

## Pathology in Practice

In collaboration with the American College of Veterinary Pathologists

# Infertility and periocular swelling in a female zebra finch (*Taeniopygia guttata*)

**Keywords:** oviduct, prolapse, mycobacterium, zebra finch, immunohistochemistry

### History

A piece of red fleshy tissue, weighing 2.84 g and measuring 3 X 1.5 X 1 cm, was found on the cage floor of group-housed, 6-month-old (adult) female zebra finches (*Taeniopygia guttata*) undergoing routine quarantine in an animal research facility. All 20 birds were bright, alert, and responsive. There were no reports of vermin, and no birds were missing. A veterinarian examined each bird, and there was no evidence of cannibalism, cloacal trauma, or other injuries. Some females were starting to lay eggs; however, none were fertilized. The piece of red fleshy tissue was submitted for evaluation. After a 3-month quarantine, birds were integrated into the main colony.

Nine months later, 1 female from this cohort was reported with a history of infertility, decreased activity, recurrent periocular swelling and chronic pododermatitis. The bird was euthanized by intracoelomic injection of sodium pentobarbital and submitted for necropsy.

### Clinical and Gross Findings

Grossly, the red tissue was spongy with an indentation in the center and focal hemorrhage on 1 side (Figure 1). No discernable feathers, hair, skin, or bone were noted. The weight of the tissue was 2.84 g post fixation.

At necropsy, gross examination revealed a visible and quiescent left ovary with the majority of the oviduct

Yao Lee, DVM, PhD, DACVP<sup>1,2</sup>; Emily G. Franklin, DVM<sup>1</sup>; Magalie Boucher, DVM, MS, DACVP<sup>1</sup>; Nathan M. Pate, DVM, DACVP<sup>1</sup>; Niora J. Fabian, DVM, MS, DACLAM<sup>1\*</sup>

<sup>1</sup>Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA

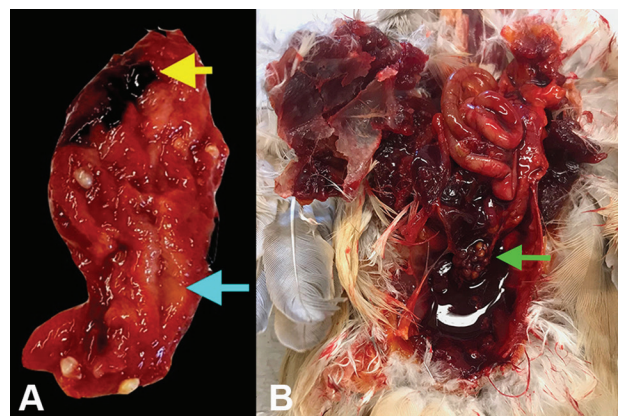
<sup>2</sup>Pathology Core, Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI

\*Corresponding author: Dr. Fabian (nfabian@mit.edu)

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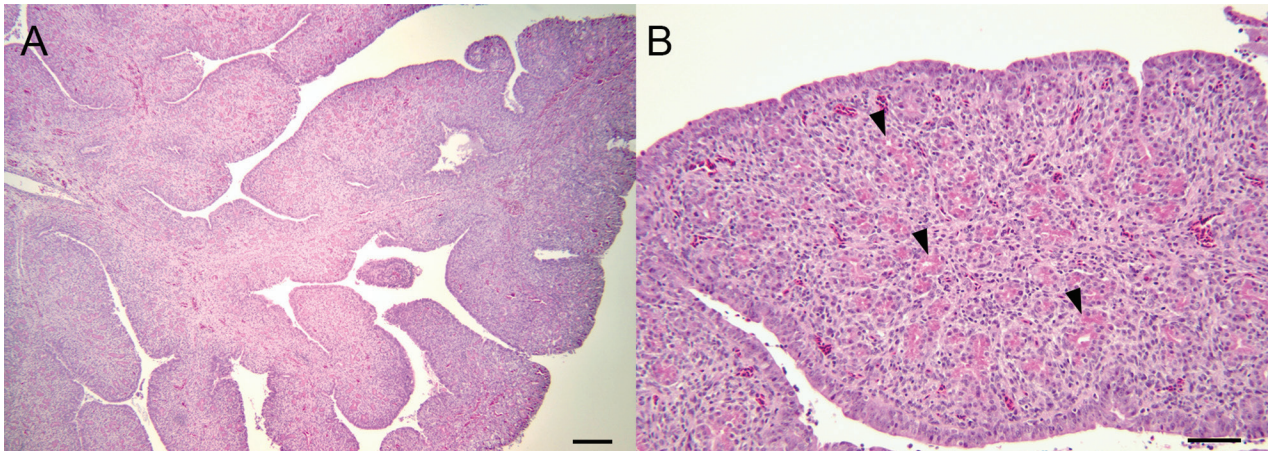
**Figure 1**—Gross pathology of the detached oviduct magnum (A), and the adult female zebra finch with a history of infertility (B). A—Detached oviduct magnum. The tissue was 3 X 1.5 X 1 cm, 2.84 g, and covered by seeds (bird feed). It was red, wet, and spongy in texture. There was an indentation in the center of the tissue (blue arrow), surrounded by numerous gyri-like structures. A focal hemorrhage was noted on the edge of the tissue (yellow arrow). B—Coelomic cavity of the finch. The keel bone and intestine were reflected toward the head. The left ovary (green arrow) was present, but the oviduct was absent. Note that in most birds, the left ovary and left oviduct develop, whereas the right ovary and right oviduct do not develop after hatching and should be barely noticeable or absent. The approximately 1.5 mL of red fluid found in the coelomic cavity was interpreted as a postmortem finding due to intracoelomic injection with euthanasia drug.

absent (Figure 1). The periocular skin was edematous with mild loss of feathers, and the skin of the metatarsal pad and tarsometatarsus was scaly bilaterally.

**Formulate differential diagnoses, then continue reading.**

### Histopathologic Findings

The tissue found at the bottom of the cage was histologically consistent with the magnum of the oviduct from a bird (Figure 2). It was composed of numerous thick folds protruding radially, lined by a columnar epithelium, supported by abundant stroma,

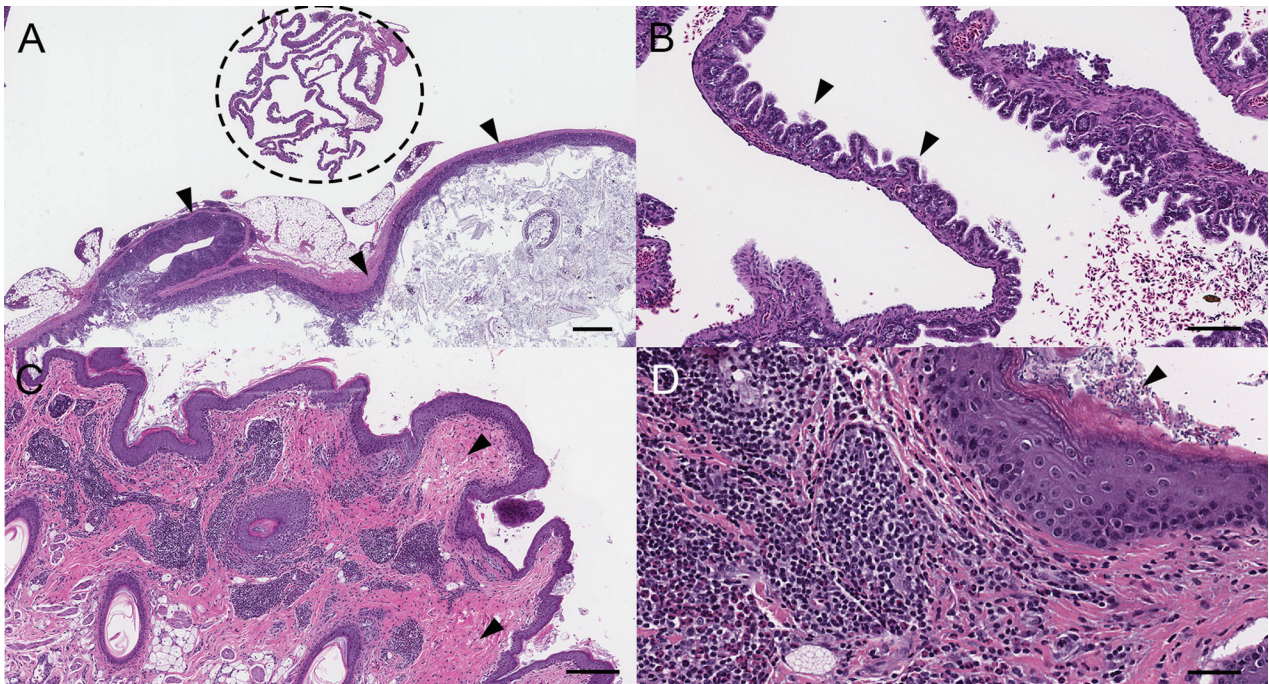


**Figure 2**—Histopathology of the detached oviduct magnum. A—Oviduct magnum; H&E stain. The oviduct magnum had folds projecting out radially (mucosa inside-out), lined by epithelium and supported by abundant fibrovascular stroma. Bar = 500  $\mu$ m. B—Oviduct magnum; H&E stain. The higher magnification showed a columnar ciliated epithelium lining on top of the abundant fibrovascular stroma with numerous albumen glands (arrowhead), which is a feature of avian oviduct magnum. Bar = 100  $\mu$ m.

embedded with many prominent albumen glands (featured by bright eosinophilic intracytoplasmic granules). The height of the fold versus the epithelium was more than 10:1. Hemorrhage was noted at the edge of the tissue. The stroma was infiltrated with minimal, multifocal lymphocytic inflammatory cells.

On histopathology (**Figure 3**) alongside the large intestine and the ovary, a remnant piece of the

oviduct consistent with the infundibulum was present and characterized by a ciliated pseudostratified columnar epithelium, supported by a thin layer of fibrovascular stroma and a tunica muscularis. The remaining portions of the oviduct were not present. The ovary had developing follicles and was microscopically unremarkable. The intestinal serosa, coelomic membrane, as well as the interstitium in

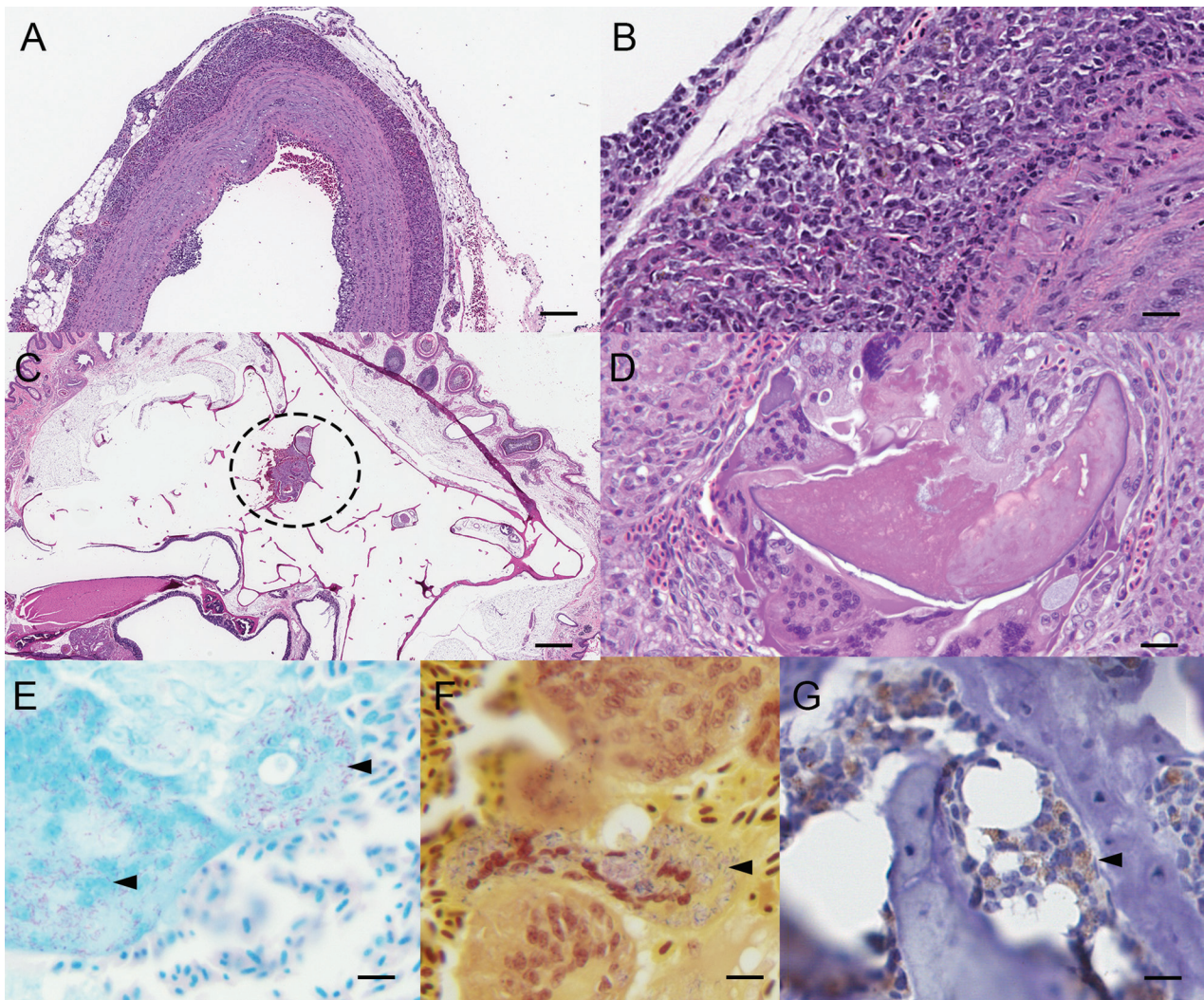


**Figure 3**—Histopathology of the bird. A—Oviduct infundibulum; H&E stain. The piece of infundibulum (dashed circle) was located between the large intestine (arrowheads) and ovary (not shown in the picture). Magnum, isthmus, and shell gland were not found in the coelomic cavity of this bird. Bar = 500  $\mu$ m. B—Oviduct infundibulum; H&E stain. The infundibulum had a pseudostratified, ciliated, columnar epithelium, supported by a thin layer of fibrovascular stroma and a tunica muscularis. Bar = 100  $\mu$ m. C—Dermatitis, periocular skin; H&E stain. The superficial dermis was expanded by fibrosis/scarring (arrowheads) and multifocal perivascular to perifollicular infiltrates. The epidermis showed marked hyperplasia and orthokeratotic hyperkeratosis. Bar = 200  $\mu$ m. D—Higher magnification for the periocular dermatitis; H&E stain. The dermal infiltrates were composed of lymphocytes, heterophils and rare macrophages. The epidermis was hyperplastic, and the surface had abundant keratin flakes mixed with coccobacilli (arrowhead). The bacteria were not invading into the tissue. No ulceration was noted. Bar = 70  $\mu$ m.

various organs such as lung and liver, had multifocal lymphocytic aggregates admixed with heterophils and rare macrophages. The dermis of the periocular skin was fibrotic (scarring) and abundant inflammatory cell infiltrates consisting of lymphocytes, heterophils, and rare macrophages were present both perifollicularly and perivascularly. The epidermis of the periocular and tarsometatarsal skin was hyperplastic with orthokeratotic hyperkeratosis. No infectious organisms were identified on Gram, Periodic acid-Schiff, and Ziehl-Neelsen acid fast stains in the coelomic cavity, and within the periocular and tarsometatarsal skin.

Upon examination of the heart and brain/skull, there was granulomatous inflammation in the aorta

and the marrow space of the os palatinum, which is a small bony structure close to palate behind the nasal cavity (**Figure 4**). In the aorta, the tunica adventitia, serosa, and to a lesser extent the tunica intima, were infiltrated by abundant macrophages and a few heterophils. Gram, Periodic acid-Schiff, and Ziehl-Neelsen acid fast stains did not reveal any pathogens in this area. In the marrow cavity of the os palatinum a focal area of osteolysis was present, characterized by bone loss, mineralized cellular debris (lytic bone), abundant macrophages and numerous multinucleated giant cells. Multinucleated giant cells were primarily considered to be osteoclasts. Macrophages and multinucleated giant cells within this lesion had numerous intracytoplasmic, gram-positive, Ziehl-Neelsen acid



**Figure 4**—Histopathology of mycobacterium-associated lesions of the bird. A—Aortitis, aorta; H&E stain. The adventitia, serosa, and intima of the aorta were thickened by inflammatory infiltrates. Bar = 200  $\mu$ m. B—Aortitis, aorta; H&E stain. The higher magnification showed that the tunica adventitia was infiltrated by abundant macrophages admixed with a few heterophils. Bar = 20  $\mu$ m. C—Osteomyelitis, os palatinum, skull; H&E stain. The marrow cavity of os palatinum (dashed circle) was occupied by inflammatory cells. Bar = 500  $\mu$ m. D—Osteomyelitis and bone resorption, os palatinum; H&E stain. The marrow space was filled with large numbers of macrophages. There was a piece of bright, eosinophilic lytic bone debris surrounded by multiple active osteoclasts. H&E stain; bar 20  $\mu$ m. E—Os palatinum, Ziehl-Neelsen acid fast stain. Abundant intracytoplasmic, positive bacilli (arrowheads) were present. Bar = 15  $\mu$ m. F—Os palatinum, Gram stain. Abundant intracytoplasmic, positive bacilli (arrowhead) were present. Bar = 15  $\mu$ m. G—Os palatinum, immunohistochemical stain for mycobacterium. There were abundant positive bacilli (arrowhead) in the macrophages in the marrow cavity. Bar = 15  $\mu$ m.

fast-positive, rod-shaped bacilli. The bacteria were also positive to the immunohistochemical (IHC) stain for *Mycobacterium* spp (Cat. No. CP140A; Biocare Medical).<sup>1</sup> The IHC result was validated by a negative control, in which an isotype antibody (IntelliPath FLX Universal Negative Control, Cat. No. IP 498 G20; Biocare Medical) was used.

## Morphologic Diagnosis and Case Summary

Morphologic diagnoses: 1) Oviduct/magnum. Oviductal hyperplasia with prolapse/detachment. 2) Periocular skin. Perivascular dermatitis and folliculitis, mild to moderate, multifocal, chronic. 3) Os palatinum/marrow. Osteomyelitis, moderate, focally extensive, chronic, granulomatous, with bone resorption, and intralesional bacilli (consistent with *Mycobacterium* spp). 4) Aorta. Aortitis, moderate, multifocal to coalescing, granulomatous.

Case summary: Oviductal prolapse/detachment, periocular and pododermatitis, aortitis, and mycobacterium-associated granulomatous osteomyelitis in an adult female zebra finch.

## Comments

Oviductal prolapse is primarily described in chickens and turkeys and has not been well documented in zebra finches. The cause of oviductal prolapse can be multifactorial, including salpingitis, eggbinding, dystocia, neoplasia, and any conditions that could cause excessive intracoelomic pressure.<sup>2</sup> In this case, there was no evidence of neoplasia, egg-binding, or salpingitis and the underlying cause of prolapse was not identified. Following prolapse, detachment likely resulted from or in combination with self-mutilation or conspecific wounding.

Nutrition and photostimulation are important factors that influence the avian endocrine system and reproductive physiology, and they should be assessed in cases of oviductal hyperplasia. The finch in this case was maintained in an AAALAC International-accredited facility in an indoor enclosure under a consistent 12:12-hour light:dark cycle, with ambient temperatures maintained between 22.8°C to 25.0°C and relative humidity between 30% to 70%. Commercial pellets for small birds and a fortified millet-canary grass seed-oat mix constituted the main diet. Cuttlebone was always available, and a high-protein supplement (moistened and mixed with minced hard-boiled eggs) was fed twice a week. Thus, nutritional imbalance was an unlikely contributor. Light exposure stimulates the avian hypothalamic-pituitary axis, resulting in secretion of follicle-stimulating hormone and luteinizing hormone that activate the ovary, promoting sexual maturity and oviductal development. Birds exposed to prolonged light are at increased risk for oviductal hyperplasia,<sup>3</sup> which potentially increases the risk of oviductal prolapse. Estradiol level was not measured, and assessment of the reproductive

tract in entirety was not possible at the time of prolapse as the affected finch could not be identified antemortem. At necropsy, the ovary of the finch was quiescent, and there was no evidence of egg-yolk-induced coelomitis, a common complication in birds with oviductal prolapse.<sup>2</sup>

*Mycobacterium genavense*, a type of slow growing mycobacterium derived from soil, is the most common isolated agent among mycobacterioses in passerines and psittacines kept in captivity.<sup>4</sup> Most birds infected with *M genavense* can maintain good body condition, with minimal pathologic lesions such as periocular and palpebral swelling, feather disorders, and brittle beak/nails.<sup>4</sup> Granuloma (tubercles) or granulomatous inflammation is the classic histopathology. Granulomatous aortitis is a possible finding in mycobacteriosis in birds, but identification of intralesional mycobacterium in the vessels has not always been positive. Polymerase chain reaction is standardly used to confirm and speciate mycobacteria in birds. However, in the present case, the PCR for mycobacterial speciation on paraffin-embedded-formalin-fixed tissues was unsuccessful, and fresh tissue was not available for testing to speciate the bacteria. The present case demonstrates that immunohistochemistry is an appropriate ancillary test to confirm the presence of mycobacterial organisms in situ, although speciation still requires PCR. In this case, mycobacteriosis resulting in osteomyelitis in the skull was confirmed, and considered unrelated to the oviductal prolapse, as there was no granulomatous inflammation in the coelomic cavity.

In the present case, periocular skin and visceral organs subjected to acid fast or mycobacterial IHC staining did not exhibit positivity. Periocular dermatitis with perivascular infiltrates and dermal fibrosis were consistent with both traumatic and inflammatory skin diseases previously recorded in psittacines.<sup>5</sup> Skin, as part of the diffuse lymphatic system in birds, can show lymphocytic inflammation and perivascular cuffing as immune responses to any infectious or noninfectious stimuli. Thus, systemic inflammation due to oviduct prolapse and mycobacteriosis, along with feather picking, were all considered to be possible causes of periocular dermatitis and multifocal lymphocytic perivascular infiltrates noted in this finch. The pododermatitis was likely due to the bird's inactivity and prolonged perching.

We concluded that this zebra finch had oviductal hyperplasia, followed by prolapse, and detachment/loss. Concurrently, this bird had periocular dermatitis and perivascular lymphocytic infiltrates, possibly related to systemic inflammation and feather picking. There was granulomatous inflammation in the os palatinum and aorta, and Gram staining, Ziehl-Neelsen acid fast staining, and IHC confirm mycobacteriosis. This case report illustrates that the inflammatory response associated with mycobacteriosis can be multisystemic, which could interfere with research studies.

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