FACT SHEET 8

Finn Pathologists *The* Veterinary Pathologists

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Laboratory Diagnosis of Ferret Adrenocortical Disease

Nodular hyperplasia, adenomas and adenocarcinomas account for 56%, 16% and 26% of adrenocortical masses in ferrets, respectively.

Ferret adrenal disease **usually affects ferrets 3 years of age or older** and there is **no sex predilection.**

Ferrets commonly have **multiple concurrent diseases**. A ferret with adrenal disease frequently also has an insulinoma or a malignant lymphoma.

Predisposing Factors

- **Artificially prolonged photoperiod that ferrets are exposed to when housed indoors and early age at gonadectomy**

  Ferrets are seasonal breeders. In the winter, when each day has less than 12 hours of light, plasma melatonin concentrations are high, resulting in a thick winter coat. High melatonin concentrations also suppress the release of GnRH from the hypothalamus. With increasing day length, this suppression is lost and GnRH is released, resulting in the pulsatile release of LH and FSH from the pituitary gland. These hormones in turn stimulate the release of oestrogen and testosterone from the gonads, which then exert negative feedback on the hypothalamus and pituitary gland, resulting in suppression of release of GnRH, LH and FSH. When ferrets are neutered, this negative feedback is lost, resulting in increased release of LH and FSH, which may promote steroidogenesis and induce non neoplastic and neoplastic adrenocortical enlargement.

  There is a significant correlation between age at neutering and age at diagnosis, suggesting that age at neutering may influence age of onset of this condition. The interval between age at neutering and age at diagnosis in a sample Dutch ferret population was 3.5 +/- 1.8 years (neutered between 6 months and 1.5 years old – median age at time of neutering of 1 year) and in a sample ferret US population was 3.3 +/-1.4 years (neutering at 6 weeks of age). Adrenocortical disease has however been reported in sexually intact ferrets.

- **Possible genetic predisposition**

  Inbreeding. In addition, and although no specific genes have been identified, the genetic profile of ferrets may explain why this condition is seen in these animals and not in other species routinely neutered.

Etiology And Pathophysiology

The pathogenesis of adrenal associated endocrinopathy involves **disruption of the negative feedback mechanism of gonadotropin-releasing hormone (GnRH) and LH release.** The disease is **pituitary independent** given the normal plasma concentrations of both adrenocorticotropic hormone (ACTH) and α-melanocytic stimulating hormone. The most likely cause is neutering and increased light exposure from ferrets being kept indoors which results in increased LH concentrations that activate LH receptors on adrenal cells. Continuous LH stimulation eventually leads to tumour formation in the adrenal cortex. A fraction of steroid producing cells of the zona fasciculata and reticularis elaborate sex steroids rather than cortisol due to chronic stimulation by persistently elevated LH levels.

Adrenal endocrinopathy is most commonly associated with **proliferative lesions such as adrenocortical hyperplasia, adrenocortical adenoma and adrenocortical carcinoma,** with reported prevalences of 56%, 16% and 26%, respectively. The proliferation of the spindle-cell component in both hyperplastic and neoplastic lesions of the adrenal cortex has been reported in ferrets and these cells have demonstrated positive immunoreactivity for oestrogen receptors. The **spindle cell component** with oestrogen receptor immunoreactivity correlated with high malignancy, a decreased disease-free interval and decreased survival time. Immunohistochemistry techniques can be used for diagnosis and to differentiate benign and malignant adrenocortical proliferative lesions. Novel genetic and epigenetic markers (e.g. insulin-like 3, forkhead box L2) were identified recently in mice and ferrets. **Adrenocortical carcinomas with myxoid differentiation** are highly malignant with an increased incidence of metastases.

Clinical Presentation

Clinical signs

- Symmetrical alopecia, often starting at the base of the tail, and then progressing cranially. It can be cyclical with seasonal recurrence resulting in permanent hair loss (reported most frequently on the dorsum adjacent to the shoulder region)
- Pruritus
- Comedones and thining of the skin
- Recurrence of sexual behaviour after neutering
- Vulvar swelling with mucoid discharge
- Mammary gland enlargement in female ferrets
- Enlarged and cystic prostate glands in male ferrets
- Dysuria, stranguria or anuria due to partial or complete urinary obstruction in males caused by periprostatic or periurethral cysts

  Different presentations of adrenal disease are possible (e.g. absence of alopecia; pruritus or stranguria only)

Physical exam

- Alopecia
- Vulvar swelling in females

- Abdominal palpation: the left adrenal gland may be found, but abdominal fat may be a limiting factor; the right adrenal gland is more difficult to palpate because it is located more cranial and lays dorsal to the caudal vena cava and caudate lobe of the liver; the contralateral adrenal gland can remain unaffected in ferrets with adrenal associated endocrinopathy

Diagnosis

Clinical signs are the most useful tool in diagnosing adrenal disease.

Clinical pathology

- **Complete blood count and serum biochemical analyses** – the results are typically within reference ranges, but may help identify concurrent disease conditions. **Bone marrow suppression** with pancytopenia and non-regenerative anaemia due to oestrogen toxicity are rare in adrenocortical endocrinopathy-affected ferrets.
- **Confirmatory diagnosis – serum hormone assay panel.**
  An increase in the serum concentration of one or more of the sex hormones (17B-oestradiol, androstenedione and 17 alpha-hydroxyprogesterone) reportedly develops in 96% of ferrets affected with adrenocortical disease.

  The University of Tennessee offers the most comprehensive ferret adrenal panel currently available (https://vetmed.tennessee.edu/wp-content/uploads/sites/4/Endo-Submission-Guidelines-and-Test-Protocols.pdf).

  The hormones which are included in the adrenal panel are elevated in **both adrenocortical disease and functional ovarian remnants** and therefore the adrenal panel will not allow differentiation between these two diseases

  Unlike dogs with hyperadrenocorticism, a high **serum cortisol concentration** is uncommon in ferrets with adrenocortical disease.
- **Urinary cortisol:creatinine ratio** can be elevated in ferrets with adrenal disease. However, this test is unable to discriminate between a ferret with adrenal disease and a functional ovarian remnant.
- **Cytological evaluation of preputial epithelial cells**
  The diagnostic value of cytological examination of preputial epithelial cells obtained via a preputial washing has been assessed. Clinically normal ferrets had a significantly lower percentage of cornified preputial epithelial cells than those with clinical signs of adrenocortical disease. No significant correlation between the percentage of cornified preputial epithelial cells and **age** or **serum 17B-oestradiol concentration** was noted though. There was a mild, but not significant correlation between the percentage of cornified preputial epithelial cells and the **serum asdrostenedione concentration**. The percentage of cornified preputial epithelial cells was significantly correlated with **serum 17 hydroxyprogesterone concentration**. There was a significant association between the percentage of cornified preputial epithelial cells and the **existence versus lack of clinical signs of adrenocortical disease**. The lack of a significant correlation between the percentage of cornified preputial epithelial cells and **serum B17-oestradiol concentration** was unexpected because the mean 17B-oestradiol concentration was higher in ferrets with clinical signs of adrenocortical disease.

Positive and negative predictive values for a cut off value of >70% cornified preputial epithelial cells were 86% (the association between ferrets having >70% cornified preputial epithelial cells and a classification of endocrinologically affected with adrenocortical disease was significant using the serum endocrine assay as the gold standard).

Because the serum endocrine hormone assay is considered highly sensitive for confirming a diagnosis of adrenocortical disease, these data suggested that a cut-off of 70% of cornified preputial epithelial cells may be a good **screening test** for detecting adrenocortical disease in castrated ferrets, even when ferrets lack clinical signs of disease.

Advanced testing

Abdominal ultrasound

Ultrasound evaluation of the size, shape and structure of the adrenal glands is considered the gold standard. Adrenal glands may be classified as abnormal when any of the following are present: rounded appearance of the gland, increased size at the cranial or caudal pole, heterogeneous structure, increased echogenicity or signs of mineralisation. In females, potential remnant ovaries can also be located and in males, the prostate can be evaluated. Finally, concurrent disease such as malignant lymphoma and insulinoma may be found.

Histopathology

In males, the **prostate** can exhibit changes such as **cysts and paraprostatic cysts** lined by squamous epithelium and filled with keratin. Squamous metaplasia of the prostate glandular epithelium and cyst formation is due to chronic stimulation by oestrogens. Squames and cellular debris can trigger **prostatitis** (subacute to chronic-active or pyogranulomatous).

Differential diagnoses

Functional ovarian remnant (= persistent oestrus)

- Clinical signs may be more obvious during the ferret's breeding season when the ovary is active and is secreting sex steroid (northern hemisphere: March/April through August/September; southern hemisphere: from August/September through March/April).
- Neutered female ferrets will show signs of being in oestrus, depending on the time of year. The vulva will swell and the ferret's odour will increase owing to sex steroid production from the ovarian remnant.
- Associated conditions include oestrogen induced bone marrow suppression with consequent anaemia and thrombocytopenia if the ovarian remnant secretes excess oestradiol for a prolonged period. Recommended CBC to check for anaemia and/or thrombocytopenia if the ferret has been showing signs of oestrus for over 3 weeks. Blood biochemistry is usually unremarkable.
- Abdominal ultrasound, exploratory laparotomy and response to human chorionic gonadotropin (hCG) stimulation can aid in diagnosis.
- The ovarian remnant may undergo hyperplasia and/or neoplasia with time.
- Upon removal of remnant ovarian tissue, clinical signs should resolve within days.

Seasonal alopecia (some clinicians speculate that seasonal alopecia is a very early sign of adrenal disease)

Food intolerance/allergy

Stress (e.g. shelters)

Prognosis and Outcome

Adrenal tumours seldom metastatise, but adrenal **cortical carcinomas with myxoid differentiation** appear to be highly malignant and an **abundant smooth muscle component (spindle cell component),** a more malignant histological grade and **oestrogen receptor expression** are significantly and positively correlated to both decreased disease-free interval and decreased survival time.

Ferrets can be managed medically for many years and most ferrets with adrenal disease will die from unrelated conditions.

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