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# Alopecia Attributed to Neoplastic Ovarian Tissue in Two Ferrets

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**Ferrets with adrenal gland dysfunction have alopecia as their most common clinical sign of disease. Two cases of alopecia in neutered female ferrets are reported that were associated instead with neoplastic tissue found at the site of an ovarian pedicle. Androstenedione and 17-hydroxyprogesterone, but not estradiol, concentrations were high in both ferrets. Following surgical resection of the abnormal tissue in one ferret, the high hormone values decreased quickly and hair regrowth ensued. In both cases, histologic examination revealed features consistent with classical sex cord-stromal (gonadostromal) tumors: prominent spindle cells, along with polyhedral epithelial cells and cells with vacuolated cytoplasm. Although similiar cell types have been described in the adrenal glands of ferrets with adrenal-associated endocrinopathy, an ovarian origin for the current neoplasms is considered likely on the basis of their anatomic location; accessory adrenal tissue has only been described close to an adrenal gland or in the cranial perirenal fat of ferrets. Immunohistochemical analysis, using an antibody against Mullerian-inhibiting substance, failed to prove definitively the source of the steroidogenic cells.**

Alopecia is the most common clinical sign of adrenal gland disease in ferrets (*Mustela putorius furo*) (1, 2). Possible differential diagnoses for hair loss in ferrets include seasonal alopecia and ectoparasites. In general, some combination of abdominal palpation or ultrasonography of an enlarged adrenal gland, high concentrations of sex steroids, intraoperative confirmation of gross adrenal irregularity, and histologic examination of resected tissue is invoked to achieve the diagnosis of adrenal-associated endocrinopathy (AAE). We report two cases of alopecia in previously ovariohysterectomized ferrets that had sex steroid assay results compatible with AAE. However, the tumor in each ferret was located caudolateral to the caudal pole of the right kidney, at the site of the ovarian pedicle, and gross adrenal gland morphology was normal. Although ectopic adrenal tissue occurs in ferrets, in published literature it has been reported in the perirenal fat ventral or cranial to the level of the renal artery (3), or otherwise in close proximity to an adrenal gland (4). In an attempt to determine whether the steroidogenic neoplasms in these female ferrets originated from gonadal or adrenal cells, immunohistochemical analysis was performed using an antibody to Mullerian-inhibiting substance (MIS; also called anti-Mullerian hormone). Anti-MIS in humans has been determined to positively identify granulosa cell tumors while failing to stain adrenocortical tumors (5).

## Case Reports

**Ferret 1.** A $2^1/_2$-year-old female ferret was evaluated because of progressive alopecia over the preceding six months. Except for ovariohysterectomy and anal sacculectomy at six months of age, the clinical history was uneventful. The ferret lived in a home where there was also another neutered female ferret, with a normal haircoat. On physical examination, the affected animal was bright and alert. Its dorsum was covered with sparse hairs (Fig. 1), and a limited pruritus was suggested by dorsal scratch marks. The vulva appeared normal. Palpation of a small mass in the ferret's mid-abdomen was equivocal; unfortunately, ultrasonography was not available. Results of examination of skin scrapings for ectoparasites, and determination of glucose and creatinine values and a complete blood count, were unremarkable. Serum was submitted for the analysis of sex hormone concentrations. At the pre-operative physical examination several weeks later, an abdominal mass was palpated distal to the normal site for an adrenal gland. Intraoperatively, a $3 \times 2 \times 2$-cm cystic mass caudolateral to the caudal pole of the right kidney (Fig. 2) was the only lesion identified; it was excised. Both adrenal glands were of expected size and shape. Five days later, sex hormone levels were again measured. A thick coat grew back within two months (Fig. 3), and the ferret was clinically normal more than two years after surgery.

**Ferret 2.** A 5-year-old female ferret was euthanized and necropsied at the conclusion of a long-term research project that had been approved by the Committee on Animal Care at the Massachusetts Institute of Technology. This ferret had undergone ovariohysterectomy two years earlier, at three years of age, to terminate persistent estrus. Truncal alopecia had been noted in the ferret's record for the preceding several months without any further workup. Bilaterally symmetrical alopecia, a small vulva, and a $5 \times 3^1/_2 \times 3$-cm cystic mass located caudolateral to the right kidney were evident at necropsy. Steroid hormone levels were assessed.

## Materials and Methods

**Steroid hormone concentrations.** Serum samples from

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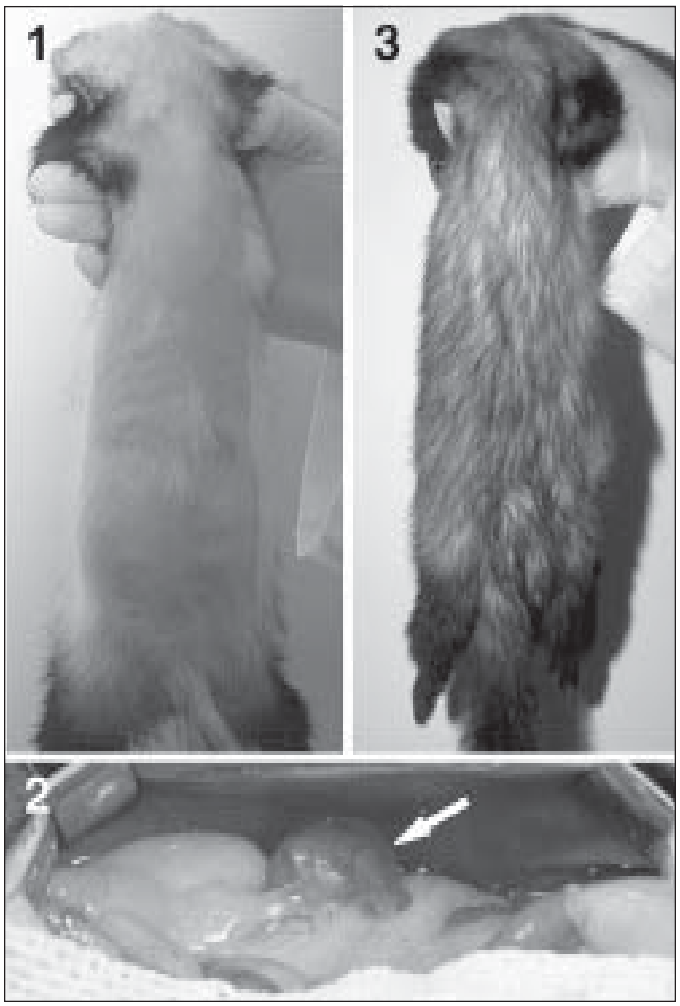


**Figure 1.** Photograph of a $2^{1}/_{2}$-year-old neutered female ferret (No. 1) evaluated because of progressive dorsal alopecia.
**Figure 2.** Intraoperative photograph of cystic mass (arrow) located caudolateral to the caudal pole of the right kidney in ferret 1.
**Figure 3.** Ferret 1 two months after surgery and removal of ovarian pedicle tumor.

ferrets 1 and 2 were sent to the University of Tennessee Endocrinology Service for measurement of estradiol, 17-hydroxyprogesterone, and androstenedione.

**Histologic examination.** Tissues were fixed in neutral-buffered 10% formalin and were processed in routine manner. Slides were stained with hematoxylin and eosin (H&E).

**Immunohistochemical examination.** The protocol for immunohistochemical examination using a polyclonal antibody raised in a rabbit to human MIS was performed as described (5). Rabbit anti-human CD3 (Dako Corporation, Carpinteria, Calif.) was used as a negative control for the tumors, although scattered T cells were labeled as expected. The anti-MIS and anti-CD3 were used at a 1:100 dilution. In addition to the two ovarian pedicle tumors, tissue sections representing normal adult ferret ovaries and adrenal glands, as well as two confirmed ferret adrenocortical tumors from our archives, were examined. One archival adrenocortical tumor came from a neutered female ferret that presented with enlarged vulva, and the other came from a neutered male ferret that had dorsal alopecia.

Each of the aforementioned tissue sections was also processed using mouse anti-human smooth muscle actin (Dako Corporation) as the primary antibody at a dilution of 1:100 for 60 min at 37°C. Antigen retrieval was not performed on these slides; otherwise the techniques for MIS labeling were followed.

**Table 1.** Sex steroid assay results for two neutered female ferrets with alopecia and tumors at ovarian pedicle sites

| | Estradiol (pmol/L) | 17-hydroxyprogesterone (nmol/L) | Androstenedione (nmol/L) |
|---|---|---|---|
| Ferret 1 | | | |
| Before surgery | 185 | 13 | 219 |
| After surgery[a] | 140 | 0.39 | 20.5 |
| Ferret 2 | 133 | 9.04 | 257 |
| Reference range for a neutered ferret[b] | 30-180 | 0-0.8 | 0-15 |

[a]Performed five days after surgery.
[b]According to the Clinical Endocrinology Service, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, Tennessee 37996. Measurements can be performed on serum or plasma, with similar results.

## Results

**Sex steroid concentrations.** In ferret 1 prior to treatment, the results of a steroid hormone panel (Table 1) were consistent with a diagnosis of adrenal gland disease. Compared with reference values for neutered ferrets as provided by the laboratory, 17-hydroxyprogesterone and androstenedione values were increased, whereas the estradiol value was essentially normal. Shortly after surgery, the previously high steroids, 17-hydroxyprogesterone and androstenedione, had decreased, even though the latter was slightly above the upper reference value limit (Table 1). The hormone values for ferret 2 (Table 1) were similar to those for ferret 1 at presentation; 17-hydroxyprogesterone and androstenedione, but not estradiol, values were high.

**Histopathologic changes.** Microscopically, the excised tissue from ferret 1 consisted of an expansile, non-invasive, encapsulated circular mass that compressed surrounding mesenteric adipose tissue. The mass was composed predominately of irregular bundles and whorls of long spindle cells that surrounded nests of polyhedral epithelial cells (Fig. 4 and 5). The spindle cells had indistinct cell borders, alternating pale and dark pink wispy cytoplasm, and were variably lined externally by a thin band of fibrillar collagen. Nuclei were oval, central, lightly stippled, and sometimes contained a prominent magenta nucleolus. Mitotic figures were rare (1/10 high-power fields [HPF]). Epithelial cells were dimorphic. Most were round to polyhedral, had indistinct cell margins, and contained a small amount of lightly eosinophilic-to-amphophilic cytoplasm. These cells had an eccentric lightly or coarsely stippled nucleus and a single deeply basophilic nucleolus. Other epithelial cells were swollen, round to oval, and had distinct cell margins surrounding cytoplasm expanded by small, pale, fairly well delineated vacuoles. Nuclei were pale, lightly granular, and rarely contained visible nucleoli. In both populations of epithelial cells, mitotic figures were rare (1/10 HPF). The mass contained little interstitial tissue, which was composed principally of thin bands of collagen. Blood vessels were few in number and located mainly near the periphery. On the basis of microscopic features and anatomic location, the mass was diagnosed as a benign sex cord-stromal (gonadostromal) or granulosa-theca cell tumor.

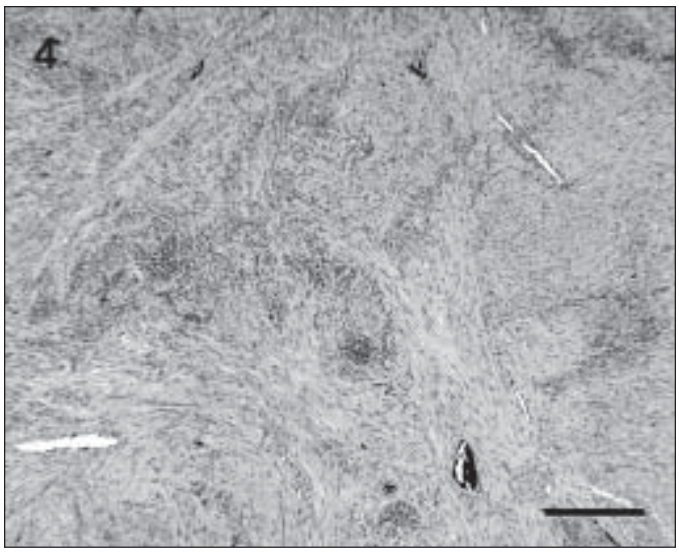


**Figure 4.** Photomicrograph of a section of the ovarian pedicle tumor from ferret 1. Notice islands and nests of round epithelioid cells separated by broad bundles and streams of fusiform cells. H&E stain; bar = 250 µm.

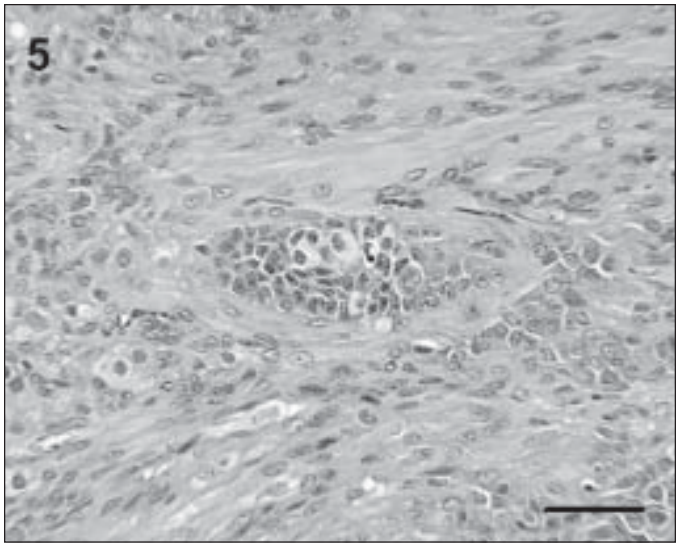


**Figure 5.** Higher magnification of the section of ovarian pedicle tumor from ferret 1. Notice island of mixed compact and plump vacuolated epithelioid cells surrounded by whorling fusiform cells, admixed with scattered and loosely aggregated epithelioid cells. H&E stain; bar = 60 µm.

Microscopic features of the mass from ferret 2 were similar to those discussed for ferret 1, except that the mass from ferret 2 was larger, incompletely circumscribed, more richly vascular, and exhibited small multifocal areas of hemorrhage and necrosis. The spindle cells were generally plumper than those in the mass from ferret 1, and they often contained one or more round-to-oval clear vacuoles. Epithelial nests contained more cells than did those of the mass from ferret 1, and were composed almost exclusively of the smaller non-vacuolated phenotype. Some nests had dropped-out centers surrounded by imperfectly palisading cells, reminiscent of Call-Exner bodies. Compared with the mass from ferret 1, mitotic figures in spindle and round cell populations were slightly more numerous (approx. 1/5 HPF); also apoptotic cells were more frequent. The second mass was diagnosed as a sex cord-stromal tumor of low-grade malignant potential.

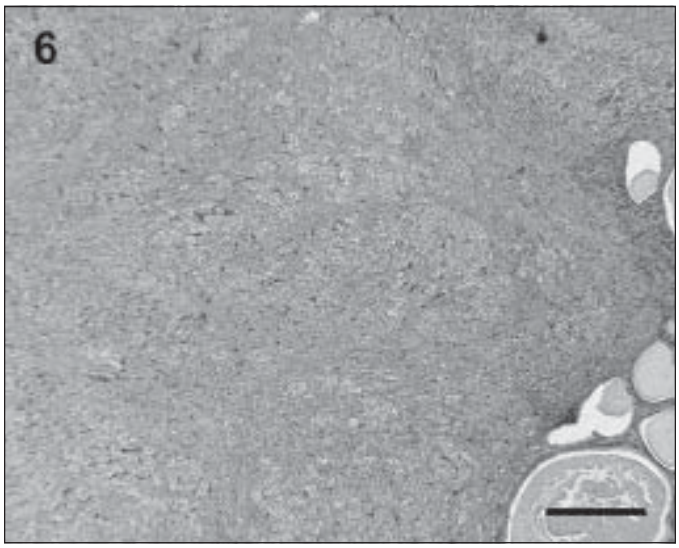


**Figure 6.** Photomicrograph of a section of an adrenal tumor from a neutered female ferret that presented with an enlarged vulva. Notice larger bundles and lobules of plump vacuolated epithelioid cells septated by relatively thin fibrovascular bands. H&E stain; bar = 250 µm.

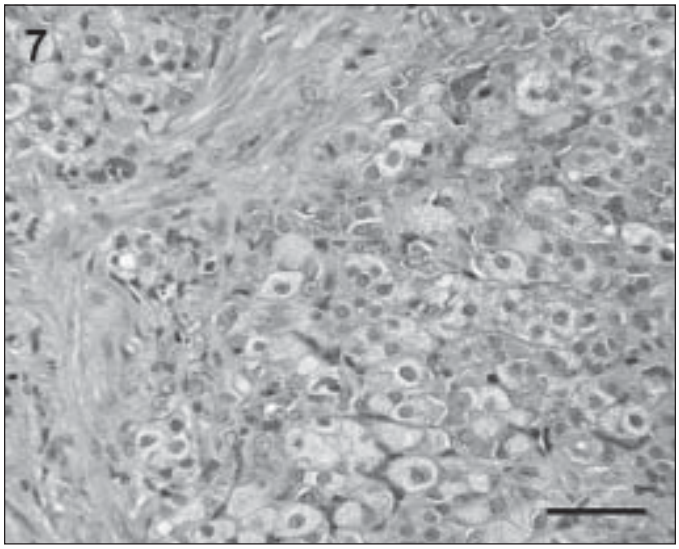


**Figure 7.** Higher magnification of the section of tumor in Fig. 6. Notice relatively uniform size and shape of microvacuolated and granular epithelioid cells, bordered by fibrocollageneous bands. H&E stain; bar = 60 µm.

Both adrenal glands from ferret 2 had very mild, focal hyperplasia. Microscopic lesions in the skin from the ferret included epidermal and follicular atrophy, sebaceous gland atrophy, mild orthokeratotic hyperkeratosis, and increased number of telogen hair follicles, all features consistent with steroid endocrinopathy.

For the purpose of comparison, intra-adrenal tumors from two other ferrets were reviewed. In both of them, expansile masses effaced most of the adrenal cortex, were well circumscribed, and were lobulated by thin to moderately broad fibrovascular and spindle-cell septae. The principal cell type was composed of round foamy-to-vacuolated cells with lightly eosinophilic cytoplasm and open nuclei, with rare mitoses (Fig. 6 and 7).

**Immunohistochemical analysis.** A biphasic pattern of immunoreactivity was observed by use of anti-MIS in ovarian and adrenal tissues from normal ferrets. In the normal adrenal gland,

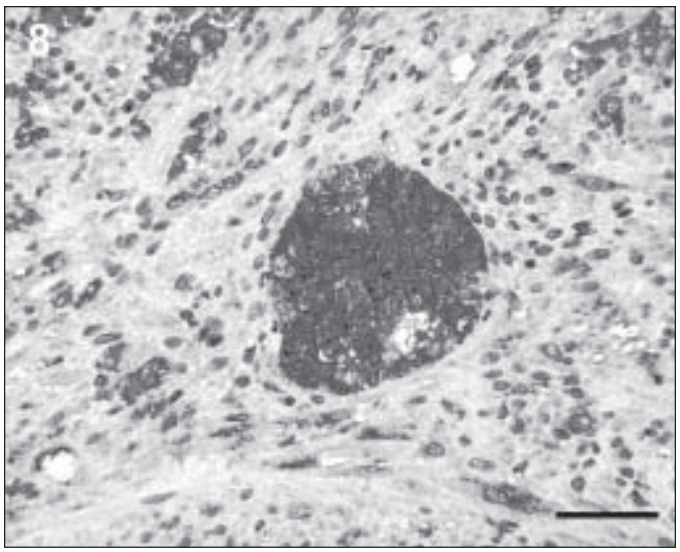

**Figure 8.** Photomicrograph of a section of the ovarian pedicle tumor from ferret 1. Immunohistochemical analysis demonstrates dense diffuse cytoplasmic labeling of epithelioid cells, and granular nuclear and perinuclear staining of fusiform cells with polyclonal antibody raised against Mullerian-inhibiting substance (MIS). Labeled streptavidin-peroxidase method, diaminobenzidine (DAB), Gill's hematoxylin counterstain. Bar = 60 µm.

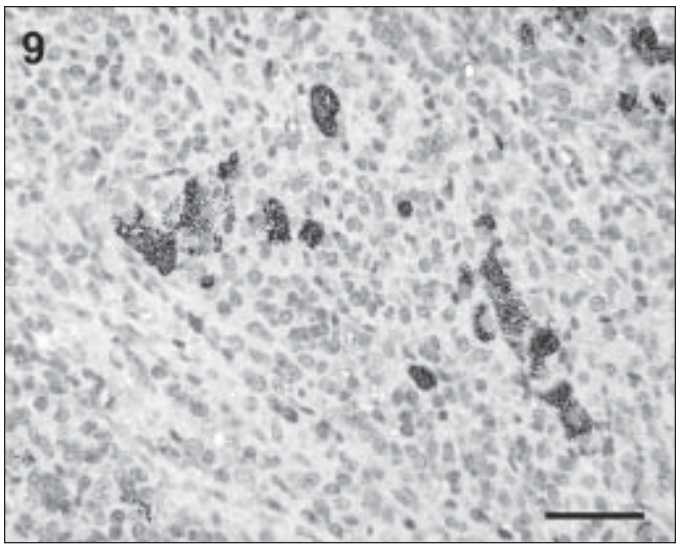

**Figure 9.** Immunohistochemical analysis of the adrenal tumor of Fig. 6 demonstrates cytoplasmic perivacuolar staining only of mature epithelioid cells with antibody against MIS. Labeled streptavidin-peroxidase method, DAB, Gill's hematoxylin counterstain. Bar = 60 µm.

most cortical cells were only faintly positive to anti-MIS; yet a subset of plump epithelial cells with more distinct vacuolation in the zona glomerulosa and outer fasciculata were labeled intensely. Likewise immunostaining of granulosa cells in the normal ferret ovary was positively correlated with degree of cytoplasmic vacuolation; the same pattern of labeling applied to normal ovarian spindle cells in the thecal cell layer of the follicles.

In the ovarian pedicle tumors from ferret 1 and 2 and the adrenal tumors, MIS labeling was also associated overall with degree of cellular vacuolation. However in the ovarian pedicle tumors, many thin spindle cells exhibited perinuclear and intranuclear labeling regardless of vacuolation state, and the epithelial nests in the tissue from ferret 1 were uniformly positive, even though most of them had little cytoplasmic vacuolation (Fig. 8). In contrast, immunoreactivity in the adrenocortical tumors was limited to well differentiated vacuolated epithelial cells (Fig. 9), and the spindle cells were not labeled.

When treated with anti-smooth muscle actin, spindle cells in the ovarian pedicle tumors from ferrets 1 and 2 were immunopositive, suggesting either a smooth muscular or thecal cell origin for the cells (not shown). The normal adrenal and ovarian tissues from ferrets were labeled appropriately for smooth muscle (e.g., muscular arteries) and thecal cells, respectively.

## Discussion

Sex steroid-producing tumors in two previously ovariohysterectomized ferrets are described that arose at the anatomic site of the ovarian pedicle, a location associated with ovarian remnants in other companion animals (6). Microscopic examination of the tumors revealed interweaving bundles of spindle cells surrounding nests of round, variably vacuolated, epithelial cells. Other authors have reported abundant spindle cells in ferret ovarian tumors and categorized them as leiomyomas (7-9) or stromal tumors (10). Additional ovarian tumors reported in reproductively intact female ferrets include granulosa cell tumor, luteoma, and thecoma (9). Clinical signs of disease, if any, were not provided for the aforementioned ovarian tumors. Tumors in neutered female ferrets that have been attributed to ovarian remnants include granulosa cell tumor, leiomyoma, and fibrosarcoma (11). In a retrospective study concerning 94 cases of hyperadrenocorticism in ferrets, retained ovarian tissue associated with alopecia, swollen vulva, or both were noted as rare (12), but the histologic appearance of the retained tissue was not discussed.

Anatomic location and histologic features compatible with ovarian tissue notwithstanding, a gonadal origin for the ferret tumors of this report is not certain. A well recognized and frequently diagnosed phenomenon in the species is adrenal gland disease causing AAE, of which alopecia and, in females, enlarged vulva are clinical hallmarks. Although accessory adrenal tissue in ferrets has been described only in the periadrenal and cranial perirenal area, the tumors of these ferrets could be composed of adrenocortical cells at an ectopic site. The chief component in the differential diagnosis for the two cases presented here was mixed tumor of ectopic adrenal origin. It is interesting, however, that a literature search did not reveal any cases where ectopic adrenal tissue in a ferret became neoplastic. Nevertheless, alopecia and sex hormone concentration increases, as observed in the two cases of this report, are typical of adrenal gland tumors in ferrets. Cell types in adrenal glands include epithelial cells with abundant, vacuolated cytoplasm, whereas spindle cells are found in blood vessel walls, and in the adrenal capsule and trabeculae. These latter cells have been termed "ovarian thecal metaplasia" in the adrenal glands of postmenopausal women because they invade the adrenal cortex and resemble ovarian cortical stroma (13). In a study in which adrenocortical tumors in ferrets were evaluated, 12 cases had a variable but sometimes predominant spindle cell component (14). The spindle cells were determined potentially to be smooth muscle cells by use of light and electron microscopy, as well as immunohistochemical staining for smooth muscle (alpha) actin. There are, however, reports of alpha actin in ovarian cells, including theca cells of several animal species and humans (15-17).

It has been hypothesized that ferret adrenal tumors frequently produce sex steroids instead of cortisol because "ectopic rests" of embryonic gonadal cells, which develop alongside adrenal cells at the urogenital ridge, are more likely to migrate with the adrenal anlage in this species (1, 18). An alternative explanation is that multipotent stem cells can migrate with both gonadal and adrenal primordia. The stem cells may then differentiate into the sex cord-stromal cell phenotype depending on the local chemical milieu.

Immunohistochemical analysis, using a polyclonal antibody against MIS, was done on the two ferret tumors and various controls to determine whether the neoplasms were derived from gonadal or adrenal cells. This antibody has distinguished between adrenal and ovarian tissue in human paraffin-embedded tissue sections (5). In our hands, however, the polyclonal antibody not only labeled ferret granulosa and thecal cells, but also normal adrenal cortical cells in the ferret. These differences may reflect a broader cross-reactivity of steroids and/or other molecules in ferret epithelial and endocrine cells with the polyclonal antibodies generated by human MIS immunization in the rabbit. It is also possible that ferrets produce MIS-like substances in a broader array of tissues and cell types than do humans.

In conclusion, two cases of sex cord-stromal tumors are evaluated that arose from the anatomic site of ovarian pedicles in neutered female ferrets with alopecia. The adrenal glands in both animals were free of enlargement or masses. Even though immunohistochemical results were inconclusive for distinguishing these tumors from adrenal tissue, their anatomic location suggests that ovarian pedicle tumors should be included in the differential diagnoses for neutered female ferrets with hair loss. A sex steroid panel will not differentiate an ovarian pedicle tumor from an adrenal gland tumor. Surgical exploration, excision, and histologic examination are necessary to achieve a definitive diagnosis. Similar cases might be more common in countries other than the United States, where ferrets are routinely neutered at six months of age or later like the ferrets of this report (19), but the prevalence of ovarian pedicle tumors in ferrets is unknown at present. Further studies are needed to determine whether long-term clinical outcome is different following surgical excision of adrenocortical versus ovarian pedicle tumors in ferrets.

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