

Title: Testicular tumor from a pet rabbit (*Oryctolagus cuniculus*)

Contributors: Javier Martínez-Caro¹, Laura Vilalta Solé^{2,3}, Antonio Meléndez-Lazo^{1,2}

¹T-CITO, Valencia, Spain

²Hospital Veterinario de Referencia UCV, Valencia, Spain

³Departamento de Medicina y Cirugía Animal, Universidad Católica de Valencia "San Vicente Mártir, Valencia, Spain

Specimen: Impression and fine needle aspiration cytology.

Signalment: A 6-year-old male pet rabbit (*Oryctolagus cuniculus*).

History and Clinical findings:

The patient presented with an enlarged right testicle. Otherwise, he had no previous clinical history, and his diet consists of a seed mixture, hay, and vegetables.

Physical examination revealed an enlarged right testicle, atrophy of the left testicle and increased body condition.

There were no lesions in the scrotal skin, but scrotal sac was increased in size due to testicular enlargement and it contacted with the perianal area and urethral opening. Otic secretion with pain on auricular palpation was noticed. Also, he had mild pododermatitis in both forelimbs and mild occlusive dental problems.

Diagnostic procedures:

Serum biochemistry and hematology were performed either as a minimum database of the patient and a preanesthetic evaluation. Hematology analysis showed a moderate lymphopenia, 460 lymphocytes per microliter (RI: 1,490-5,210 per microliter) and serum biochemistry results were within reference intervals. Ultrasound examination revealed an increase size of the right testicle (6 cm in diameter), the majority of the testicular parenchyma has been replaced by hypoechoic to anechoic foci of various sizes and shapes with abnormal Doppler color signal and mineralized areas. The left testicle was atrophied but with normal architecture. Prostatic abnormalities were not seen. On thoracic radiographs, metastatic lesions were not evident. Cytological examination of the otic secretion revealed the presence of *Malassezia* sp., for its morphology, most likely *M. cuniculi*.

Surgical procedure and treatment:

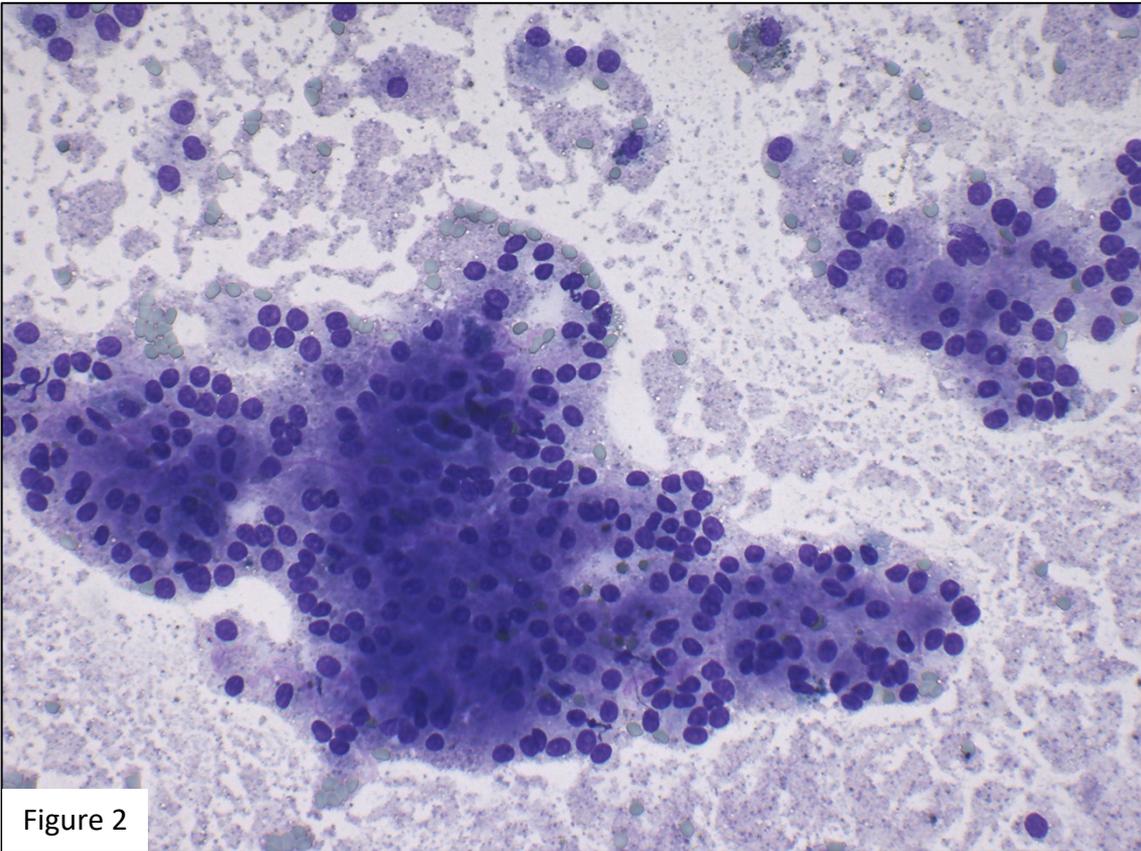
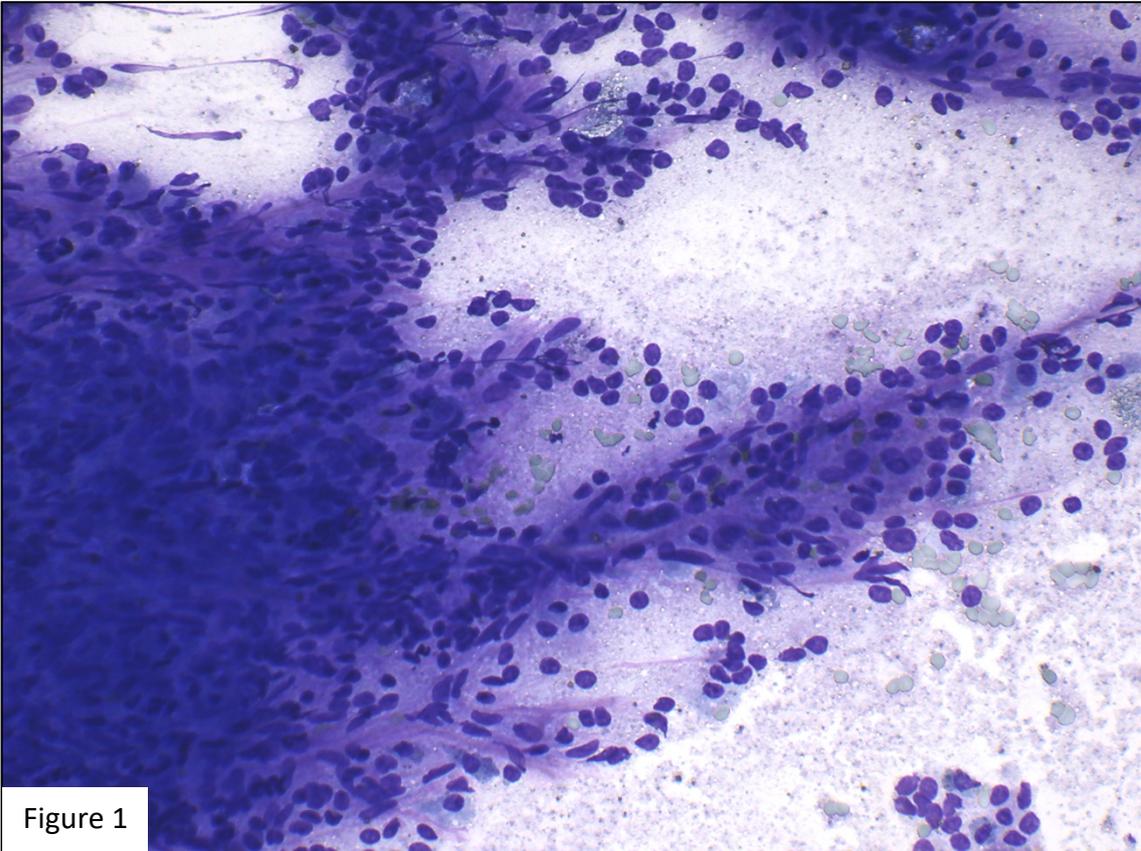
Because neoplasia was suspected, bilateral orchiectomy and scrotal ablation was performed using an open prescrotal approach under general anesthesia. Postsurgical treatment included intravenous fluid therapy, analgesia with opioid drugs (buprenorphine), NSAID (meloxicam), antibiotherapy (enrofloxacin) and prokinetics (metoclopramide).

Cytologic specimens (figures 1 to 5) were prepared using fine needle aspiration and impression techniques before formalin fixation for histopathological examination.

Questions:

1. What is your description of the cytologic findings?
2. What is your interpretation?
3. Which further tests would you recommend in order to confirm the preliminary diagnosis?

Figures 1 and 2: cytological photomicrograph of the testicular mass. Aqueous Romanowsky stain, x20 objective.



Figures 3 and 4: cytological photomicrograph of the testicular mass. Aqueous Romanowsky stain, ×40x objective.

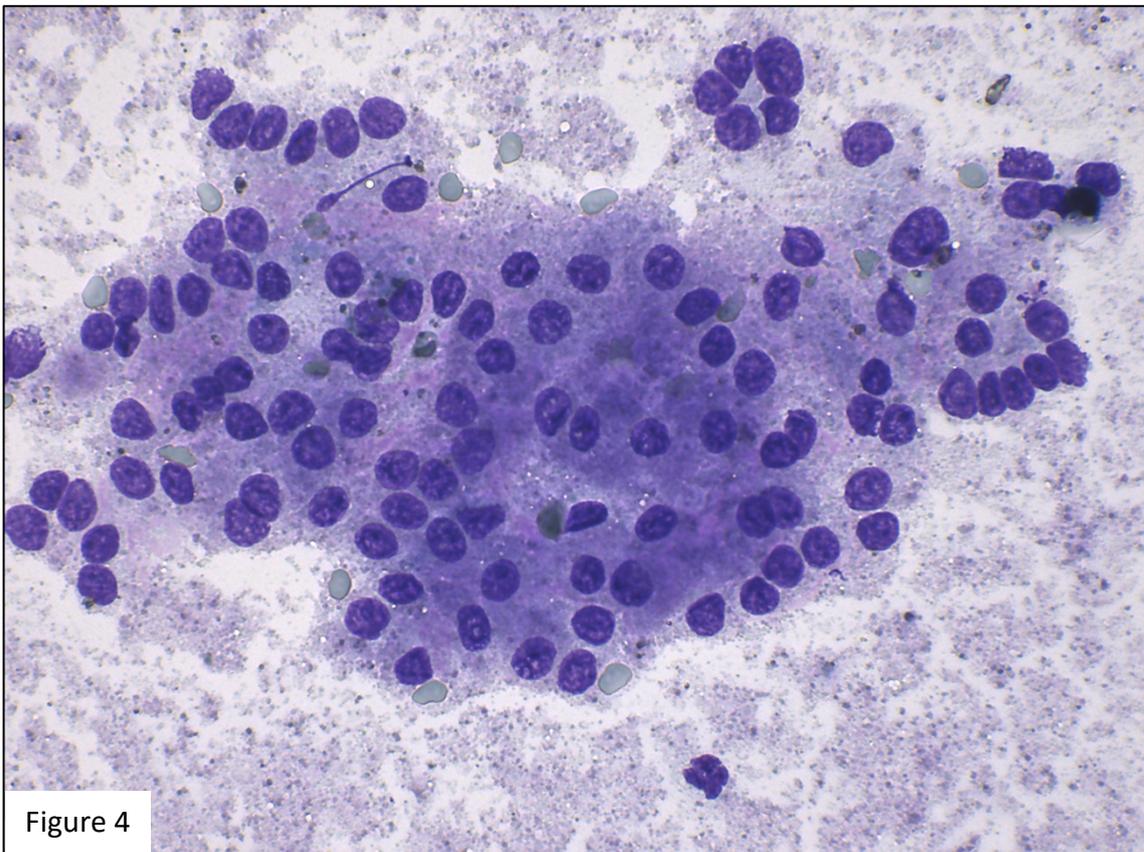
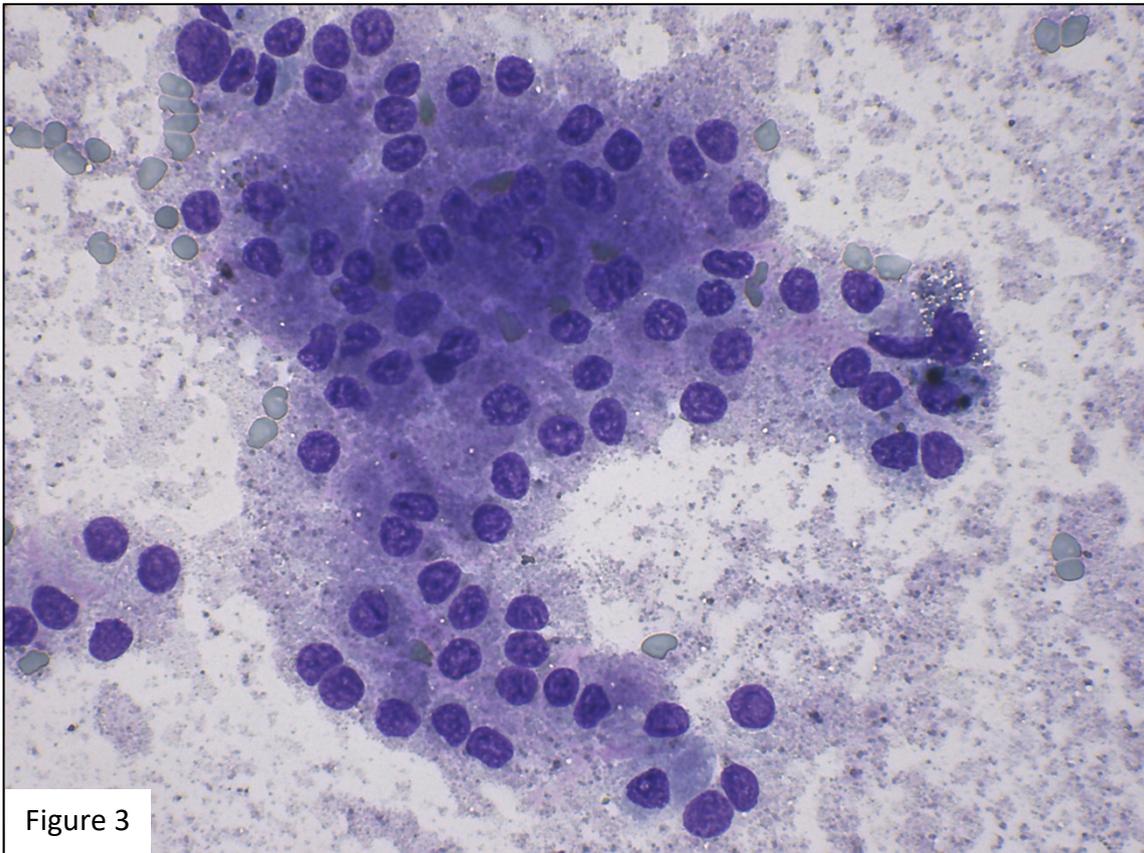


Figure 5: cytological photomicrograph of the testicular mass. Aqueous Romanowsky stain, ×100x objective.

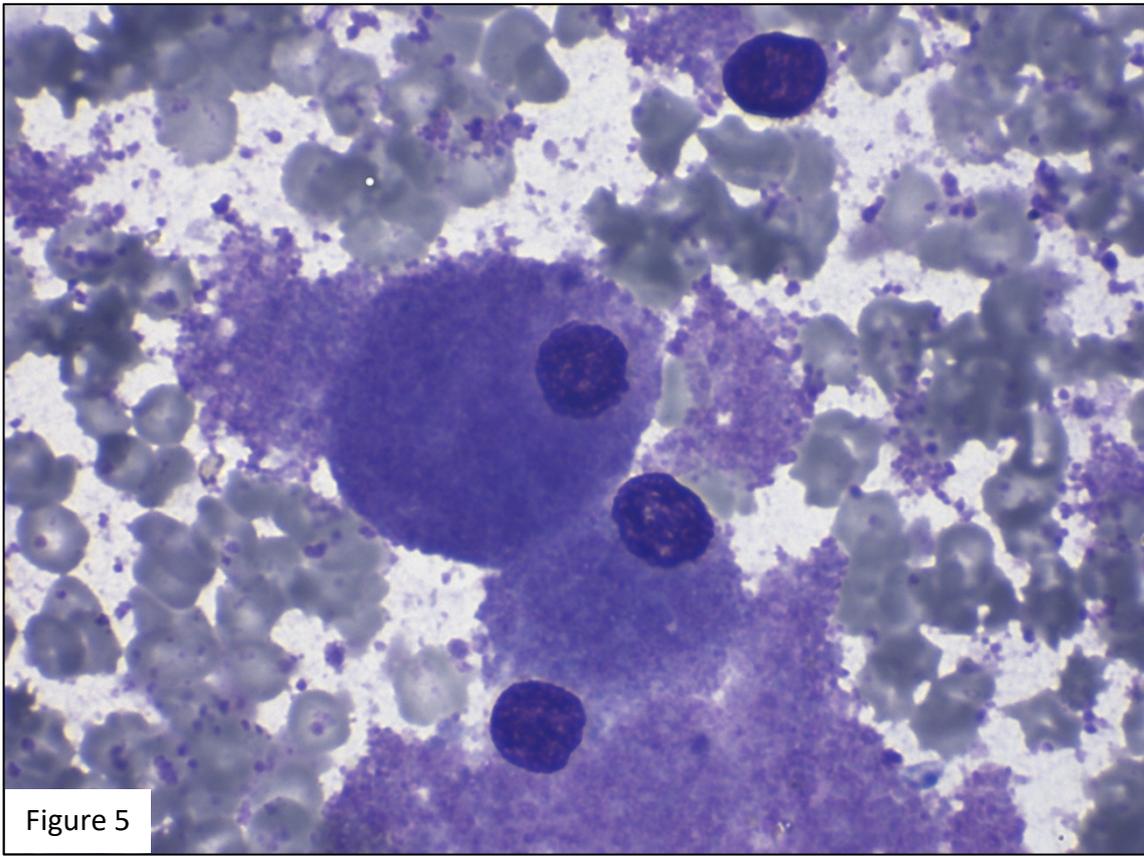


Figure 5

1. Description:

The smears are of moderate to high cellularity and moderate cell preservation. The background is bluish, with mild blood contamination, frequent smudge cells and high numbers of pinkish granules.

There is a cell population of round to polygonal cells, distributed in moderately cohesive aggregates or isolated. The intact cells have poor to moderately defined cell borders and a moderate to abundant amount of cytoplasm, filled with pink to amphophilic granules. Nuclei are round to oval in shape and placed in central to paracentral position. They have finely stippled chromatin pattern and single, round nucleoli can be occasionally seen. Anisocytosis and anisokaryosis are mild to moderate and the nucleus-to-cytoplasmic ratio is low to moderate. Mitoses are not seen.

In low numbers, there are mesenchymal cells with minimal atypia, possibly the stroma component of the tumor. There are occasional macrophages with variable amount of clear cytoplasmic vacuoles and phagocytosed cellular debris.

No microorganisms are detected in the examined cytological preparations.

2. Interpretation:

Consistent with testicular granular cell tumor

3. Further recommended tests:

The main recommendation is to perform histopathological examination in order to confirm the cytological interpretation. Further ancillary techniques would be preferred, for example histochemistry, as the Periodic acid–Schiff (PAS) stain to demonstrate the granular cell origin, or immunohistochemistry profile including melan-A.

Complementary procedures:

Histopathological exam of the right testicle revealed cavitated, neoplastic proliferation. It was partially encapsulated and extended over the resection borders. Cells were organized in nests separated by a scant fibrovascular stroma. They were polygonal in shape, medium to large in size, with an abundant eosinophilic cytoplasm and numerous small clear vacuoles. Nuclei were large and round with a finely stippled chromatin pattern and a visible, central nucleolus. Anisocytosis and anisokaryosis were mild and the mitotic index was 1-2/10HPF. Seminiferous tubules were seen compressed in the periphery of the tumor.

The contralateral testicle showed a marked atrophy of seminiferous tubules, and spermatogenic cells were absent. There was moderate interstitial fibrous proliferation and epididymis contained a low number of spermatozoa.

The differential diagnosis of histopathological examination included interstitial cell tumor (ICT) or granular cell tumor (GCT) in the right testicle, and atrophy of contralateral testicle. However, after the special stain Periodic acid-Schiff (PAS), the interpretation was consistent with testicular granular cell tumor.

Discussion:

The prevalence of testicular neoplasms in rabbits is low, estimated in 1.93%, with mean age at presentation of 7.5-year-old.¹ Before 2019, the predominant neoplasm reported in bibliography was ICT, but most of the cases histologically diagnosed as ICT were reclassified into GCT after the stained with PAS.^{1,2}

In a retrospective study with 57 testicular tumors in rabbits, 63% were GCT, followed by seminomas (14%), ICT (12%), Sertoli cell tumors (7%) myxosarcoma (2%) and undetermined (2%).² In other study, all reviewed ICT were reclassified into GCT and account for 71% of all testicular neoplasms.¹

Cytological examination of GCT specimens usually allow to make a diagnosis with high confidence degree. The reported findings, like described in our case, include a monotonous population of cells with a high number of pink to purple/magenta cytoplasmic granules, that sometimes difficult nucleus visualization.³

Misdiagnoses between ICT and GCT are frequent in hematoxylin-eosin, stained tissue sections, since both are quite similar in appearance. However, slight differences may be seen and the cytoplasm of GCT is more granular than that of ICT, which is often vacuolated and exhibits a larger nucleus.² The use of the PAS reaction is a simple and quick method to differentiate GCT and ICT. Granules of GCT is PAS positive and stain light to moderately pink, but ICT are PAS negative.^{2,3}

Reported immunohistochemistry cases of testicular GCT described an intense immunolabeling for melan-A. Multifocally, neoplastic cells expressed neuron-specific enolase, periaxin, S100 protein and vimentin. Granular cells were negative for cytokeratin, α -SMA, GFAP and MBP. For the immunohistochemistry results, GCT has been hypothesized to be of neuroectodermal origin.^{2,3}

Transmission electron microscopy can also be used to differentiate ICT from GCT, which contain numerous secondary lysosomes.^{2,3}

Testicular GCT has a benign biological behavior in rabbits, with a median survival time of 599 days with castration. After surgical excision, prognosis is excellent, and patients do not require further treatments.¹

In conclusion, GCT is the most prevalent testicular neoplasm in rabbits, a cytological diagnosis may be achieved, and it usually has a benign behavior.

References:

1. Webb JK, Reavill DR, Garner MM, Kiupel M, Graham JE. Characterization of Testicular Granular Cell Tumors in Domestic Rabbits (*Oryctolagus cuniculus*). *Journal of Exotic Pet Medicine*. 2019;29:159-165.
2. Reineking W, Seehusen F, Lehmbecker A, Wohlsein P. Predominance of Granular Cell Tumours among Testicular Tumours of Rabbits (*Oryctolagus cuniculi f. dom.*). *Journal of Comparative Pathology*. 2019;173:24-29.
3. Irizarry-Rovira AR, Lennox AM, Ramos-Vara JA. Granular Cell Tumor in the Testis of a Rabbit: Cytologic, Histologic, Immunohistochemical, and Electron Microscopic Characterization. *Veterinary Pathology*. 2008;45:73-77.