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Short Communication

Ovarian teratoma in an adult female *Zoogoneticus tequila* (Webb & Miller 1998): histological and immunohistochemical features

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The Mexican Goodeid, *Zoogoneticus tequila* (Webb & Miller 1998), is considered nearly extinct in the wild, and it is maintained in captivity by the non-profit international 'Goodeid Working Group' represented by zoological institutions and aquarists in North America, Mexico and Europe. The unique, currently existing Italian colony was founded in 2007 by one of the authors (Arbuatti, Della Salda & Romanucci 2013), and it has produced about 240 fish so far. Information concerning the anatomy and biology of *Z. tequila* is extremely limited (Webb & Miller 1998; De La Vega-Salazar, Avila-Luna & Macìas-Garcia 2003). A single pathology survey on the unique Italian colony was also previously published by the authors (Arbuatti *et al.* 2013), but no other literature reports regarding pathologies of this species are available. *Z. tequila* is a viviparous species, and females give birth to free-swimming fry after an intraovarian gestation.

The present case describes an adult female showing a progressive coelomic distention, suggestive of gestation. However, after a period of anorexia of 2-day duration, the fish died and was immediately submitted for necropsy examination. Macroscopical examination confirmed the abdominal enlargement, associated with the presence of a mucous secretion from the genital pore (Fig. 1a). A large, oval, brownish to grey, multilobulated mass (2 × 1.5 × 1 cm) occupied most of the coelomic cavity (Fig. 1b,c), causing intestinal occlusion by compression of the intestine without infiltration. Samples of the mass and representative tissues of all major organs were fixed in 10% neutral-buffered formalin, embedded in paraffin, cut in 5-μm-thick sections, stained with haematoxylin and eosin (H&E) and examined by light microscopy. Additional sections were also processed for immunohistochemistry (IHC) using the standard avidin–biotin–peroxidase method and specific antibodies against pan-cytokeratin (CK), vimentin, neuron-specific enolase (NSE) (Dako) and glial fibrillary acidic protein (GFAP) (Chemicon International). Negative controls were performed in all instances by omitting the primary antibody from the staining schedule or replacing it by an antibody of irrelevant specificity. Sections of canine skin and brain were used as positive controls for CK and vimentin, and for NSE and GFAP, respectively.

Histologically, the mass consisted of a complex variety of tissues derived from the three germ cell layers, suggesting a diagnosis of tridermic teratoma. Structures of ectodermal origin included multifocal, keratinizing squamous epithelium, and large areas of nervous system tissue resembling cerebrum (Figs 2 & 3). Mesenchymal tissues (mesodermal origin) consisted of skeletal muscle, cartilage, adipose tissue and fibrous connective tissue, with multifocal sheets of melanomacrophages

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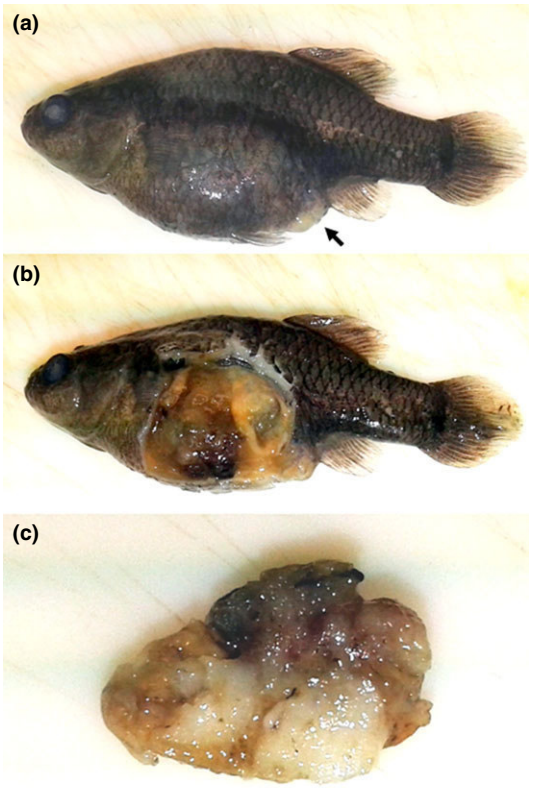

Figure 1 (a) Adult female *Zoogoneticus tequila* showing coelomic distention, with a mucous secretion from the genital pore (arrow). (b) Coelomic cavity occupied by a large, oval, brownish to grey, multilobulated mass. (c) Higher magnification of the cut section of the abdominal mass.

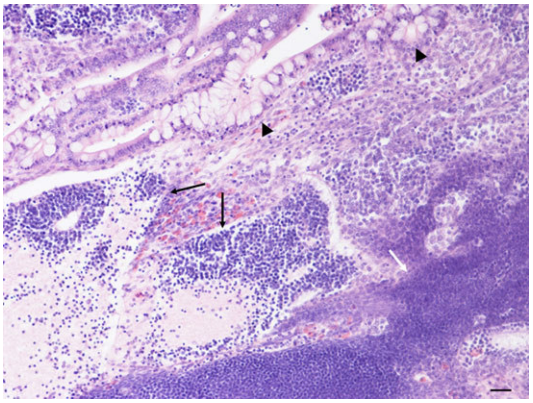

Figure 2 Section of the ovarian teratoma showing a mixture of large areas of nervous system tissue (black arrows), glandular structures lined by intestinal-type epithelium (arrowheads) and densely cellular islands of undifferentiated tissue, resembling primitive mesenchyme (white arrow). H&E, Bar = 25 μm.

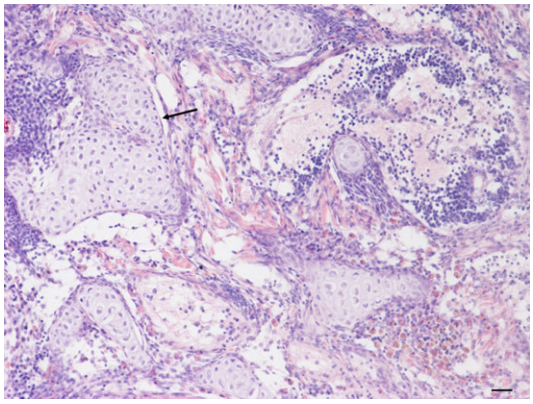

Figure 3 Section of the ovarian teratoma showing nervous system tissue and multiple islands of cartilaginous tissue (arrow), with admixed fibrous connective tissue fibres and melanomacrophages. H&E, Bar = 25 μm.

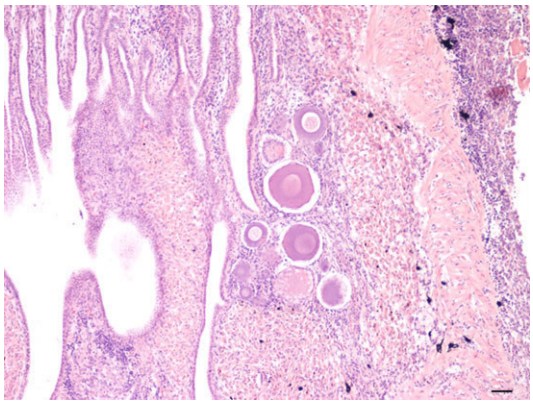

Figure 4 Section of the ovarian teratoma showing the presence of residual folds of ovarian tissue (on the left) and a group of oocytes in different stages of development (at the centre), surrounded by fibrous connective tissue fibres and melanomacrophages. H&E, Bar = 45 μm.

(Fig. 3). Numerous, simple-to-branching glandular structures lined by intestinal-type epithelium (endodermal origin) were also admixed (Fig. 2). The presence of sparse, residual folds of ovarian tissue with admixed residual oocytes in different stages of development (Fig. 4) indicated an ovarian origin of the tumour. On IHC, a mixture of CK- (Fig. 5a) and vimentin-positive (Fig. 5b) tissues was observed. All the epithelial components were CK positive, whereas extensive neuronal and glial differentiation was confirmed by multifocal to diffuse, intense immunoreactivity for GFAP (Fig. 5c) and, to a lesser extent, for NSE (Fig. 5d). GFAP showed both discrete fibrillary cell process and cell body staining.

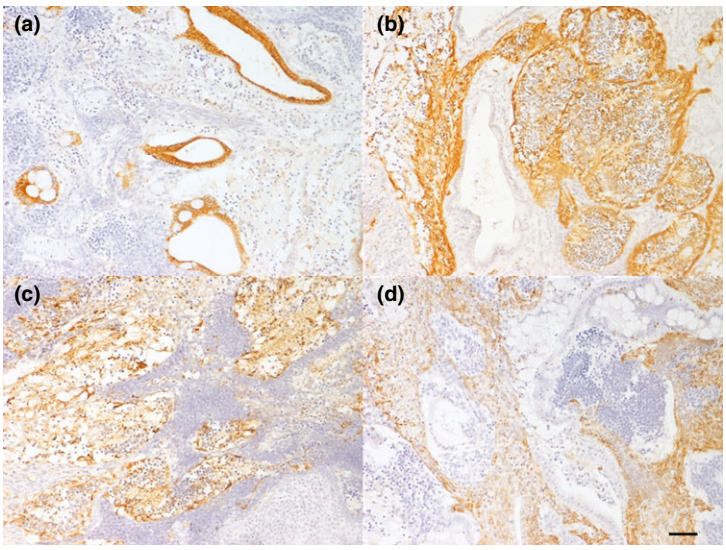

Figure 5 Immunohistochemistry (IHC) of the ovarian teratoma: (a) Intensely CK-positive epithelial components, constituted by glandular structures lined by intestinal-type epithelium (at the bottom) and keratinizing squamous epithelium. (b) Multifocal to coalescing, strongly vimentin-positive tissues. (c) Coalescing areas of GFAP-stained glial tissue, admixed with densely cellular islands and trabeculae of negative, undifferentiated tissue. (d) Multifocal to coalescing areas of NSE-stained nervous tissue. Mayer's haematoxylin counterstain, Bar = 45 μm.

In addition, multiple, densely cellular islands and branching trabeculae of undifferentiated tissue, resembling primitive mesenchyme, characterized by mild cellular and nuclear atypia, and sparse mitotic figures, were observed (Fig. 2). Such tissue did not show an obvious cell line of differentiation by IHC, although isolated histological aspects of slight chondroid differentiation were detectable.

Histopathologic examination did not reveal significant microscopical lesions in the other organs examined.

Teratomas are neoplasms composed of multiple tissues derived from more than one germ cell layer, possibly arising from pluripotential germ cells that have undergone differentiation (Linder, McCaw & Hecht 1975; MacLachlan & Kennedy 2002). They mostly arise in the gonads, although occasionally, due to an aberrant germ cell migration during early embryogenesis, they are distributed in extragonadal sites (MacLachlan & Kennedy 2002; Palmieri *et al.* 2012).

They may be also classified as benign (mature) or malignant (immature) depending on the degree of anaplasia, the presence of embryonic-like undifferentiated elements or regional overgrowth of a single tissue type (MacLachlan & Kennedy 2002; Newman, Brown & Patnaik 2003).

Teratomas have been occasionally described in fish, especially in viviparous species (Stolk 1959; Hisaoka 1961, 1963; Harshbarger 2001), even though they are usually poorly characterized at the histological and immunohistochemical levels.

In the present case, even though metastatic or implanted masses were not noted, the presence of poorly differentiated elements in addition to mature structures indicates a definitive diagnosis of tridermic ovarian immature teratoma. IHC was useful to reveal a predominant neuroglial differentiation.

Intracoelomic teratomas usually appear to originate from the ovary in female fish, with a dorsocaudal localization in the coelomic cavity. However, they may be large, poorly differentiated tumours at the time of diagnosis, occasionally resulting in the rupture of the body wall, and the site of origin may be difficult to recognize. The difficulty in identifying these tumours early is that, during breeding season, viviparous female fish commonly show coelomic distention (Reavill 2010).

To our knowledge, this is the first report of a tridermic ovarian immature teratoma in an adult female *Z. tequila*, in which a detailed histological and immunohistochemical examination was performed and in which an extensive neural and glial differentiation was demonstrated.

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