

## Chapter 5

### **Urinary Corticoid/Creatinine Ratios in Healthy Ferrets and Ferrets with Hyperadrenocorticism**

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Submitted

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## Summary

Hyperadrenocorticism in ferrets is usually associated with unaltered plasma concentrations of cortisol and ACTH, although the urinary corticoid/creatinine ratio (UCCR) is usually elevated. In this study cortisol metabolism and urinary steroid profiles in healthy ferrets and in ferrets with hyperadrenocorticism were investigated, as well as possible seasonal fluctuations in the UCCR.

In healthy ferrets and in one ferret with hyperadrenocorticism, approximately 10% of plasma cortisol and its metabolites was excreted in the urine. High-performance liquid chromatography revealed one third of the urinary corticoids to be unconjugated cortisol; the other peaks mainly represented cortisol conjugates and metabolites.

In 21 healthy sexually intact ferrets, the UCCR started to increase by the end of March and declined to initial values halfway the breeding season (June). In healthy neutered ferrets there was no significant seasonal influence on the UCCR. In two neutered ferrets with hyperadrenocorticism the UCCR was raised, primarily during the breeding season.

In 27 of 31 privately owned ferrets with hyperadrenocorticism, the UCCR was higher than the upper limit of the reference range ( $2.1 \times 10^{-6}$ ). In 12 of 14 healthy neutered ferrets dexamethasone administration decreased the UCCR by more than 50%, whereas in only one of the 28 hyperadrenocorticoid ferrets did the UCCR decrease by more than 50%.

We conclude that the UCCR in ferrets primarily reflects cortisol excretion. In healthy sexually intact ferrets and in ferrets with hyperadrenocorticism the UCCR increases during the breeding season. The increased UCCR in hyperadrenocorticoid ferrets is resistant to suppression by dexamethasone, indicating ACTH-independent cortisol production.

### Introduction

Hyperadrenocorticism leading to a glucocorticoid-induced catabolic state, characterized by muscle weakness, skin atrophy, and centripetal obesity, occurs in humans,<sup>1</sup> dogs,<sup>5,16</sup> cats,<sup>13,15</sup> and horses.<sup>11</sup> The glucocorticoid excess may be due to an adrenocortical tumor or over-stimulation of the adrenal cortex. In pet ferrets (*Mustela putorius furo*) the situation is different. In this species hyperadrenocorticism is characterized by signs of excessive production of steroids (androstenedione, 17 $\alpha$ -hydroxyprogesterone, dehydroepiandrosterone sulfate, and/or estradiol), leading to symmetrical alopecia, vulvar swelling in neutered female ferrets (jills), and recurrence of sexual behavior after neutering in male ferrets (hobs).<sup>10,19,20,21,24,35</sup> Plasma concentrations of cortisol are only increased in a minority of cases.<sup>20</sup>

In humans, dogs, cats, and horses, the assessment of basal adrenocortical function usually includes the measurement of urinary corticoids relative to the urinary creatinine concentration,<sup>1,7,18,31</sup> and is expressed as the urinary corticoid/creatinine ratio (UCCR). The main advantage of the UCCR over plasma cortisol concentrations is that urinary corticoid excretion reflects the free plasma cortisol concentration over a period of time, and is thus less influenced by the pulsatile secretion of this hormone than is the plasma cortisol level. A practical advantage of this measurement is that urine samples can be collected at home without the stress of a clinic visit and the blood sampling, thereby avoiding iatrogenic elevations.

In ferrets the UCCR has also been assessed for diagnosing hyperadrenocorticism, with a UCCR higher than  $1.6 \times 10^{-6}$  being regarded as diagnostic for hyperadrenocorticism.<sup>8</sup> However, other have questioned whether measurement of the UCCR is an appropriate way to diagnose hyperadrenocorticism in ferrets,<sup>19</sup> because hypercortisolism is not considered to play an important role in hyperadrenocorticism in ferrets.<sup>20</sup>

Recently, we reported that urinary corticoid levels are increased during the breeding season in ferrets with hyperadrenocorticism.<sup>25</sup> This increase coincided with the reported increase in plasma luteinizing hormone (LH) concentrations.<sup>9</sup> It is not clear whether the increased UCCR associated with hyperadrenocorticism in ferrets is due to hypercortisolism or the result of cross-reaction of other urinary steroids in the cortisol assay.<sup>25</sup>

Here, we report on the results of studies of cortisol metabolism and urinary steroid profiles in healthy ferrets and ferrets with signs of hyperadrenocorticism. In addition, we present the results of UCCR measurements performed at 2-week intervals in intact and neutered ferrets for a period of 19 months.

### Materials and Methods

#### HPLC and radioimmunoassay

Urine samples (10-15 ml) for high-performance liquid chromatography (HPLC) were extracted on a C18 SepPak cartridge (Millipore, Bedford, MA). Urine samples were passed

over the cartridge followed by a wash step with water. Steroids were eluted with 100% methanol, evaporated to dryness, and dissolved in mobile phase before chromatography.

Steroids were separated by HPLC (Pharmacia, Uppsala, Sweden) on a reversed phase C18 column (Merck, Darmstadt, Germany; particle size, 5  $\mu\text{m}$ ) using a mobile phase of HPLC-quality MeOH (Labsan Ltd, Dublin, Ireland) and double-distilled water (60:40 v/v) at a flow rate of 1 ml/min. Retention times of synthetic steroids (Sigma-Aldrich, St. Louis, USA) were determined using UV detection at 254 nm and were: aldosterone, 4.5 min; dehydrocorticosterone, 6 min; hydrocortisone (cortisol), 6.5 min; corticosterone, 9 min; 11-deoxycortisol, 9.5 min; androstenedione, 14 min; 17 $\beta$ -estradiol, 15.5 min; progesterone, 21.5 min and 17 $\alpha$ -hydroxyprogesterone, 33 min. Fractions were collected every 30 sec for 45 min for the measurement of their immunoreactivity in the radioimmunoassay (RIA) for cortisol, as described previously.<sup>18</sup> The cortisol antiserum was raised in rabbits against a cortisol-21-hemisuccinate-bovine serum albumin conjugate. This antiserum is known to cross-react with other steroids such as 21-deoxycortisol (62%), corticosterone (11%), cortisone (2%), 11-deoxycortisol (1.3%), deoxycorticosterone (1.3%), and 17 $\alpha$ -hydroxyprogesterone (0.1%).<sup>30</sup> The urinary corticoid concentration was related to the urinary creatinine concentration (Jaffé kinetic method, initial rate reaction) by calculation of its quotient ( $\times 10^{-6}$ ).<sup>27</sup>

### Urinary excretion of corticoids

#### *Animals*

For this study, which was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University, three male and three female 5-year-old ferrets were used. All ferrets had been neutered. One of the neutered hobs had hyperadrenocorticism diagnosed on the basis of increased plasma concentrations of androstenedione and 17 $\alpha$ -hydroxyprogesterone, and ultrasonography of the adrenals. There was a (histologically confirmed) adenoma in the left adrenal gland.

All ferrets were individually housed in outdoor suspended cages with a night box. Water and ferret pellets (FerRet®, Hope Farms, Woerden, The Netherlands) were available ad libitum.

#### *Experimental procedure*

The ferrets were sedated with an intramuscular injection of medetomidine (100  $\mu\text{g}/\text{kg}$ ; Domitor®, Pfizer Animal Health BV, Capelle a/d IJssel, The Netherlands) for placement of a 24-gauge cannula with an injection port (Vasofix® Braunüle®, Braun, Melsungen, Germany) in the cephalic vein. Ferret plasma mixed with 5  $\mu\text{Ci}$  [1,2,6,7-<sup>3</sup>H]-cortisol (Amersham, Buckinghamshire, United Kingdom) was injected through the cannula which subsequently was flushed with 1 ml saline. Sedation was reversed with atipamazole (400  $\mu\text{g}/\text{kg}$ ; Antisedan®, Pfizer Animal Health BV, Capelle a/d IJssel, The Netherlands).

For the next 4 days urine was collected once daily by means of propylene litter boxes with macrolon plates placed underneath the cages. A 2-mm space between the plate and the wall of the litter box allowed urine to drain away from the feces.<sup>14</sup> Ten 1-ml aliquots of each urine sample were mixed with 4 ml scintillation liquid (Ultimagold; Packard Instrument company, Downers Grove, IL), and after thorough mixing radioactivity was

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measured in a  $\beta$ -counter (Rackbeta liquid scintillation counter; LKB, Bromma, Sweden). The percentage of [ $^3\text{H}$ ]cortisol excreted in the urine was calculated as  $[(\sum^3\text{H}/10) \text{ (dpm/ml)} \times \text{U volume (ml)} / ^3\text{H administered (dpm)}] \times 100$ .

### **Seasonal influence on urinary corticoid/creatinine ratios (UCCR)**

The UCCRs of 21 healthy, intact ferrets (7 male, 14 female) and 9 gonadectomized ferrets (5 male, 4 female) were measured from February 2000 until September 2001. In February 2000 the ferrets were 2 to 3 years old. Eight ferrets had been gonadectomized at 6 weeks of age and one hob at 9 months of age. The ferrets were housed and fed as described above.

Although no signs of hyperadrenocorticism were present in any of the animals, two of the gonadectomized hobs appeared to have hyperadrenocorticism, based on increased plasma concentrations of androstenedione and  $17\alpha$ -hydroxyprogesterone and ultrasonographic findings. Later on, this was confirmed by histological examination of the adrenal glands. The UCCR of the 9 gonadectomized ferrets, recorded over a year, have been published previously.<sup>25</sup>

Overnight urine samples were collected in litter boxes (see above) which were underneath the cages from 17:00 – 8:00 hours. Urine was stored in tubes at 4°C pending analysis, which was performed within 5 days of urine collection.

### **UCCRs with HDDST in healthy neutered ferrets**

Between March 15 and September 29 2001, the owners of 17 (8 male, 9 female) neutered ferrets (median age 3.5 years, range 1.5 to 8 years) collected urine samples from their ferrets. These ferrets were housemates of ferrets that had been presented with signs of hyperadrenocorticism. On physical examination no abnormalities were found in these ferrets. In 12 of these ferrets plasma concentrations of androstenedione and  $17\alpha$ -hydroxyprogesterone were within the reference ranges.<sup>25</sup>

The owners were requested to collect the first morning urine produced on 3 consecutive days. The ferrets were allowed to urinate on a smooth floor from which the urine could easily be collected. After the second urine collection, the owners administered 0.1 mg dexamethasone orally, 3 times at 8-hour intervals (high-dose dexamethasone suppression test; HDDST). The next morning the third urine sample was collected. Urine samples were stored in the refrigerator and sent to the laboratory of the Department of Clinical Sciences of Companion Animals of Utrecht University for analysis within one week.

Two consecutive morning urine samples were also collected in seven 4-year-old neutered ferrets (2 male, 5 female) kept under laboratory conditions.

### **UCCRs with HDDST in privately owned ferrets with hyperadrenocorticism**

From 1997 through 1999, hyperadrenocorticism was diagnosed in 31 neutered ferrets (25 male, 6 female; median age 5 years, range 2 to 8 years). The history included symmetrical alopecia (27 of 31 ferrets), an enlarged vulva (5 of 6 jills), and increased libido (6 of 25 hobs). An enlarged adrenal gland was palpated in 8 ferrets. In 22 of 28 ferrets in which ultrasonography was performed, the results were consistent with surgical findings.

Histological examination of the adrenal glands collected at surgery or at post mortem examination revealed hyperplasia (n=10), adenoma (n=16), and adenocarcinoma (n=5).

Urine samples were collected as described above for the healthy ferrets. A HDDST was performed in 28 of 31 ferrets.

### Calculations and statistics

In the serial measurements of UCCRs, the values are expressed as mean ratios  $\pm$  standard error of the mean (SEM). The paired Student's *t* test was used to compare the mean UCCRs of intact ferrets in April with those in December, and the serial UCCR measurements for the 2 ferrets with hyperadrenocorticism with those of the 7 healthy ferrets during both the breeding season (March until September) and the non-breeding season (September until March). Statistical significance was assumed at  $P < 0.05$ .

In the diagnostic assessment of the UCCR, the mean of the ratios of the samples collected on two consecutive days was used. The percentile method was used to establish the reference range for the UCCR in healthy ferrets.<sup>4</sup> The percentiles  $P_{2.5}$  and  $P_{97.5}$  were determined with a probability of 95%.

## Results

### Urinary excretion of corticoids

Within two days of intravenous administration of [1,2,6,7-<sup>3</sup>H]cortisol in the 5 healthy ferrets, 10.1% (range, 1.7% – 22.5%) of the administered cortisol was cleared in the urine. The urinary excretion of [<sup>3</sup>H]cortisol in the ferret with hyperadrenocorticism was 10.4%. Urine samples collected on the two following days contained practically no radioactivity, indicating that all [<sup>3</sup>H]cortisol had been excreted in two days.

The HPLC fraction eluting at the retention time of cortisol ( $t = 6.5$  min) contained  $33.2 \pm 4.5\%$  (mean  $\pm$  SEM,  $n=10$ ) of the total urinary corticoids (measured by RIA). The other two peaks with retention times of 3.5 and 5.5 min were probably conjugated or hydroxylated cortisol metabolites. No immunoreactivity eluted with retention times longer than 10 min, indicating that other steroids, such as androstenedione, 17 $\beta$ -estradiol, progesterone, or 17 $\alpha$ -hydroxyprogesterone, did not interfere with the assessment of the UCCR.

### Seasonal influence on urinary corticoid/creatinine ratios

*Intact ferrets:* - In both years of the study the UCCRs of the 21 intact healthy ferrets started to increase toward the end of March, reaching a peak in April. The ratios returned to initial values by the end of June (Figure 1). These UCCR peaks at the beginning of the breeding season were similar in both genders; at the end of April 2000 and 2001 the UCCR ( $\pm$  SEM) for 7 intact hobs was  $5.9 \pm 0.7 \times 10^{-6}$  and  $4.3 \pm 1.0 \times 10^{-6}$ , respectively, and that for 14 intact jills was  $6.0 \pm 0.7 \times 10^{-6}$  and  $3.2 \pm 0.3 \times 10^{-6}$ , respectively. At the end of April 2000 and 2001, the UCCR in all healthy ferrets ( $6.0 \pm 0.5 \times 10^{-6}$  and  $3.5 \pm 0.4 \times 10^{-6}$ , respectively) was significantly higher than at the end of December 2000 ( $1.4 \pm 0.1 \times 10^{-6}$ ).

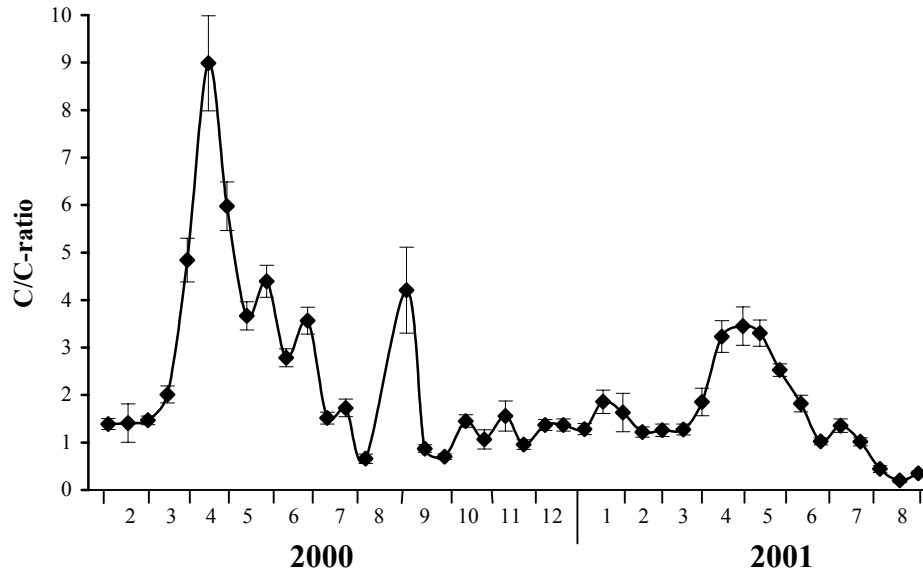


Figure 1. The mean ( $\pm$  SEM) urinary corticoid/creatinine ratio ( $\times 10^{-6}$ ) of 21 healthy intact ferrets from February 2000 until September 2001.

*Neutered ferrets:* - The UCCR was not affected by seasonal influences in healthy neutered ferrets. The mean value ( $1.3 \pm 0.1 \times 10^{-6}$ ) was not significantly different from that of the intact ferrets in the non-breeding season ( $1.5 \pm 0.2 \times 10^{-6}$ ). During the breeding seasons, the UCCR ( $\pm$  SEM) of the two hyperadrenocorticotrophic ferrets ( $3.5 \pm 0.2 \times 10^{-6}$  and  $2.9 \pm 0.2 \times 10^{-6}$ ) was significantly higher than that of the neutered control animals ( $1.0 \pm 0.2 \times 10^{-6}$ ). Early in the non-breeding season, the UCCR of one of the hyperadrenocorticotrophic ferrets was still significantly higher ( $2.9 \pm 0.3 \times 10^{-6}$ ) than that of the healthy control animals ( $1.6 \pm 0.3 \times 10^{-6}$ ). In November and December, however, the UCCR of this ferret was no longer higher than that of the other animals ( $1.9 \pm 0.4 \times 10^{-6}$  versus  $1.8 \pm 0.2 \times 10^{-6}$ ) (Figure 2).

**UCCRs and HDDST in healthy neutered ferrets**

The mean UCCR of two consecutive morning urine samples in 24 healthy neutered ferrets ranged from  $0.35$  to  $2.6 \times 10^{-6}$  (median,  $1.3 \times 10^{-6}$ ). The reference range (percentiles  $P_{2.5}$  and  $P_{97.5}$  with a probability of 95%) was  $0.6 - 2.1 \times 10^{-6}$ . Oral dexamethasone administration to 14 healthy neutered ferrets resulted in a more than 50% decrease in the UCCR in 12 ferrets, a 30% decrease in one ferret, and a 6-fold increase in another ferret (Figure 3).

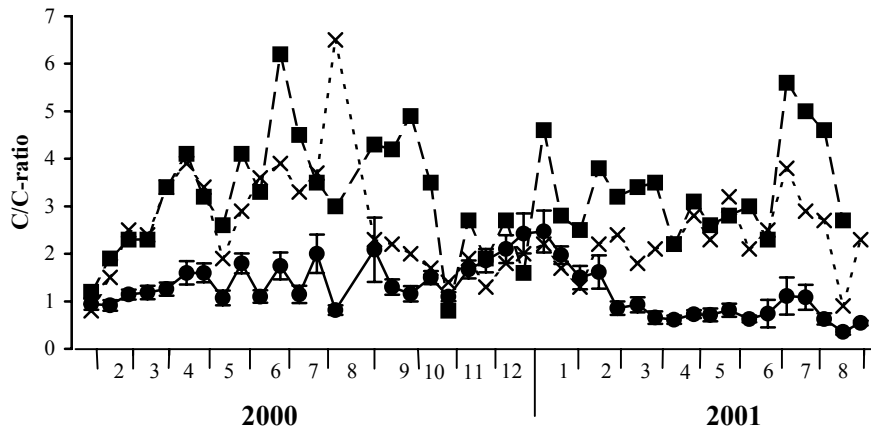


Figure 2. The mean ( $\pm$  SEM) urinary corticoid/creatinine ratio (UCCR) ( $\times 10^{-6}$ ) of 7 healthy neutered control ferrets ( $\bullet$ ), and the UCCR of 2 neutered ferrets ( $\blacksquare$ ,  $\times$ ) with hyperadrenocorticism from February 2000 until September 2001. During both breeding seasons (March to August) the mean UCCR of both ferrets with hyperadrenocorticism was significantly higher than the mean UCCR of the 7 control ferrets ( $P < 0.025$ ).

#### UCCRs and HDDST in privately owned ferrets with hyperadrenocorticism

The median UCCR in ferrets with hyperadrenocorticism was  $6.8 \times 10^{-6}$  (range,  $1.5 - 96 \times 10^{-6}$ ). In 27 of 31 ferrets (87%) the UCCR was higher than the upper limit of the reference range (see above). Oral dexamethasone administration to 28 hyperadrenocorticoïd ferrets resulted in an increase in the UCCR in 12 ferrets. In one ferret no change was seen while in 15 ferrets a decrease occurred. In only one of the latter ferrets was the decrease more than 50% (Figure 3).

### Discussion

The present results indicate that in ferrets approximately 10% of cortisol and its metabolites is excreted in the urine, which is higher than the 2% found in cats,<sup>7</sup> but much lower than the 55% in dogs.<sup>17</sup> Apparently in ferrets, as in cats, the majority of cortisol is cleared by hepatobiliary excretion. Nevertheless, urinary cortisol concentrations can be measured by RIA.

Plasma cortisol concentrations in ferrets with hyperadrenocorticism are similar to those of healthy ferrets.<sup>20</sup> In contrast, UCCRs of hyperadrenocorticoïd ferrets are significantly higher than those of healthy ferrets.<sup>8</sup> It has been hypothesized that urinary steroids or their metabolites, other than cortisol, cross-react in the cortisol assay.<sup>25</sup> For example, estrogens or androgens of adrenal origin and/or their precursors might cross-react in the urinary

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cortisol assays. However, although the antibody used in the cortisol RIA cross-reacts with other steroids, no immunoreactivity was found in HPLC fractions corresponding with the elution of androstenedione,  $17\beta$ -estradiol, progesterone, and  $17\alpha$ -hydroxyprogesterone. Thus the UCCR in ferrets mainly reflects urinary cortisol excretion. In our study, the upper limit of the reference range for the UCCR was  $2.1 \times 10^{-6}$ , which is slightly higher than the  $1.6 \times 10^{-6}$  reported previously.<sup>8</sup> Different methodology, e.g. the antibody used in the measurement of cortisol, might explain this difference.

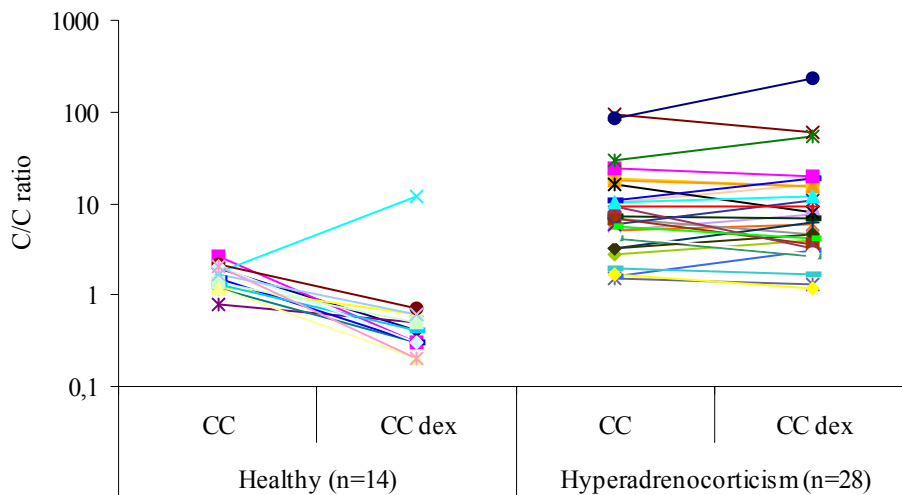


Figure 3. Logarithmic representation of the mean urinary corticoid/creatinine ratio ( $\times 10^{-6}$ ) measured in two consecutive morning urines (CC) from healthy ferrets and ferrets with hyperadrenocorticism. After the collection of these two urine samples the ferrets received three oral doses of dexamethasone (0.1 mg/kg) at 8-h intervals. The next morning a third urine sample was collected (CC dex). In ferrets with hyperadrenocorticism, the suppression of urinary corticoid excretion by dexamethasone was less than 50%, while in almost half of the cases a paradoxical rise occurred.

Interestingly, in healthy intact ferrets a distinct seasonal variation in the UCCR was observed. The ratio increased significantly in the middle of March, peaked at the beginning of April, and returned to basal values by the end of June. Similar variations in cortisol (metabolite) excretion have been found in non-human primates during periods of increased sexual activity.<sup>12,29</sup>

From a mechanistic point of view, there are several possible explanations for the increase in cortisol levels during the breeding season. First, there is the possibility that the elevated UCCR is due to activation of the pituitary-adrenocortical axis associated with the increased sexual activity. Our hobs and jills were housed in each other's vicinity without having the possibility to mate, and thus the heightened arousal may well have increased the UCCR. From studies of healthy pet dogs it is known that minor stresses, such as being briefly in a consultation room and hospitalization, can increase the UCCR.<sup>32</sup> In support of

this possibility is the fact that during the breeding season plasma adrenocorticotrophic hormone (ACTH) concentrations are higher in intact jills than in neutered jills.<sup>23</sup> However, in intact hobs plasma ACTH concentrations are similar to those of neutered hobs.<sup>23</sup> This may be due to the effect of testosterone, which is known to inhibit the response of the hypothalamus-pituitary-adrenocortical axis to stress.<sup>33</sup> Since the peak UCCR at the end of April was similar in both genders, this rise cannot fully be ascribed to sexual arousal.

Another explanation for the elevated UCCR in the breeding season of ferrets concerns LH. The sexual maturation of ferrets, under the influence of stimulatory photoperiods, is associated with increased LH secretion.<sup>22</sup> Moreover, LH-receptors (LH-Rs) have been detected in the adrenal cortex of ferrets,<sup>25</sup> and *in vitro* stimulation tests have shown that LH stimulates adrenocortical cortisol secretion (Schoemaker *et al.*, in preparation). One would expect, however, that the LH-R-mediated cortisol secretion would suppress endogenous plasma ACTH concentrations during the breeding season, which is not the case.<sup>23</sup> In addition, administration of a GnRH analogue to healthy (neutered) ferrets does not increase plasma concentrations of adrenocortical steroids.<sup>25</sup>

A third explanation for the increased cortisol excretion during the breeding season is that altered concentrations of sex steroids might influence of cortisol metabolism, for example, the capacity of cortisol-binding globulins (CBG) in plasma. In male sugar gliders, an inverse relationship has been found between testosterone concentrations and the CBG-binding capacity, resulting in increased plasma concentrations of free cortisol during the breeding season.<sup>2</sup> Changes in the concentration of carrier globulins may affect urinary cortisol excretion, as observed in a woman with clinical Cushing's disease and CBG deficiency. In this woman, total plasma cortisol concentrations were within the normal range, whereas plasma concentrations of free cortisol and the urinary corticoid excretion were elevated.<sup>34</sup>

In the healthy neutered ferrets there were no seasonal changes in the UCCR. The ratio remained constant at the same low level as in the intact ferrets during the non-breeding season. In contrast, two neutered hyperadrenocorticotrophic ferrets had an elevated UCCR during the breeding season. It is possible that under the influence of the castration-induced increased of gonadotropic hormones, the adrenocortical cells had become receptive to stimulation, e.g. by increased expression or a regain of functionality of LH-receptors.<sup>26</sup> As in sexually intact ferrets, plasma LH concentrations, which are influenced by the photoperiods,<sup>3</sup> may have been low during the non-breeding season and high during the breeding season. The latter, in combination with adrenocortical responsiveness, may have led to an increase in the secretion of steroids. In turn, this may have led to an increase in the plasma free cortisol concentration and consequently to an increased UCCR. However, this explanation should be considered with some reservation. While LH-R-mediated cortisol production may contribute to the elevated UCCR in the breeding season, in the two hyperadrenocorticotrophic ferrets, hCG administration caused plasma concentrations of androstenedione and 17 $\alpha$ -hydroxyprogesterone to rise without a concomitant increase in plasma cortisol concentrations.<sup>25</sup> However, sexual arousal and lowering of the CBG levels may have contributed to cortisol excretion, as described above for the sexually intact ferrets.

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In 27 of the 31 privately owned ferrets with hyperadrenocorticism, the UCCR was higher than the upper limit of the reference range, confirming the previously reported increase in the UCCR in ferrets with hyperadrenocorticism.<sup>8</sup> In almost all of the healthy neutered ferrets, dexamethasone administration decreased the UCCR by more than 50%, whereas in the great majority of clinical cases there was resistance to suppression. In some cases, the UCCR even increased after dexamethasone administration. Such paradoxical increases, which have also been observed in humans<sup>28,36</sup> and dogs<sup>6</sup> with adrenocortical tumors, have been ascribed to a direct effect of dexamethasone on the adrenocortical tumor.<sup>6,36</sup>

Dexamethasone resistance is compatible with autonomy at adrenocortical level, although it may also be associated with some pituitary tumors. Autonomy at a pituitary level is very unlikely because hyperadrenocorticism is not associated with corticotrophic adenomas in ferrets.<sup>19, Schoemaker *et al.*, submitted</sup> Therefore ACTH-independent production of corticosteroids has to be assumed, for instance, LH-dependent production of adrenal steroids, including cortisol. The initially LH-dependent production of adrenal steroids may become LH-independent as the adrenal gland undergoes neoplastic transformation. Irrespective of which mechanism is operational, yet another mechanism has to be considered as explanation for the combination of an elevated UCCR, normocortisolemia, and unaltered plasma ACTH concentrations,<sup>23</sup> namely an increased production of steroids. These would affect the CBG concentration, leading to elevated free cortisol concentrations without an elevation of total cortisol levels. It is also possible that ACTH levels are not suppressed by the LH-dependent or autonomously hypersecreting adrenal lesions, because cortisol secretion was only marginally elevated.

In conclusion, our results indicate that the UCCR in ferrets primarily reflects cortisol excretion. In healthy ferrets and in ferrets with hyperadrenocorticism, the UCCR increases during the breeding season. The increased UCCR in hyperadrenocorticoid ferrets is resistant to suppression by dexamethasone, indicating ACTH-independent cortisol production.

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