

# Assessment of cytologic evaluation of preputial epithelial cells as a diagnostic test for detection of adrenocortical disease in castrated ferrets

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**Objective**—To determine whether results of cytologic evaluation of preputial epithelial cells correspond to results of a serum endocrine hormone assay and clinical signs associated with adrenocortical disease in castrated ferrets.

**Animals**—13 clinically normal ferrets and 8 ferrets with signs of adrenocortical disease.

**Procedures**—Blood and preputial lavage samples were collected from each ferret. Serum samples were submitted to the University of Tennessee Veterinary Diagnostic Laboratory for performance of an endocrine hormone assay. Differential epithelial cell counts were performed on preputial lavage samples to determine the percentage of cornified cells. Results of cytologic evaluation were compared with results of the endocrine hormone assay and clinical status of ferrets.

**Results**—The percentage of cornified preputial epithelial cells was not significantly correlated with serum 17 $\beta$ -estradiol or androstenedione concentration but was significantly correlated with serum 17-hydroxyprogesterone concentration ( $r = 0.60$ ). The percentage of cornified preputial epithelial cells was higher in ferrets with clinical signs of adrenocortical disease (mean  $\pm$  SD, 71.3  $\pm$  16.9%) than in clinically normal ferrets (55.5  $\pm$  19.0%).

**Conclusions and Clinical Relevance**—Cornification of preputial epithelial cells was correlated with an increase in serum 17-hydroxyprogesterone concentration as well as clinical signs of adrenocortical disease in castrated ferrets. Additional investigation is needed to elucidate the mechanism of preputial epithelial cell cornification in castrated ferrets. (*Am J Vet Res* 2009;70:619–623)

Adrenocortical disease is one of the most common diseases in ferrets (*Mustela putorius furo*),<sup>1</sup> with a prevalence reportedly as high as 25% in the United States.<sup>2</sup> The pathophysiologic mechanisms of adrenal gland disease have not been definitively established, although it is believed that early age gonadectomy of ferrets results in an increase in stimulation of the adrenal glands by luteinizing hormone because of a lack of negative feedback from hormones usually released by the gonads. Constant stimulation of the adrenal glands by luteinizing hormone results in adrenocortical hyperplasia or development of adrenal neoplasia.<sup>3</sup> Early age at time of gonadectomy has been associated with adrenocortical neoplasia in several other animal species, including mice, rats, hamsters, and guinea pigs.<sup>4</sup> Most commercially raised ferrets in the

ABBREVIATION	
17-HPG	17-hydroxyprogesterone

United States undergo gonadectomy at approximately 6 weeks of age.<sup>4</sup> Other reasons suggested for the high prevalence of adrenocortical disease in ferrets include the artificially prolonged photoperiod that ferrets are exposed to when housed indoors as well as a possible genetic predisposition.<sup>4</sup>

The most common clinical signs of adrenocortical disease in male ferrets include bilaterally symmetric alopecia, return to male sexual behavior, epidermal thinning, cachexia, potbellied appearance, and dysuria.<sup>5,6</sup> Some affected ferrets may have these signs, or the signs may be subtle and go unnoticed by the owner until the disease is more advanced.<sup>2</sup> Abdominal palpation may be useful for confirming a suspicion of disease when the affected adrenal gland is large.<sup>7</sup> Complete blood counts and serum biochemical analyses are unreliable diagnostic tools because results are typically within reference ranges in ferrets with adrenocortical disease; however, these tests may help identify concurrent disease conditions.<sup>8,9</sup>

In the United States, a diagnosis of adrenocortical disease in ferrets is supported by results of transabdominal ultrasonographic evaluation of the adrenal glands or an endocrine hormone assay (ferret adrenal

Received May 15, 2008.

Accepted August 6, 2008.

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Supported by the Morris Animal Foundation.

Presented in poster form at the Morris Animal Foundation Annual Meeting, Denver, June 2008, and at the International Symposium for Canine and Feline Reproduction, Vienna, July 2008.

The authors thank Dr. Elena Gorman for assistance with cytologic classification and Dr. Aurora Villarroel for assistance with statistical analyses.

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hormone panel) performed only at the University of Tennessee Veterinary Diagnostic Laboratory. Ultrasonographic evaluation is not always an option for veterinary practitioners, and accurate diagnosis relies heavily on the skill of the clinician. Furthermore, ultrasonography is not a reliable way of detecting adrenal hyperplasia in ferrets lacking clinical signs of adrenal disease.<sup>8</sup> Dissimilar from the situation in dogs with hyperadrenocorticism, a high serum cortisol concentration is uncommon in ferrets with adrenocortical disease.<sup>10,11</sup> The aforementioned endocrine hormone assay is used to measure serum concentrations of 17 $\beta$ -estradiol, androstenedione, and 17-HPG.<sup>10</sup> An increase in the serum concentration of one or more of these hormones reportedly develops in 96% of ferrets affected with adrenocortical disease.<sup>10</sup> However, this endocrine hormone assay is expensive for clients, and delivery of results may be delayed for 1 week or longer.

Cytologic characteristics of exfoliative vaginal epithelial cells have been evaluated in ferrets. In 76% of estrual female ferrets, cytologic characteristics indicative of estrus ( $\geq 90\%$  cornified epithelial cells) result as a response to a high serum 17 $\beta$ -estradiol concentration.<sup>12</sup> Similarly in dogs, vaginal and preputial epithelia respond to a high serum 17 $\beta$ -estradiol concentration by undergoing hyperplasia and cornification,<sup>13</sup> such as takes place in dogs with Sertoli cell tumors.<sup>13</sup> Serum 17 $\beta$ -estradiol concentration is reportedly high in 36%<sup>10</sup> to 88%<sup>14</sup> of ferrets with adrenal tumors. It is unknown whether preputial epithelium in ferrets responds to a high serum 17 $\beta$ -estradiol concentration in a manner similar to preputial or vaginal epithelium in dogs and vaginal epithelium in ferrets. Hyperestrogenism in female ferrets with adrenocortical disease results in vulvar enlargement, which may explain the finding that more than twice as many ferrets treated for adrenocortical disease are female rather than male,<sup>10</sup> even though there is no apparent sex predisposition for the condition.<sup>1,15</sup>

The purpose of the study reported here was to develop a method for collecting preputial epithelial cells from ferrets and to evaluate differences in percentages of cornified preputial epithelial cells between clinically normal castrated males and those with clinical evidence of adrenocortical disease. Specifically, we sought to determine whether results of in-house cytologic evaluation of preputial epithelial cells would correspond to results of a serum endocrine hormone assay and clinical signs associated with adrenocortical disease in castrated ferrets.

## Materials and Methods

**Animals**—Castrated ferrets were obtained from local shelters. A physical examination was performed to determine whether each ferret had one or more of the following clinical signs of adrenocortical disease: bilaterally symmetric alopecia, return to male sexual behavior, epidermal thinning, cachexia, and potbellied appearance. Ferrets that met these criteria were considered to have adrenocortical disease and classified as clinically affected; the remainder were classified as clinically normal.

**Blood sample collection**—All samples were collected within the natural ferret breeding season (March

through August in the Northern Hemisphere), when reproductive hormone concentrations are reportedly higher than at other times of the year.<sup>16</sup> Ferrets were manually restrained ( $n = 15$ ) or anesthetized with sevoflurane (7), depending upon temperament of the ferret and availability of anesthetic equipment. A jugular venous blood sample (2 mL) was collected from each ferret by use of a 22-gauge, 2.5-cm needle and a 3-mL syringe. Samples were allowed to clot, and harvested serum was submitted to the University of Tennessee Veterinary Diagnostic Laboratory for determination of serum concentrations of 17 $\beta$ -estradiol, androstenedione, and 17-HPG by means of radioimmunoassay. Respective laboratory reference ranges for healthy castrated ferrets are 30 to 180 pmol/L,  $< 0.1$  to 15 nmol/L, and  $< 0.1$  to 0.8 nmol/L.<sup>10</sup>

**Preputial epithelial cells sample collection**—For collection of preputial epithelial cells, each ferret was positioned in dorsal recumbency by use of minimal restraint. The ferret's attention was redirected during the procedure by feeding of a highly palatable nutritional supplement.<sup>a</sup> The external preputial opening was gently cleansed prior to sample collection. Preputial lavage was accomplished with 20  $\mu$ L of sterile physiologic saline (0.9% NaCl) solution, which was introduced into the external preputial opening with a flame-smoothed pipette tip and subsequently aspirated several times. The preputial lavage fluid was dispensed onto a glass microscope slide, allowed to dry, and then stained with Romanowski stain.<sup>b</sup> Slides were examined by use of light microscopy at 400 $\times$  magnification, and differential cytologic counts were performed to determine the percentage of cornified cells within the sample. One author (HJP) performed all differential cell counts without knowledge of ferret identity. Superficial epithelial and anuclear squamous cells were included in the counts.

**Statistical analysis**—Comparisons of mean age, percentage of cornified epithelial cells, and serum 17 $\beta$ -estradiol, and 17-HPG concentrations were made between clinically normal and clinically affected ferrets by use of the 2-tailed Student *t* test. Median androstenedione concentrations were compared by means of the Mann-Whitney *U* test. Relationships of serum hormone concentrations and ferret age with percentage of cornified preputial epithelial cells were evaluated by calculating the Pearson correlation coefficient. The association between percentage of cornified preputial epithelial cells and clinical signs of adrenocortical disease was determined by use of univariate and bivariate regression analyses. The predictive value of the percentage of preputial epithelial cell cornification as a diagnostic test for adrenocortical disease in ferrets was determined with a Fisher exact test, with ferrets grouped according to clinical presentation (clinically normal or clinically affected). Ferrets were reclassified on the basis of results of the endocrine hormone assay (endocrinologically normal or endocrinologically affected), and a similar comparison was made to determine the predictive value of the degree of preputial epithelial cell cornification as a diagnostic test for adrenocortical disease. Data are reported as mean  $\pm$  SD. A value of  $P < 0.05$  was considered significant.

## Results

**Animals**—Twenty-seven castrated male ferrets from local animal shelters were initially included in the study. Of these, 17 were clinically normal. The remaining 10 ferrets comprised the clinically affected group, which consisted of 1 ferret with an ultrasonographically confirmed adrenal mass, 1 with a histologically confirmed metastatic adrenal adenocarcinoma, and 4 others that had undergone unilateral adrenalectomy and developed clinical signs of adrenal disease again. None of the ferrets had received medical treatment for adrenocortical disease with the exception of one that received a melatonin implant administered SC 1 day prior to sample collection. Five ferrets from which samples were collected were excluded from the study because they were neutered at a significantly later age (1 to 5 years old) than the other ferrets in the study (neutered at approx 6 weeks old). One of these excluded ferrets was considered clinically affected, and the other 4 were considered clinically normal. In addition, 1 clinically affected ferret (neutered at approx 6 weeks) was excluded from the study because of an unusually high serum 17-HPG concentration (79.4 nmol/L) and was considered a statistical outlier. Ferrets in the clinically affected group (mean  $\pm$  SD age, 55  $\pm$  21 months; n = 8) were significantly older than those in the clinically normal group (32  $\pm$  23 months; 13).

**Characteristics of preputial epithelial cells**—Epithelial cells collected via preputial lavage were morphologically similar to vaginal epithelial cells in female

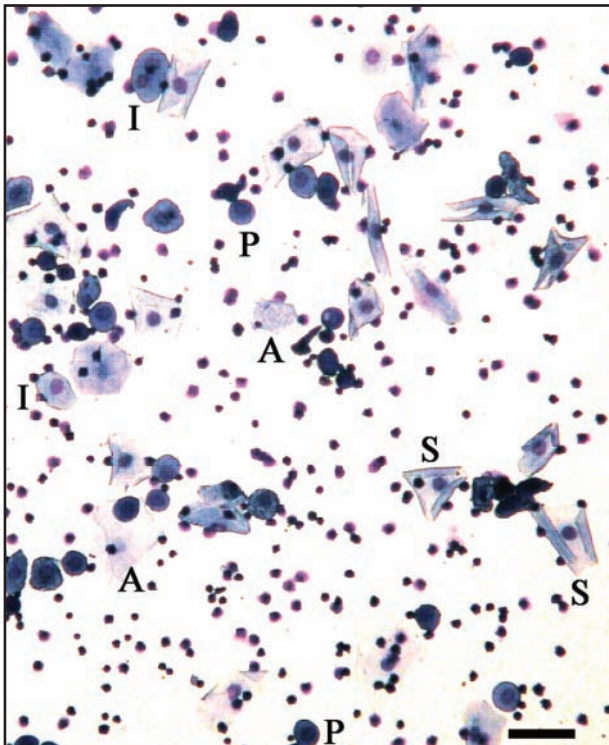


Figure 1—Representative photomicrograph of results of cytologic evaluation of preputial epithelial cells obtained from a castrated ferret (*Mustela putorius furo*) via preputial lavage. Parabasal (P), intermediate (I), superficial (S), and anuclear squamous (A) cells are evident. Romanowski stain; bar = 40  $\mu$ m.

ferrets.<sup>17</sup> Parabasal cells, intermediate cells, superficial cells, and anuclear squamous cells were detected in all samples, although the number of each cell type varied among samples (Figure 1). Parabasal and intermediate cells were deeply basophilic, whereas superficial cells were lighter stained, contained evidence of degenerative nuclear changes (pyknosis or karyorrhexis) with angled cytoplasmic margins, and had a larger cytoplasm-to-nucleus ratio. Anuclear squamous cells were larger, had angled cytoplasmic margins, and lacked nuclei. Clinically normal ferrets had a significantly lower percentage of cornified preputial epithelial cells than those with clinical signs of adrenocortical disease (Table 1).

**Serum endocrine hormone assay**—Ferrets in the clinically normal group had significantly lower serum concentrations of 17 $\beta$ -estradiol, androstenedione, and 17-HPG than those with clinical signs of adrenocortical disease (Table 1). No ferret had a serum 17 $\beta$ -estradiol concentration that exceeded the reference range for healthy castrated ferrets.

In 1 of 8 ferrets with clinical signs of adrenocortical disease (alopecia and potbellied appearance), serum concentrations of 17 $\beta$ -estradiol, androstenedione, and 17-HPG were within respective reference ranges. Approximately 68% of this clinically affected ferret's preputial epithelial cells were cornified. One of the 13 clinically normal ferrets had a serum 17-HPG concentration that exceeded the reference range (0.82 nmol/L). The percentage of cornified preputial epithelial cells in this clinically normal ferret was high (76%), which is characteristic of ferrets with clinical signs of adrenocortical disease. In 5 ferrets (2 clinically affected and 3 clinically normal), preputial epithelial cell samples for cytologic evaluation were collected a second time 40 days after the initial collection to assess repeatability of results. Results of a paired Student *t* test indicated there was no significant ( $P = 0.66$ ) difference in the percentage of cornified epithelial cells between the 2 collection points.

**Associations among variables**—Results of Pearson correlation analyses indicated there were no significant correlations between the percentage of cornified preputial epithelial cells and age or serum 17 $\beta$ -estradiol concentration in the ferrets. Percentage of cornified preputial epithelial cells and serum androstenedione concentration appeared to be mildly correlated ( $r = 0.38$ ); however, the correlation was not significant ( $P = 0.09$ ). On the other hand, the percentage of cornified

Table 1—Mean  $\pm$  SD percentages of cornified preputial cells and serum concentrations of endocrine hormones in castrated ferrets (*Mustela putorius furo*) classified as clinically normal (n = 13) and clinically affected with adrenocortical disease (8).

Variable	Clinically normal	Clinically affected
Cornified epithelial cells (%)	55.5 $\pm$ 19.0	71.3 $\pm$ 16.9
17 $\beta$ -estradiol (pmol/L)	96.6 $\pm$ 29.9	133.1 $\pm$ 29.1
17-HPG (nmol/L)	0.41 $\pm$ 0.25	4.3 $\pm$ 4.6
Androstenedione (nmol/L)	7.2 $\pm$ 2.8	95.6 $\pm$ 100.9

Values for every variable are significantly ( $P < 0.05$ ) different between groups.



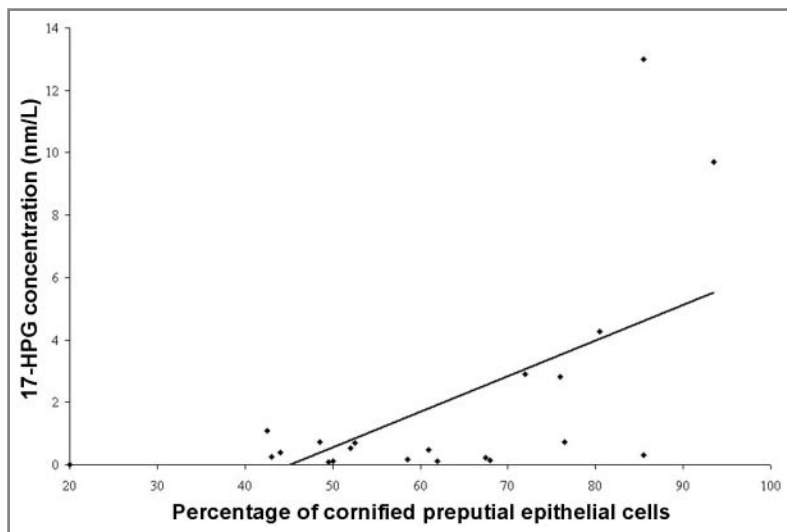


Figure 2—Graph of the correlation between serum 17-HPG concentration and percentage of cornified preputial epithelial cells in 21 castrated ferrets. The correlation was moderate ( $r = 0.60$ ) and significant ( $P = 0.004$ ).

preputial epithelial cells was significantly ( $P = 0.004$ ) correlated with serum 17-HPG concentration ( $r = 0.60$ ; Figure 2). Results of univariate regression analysis indicated there was a significant ( $P = 0.004$ ) association between percentage of cornified preputial epithelial cells and existence (vs lack) of clinical signs of adrenocortical disease. Similar results were obtained when bivariate analysis was used to control for the effect of age on disease status ( $P = 0.04$ ).

**Predictions of adrenocortical disease**—A Fisher exact test was used to determine whether a cutoff value of  $> 70\%$  cornified preputial epithelial cells was a reliable predictor of ferrets being clinically affected with adrenocortical disease (vs clinically normal). This cutoff was selected on the basis of the mean percentage of cornified preputial epithelial cells in the group of clinically affected ferrets. The positive predictive value of the  $> 70\%$  cutoff value was 71%, and the negative predictive value was 79%.

When ferrets were reclassified as endocrinologically normal or endocrinologically affected on the basis of results of the serum endocrine hormone assay, only 1 clinically normal ferret had  $> 70\%$  cornified preputial epithelial cells and 2 clinically affected ferrets had  $< 70\%$  cornified preputial epithelial cells. Therefore, the positive and negative predictive values calculated by use of the  $> 70\%$  cutoff became 86%. The association between ferrets having  $> 70\%$  cornified preputial epithelial cells and a classification of endocrinologically affected with adrenocortical disease was significant ( $P = 0.003$ ).

## Discussion

In the study reported here, we performed differential cytologic evaluation of preputial epithelial cells in clinically normal castrated ferrets and castrated ferrets with signs of adrenocortical disease. Percentages of cornified epithelial cells in preputial samples were highly variable in both groups of ferrets. Ferrets with signs of

adrenocortical disease had a significantly higher percentage of cornified preputial epithelial cells than clinically normal ferrets. However, clinically affected ferrets were also significantly older than clinically normal ferrets. Additional investigation with age-matched controls may help clarify the association between percentage of preputial epithelial cornification and disease status by eliminating any potential confounding effect of age. However, there was no significant correlation between age and percentage of cornified preputial epithelial cells, so it is unlikely that aging alone (without the existence of adrenocortical disease) is responsible for the increase in cornified preputial epithelial cells.

Isoflurane anesthesia may cause an increase in plasma concentration of  $\alpha$ -melanocyte-stimulating hormone; however, manual restraint and isoflurane anesthesia reportedly do not alter concentrations of adrenal-associated reproductive steroid hormones.<sup>18</sup> In the study reported here, ferrets were manually restrained or anesthetized with sevoflurane, depending on temperament of the ferret and availability of anesthetic equipment. Our results were similar to those of another study<sup>3</sup> in that serum endocrine hormone concentrations were not significantly different between these restraint methods (data not shown).

In 1 of 8 ferrets with clinical signs of adrenocortical disease (alopecia and potbellied appearance), serum concentrations of  $17\beta$ -estradiol, androstenedione, and 17-HPG were all within respective reference ranges for clinically normal castrated ferrets. Given that 96% of ferrets affected with adrenocortical disease may have high values for one or more of these hormones,<sup>10</sup> other differential diagnoses that might explain the clinical signs include stress, ectoparasites, seasonal alopecia, abdominal disease (eg, hemoabdomen, ascites, or neoplasia), or exogenous sex hormone administration. No evidence of ectoparasites or overt abdominal disease existed in the aforementioned ferret, and exogenous sex hormones were not being administered. However, the shelter environment can be stressful on ferrets, and ferrets can develop seasonal alopecia, with males more commonly affected than females,<sup>7</sup> so it is possible this ferret was affected by a condition other than adrenocortical disease. Because the ferrets used in the present study were shelter-owned and available for adoption during the study period, no follow-up testing was possible.

The lack of a significant correlation between percentage of cornified preputial epithelial cells and serum  $17\beta$ -estradiol concentration was unexpected because the mean  $17\beta$ -estradiol concentration was higher in ferrets with clinical signs of adrenocortical disease. This lack of a correlation may be attributable to the fact that none of the ferrets in the present study had a serum  $17\beta$ -estradiol concentration that exceeded the reference range. Another unexpected finding was the significant, positive correlation between serum 17-HPG concentrations and percent-

age of cornified preputial epithelial cells. These findings suggest that a different mechanism for cornification of the preputial epithelium might exist in male castrated ferrets, compared with that in dogs,<sup>13</sup> and additional research is needed to clarify this mechanism.

Seven of 8 ferrets with clinical signs of adrenocortical disease had a serum 17-HPG concentration that exceeded the reference range. In contrast, only 1 of 13 clinically normal ferrets had a high 17-HPG concentration. It is interesting that this 1 clinically normal ferret was 1 of 2 clinically normal ferrets that had > 70% cornified preputial epithelial cells. This finding likely indicated that the ferret had adrenocortical disease but had not yet developed any clinical signs and provided evidence that cytologic evaluation of preputial epithelial cells may be useful as a diagnostic test for adrenocortical disease. Because blood and preputial epithelial cell samples were collected during the season when reproductive hormone concentrations were at their peak, additional investigation during other seasons is required to more completely evaluate seasonal influences on cytologic characteristics of preputial epithelial cells in castrated ferrets.

When ferrets were reclassified as affected with adrenocortical disease on the basis of results of the endocrine hormone assay, the positive and negative predictive values were both 86%. Because the serum endocrine hormone assay is considered highly sensitive for confirming a diagnosis of adrenocortical disease,<sup>10</sup> these data suggested that a cutoff of > 70% for percentage 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.

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- a. FerreTone, Eight-in-One Pet Products, Hauppauge, Pa.  
 b. Diff-Quik, Siemens Healthcare Diagnostics, Deerfield, Ill.
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