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Case Report

Diffuse unilateral seminoma in a fighting rooster (*Gallus gallus*)

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Abstract

The aim of this study was to report a diffuse unilateral seminoma in a 4-year-old fighting rooster (*Gallus gallus*) by considering the anatomic, pathological and ultrastructural features. A significant decline in the viability of the bird's embryos was reported during the last year. The bird died with a marked abdominal distention. During necropsy, a firm ovoid neoplasm of approximately 9 cm long, 8 cm wide and 5 cm thick was found in the region adjacent to the pronephros, replacing the left testicle. The neoplasia was soft and encapsulated, and the cut surface showed extensive areas of haemorrhage interspersed with necrotic areas. Histologically, the neoplastic cells formed large mantles or cords with moderate and eosinophilic cytoplasm. The nuclei were pleomorphic, and some of them were central, while others were eccentric. Mitoses were scarce. Some important findings in the ultrastructural study were nuclei exhibited an elongated, electrodense, rope-like nucleoli and the presence of scarce intracytoplasmic glycogen. The anatomo-pathological and ultrastructural findings of the tumour were consistent with a seminoma. The ultrastructural study provided important support for the diagnosis, as other tumours were ruled out. The early diagnosis of malignant or benign testicular tumours in birds is the key for a timely treatment, as these tumours can metastasize or grow to such an extent that they compromise the lives of birds, as in the present case.

Key words: *Gallus gallus*, testis, seminoma, ultrastructure.

Introduction

The incidence of testicular tumours is higher in dogs than in other animal species (2). These tumours are rare in birds; however, they have been described in parakeets (*Melopsittacus undulatus*) and nymphs (*Nymphicus hollandicus*), (13, 14, 15), pigeons (*Columba livia*) (1, 4, 12, 17, 18), ducks (Anas platyrhynchos) (6, 10, 11), guinea fowl (*Numida meleagris*) (7, 9), black swans (*Cygnus atratus*) (8), geese (*Anser cygnoides domesticus*) (16, 19) and common mynah (*Acridotheres tristis*) (3). The predisposing factors for the development of testicular tumours are not known in birds; however, one of them could be monorchidism (9). The three main types of testicular tumours described in animals, including birds, are seminoma (germinal sperm epithelium), Sertoli cell tumour and interstitial cell tumour (sexual cords and gonadal stroma), which may be unilateral or bilateral (2). Mixed testicular germ cell-sex cord stromal tumours have also been described in birds (10). Metastasis is rare; however, it has been reported in cases of malignant seminomas in a nymph (*Nymphicus hollandicus*) (14), a duck (*Anas platyrhynchos*) (11) and a guinea fowl (*Numida meleagris*) (7). Because of the rarity of the case, the aim of this study was to describe a diffuse unilateral seminoma in a fighting rooster (*Gallus gallus*) by

considering the anatomo-pathological and ultrastructural features.

Case Report

A 4-year-old fighting rooster (*Gallus gallus*) from a breeding farm in the State of Mexico, which was used as a breeder for two years, was referred to the Laboratory of Diagnosis and Research in Bird Diseases of the Veterinary Medicine and Animal Science Faculty from the National Autonomous University of Mexico, for presenting abdominal distension, dyspnea, dehydration and decreased muscular mass. The owner informed a significant decline in the viability of his offspring as well as in the attempts to breed with the hens. During the last 4 months before its death, food consumption and rooster activity declined. Fluid therapy was given, nevertheless the rooster died 6 hours after admitted to the hospital. Post-mortem examination revealed evident abdominal distention. In the region adjacent to the pronephros, a soft, ovoid mass of 9 cm long, 8 cm wide and 5 cm thick was found attached to the intestinal loops and replacing the left testicle (Fig. 1). The mass was white and encapsulated; the cut surface was irregular and presented extensive haemorrhage areas intermixed with friable yellow areas consistent with necrosis (Fig. 2). The collateral testis was not identified. Representative tissue samples were collected and fixed in 10% neutral buffered formalin. The samples were routinely processed for histopathologic exam. The tissue sections were stained with haematoxylin and eosin (HE) and Periodic acid-Schiff (PAS). On the histopathology, the tumour was partially delimited by connective tissue. The architecture of the seminiferous tubules was not clearly distinguishable. Neoplastic cells formed large mantles or cords (Fig. 3). Cytoplasm was moderately eosinophilic and cytoplasmic edges were not well defined. Nuclei were pleomorphic and central or eccentric. Some nucleoli were prominent, and mitosis figures were scarce (Fig. 4). Additionally, extensive necrotic and haemorrhagic areas were observed. PAS staining was negative. For transmission electron microscopy (TEM), the samples were fixed in 2.5% glutaraldehyde (Electron Microscopy Sciences., México) for 24 hours, post-fixed in 1% osmium tetroxide for 1 hour, dehydrated with increasing concentrations of acetone (J.T. Baker., México), embedded in epoxy resin (Epon 812, Electron Microscopy Sciences., México) and acetone and enclosed in gelatin capsules and polymerized at 60°C for 24 hours. Ultrathin sections stained with uranyl acetate and lead citrate were examined with an electron microscope (60 kV, Jeol 1010 TEM, Akishima, Tokyo). Ultrastructurally, the cells possessed a moderate cytoplasm, in which the rough endoplasmic reticulum (RER) was scarce and presented different dilation degrees. The mitochondria also exhibited variable degrees of swelling and loss of the mitochondrial ridges, although the possibility that these changes are attributed to autolysis is not discarded. Scarce intermixed glycogen electrodense aggregates were observed. A few small desmosomes were also found (Fig. 5). The nuclei were irregular and exhibited marked irregularities in their nuclear membranes, with sparse electrodense heterochromatin attached to the nuclear inner envelope. Some neoplastic cells in the nuclei exhibited an elongated, electrodense, rope-like nucleoli (Fig. 6). According to the anatomo-pathological and ultrastructural characteristics, the definitive diagnosis for this neoplastic tissue was of a unilateral, diffuse seminoma.

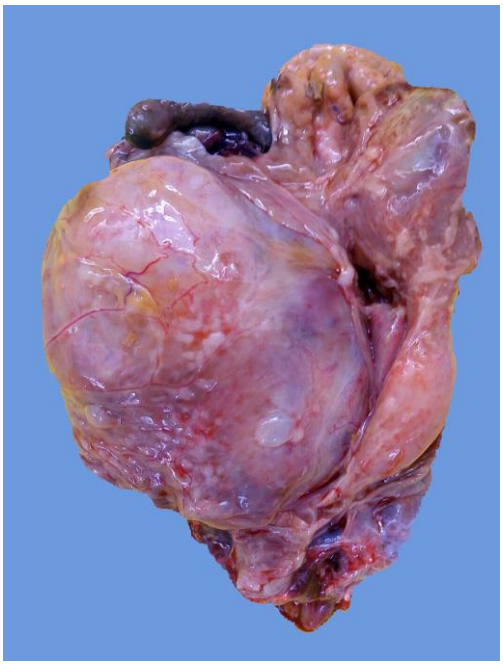

Figure 1. Macroscopic appearance of an ovoid, encapsulated, soft, whitish tumour replacing the left testicle.

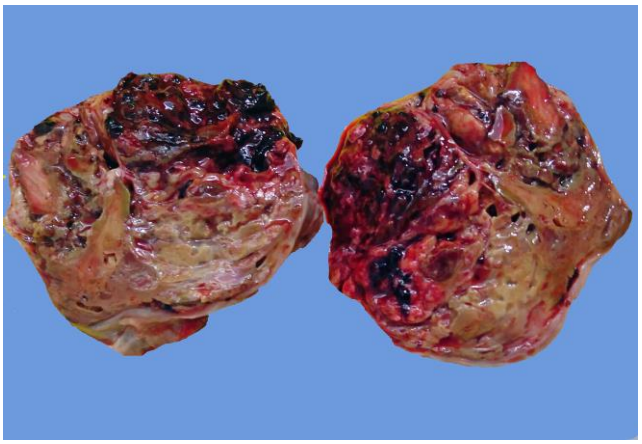

Figure 2. Testicular tumour with an irregular cut surface showing extensive areas of haemorrhage interspersed with friable yellow areas consistent with necrosis.

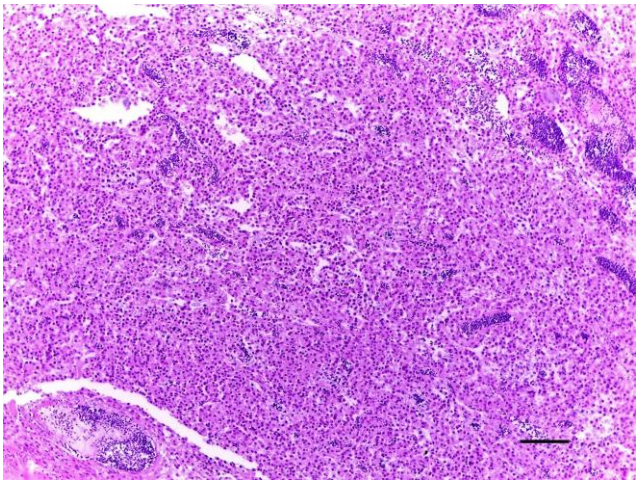

Figure 3. Histological appearance of the left testis demonstrating testicular seminoma. Neoplastic tissue showing large and pleomorphic cells, arranged in mantles or cords. H&E staining. Bar = 100 µm.

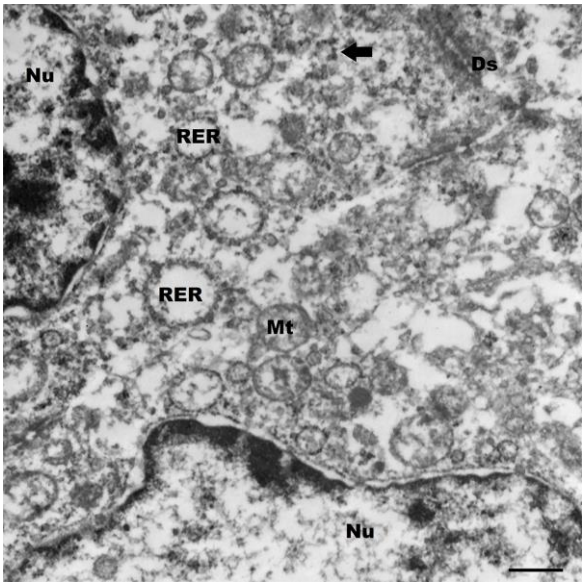

Figure 5. Ultrastructure of two neoplastic cells. Two nuclei are observed (Nu). Note that the cytoplasm exhibits a dilated, rough endoplasmic reticulum (RER), in addition to glycogen aggregates (arrow). Mitochondria appear swollen with the loss of their mitochondrial ridges (Mt), and desmosomes are small (Ds). Contrast technique with uranyl acetate and lead citrate. Bar = 500 nm.

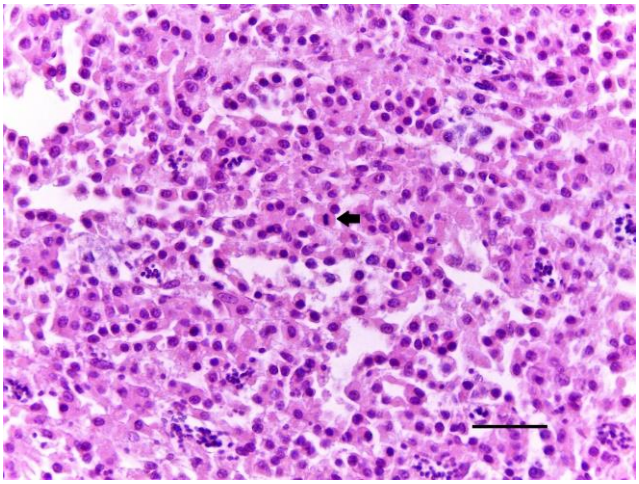

Figure 4. Detail of the neoplastic cells exhibiting moderate and eosinophilic cytoplasm, pleomorphic nuclei (some central and other eccentric) with dense or granular chromatin. Some nucleoli are prominent. The arrow shows a mitotic figure (arrow). H&E staining. Bar = 40 µm.

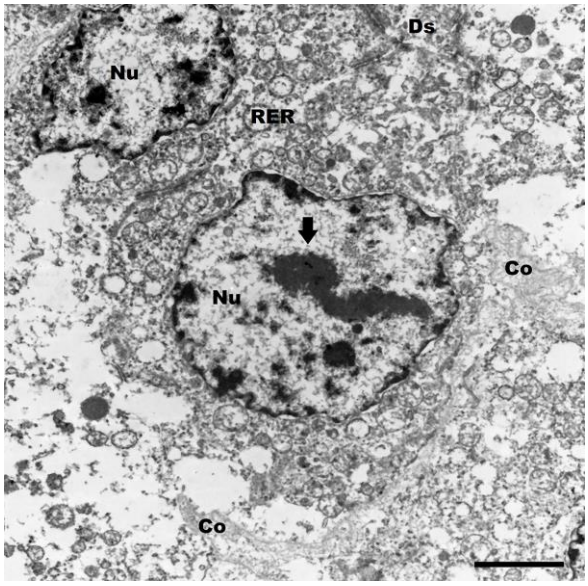

Figure 6. Ultrastructure of a neoplastic cell, showing in the cytoplasm small desmosomes (Ds), fragments of slightly electrodense collagen (Co), and dilated roughened endoplasmic reticulum (RER). The nucleus (Nu) exhibits irregularities on its nuclear membrane as well as an electrodense heterochromatin in clusters. Two nucleoli are visible inside; one is round, and the other exhibits rope-like form (arrow). Contrast technique with uranyl acetate and lead citrate. Bar = 2 µm.

Discussion

It is worth mentioning that there were no reports on testicular tumours in fighting roosters (*Gallus gallus*); therefore, the presence of these tumours in this bird species may be infrequent or poorly reported due to the fact that it is not customary to refer this type of bird with some pathology to medical examination, and, therefore, they are not diagnosed. The anatomopathological characteristics of the tumour were similar to those described for seminomas in dogs and other species (2). It has been previously reported that seminomas in dogs are positive for PAS staining due to the presence of glycogen. However, in this case, the staining was possibly negative as was observed in cases of spermatocytic seminomas in humans (2) or because of the scarce amount of glycogen produced by the neoplastic cells, although it was detected during the ultrastructural study. The use of immunohistochemistry for the diagnosis of tumours is still not common in birds; however, some immunohistochemical markers were successfully used in a common mynah (*Acridotheres tristis*) with a Sertoli cell tumour (3). It should be noted that the ultrastructural appearance of this tumour was also consistent with a seminoma due to the presence of electrodense rope-like nucleoli and the presence of scarce intracytoplasmic glycogen. Further, desmosomes were scarce and small, and the Golgi complexes were not identified as previously reported in some human seminomas (5). In addition to supporting the seminoma diagnosis, no ultrastructural structures were observed, such as lumina lined by microvilli, junctional complexes, numerous desmosomes, basal lamina and abundant SER as in Sertoli cell tumours in humans (5). On the other hand, neither lipid vacuoles, mitochondria with tubular cristae, nor Reinke crystals were observed, which are ultrastructural characteristics reported for interstitial cell tumors, also in humans (5). In this case, the tumour was determined to be benign because of its histological characteristics and because no metastasis was detected. However, the neoplasia replaced the entire testicle and occupied a large part of the coelomic cavity causing organ compression. The contralateral testis was not identified, possibly due to severe atrophy or because the bird was monorchid, as has been reported in a guinea fowl (*Numida meleagris*) with seminoma (9). We conclude that the ultrastructural study provided important support for the diagnosis because it confirmed that the neoplasia corresponded to a seminoma. It is also important to note that the early diagnosis of testicular tumours in birds is the key for a timely treatment; this is because these tumours, although benign, may grow to such an extent that they can compromise the birds' lives, as in this case, and if they are malignant, they can cause metastasis.

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