Testicular leiomyosarcoma and marked alopecia in a cryptorchid ferret (*Mustela putorius furo*)

P. Kammeyer; S. Ziege; S. Wellhöner; S. Cichowski; W. Baumgärtner

1Department of Pathology, University of Veterinary Medicine Hannover, Hanover, Germany; 2Small Animal Practice, Dissen a. TW, Germany

### Key words
Mustelids, testicular tumour, α-smooth muscle actin, desmin

### Summary
A 3.5-year-old male ferret, bought as male castrated, was presented to the veterinarian with marked alopecia of back, neck, abdomen and tail, a pronounced sexual behaviour and weight loss. An inguinal mass of about 2.5 cm in diameter was diagnosed as potentially tumorous inguinal testicle by ultrasound and fine-needle aspiration. Adrenal glands and prostate were ultrasonographically unremarkable. The surgically removed cryptorchid testicle contained a greyish tumour that was histologically composed of spindle-shaped cells with elongated nuclei, embedded in a fibro-vascular stroma. Up to two mitotic figures per high power field were noted. Additionally, an interstitial cell hyperplasia and marked reactive proliferation of a collagen-rich fibrous tissue were observed. Tumour cells were positive for α-smooth muscle actin, desmin, and occasionally vimentin and S-100, leading to the diagnosis of an intratesticular leiomyosarcoma. As an adrenal-associated endocrinopathy was excluded and a complete fur recovery was observed after removal of the cryptorchid testicle the alopecia was eventually due to hormones produced by the hyperplastic interstitial (Leydig) cells.

### Introduction
Due to the early neutering of male ferrets reports on testicular neoplasms are rare in this species. So far, Sertoli cell tumours, an interstitial cell tumour, a mixed interstitial and Sertoli cell tumour in the same testicle as well as an intratesticular peripheral nerve sheath tumour have been described in non-castrated ferrets (2, 4, 6, 8). Reports on tumours in cryptorchid testicles of ferrets are even rarer. A testicular Sertoli cell tumour has been reported in a presumably neutered male ferret with additional adrenocortical hyperplasia and prostatic abscesses (18). In the present case, a multilobular, partly infiltrating testicular leiomyosarcoma with interstitial cell hyperplasia is described in a cryptorchid ferret. Additionally, the animal showed a marked alopecia and pronounced sexual behaviour, whose pathogenesis is discussed.
Case report
Clinical history
A 3.5-year-old male ferret was presented to the referring veterinarian with marked alopecia of back, neck, abdomen (Fig. 1a) and tail (Fig. 1b) as well as a tentative diagnosis of hyperadrenocorticism. In few abdominal areas the hairless skin showed a mild redness and single small pustules. Alopecia was first reported about one year before, restricted to the tail, vanishing 6 months later and re-occurring in the above mentioned localisations 3 months prior to presentation. The animal was bought at the age of one year as male-neutered but nevertheless showed a pronounced sexual behaviour, hyperactivity, increased vocalizations and a strong sex-related smell. Additionally, a marked weight loss (from 1.8 to 1.4 kg) despite an unchanged feed intake was observed for 3 months.

Abdominal palpation revealed a firm, mobile, painless inguinal mass of about 2.5 cm in diameter on the left side. Ultrasound was performed and a potentially neoplastic inguinal testicle was suspected. Fine-needle aspiration of the mass supported this assumption. Additionally, an atrophic testicle was suspected in the right scrotum. Adrenal glands were of normal shape and size with a length of 6.0 mm and a width of 2.7 mm. The prostate gland was ultrasonographically unremarkable. Hematologic and biochemical blood parameters were within normal ranges. Hormone levels were not determined for financial reasons.

The inguinal mass, measuring 2.3 x 1.5 x 1.0 cm, was surgically removed under general anesthesia, formalin-fixed and submitted for histopathology. At the same time, a very small intrascrotal right testicle (0.8 x 0.5 x 0.5 cm) was removed. This apparently atrophic testicle showed an unremarkable macroscopic appearance in transverse sections, which is why it was not further investigated.

Following surgery the ferret showed good recovery under antibiotic treatment (amoxicillin/clavulanic acid, 10 mg/kg for 5 days, oral administration). Signs of mild dermal inflammation vanished and new hair growth was noted about 14 days after castration resulting in a complete coat recovery (Fig. 1c). Additionally, the animal became much calmer and the gender-related smell almost completely vanished.

Histopathological and immunohistochemical examination
For microscopic examination, the formalin-fixed sample was trimmed routinely, embedded in paraffin wax, sectioned at 5 µm, and stained with haematoxylin and eosin (HE).

To further characterize the neoplastic cells, immunohistochemistry was performed using the avidin-biotin-peroxidase method. Antibodies directed against α-smooth muscle actin (α-SMA, 1:200 monoclonal mouse anti-human), desmin (1:100, monoclonal mouse anti-human), pancytokeratin (1:2500, monoclonal mouse anti-human), cytokeratin AE1/AE3 (1:500, monoclonal mouse anti-human), vimentin (1:100, monoclonal mouse anti-porcine), Melan-A (1:600, Clone A103, monoclonal mouse anti-human; all DakoCytomation, Glostrup, Denmark) and S-100 (1:800, polyclonal rabbit anti-human, Sigma Aldrich, Taufkirchen, Germany) were used. 3.3-diaminobenzidine (DAB) served as chromogen. Positive immunohistochemical controls included appropriate cells of normal ferret organs. Non-neoplastic testicular tissue of a male ferret was used for comparison.
Results

The submitted mass consisted of testicular and epididymal tissue. Testicular tissue was largely replaced by a moderately well demarcated, partly infiltrating greyish mass of 0.8 cm in diameter, which did not extend to the cut borders or penetrate the tunica albuginea. The mass consisted of a multilobular, partly encapsulated cell-rich neoplastic proliferation composed of spindle-shaped cells arranged in densely packed streams (▶Fig. 2a, g). Tumour cells showed indistinct cell borders and abundant eosinophilic cytoplasm. The often centrally located nuclei were elongated to oval with finely stippled chromatin and up to two partly prominent nucleoli (▶Fig. 2g). A moderate anisocytosis and anisokaryosis and up to two mitotic figures per high power field were noted. In addition, a moderately developed fibro-vascular stroma was present. Pre-existing tissue surrounding the tumour was characterized by severe compression atrophy with loss of seminiferous tubules and aspermia. Additionally, areas of hyperplastic, finely vacuolated in-

![Fig. 2](cryptorchid-testicle.png)

**Fig. 2** Cryptorchid testicle. a) Leiomyosarcoma (L), adjacent hyperplastic interstitial (Leydig) cells (I) and marked reactive collagen-rich fibrous tissue (F). b) α-SMA-positive tumour cells. c) Positive desmin immunoreactivity of a high number of tumour cells. d) Weakly positive vimentin expression of some tumour cells and of collagen-rich fibrous tissue. e) Negative pancytokeratin immunoreactivity of tumour cells. f) Melan-A-negative tumour cells adjacent to Melan-A-immunoreactive hyperplastic interstitial cells. g) Spindle-shaped neoplastic cells arranged in densely packed streams showing indistinct cell borders and abundant eosinophilic cytoplasm as well as elongated to oval nuclei. h) Hyperplastic, finely vacuolated interstitial cells. i) Prominent Melan-A immunoreactivity of hyperplastic interstitial cells. (a, g, h) HE-stain; (b–f, i) immunohistochemistry, DAB as chromogen. Bar (a–f) 250 µm, (g–i) 25 µm.

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terstitial (Leydig) cells were noted (Fig. 2a, h) as well as areas with a lower cell density and a high amount of reactive collagen-rich fibrous tissue (Fig. 2a). The epididymal tissue showed a marked fibrosis while neoplastic cells and spermatozoa were not detectable. Multifocally, a mild infiltration of lymphocytes and macrophages was noted.

Tumour cells were positive for \( \alpha \text{-SMA} \) (Fig. 2b) and, less prominent, for desmin (Fig. 2c), which was not found in normal ferret testicular tissue. Few tumour cells additionally stained weakly positive for vimentin, which also labelled the reactive collagen-rich fibrous tissue (Fig. 2d), and S-100. Neoplastic cells were negative for pancytokeratin (Fig. 2e) and cytokeratin AE1/AE3. The latter could both be found in epithelial cells of deferent ductules and epididymis instead. Melan-A-immunoreactivity was not detectable in tumour cells but prominent in hyperplastic interstitial cells of the adjacent parenchyma (Fig. 2f, i). Interstitial cells of normal ferret testicular tissue occasionally showed a weak, finely granular Melan-A immunoreactivity.

**Discussion**

Regarding the clinical signs like alopecia and seasonal sexual behaviour an adrenal-associated endocrinopathy (AAE), which is common in ferrets and may be induced by adrenocortical adenoma, carcinoma, or nodular hyperplasia, was initially suspected in the present case (11, 21, 22, 23). However, adrenocortical lesions were excluded after ultrasonographic examination, revealing adrenal glands of normal shape and size (15, 20). Additionally, pruritus, which is often noted in adrenal disease, has not been observed (20).

Instead, an inguinal mass representing a neoplastic cryptorchid testicle was found. The microscopic and immunohistochemical findings, such as positive reaction for \( \alpha \text{-SMA} \) and desmin and negative cytokeratin labeling, indicated that the neoplastic cells were of smooth muscle origin (1, 7, 12, 17). This led to the diagnosis of a multinodular, partly infiltrating testicular leiomyosarcoma with compression of pre-existing tissue, interstitial cell hyperplasia, and marked reactive proliferation of collagen-rich fibrous tissue.

Testicular leiomyosarcoma is a very rare finding in domestic animals and man. Actually, only few reports exist for humans and a cryptorchid stallion (1, 7, 10, 14). Due to the small number of reported cases, the biological behaviour of these tumours is difficult to predict (10). Soft tissue and bone metastases have been described in a human patient (7). However, orchidectomy seems to be curative and, combined with a close follow-up, the treatment of choice in most individuals (10). In the present case there were no indications for recurrence or metastases up to 8 months post surgery. Intratesticular leiomyosarcoma may arise from smooth muscle cells of testicular blood vessels, contractile cells of the seminiferous tubules, or the tunica albuginea (7, 9). Paratesticular tumours arising from the spermatic cord and the epididymis, which are more frequently seen, should be excluded by macroscopic and histologic examination as it was done in this case (9).

A prominent clinical finding in the present case was the marked alopecia, accentuated on back and tail. In ferrets, alopecia is most often associated with elevated androgen and/or estrogen levels due to AAE (21, 22). Additionally, hormone producing tumours of the genital tract or endometrial hyperplasia associated with elevated estrogen levels could potentially lead to alopecia in ferrets, even though this was not an obvious finding in the so far reported cases (2, 3, 4, 6). As hormone secretion is not a typical feature of leiomyosarcomas, the cause of the alopecia and the intense sexual behaviour of the ferret remains speculative. The fact that a complete fur recovery and a normalization of behaviour were observed after surgical removal of the neoplastic testicle substantiates the suspicion that the reported clinical signs were a consequence of hormonal imbalances related to the cryptorchid testicle. Maybe the diagnosed interstitial cell hyperplasia, which could have led to an increased androgen and/or progesterone production, may have caused the alopecia and increased sexual behaviour in the present case (5, 16, 24). This assumption is supported by the prominent positive reaction of hyperplastic interstitial cells for Melan-A. The latter is known to indicate steroid-production in interstitial cells and steroid-producing tumours of dogs and cats (13, 19). In contrast, interstitial cells of a control animal only showed occasional weak Melan-A immunoreactivity. Regrettably, hormone levels have not been determined in the present case. This would have been helpful in explaining the observed clinical signs.

Interestingly, alopecia was also a clinical finding in a case of intra-abdominal leiomyosarcoma of a female ferret (17). This raises the question, whether these tumours could have the potential to secrete hormones, leading to the described clinical signs in ferrets. Unfortunately, the primary site of the intra-abdominal tumour could not be determined and hormone levels were not stated by the authors (17).

**Conclusion for practice**

Even in presumably neutered ferrets cryptorchid testicles should be kept in mind when an animal is presented with the described clinical signs and unremarkable adrenal glands. A potentially tumorous process associated with hormonal activity stresses the importance to remove the cryptorchid testicle surgically. As in the present case, this may result in a complete fur recovery and normalization of sexual behaviour. Besides the rather common Sertoli and interstitial cell tumours, unusual testicular neoplasms like leiomyosarcomas should be added to the list of differential diagnoses in this species. Furthermore, determination of hormone levels might be useful to correlate histopathological findings with clinical signs and should be included in the diagnostic work-up of such cases.
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Conflict of interest
None of the authors have any conflicts of interest to declare.

References