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*Case Report*

# TESTICULAR GRANULAR CELL TUMOR AND METACHRONOUS BILIARY ADENOMA IN A DOMESTIC RABBIT (*Oryctolagus cuniculus*)

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**Abstract**

A nine-year-old intact male domestic rabbit (*Oryctolagus cuniculus*) was presented with an enlarged left testis. Following surgical castration, histopathologic examination of the affected testis revealed a granular cell tumor. The tumor cells tested positive for PAS and Melan A. Testicular hypoplasia was diagnosed in the contralateral testis. The animal recovered uneventfully. More than a year later, the animal died from unrelated cause. Necropsy revealed a hepatic neoplasm, along with pulmonary and renal changes. There was no evidence of metastasis from the testicular granular cell tumor. Microscopic examination identified a biliary adenoma with multifocal biliary hyperplasia in the liver, moderate interstitial pneumonia, hyperplastic changes in the pulmonary arterial blood vessels, and end stage kidney disease. This case report supports existing literature indicating that testicular granular cell tumors in rabbits are benign and that surgical castration is curative. It remains unclear whether hypoplasia of the contralateral testis influenced neoplastic proliferation. The biliary adenoma was an incidental necropsy finding and could have progressed from biliary hyperplasia.

**Key Words:** biliary adenoma, granular cell tumor, rabbit

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## CASE PRESENTATION

A nine-year-old intact male domestic rabbit (*Oryctolagus cuniculus*) was presented for examination after the owners noticed an enlarged left testis, which they reported had been increasing in size over the past month. The owners also stated that the rabbit exhibited no changes in behavior or appetite.

The rabbit was in good body condition. Clinical examination revealed an enlarged left testis and right testis of decreased size. Body temperature was normal, and hematologic and biochemical parameters were within the reference range (blood was collected under general anaesthesia from the cephalic vein). Palpation of the enlarged testis did not elicit a pain response. Thoracic radiographs showed no abnormalities, but visualization of the abdominal organs was limited due to gastrointestinal content. Surgical castration was elected.

Thirty minutes prior to anesthesia meloxicam (Movalis, Boehringer Ingelheim, Germany) 0.5 mg $kg^{-1}$ was administered subcutaneously (SC). For anesthesia ketamine (Ketamidor, Richter Pharma AG, Austria), 15 mg $kg^{-1}$ and medetomidine (Domitor, Pfizer, Sandwich, Kent, UK), 0.25 mg $kg^{-1}$, were administered SC. The rabbit was returned to its basket, which was gently tilted every 60 seconds to assess the loss of righting reflex. After that, supplemental isoflurane anesthesia was administered via a mask (2%). Both testicles were removed through a scrotal incision. After castration, the patient received atipamezole (Antisedan, Pfizer, Sandwich, Kent, UK), 0.5 mg $kg^{-1}$, SC 30 minutes after discontinuation of isoflurane. Also, enrofloxacin (Baytril, Bayer Animal Health, Germany), 10 mg $kg^{-1}$, metoclopramide (Klometol, Galenika, Serbia), 0.5 mg $kg^{-1}$, and 15 mL of saline was administered SC. Postoperative therapy, i.e., an antibiotic and an analgesic, was administered for seven days. The rabbit woke up in an incubator, with body temperature monitored until it was fully awake. Recovery was uneventful. Both testes were submitted for histopathologic examination.

Grossly, the submitted left testis was oval, measuring 2.8×2×1.8 cm. It was nodular, firm, with a few foci of haemorrhage, and a white, bulging cut surface (Figure 1a).

Microscopically, the affected testis consisted of lobules of neoplastic cells separated by fine fibrocollagenous septa. The tumor cells were large, oval to polygonal, with varying amounts of granular eosinophilic cytoplasm (Figure 1b). They contained a single, round to oval, euchromatic nucleus with a prominent nucleolus. Anisokaryosis was mild to moderate, with larger nuclei appearing polygonal, hypochromatic with dispersed chromatin, and a large, central, magenta nucleolus. The neoplastic cells formed sheets and lobules subdivided by fine, inconspicuous fibrous cords. The mitotic count was three mitotic figures per 2.37 $mm^2$.

Multifocal cholesterol clefts were present, surrounded by macrophages and lymphocytes. At the periphery of the tissue section, a small amount of normal testicular parenchyma was observed, showing atrophy and degeneration of the seminiferous epithelium. In

the epididymal ducts, epithelial cells exhibited shortening, vacuolar degeneration, and loss of cilia, with no spermatozoa present.

Tumor cells showed variable positivity with Periodic acid–Schiff (PAS) staining (PAS kit, Biognost, Zagreb, Croatia) and diffuse immunolabeling with anti-Melan A antibodies (NCL-L-Melan A, Novocastra, Leica Biosystems Newcastle, UK) (Figure 1c, d). Based on cell morphology, PAS, and Melan A positive staining, a diagnosis of testicular granular cell tumor (GCT) was made.

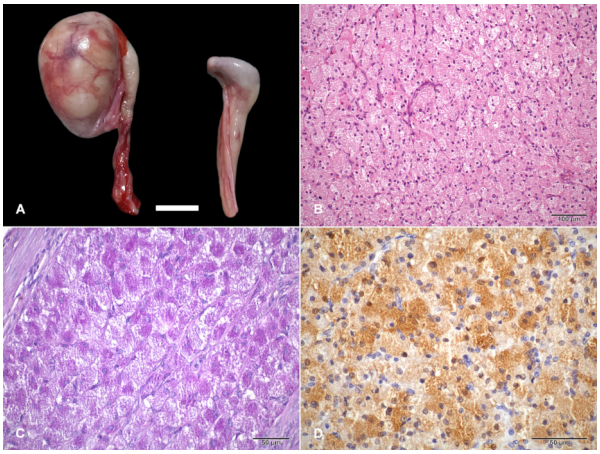


**Figure 1.** Granular cell tumor (GCT) in the testis of a domestic rabbit. (**A**) Macroscopic appearance of an enlarged, nodular left testis with GCT and a small, irregular, hypoplastic right testis (white bar = 1 cm). (**B-D**) Photomicrographs of GCT: (**B**) diffuse proliferation of oval to polygonal cells with eosinophilic granular cytoplasm and round to oval nuclei (HE); (**C**) neoplastic cells showing variable positivity with Periodic acid–Schiff staining; (**D**) neoplastic cells exhibiting moderate to strong cytoplasmic immunolabeling for anti-Melan A.

The right testis was irregular in shape, measuring 1.2×0.5×0.4 cm. It was white, soft, and lacked recognizable parenchyma on the cut surface. Histologically, it consisted primarily of adipose tissue with a few foci of seminiferous tubules containing dystrophic germinal and stromal cells. A diagnosis of testicular hypoplasia was made.

One year later, the rabbit was rehomed when became apathetic and anorexic. The previous owners brought the animal to the clinic. Physical examination revealed pale mucous membranes, a complete lack of response to environmental stimuli, and painful reactions to palpation of the abdomen and kidneys. Blood was collected under general anaesthesia from the cephalic vein, and biochemical analysis indicated liver and kidney dysfunction (Table 1). The owners declined further diagnostic testing. A few days later, following rapid deterioration, the rabbit was euthanised. The owners consented to a necropsy.

**Table 1.** The results of serum biochemical analysis

| Analyte | Value | Reference Interval |
|---|---|---|
| **Albumin, g/dL** | 1.2 | 2.8–4.0 |
| **Alkaline phosphatase, U/L** | 27.3 | 6–14 |
| **Alanine transferase, U/L** | 520 | 52–80 |
| **Aspartate aminotransferase, U/L** | 327.53 | 48–96 |
| **Creatine kinase U/L** | 968 | 23–247 |
| **Creatinine, mg/dL** | 4.8 | 1.0–2.2 |
| **Urea nitrogen, mg/dL** | 54.2 | 9–29 |
| **Phosphorous, mg/dL** | 9.4 | 3.0–6.2 |

Necropsy revealed poor body condition, dry hair and perineal soiling. Subcutaneous adipose tissue was scarce. Approximately 15 mL of transparent, red tinged serous fluid was present in both the abdominal and thoracic cavities. The lungs failed to collapse and appeared red, rubbery, with multifocal emphysematous areas. The liver was enlarged, pale, with an accentuated lobular pattern and a markedly dilated gallbladder. A distinct irregular, white subcapsular lesion measuring approximately 1.3×7 mm was observed (Figure 2a). The kidneys were pale, firm, with a finely granular surface, several small cysts, and a poorly defined corticomedullary junction.

Microscopic examination of the liver mass revealed a biliary adenoma (Figure 2b). The tumor consisted of proliferating biliary ducts of varying sizes and shapes, surrounded by fibrous tissue that was multifocally infiltrated with small to moderate number of mononuclear cells.

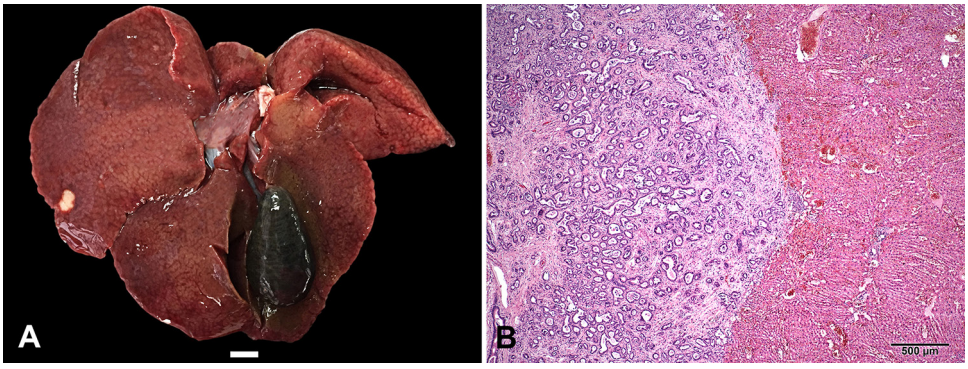


**Figure 2.** Biliary adenoma in the liver of a domestic rabbit. (**A**) Macroscopic appearance of a small, distinct white mass in the left lateral lobe (white bar = 1 cm). (**B**) Photomicrograph showing an unencapsulated, well demarcated neoplasm composed of proliferating biliary ducts lined by well differentiated epithelial cells (HE).

The ducts were lined by cuboidal to columnar epithelial cells, with small amount of eosinophilic cytoplasm, round to oval nuclei and inconspicuous nucleoli. No mitotic figures were seen. The tumour mass was sharply demarcated from the surrounding liver parenchyma. In addition to the adenoma, multifocal biliary hyperplasia with marked mononuclear infiltration and cholestasis was present.

Histologic examination of the lungs revealed moderate interstitial pneumonia with type II pneumocyte hyperplasia and multifocal infiltration by varying numbers of lymphocytes, macrophages, and heterophils, occasionally forming peribronchiolar and perivascular aggregates. There were areas of alveolar emphysema. Many medium and some small arteries were hyperplastic. Segmental proliferation of intimal cells in medium arteries resulted in a reduced lumen diameter, with some arteries being nearly occluded. Small arteries showed circumferential intimal proliferation. Occasionally, smooth-muscle cell proliferation was observed in the arterial media, with many of these cells appearing pale and vacuolated.

Histologic examination of the kidneys showed features consistent with end-stage renal disease, including interstitial fibrosis, glomerular sclerosis, tubular atrophy, and scattered mononuclear infiltrates.

## DISCUSSION

There are few reports of spontaneous multiple tumors in rabbits involving testicular GCT or biliary adenoma. Reported cases include ovarian luteoma and biliary cystadenoma (Nasrin et al., 2012), mandibular fibrosarcoma and bile duct adenoma (Thas et al., 2014), and scrotal malignant melanoma and testicular GCT (Mack et al., 2021). Additionally, biliary cystadenoma (DeCubellis et al., 2010; Sabater et al. 2014) and testicular GCT (Irizarry-Rovira et al., 2008) have been documented as solitary tumors, along with two case studies specifically describing GCTs (Reineking et al., 2019; Webb et al., 2019). This case report describes a biliary adenoma in a rabbit with a prior history of testicular GCT.

GCTs have been reported in several animal species and can occur in various anatomical locations (Patnaik, 1993). They are easily identifiable with light microscopy due to their characteristic appearance. Histologically, GCTs consist of oval to polygonal cells with distinct margins, varying amounts of eosinophilic granular cytoplasm, and typically, a central nucleus (Patnaik, 1993; Irizarry-Rovira et al., 2008; Reineking et al., 2019; Webb et al., 2019). The literature is inconsistent regarding GCT histogenesis. Although usually designated as of neural crest origin, studies have shown that GCTs can be of different cellular origins (Patnaik, 1993). In rabbits GCTs were reported only in testis, and some authors suggest that they could derive from Leydig cells (Irizarry-Rovira et al., 2008).

The main differential diagnosis for testicular GCT is an interstitial (Leydig) cell tumor (ICT). Since the first description of GCT in a rabbit (Irizarry-Rovira et al., 2008), two

studies have shown that many tumors previously diagnosed as ICTs were actually GCTs (Reineking et al., 2019; Webb et al., 2019). One of these studies even found that all tumors classified as ICTs were, in fact, GCTs (Webb et al., 2019). The misclassification likely occurred because these tumors closely resemble each other histologically, and GCTs were rarely diagnosed in rabbits until recently.

Distinguishing ICTs from GCTs based on histology alone is challenging, as both tumors can exhibit similar architecture and cytomorphology. Additionally, they share certain immunohistochemical characteristics, as both are positive with anti-Melan A staining (Irizarry-Rovira et al., 2008). In our case, cells were diffusely positive to Melan-A. Therefore, PAS staining is recommended for definitive diagnosis. In our case, the tumor cells exhibited variable but consistent PAS positivity, similar to previous reports, where a "weak to focally strong positive reaction" was described (Webb et al., 2019).

The tumor showed no signs of malignancy, and one year after surgery, there was no evidence of metastatic disease. This finding supports previous reports stating that surgical removal is curative in cases of testicular GCTs in rabbits (Webb et al., 2019). However, GCTs can be malignant in other animal species (Patnaik, 1993).

Testicular hypoplasia is rarely reported in animals and is generally considered clinically insignificant. In this case, it is unclear whether the testicular hypoplasia may have been related to the neoplastic proliferation in the other testis. We note that there are no literature data to support such a connection.

Bile duct adenomas were once considered the fourth most common spontaneous tumor in rabbits (Weisbroth, 1974), but their reported incidence appears to be much lower today. Also known as cholangiocellular adenomas, these tumors typically present as solitary, well-demarcated, white to tan masses, distinct from normal liver tissue, as observed in this case. Microscopically, they consist of tubules lined with a single layer of well-differentiated biliary epithelial cells, accompanied by a variable amount of fibrous stroma. When tubular cystic changes are present, these tumors are classified as cystadenomas.

It has been suggested that bile duct adenomas could arise from the reactive proliferation of biliary epithelium secondary to noxious stimuli, particularly infections, ischemia, and carcinogenic agents (Nasrin et al., 2012). Additionally, Tinkey et al. (2012) speculated that bile duct tumors could originate from initially hyperplastic epithelium. This hypothesis is relevant in the present case, as marked multifocal biliary hyperplasia was observed throughout the liver tissue.

The primary differential diagnosis for bile duct adenoma included biliary hamartoma and biliary hyperplasia, which can be challenging to distinguish histologically. Although biliary adenomas can sometimes be multiple, multifocal lesions are more likely to be hamartomas or hyperplasia. In most cases, all these lesions are incidental findings at necropsy and have no clinical significance.

Biochemical analysis revealed significant alterations in multiple parameters, including elevated AST, ALT, and creatinine levels, indicating widespread organ dysfunction.

However, due to the markedly deteriorated general condition of the rabbit at the time of presentation, these changes are not considered organ-specific but rather a reflection of multisystemic failure. The severity of the clinical signs suggested advanced systemic disease, making it difficult to attribute the alterations in biochemical parameters to a single organ system.

Spontaneous lung lesions in aging non-laboratory rabbits are not well documented. The lung lesions observed in our case were found by Cooper et al. (2017) to be common in rabbits over two years of age. Their study reported fibromuscular intimal hyperplasia of pulmonary arteries to a variable extent in all examined animals (36/36) and pulmonary emphysema in nearly all cases (30/36), despite the absence of clinical signs of respiratory disease, even in severely affected animals. Similarly, the rabbit from our case exhibited no signs of respiratory disease.

Frequent findings of type II pneumocyte hyperplasia (adenomatosis) by Cooper et al. (2017) were associated with surfactant pneumonia. In our case, this lesion may have resulted from interstitial pneumonia, as there was no histological evidence of surfactant pneumonia. However, it is worth noting that Cooper et al. (2017) observed these findings specifically in an inbred laboratory rabbit strain (EIII/JC audiogenic strain), which may limit direct comparisons to non-laboratory rabbits.

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**Authors' contributions**

MA: Conceptualization, Writing, Reviewing and Editing; IV and SN: Methodology and Resources; VM: Processing; AP: Writing - Original Draft, MV: Reviewing and Editing.

**Competing interests**

The authors declare that they have no competing interests.

**Ethical statement**

The owner of the animal was fully informed about all premortal and postmortal procedures that were to be conducted. Written informed consent was obtained prior to the procedures, in accordance with ethical standards and institutional guidelines.

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# TUMOR GRANULARNIH ĆELIJA TESTISA I METAHRONI BILIJARNI ADENOM DOMAĆEG KUNIĆA (*Oryctolagus cuniculus*)

Milan ANIČIĆ, Ivana VUČIĆEVIĆ, Vladimir MAGAŠ, Slađan NEŠIĆ, Ana PEŠIĆ, Miloš VUČIĆEVIĆ

**Kratak sadržaj**

Kunić, mužjak, devet godina star, nekastriran (*Oryctolagus cuniculus*) donešen je na pregled zbog uvećanog levog testisa. Nakon hirurške kastracije, histopatološkim pregledom tkiva izmenjenog testisa dijagnostikovan je tumor granularnih ćelija. Tumorske ćelije su bile pozitivne na PAS i Melan A bojenje. Na drugom testisu utvrđena je hipoplazija. Oporavak životinje je protekao bez komplikacija, a više od godinu dana kasnije, kunić je uginuo usled stanja nepovezanog sa primarnom dijagnozom. Na obdukciji je ustanovljen tumor jetre, uz promene na plućima i bubrezima. Nije bilo dokaza o metastaziranju tumora granularnih ćelija testisa. Mikroskopski je identifikovan bilijarni adenom sa multifokalnom bilijarnom hiperplazijom u jetri, umerena intersticijalna pneumonija i hiperplastične promene u plućnim arterijama, kao i terminalna bolest bubrega. Ovaj prikaz slučaja potvrđuje prethodne navode u literaturi da su tumori granularnih ćelija testisa kunića benigni i da je hirurška kastracija metoda izbora. Ostaje nejasno da li je hipoplazija kontralateralnog testisa uticala na neoplastičnu proliferaciju. Bilijarni adenom predstavlja slučajan nalaz na obdukciji i moguće je da je nastao progresijom bilijarne hiperplazije.

**Ključne reči:** bilijarni adenom, tumor granularnih ćelija, kunić