

What's Your Diagnosis

A Focal Ophthalmic Lesion in a New Zealand White Rabbit (*Oryctolagus cuniculus*)

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DOI: 10.30802/AALAS-JAALAS-25-086

History

An 18-mo-old female New Zealand White rabbit (*Oryctolagus cuniculus*) received bilateral imaging-guided subretinal injections (30-gauge, ½-in. needle) of Matrigel (20 µL, Corning, Corning, NY) and vascular endothelial growth factor (VEGF; 7.5 µL, 100 µg/mL, Shenandoah Biotechnology, Warminster, PA) to create choroidal neovascularization and model human wet macular degeneration.¹ Both photography (50° color fundus camera, Topcon 50EX, Topcon, Tokyo, Japan) and tomographic examination (Ganymede-II-HR optical coherence tomography, Thorlabs, Newton, NJ) of the fundus before and after injection were unremarkable bilaterally, and they demonstrated the expected subretinal fluid bleb after injection (Figure 1, Procedure). This procedure was performed with an aseptic technique and under injectable general anesthesia (ketamine [40 mg/kg] and xylazine [5 mg/kg] IM).¹ Twenty days following injections the animal presented for veterinary care to evaluate an opacity in the right eye (OD). Ophthalmologic examination identified a central, well-demarcated, smooth, pale-tan irregularity at the level of the lens that obstructed the view of the fundus (Figure 1, Presentation). Intraocular pressure (IOP; iCare USA, Raleigh, NC), a Schirmer tear test (STT), and corneal fluorescein staining were within normal published limits.^{2,3} Right-sided visual acuity appeared decreased but present based on head-turning in response to silent visual stimuli. Epiphora, blepharospasm, and anterior chamber aqueous flare or cellular debris were not noted. Examination of the left eye, including direct fundus examination, was unremarkable. Systemically, the rabbit was normal and appeared comfortable; she was bright, alert, and responsive with ears in a normal position and no observed orbital tightening or alterations in nose or whisker positioning.⁴

The colony underwent regular health surveillance quarterly for selected agents of concern, and required strict personal protective equipment use.

Differential Diagnosis

A cataract was considered the most likely differential for the intraocular opacity. Rank-ordered differential diagnoses for the lenticular opacity included traumatic (lens rupture, lens luxation) or degenerative (lens degeneration) etiologies, metabolic derangements (diabetes, xanthomatosis), infectious/inflammatory processes causing uveitis or hypopyon (*Pasteurella multocida*, *Encephalitozoon cuniculi*, toxocariasis), congenital (persistent hyperplastic primary vitreous or congenital cataract), retinal detachment, and neoplastic (lymphoma, amelanotic melanoma, retinoblastoma, astrocytic hamartoma, or metastatic) etiologies.

Treatment and Management

Due to health surveillance with strict biosecurity, exposure to *E. cuniculi*, *P. multocida*, or toxocariasis was considered less likely. Sterile aseptic technique was used for injection with 5% ophthalmic betadine preparation. This, alongside the lack of aqueous flare, hypopyon, vascular injection, erythema, discharge, and behavioral signs of pain, decreased suspicion for endophthalmitis. The animal was confirmed to be a bred inhouse wild-type New Zealand White rabbit, making hyperlipidemic-associated lesions such as xanthomatosis also less likely. The rapid onset and lack of systemic findings made neoplastic etiologies less likely. Altogether, traumatic and degenerative cataract etiologies were considered to be most likely. Over the next 2 wk, the opaque lesion increased in surface area, IOP decreased to 7 mm Hg, STT increased to 10 mm/min, and aqueous flare developed alongside epiphora, all of which suggested the development of uveitis. During this period, OS remained unremarkable with a high-normal IOP of 23 mm Hg and STT of 5 to 8 mm/min. Fluorescein stain uptake was not noted in either eye throughout the duration of presentation.

A course of neomycin-polymyxin-dexamethasone was initiated (topical [~1- to 2-mm strip] daily application OD for 7 d) for uveitis presumed secondary to lens trauma. Within a week of treatment epiphora resolved and IOP and STT became more normal (10 to 15 mm Hg and 1 to 5 mm/min bilaterally, respectively). Throughout the remainder of the study, 2 more incidents of decreased IOP (5 to 10 mm Hg) in the right eye occurred. Both incidents occurred ~2 mo apart and responded similarly to the same medical management regime (neomycin-polymyxin-dexamethasone). The opacity remained

Submitted: 20 May 2025. Revision requested: 09 Jun 2025. Accepted: 04 Jul 2025.

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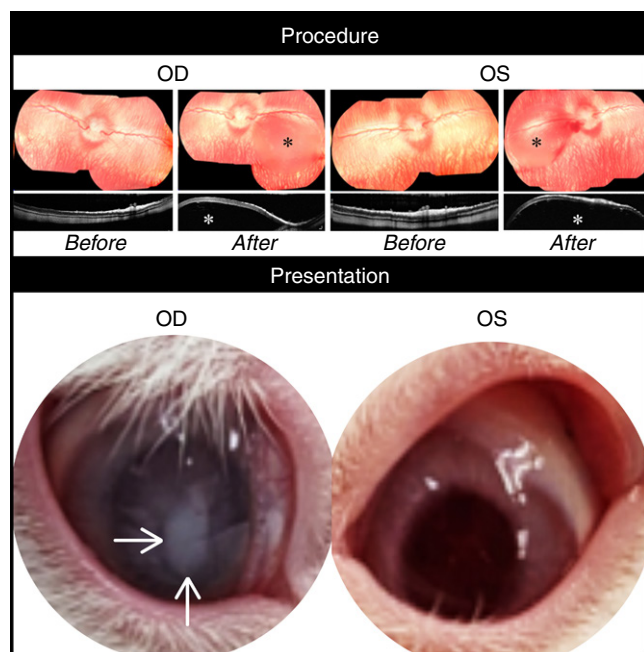


Figure 1. Direct fundus and optical coherence tomography examination of the right (OD) and left (OS) eyes following experimental image-guided subretinal injection. The injection bleb (*) is recognized as a smooth rounded swelling grossly, or as a hyporeflective focus elevating the hyperreflective retina on optical coherence tomography. On presentation to veterinary staff, clinical ophthalmic examination 20 d after injection revealed the pictured lesion (arrows) OD, and no remarkable changes OS.

unchanged throughout medical management and the animal remained comfortable, as indicated by the lack of grimace or generalized systemic signs and was euthanized at the intended experiment endpoint 7 mo after presentation.

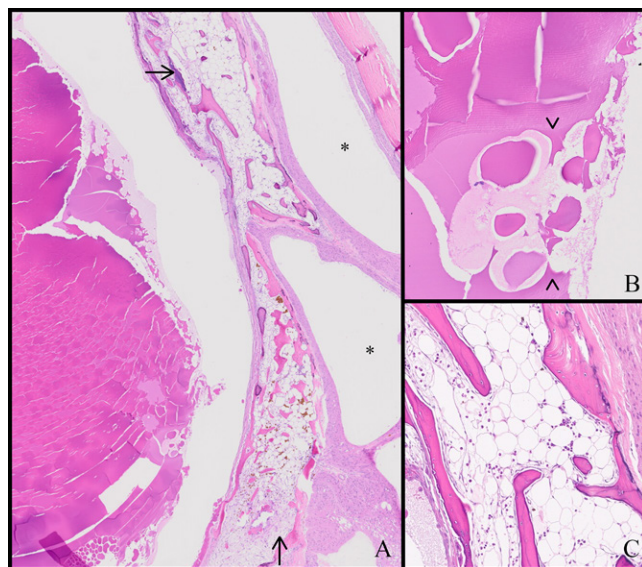


Figure 2. Histologic evaluation of the right eye with persistent opaque lesion. (A) The posterior segment of the eye shows disruption of the lens, adherence of the posterior lens capsule to the fundus/heterotopic ossification (arrows), and cystic cavitation of the choroid (*) related to the choroidal neovascularization model. (B) Higher magnification of the lens shows degeneration of lens fibers with replacement by Morgagnian globules (arrowhead), consistent with cataract development. (C) Heterotopic ossification is characterized by thin, well-differentiated trabeculae of bone separated by mature marrow elements including adipose tissue and low numbers of erythroid and myeloid cells.

Histology confirmed clinical suspicions of lens degeneration with cataract formation as the source of the persistent opacity (Figure 2B). Unexpectedly, the opaque lens had hidden a posterior growth of histologically normal mature bone, complete with well-differentiated marrow (Figure 2C), prompting an additional diagnosis of intraocular heterotopic ossification (HO).

Discussion

HO is a condition where nonneoplastic bone forms in extraskeletal locations. The growth is differentiated from extraskeletal osteosarcoma by the presence of organized bone maturation and the inclusion of mature bone elements, such as bone marrow.⁵ Penetrating eye injuries and chronic inflammation are recognized as risk factors for development of HO in other species, including guinea pigs, birds, and humans.⁵⁻⁷ Lens fibers are inflammatory by themselves when they leak out of the capsule, so even when an injury to the lens is sterile, the persistent or intermittent leakage of lens material can lead to chronic eye inflammation (uveitis/ophthalmitis). Rabbits are often a favored model for ocular study, but the large size of their lens relative to their globe makes intravitreal or subretinal injection technically challenging, potentially predisposing rabbits to lens trauma. Surgical microscopes and image-based guided methods have helped reduce these challenges,^{1,8} but as this case highlights, the risk still exists.

Also of note is the experimental model of choroidal neovascularization that this rabbit underwent, in which subretinal VEGF and Matrigel were injected bilaterally. Matrigel is a complex mixture of matrices and growth factors that is commonly used as an injectable scaffold to provide a supportive environment for cell attachment, growth, differentiation, and migration. In mice (*Mus musculus*), Matrigel has been used to model HO by enhancing localization and survival of osteogenic cells or delivery of osteoinductive factors.⁹⁻¹¹

Mesenchymal stem cells expressing VEGF,¹² or VEGF-c (primarily lymphangiogenic),¹³ have also been shown to contribute to the formation of HO following soft tissue trauma. In this case, the combined utilization of Matrigel and VEGF in addition to the lens injury and recurrent uveitis may have further predisposed this rabbit to developing HO.^{1,8,13-15}

From a comparative medicine perspective, ophthalmic HO is a rare but well-recognized lesion in guinea pigs (*Cavia porcellus*).¹⁶ It has been suggested that ~8 of 1000 guinea pigs develop HO of the ciliary body. This typically begins around the outer margin of the iris and progresses centrally as a white opacity. One suggested etiopathogenic hypothesis points toward the relatively concentrated amounts of vitamin C within the aqueous humor of guinea pigs.¹⁶ It is thought that this promotes localized mineralization and bone formation, as vitamin C has been associated with trabecular bone remodeling by influencing expression of bone matrix deposition genes in osteoblasts.¹⁷ Rabbits have also been documented to concentrate high levels of vitamin C within their aqueous humor, but a similar rate of occurrence for ophthalmic HO has not been documented.^{7,16} The occurrence of HO in this case is further contrasted by its anatomic location. While HO of guinea pigs is typically in the anterior segment (ciliary body) of the eye (corresponding with aqueous humor), HO in this rabbit developed posterior to the lens on the retina. So, while the role of vitamin C in the aqueous chamber and the development of HO may be an interesting topic for investigation in rabbits, it is probably of lesser importance in this case.

Because HO was posterior in this case, it is also difficult to delineate its impact on decreased visual acuity and that

of the cataract, which obstructed the view of the fundus. If HO was more anterior, such as associated with the iris, it may have a larger clinical impact on vision. An iridal location may also have more impact on the fluid dynamics of the eye and associated intraocular pressures, as blockage may lead to glaucoma. However, clinicians should also account for reduction in IOP related to uveitis when making determinations on fluid dynamic pathology. Previous reports have documented intraocular pressures ranging from 15 to 23 mm Hg,² and STT values from 5.3 to 10 mm/min³ in clinically normal rabbits. As was done in this case, utilization of a patient's baseline with serial or contralateral measurements can often provide greater insights into the progression of the disease course than comparison to reference intervals alone.

This case of chronic recurrent uveitis and traumatic cataract with posterior adhesion and heterotopic ossification of the retina not only highlights a potential adverse consequence to a commonly used experimental model but also demonstrates that even when the fundus cannot be seen, there may still be interesting, though obscured, pathologic changes. It reminds us that disease rarely occurs in a vacuum, and it is important to recognize there can be potential interactions between an individual animal's experiences and the intended experimental outcomes. As laboratory animal clinicians it is therefore essential to communicate with researchers to ensure that we account for the whole picture of clinical signs, experimental history, and species variation.

Acknowledgments

Aspects of the case were previously presented orally at the 75th AALAS National Meeting, November 3 to 7, 2024, Nashville, TN. Special thanks to Dr. Yuqing Chen, as well as the University of Michigan CAMTraST for donating rabbits for this research.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

The Unit for Laboratory Animal Medicine at the University of Michigan provided internal funding of this case report. This project was also supported by a grant from the National Eye Institute (YMP: 1R01EY034325).

Ethics Statement

All procedures were performed within an AAALAC-accredited facility, with the approval of the University of Michigan IACUC, and in accordance with the *Guide for the Care and Use of Laboratory Animals* as well as the Animal Welfare Act.

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