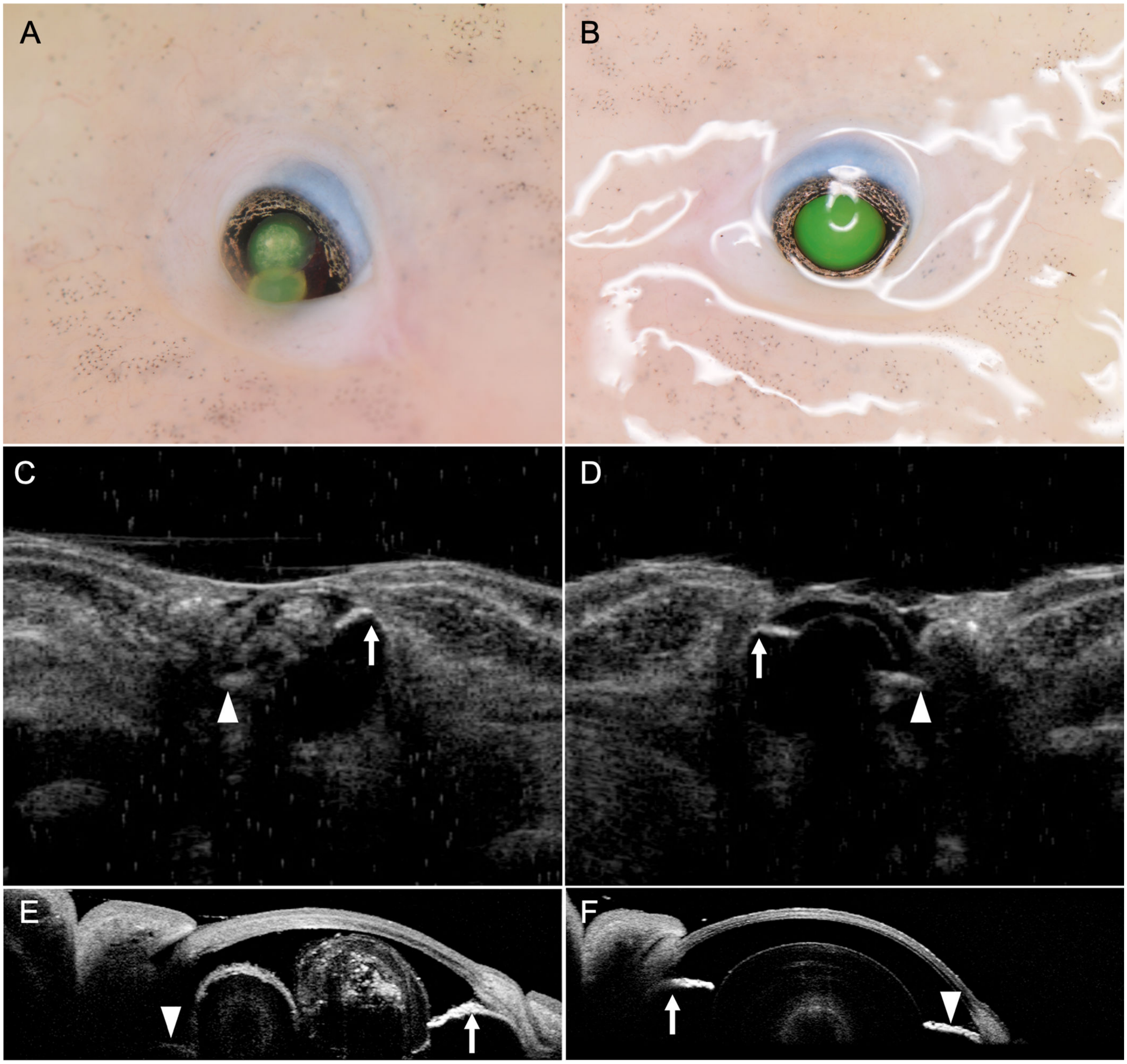

Figure 1.

Clinical diagnostics of lens abnormality in an axolotl. **A** Two lens-like structures are visible in the anterior chamber, one dorsally and one ventrally in the right eye (OD). **B** Normal left eye (OS). The green fundus reflex in axolotls is a normal finding. **C,D** Corresponding ultrasound biomicroscopy (UBM) of OD and OS respectively. Note the two, hyperechoic spherical structural between the linear iris leaflets OD compared to the normal lens OS. **E,F** Anterior segment optical coherence tomography images of the affected and normal eyes. Note the enhanced detail of these images compared to the UBM images with similar findings. Arrowheads represent the ventral iris leaflet, whereas arrows indicate the dorsal leaflet.