

## Chapter 8

### **The Role of Luteinizing Hormone in the Pathogenesis of Hyperadrenocorticism in Neutered Ferrets**

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### Summary

Four studies were performed to test the hypothesis that gonadotropic hormones, and particularly luteinizing hormone (LH), play a role in the pathogenesis of hyperadrenocorticism in ferrets: (I) adrenal glands of ferrets with hyperadrenocorticism were studied immunohistochemically to detect LH receptors; (II) gonadotropin-releasing hormone (GnRH) stimulation tests were performed in 10 neutered ferrets, with measurement of androstenedione, 17 $\alpha$ -hydroxyprogesterone and cortisol as endpoints; (III) GnRH stimulation tests were performed in 15 ferrets of which 8 had hyperadrenocorticism, via puncture of the vena cava under anesthesia; and (IV) urinary corticoid/creatinine ratios were measured at 2-week intervals for 1 year in the same ferrets as used in study II.

Clear cells in hyperplastic or neoplastic adrenal glands of hyperadrenocorticoïd ferrets stained positive with the LH receptor antibody. Plasma androstenedione and 17 $\alpha$ -hydroxyprogesterone concentrations increased after stimulation with GnRH in 7 out of 8 hyperadrenocorticoïd ferrets but in only 1 out of 7 healthy ferrets. Hyperadrenocorticoïd ferrets had elevated urinary C/C ratios during the breeding season.

The observations support the hypothesis that gonadotropic hormones play a role in the pathogenesis of hyperadrenocorticism in ferrets. This condition may be defined as a disease resulting from the expression of LH receptors on sex steroid-producing adrenocortical cells.

## Introduction

The most prominent, and initially seasonal, symptoms of hyperadrenocorticism in ferrets are symmetrical alopecia (Fig. 1), vulvar swelling in neutered jills, and recurrence of sexual behavior in neutered males.<sup>15</sup> There is no sex predilection.<sup>18</sup> The diagnosis is based upon increased plasma concentrations of androstenedione, 17 $\alpha$ -hydroxyprogesterone, dehydroepiandrosterone sulfate, and/or estradiol. In contrast plasma concentrations of cortisol are increased in a minority of cases.<sup>16</sup> Measurement of urinary corticoid/creatinine ratios<sup>4</sup> and ultrasonography of the adrenals<sup>15</sup> may contribute to the diagnosis. In approximately 85% of ferrets with hyperadrenocorticism only one adrenal gland is enlarged, without atrophy of the contralateral adrenal gland, and in the remaining 15% there is bilateral involvement.<sup>17,24</sup> After unilateral adrenalectomy there may be recurrence of the disease due to enlargement of the contralateral adrenal gland.<sup>24</sup> The histologic changes of the adrenals range from (nodular) hyperplasia to adenoma and adenocarcinoma.<sup>17,24</sup>



Figure 1. Symmetrical alopecia in a ferret with hyperadrenocorticism

These characteristics of hyperadrenocorticism in ferrets resemble those seen in some strains of mice in which nodular adrenocortical hyperplasia and adrenocortical tumors occur after neutering at an early age.<sup>3,11,19</sup> In recent years, independent observations have provided suggestive evidence that castration is an important risk factor in the development of hyperadrenocorticism in ferrets. First, in the USA and in The Netherlands hyperadrenocorticism is a common disease in ferrets,<sup>15,18</sup> whereas in the United Kingdom the condition is seldom diagnosed. This difference in incidence may be ascribed to the fact

that ferrets are usually not neutered in the United Kingdom,<sup>10</sup> whereas this is common practice in the USA and in the Netherlands. Second, a significant correlation has been found between the age at neutering and age at onset of hyperadrenocorticism in ferrets.<sup>18</sup> The observation that initially signs of hyperadrenocorticism occur only during the breeding season,<sup>15</sup> when plasma concentrations of gonadotropic hormones are high,<sup>6</sup> and the recently reported beneficial effects of treatment with leuprolide acetate<sup>23</sup> have led to the hypothesis that hyperadrenocorticism in ferrets is mediated by gonadotropic influences, for which castration may play a precipitating role.<sup>9,17,18</sup>

To test this hypothesis four studies were performed: (I) adrenal glands of ferrets with hyperadrenocorticism were studied immunohistochemically to detect luteinizing hormone receptors (LH-R); (II) a gonadotropin-releasing hormone (GnRH) stimulation test was performed in neutered ferrets via implanted venous catheters; (III) a GnRH stimulation test was performed in neutered ferrets via puncture of the vena cava under anesthesia; and (IV) urinary corticoid/creatinine (C/C) ratios were measured at 2-week intervals for 1 year in the ferrets of study II to investigate possible seasonal fluctuations.

## Materials and Methods

### (I) Immunohistochemical staining for the presence of LH receptors

#### *Tissues*

Adrenal glands of 6 neutered ferrets with signs of hyperadrenocorticism, and histologically diagnosed hyperplasia or adenoma, were examined for the presence of LH receptors. The adrenal glands were obtained during surgery. In all cases the contralateral adrenal gland appeared to be unaffected. Signs of hyperadrenocorticism disappeared after surgery in all cases. Two histologically normal adrenal glands of 2 intact ferrets without signs of hyperadrenocorticism were also examined for the presence of LH receptors. Ovaries and testes of healthy 6 to 9-month-old ferrets were used as positive controls.

#### *Antibody and immunohistochemical staining*

The murine LH-R monoclonal antibody (P1B4) was a gift from Dr. Wimalasena (Dept. Obstetrics and Gynecology, University of Tennessee, Knoxville, TN). The antibody had been raised against purified rat LH receptors, as described by Indrapichate *et al.*<sup>5</sup> The antibody binds specifically to LH receptors in various tissues of different species.<sup>1,13</sup>

All tissues were fixed in 4% buffered formalin. After at least 24 h of fixation the tissues were embedded in paraffin and cut into 4- $\mu$ m sections. One section was routinely stained with hematoxylin and eosin. For immunohistochemical staining, sections were deparaffinized and endogenous peroxidase was blocked with 1% H<sub>2</sub>O<sub>2</sub> in methanol for 30 min. The slides were washed in 0.01 M Tris-buffered saline (TBS, pH 7.4), incubated with 0.75% glycine in TBS for 30 min, and rinsed with TBS. The sections were blocked with 10% normal goat serum in TBS for 30 min, and then incubated overnight at 4 °C with the LH-R P1B4 monoclonal antibody at a 1:5000 dilution in TBS to which 0.05% acetylated BSA (BSA-c) was added (Aurion, Wageningen, The Netherlands). The next day the slides were rinsed with TBS and incubated for 60 min with a biotinylated goat-anti-mouse

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antibody (Vector Laboratories, Burlingame, CA) [1:200 dilution in TBS with 0.05% BSA-c] at room temperature. Again, the slides were rinsed in TBS and subsequently incubated with the avidin (A) biotin (B) complex of the ABC staining kit (Vector Laboratories) for 60 min in a dilution of 1:1500 in TBS with 0.05% BSA-c. The ABC solution was prepared at least 15 min prior to use, to allow complex formation. Slides were rinsed again in TBS followed by Tris-HCl (0.05M, pH 7.6). Bound antibody was visualized using a 0.6 mg/ml solution of 3,3' - Diaminobenzidine tetrachloride (DAB, Sigma Chemical Co., St. Louis, MO) in Tris-HCl, to which 0.03% H<sub>2</sub>O<sub>2</sub> was added. Sections were incubated with the DAB solution for approximately 50 sec and counterstained with Mayer's hematoxylin.

In control experiments the primary antibody was replaced by normal mouse serum.

### (II) GnRH stimulation test in catheterized ferrets

#### *Animals*

For this study, which was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University, 5 male and 5 female healthy ferrets were used in July and August 1999. All but one ferret were 2 years of age and had been gonadectomized at 6 weeks of age. One ferret, a 1-year-old male, had been gonadectomized at 9 months of age. The ferrets were either purchased at 6 weeks of age from a breeder or born and raised at the Department of Clinical Sciences of Companion Animals. The ferrets were individually housed in outdoor suspended cages with a night box. Both water and ferret pellets (FerRet, Hope Farms, Woerden, The Netherlands) were available *ad libitum*.

Unexpectedly, in two ferrets elevated basal plasma androstenedione and 17 $\alpha$ -hydroxyprogesterone concentrations, as well as unilaterally enlarged adrenal glands visualized on ultrasonographic examination, indicated the presence of hyperadrenocorticism.

#### *Catheterization*

A jugular catheter was placed under isoflurane anesthesia in all ferrets. The catheters were tunneled subcutaneously to the base of the skull and connected to a 20-gauge cannula with an injection port (Vasofix® Braunüle®, Braun, Melsungen, Germany). The injection port was attached to the skin with polyglactin 910 (Vicryl®, Ethicon, Norderstedt, Germany) 2-0 USP sutures. The catheters (Silastic® Medical Grade Tubing [602-155], Down Corning Co. Midland, Michigan, U.S.A) had an inner diameter of 0.025 inch (0.64 mm) and an outer diameter of 0.047 inch (1.19 mm). The catheter was filled with a polyvinyl pyrrolidone (PVP) and heparin mixture (60 g PVP / 54 ml 0.9% NaCl + 6 ml heparin [5,000 IU/ml]) to keep it patent.

#### *Sampling*

Blood samples were collected 2 days after placement of the jugular catheter at -5, 0, 30, 60, 90, 120, 240, and 480 min after intravenous injection of 10  $\mu$ g of a synthetic GnRH analogue (Fertagyl®, Intervet Nederland B.V., Boxmeer, The Netherlands), placed in pre-chilled EDTA-coated tubes and centrifuged. Plasma was divided into two portions and stored at -20 °C pending analysis. After the collection of each blood sample the catheter was flushed with 0.3 ml heparin solution (50 IU/ml).

### **(III) GnRH stimulation test with blood collection under anesthesia**

We used a modified GnRH stimulation test for privately owned ferrets thought to have hyperadrenocorticism. Blood samples were collected under isoflurane anesthesia by puncture of the cranial vena cava, immediately before and 30 min after intravenous injection of 10 µg Fertagyl®. In between blood collections the ferrets were allowed to recover from anesthesia, which usually occurred within 5 minutes. Blood samples were placed in pre-chilled EDTA-coated tubes and centrifuged. Plasma was stored at – 20 °C pending analysis.

#### *Animals*

GnRH stimulation tests with blood collection under isoflurane anesthesia were performed in 9 of the gonadectomized ferrets of study II (5 male and 4 female), including the 2 ferrets with hyperadrenocorticism, and in 6 privately owned neutered ferrets (4 male and 2 female; 3 – 7 years of age) with hyperadrenocorticism. The diagnosis of hyperadrenocorticism in the latter cases was based upon history, physical changes, and ultrasonographic examination of the adrenal glands. In the privately owned ferrets only plasma concentrations of androstenedione were measured because blood was needed for routine laboratory tests and the animals had to undergo surgery.

#### *Reference plasma concentrations of androstenedione and 17α-hydroxyprogesterone*

For the determination of reference values for basal plasma concentrations of androstenedione and 17α-hydroxyprogesterone, blood was collected between March 15 and September 29, 2000, from 18 healthy, privately owned ferrets, and 14 healthy ferrets kept under laboratory conditions. All ferrets (20 female and 12 male) had been neutered and were between 1.5 and 8 years old (median 3 years).

#### *Hormone determinations*

Androstenedione concentrations were measured by radioimmunoassay (RIA) as described previously.<sup>22</sup> The lower limit of detection was 0.1 nmol/l and the interassay coefficients of variation were 10.5%, 9.3%, and 11.6% at 1.43, 4.82, and 11.76 nmol/l, respectively. Steroids cross-reacted in this assay as follows: 0.4 % for testosterone, 0.3 % for dihydrotestosterone, 3.6 % for 11β-OH-androstenedione, 2.2 % for adrenosterone (11-keto-androstenedione), 5.5 % for 5α-androstenedione, and 2.2 % for 5β-androstenedione.

17α-Hydroxyprogesterone concentrations were measured after toluene extraction using a competitive RIA and a polyclonal anti-17α-hydroxyprogesterone-antibody (UCB i903, UCB Bioproducts, Brussels, Belgium). 17α-Hydroxy[1,2,6,7-<sup>3</sup>H]-progesterone (TRK 611, Amersham Pharmacia Biotech Benelux, Roosendaal, The Netherlands) was used as a tracer after chromatographic purification. The lower limit of detection was 0.2 nmol/l and interassay coefficients of variation were 9.0%, 7.4%, and 9.9% at 0.89, 5.13, and 26.02 nmol/l, respectively. Steroids cross-reacted in this assay as follows: 0.4 % for progesterone, 0.3 % for pregnenolone and 20 % for 17α-OH-pregnenolone.

Cortisol concentrations were measured by RIA (Coat-A-Count® Cortisol, Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was 1 nmol/l and the interassay coefficient of variation was between 4.0% and 6.4%.

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### (IV) Serial measurements of urinary corticoid/creatinine (C/C) ratios

#### *Animals*

One year after the GnRH stimulation test, 9 of the gonadectomized ferrets of study II (5 male and 4 female), including the 2 ferrets with hyperadrenocorticism, were used to monitor the urinary C/C ratios for 1 year. One of the ferrets with hyperadrenocorticism died 7 months after the last urine sample was collected. On postmortem examination a large (metastasized) adrenocortical tumor was found.

#### *Sampling*

Propylene litter boxes with Macrolon plates were placed underneath the cages for collection of overnight urine samples at 2-week intervals for a period of 1 year. A 2-mm space between the plate and the wall of the litter box allowed urine to drain away from the feces.<sup>12</sup> The litter boxes were underneath the cages from 17:00 – 8:00 hours. Urine was transferred to tubes and stored at 4 °C pending analysis, which was performed within 5 days after collection of the sample.

#### *Hormone determination*

Urinary corticoid concentrations were measured by RIA for cortisol as described previously.<sup>14</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 endogenous corticosteroids such as 21-deoxycortisol (62%), corticosterone (11%), cortisone (2%), 11-deoxycortisol (1.3%), deoxycorticosterone (1.3%), and 17 $\alpha$ -hydroxyprogesterone (0.1%).<sup>21</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>20</sup>

#### *Statistics*

The increase in plasma androstenedione concentrations in the GnRH stimulation test in the two hyperadrenocorticoid ferrets was compared with the (mean + 2sd) increase in plasma androstenedione concentrations in the 8 ferrets with normal basal plasma androstenedione concentrations (study II). Significance at  $P < 0.025$  was assumed when the increase in plasma androstenedione concentrations in one of the hyperadrenocorticoid ferrets was higher than the mean increase + 2sd in the 8 other ferrets.

The reference values for plasma androstenedione and 17 $\alpha$ -hydroxyprogesterone concentrations were established in percentiles.<sup>2</sup> The inner limits of the percentiles  $P_{2.5}$  and  $P_{97.5}$  are presented with a probability of 95%.

Student's *t*-test was used to compare the mean corticoid/creatinine ratio of the 2 ferrets with hyperadrenocorticism with the mean ratio for the 7 healthy ferrets during both the breeding and the non-breeding season. Statistical significance was assumed at  $P < 0.05$ .

## Results

### (I) Immunohistochemical staining for the presence of LH receptors

Thecal cells in the ovaries and Leydig cells in the testes of healthy control animals stained positively with the LH-R antibody. In the adrenal glands of the healthy ferrets there was positive staining for LH-R in the zona glomerulosa and a slightly less clear staining in the zona fasciculata (not shown).

The adrenal glands of ferrets with hyperadrenocorticism had a heterogeneous appearance on histology. Cells were either small with pyknotic nuclei or large with a clear cytoplasm. The latter cells are referred to as clear cells. Spindle-shaped cells were also observed in the adenomas. Cells staining positive for the LH receptor were seen in the hyperplastic and neoplastic adrenal glands of the ferrets with hyperadrenocorticism. These cells were mostly clear cells (**Color section**; Fig. 6).

### (II) GnRH stimulation test in catheterized ferrets

No significant response was seen after the administration of GnRH to the 8 healthy ferrets. In the 2 ferrets with hyperadrenocorticism basal plasma concentrations of androstenedione (1.5 and 3.8 nmol/l) and 17 $\alpha$ -hydroxyprogesterone (1.7 and 1.9 nmol/l) were significantly higher than those of the healthy ferrets. Moreover, the plasma concentrations of these hormones rose significantly ( $P < 0.025$ ) 30 min after GnRH administration, and returned to basal concentrations within 60 – 90 min. In all ferrets, including the ferrets with hyperadrenocorticism, plasma cortisol concentrations did not change significantly after GnRH stimulation (Fig. 2).

### (III) GnRH stimulation test with blood collection under anesthesia

#### *Reference plasma concentrations*

Basal plasma androstenedione concentrations in 32 healthy neutered ferrets ranged from 0.1 to 0.5 nmol/l (median 0.2 nmol/l;  $P_{2.5} - P_{97.5}$ : 0.1 – 0.4 nmol/l). Basal plasma 17 $\alpha$ -hydroxyprogesterone concentrations in the same ferrets ranged from 0.3 to 1.2 nmol/l (median 0.4 nmol/l;  $P_{2.5} - P_{97.5}$ : 0.3 – 0.7 nmol/l).

#### *Ferrets used in study II*

Basal plasma androstenedione and 17 $\alpha$ -hydroxyprogesterone concentrations of the two ferrets with hyperadrenocorticism (number 4 and 7) were higher than the respective reference range. Concentrations of these hormones were significantly higher at 30 min after stimulation with GnRH. In the other 7 ferrets, basal plasma androstenedione and 17 $\alpha$ -hydroxyprogesterone concentrations were all within the reference range (Table 1). The plasma androstenedione concentration was unchanged 30 min after stimulation with GnRH in 3 of the ferrets; it increased slightly, but remained within the reference range in 3 ferrets; and in 1 ferret it rose above the reference range (Table 1). The plasma 17 $\alpha$ -hydroxyprogesterone concentration remained unchanged 30 min after stimulation with GnRH in 4 ferrets; it increased slightly, but remained within the reference range, in 1 ferret; and it rose above the reference range in 2 ferrets (Table 1).

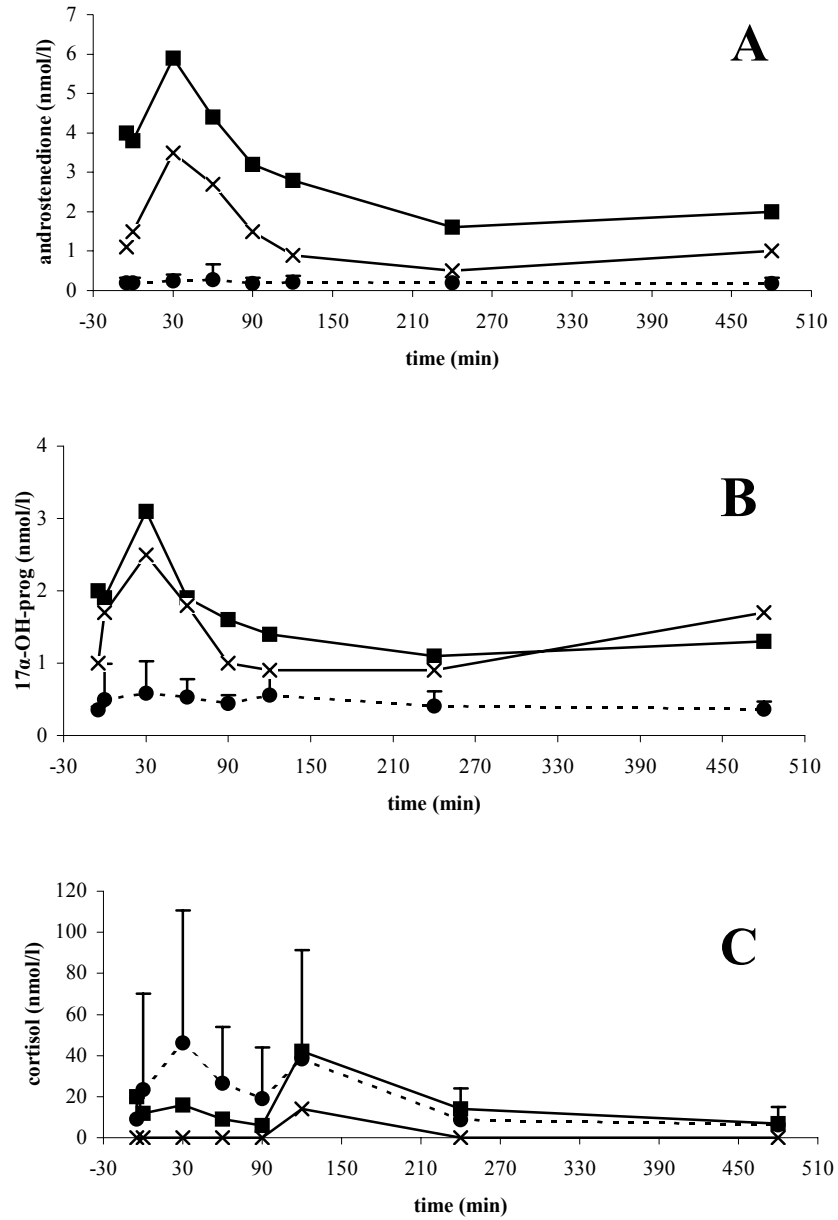


Figure 2. Mean ( $\pm$  sd) plasma concentrations of androstenedione (A), 17 $\alpha$ -hydroxyprogesterone (B), and cortisol (C) before and after stimulation with 10  $\mu$ g Fertagyl® (GnRH analogue) in 8 healthy neutered ferrets (●) and in 2 neutered ferrets (■, x) with hyperadrenocorticism.

Table 1. Plasma concentrations of androstenedione and 17 $\alpha$ -hydroxyprogesterone (nmol/l) in 9 ferrets used in study II (1 – 9) and 6 privately owned ferrets with hyperadrenocorticism (C1 – C6) before and 30 min after intravenous injection with 10  $\mu$ g Fertagyl® (GnRH analogue). Blood samples were collected under isoflurane anesthesia. The same 2 ferrets which responded to GnRH stimulation when catheterized (number 4 and 7) responded in this modified stimulation test.

Ferret number	androstenedione		17 OH prog	
	t = 0	t = 30	t = 0	t = 30
1	0.2	0.2	0.4	0.4
2	0.2	0.3	0.4	0.3
3	0.2	0.2	0.4	0.4
4	8.5	13.8	5.1	10.5
5	0.2	0.2	0.4	0.4
6	0.3	0.4	0.4	0.5
7	4.2	16.9	7.5	35.0
8	0.3	0.4	0.5	0.8
9	0.4	1.3	0.6	1.0
C1	0.1	1.3		
C2	0.3	0.4		
C3	11.7	33.3		
C4	0.7	5.4		
C5	0.3	0.6		
C6	0.5	0.7		

*Privately owned ferrets*

In 3 of the 6 privately owned hyperadrenocorticoid ferrets, basal plasma concentrations of androstenedione were within the reference range. The plasma androstenedione concentration was within the reference range 30 min after stimulation with GnRH in 1 ferret; it was higher than the reference range in 2 ferrets (Table 1); and it was higher than the reference range at baseline and increased further after stimulation with GnRH in 3 ferrets (Table 1).

**(IV) Serial measurements of urinary corticoid/creatinine ratios**

During the breeding season (March to August) and the non-breeding season (September to February), the urinary corticoid/creatinine (C/C) ratio was measured on 12 occasions (Fig. 3). During the breeding season the mean ( $\pm$  sd) C/C ratio of the 2 ferrets with hyperadrenocorticism (both  $3.5 \pm 1.1 \times 10^{-6}$ ) was significantly higher than that of the neutered control animals ( $1.4 \pm 0.5 \times 10^{-6}$ ); however, during the non-breeding season the mean C/C ratio of only one ferret was significantly higher ( $3.0 \pm 1.3 \times 10^{-6}$  and  $1.8 \pm 0.3 \times 10^{-6}$  versus  $1.8 \pm 0.7 \times 10^{-6}$  in the healthy control animals). The urinary C/C ratio of the latter ferret decreased at the end of October, after which the mean C/C ratio was no longer significantly higher than that of other animals ( $2.5 \pm 1.1 \times 10^{-6}$  versus  $1.9 \pm 0.7 \times 10^{-6}$ ).

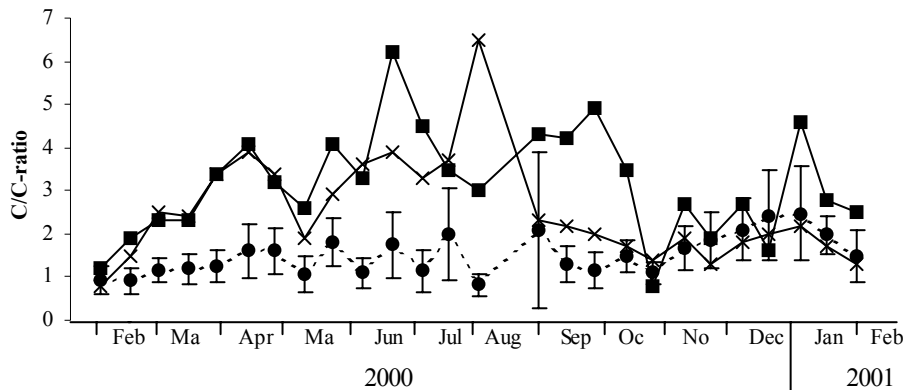


Figure 3. The mean ( $\pm$  sd) urinary corticoid/creatinine (C/C) ratio ( $\times 10^{-6}$ ) of 7 healthy neutered control ferrets ( $\bullet$ ) and the urinary C/C ratios of 2 neutered ferrets ( $\blacksquare$ ,  $\times$ ) with hyperadrenocorticism. During the breeding season (March to August) the mean C/C ratio in both ferrets with hyperadrenocorticism was significantly higher than the mean C/C ratio in the 7 control ferrets ( $P < 0.025$ )

## Discussion

The detection of LH receptors in hyperplastic and/or neoplastic adrenal glands of ferrets with hyperadrenocorticism supports the hypothesis that LH plays a role in the pathogenesis of hyperadrenocorticism in these animals, and that this hormone is involved in the production of androstenedione and  $17\alpha$ -hydroxyprogesterone. The adrenal cortices of young intact healthy ferrets also stained positively for LH-R proteins. Since the LH-R antibody used in this study reacts with both intact LH-R protein and LH-R protein fragments,<sup>5</sup> it cannot be excluded that the immunohistochemical reactivity was due to staining of LH-R fragments, i.e. truncated forms of the receptor that are not functional. To investigate the functionality of this receptor, GnRH or LH stimulation tests should be performed either in vivo or in tissue culture.

Human chorionic gonadotropin (hCG) has been used for stimulation tests in women with endocrine tumors. In a woman with a virilizing adrenal adenoma testosterone levels increased after hCG,<sup>8</sup> and in a woman with LH-dependent Cushing's syndrome plasma cortisol levels increased after hCG.<sup>7</sup> However, we used GnRH as stimulating hormone, because it is not known which gonadotropic hormone stimulates the adrenal cortex in ferrets. Only the two ferrets (study II) with high basal androgen levels responded to stimulation with GnRH. Plasma concentrations of androstenedione and  $17\alpha$ -hydroxyprogesterone increased upon stimulation, whereas plasma cortisol concentrations

remained unchanged. The observation that the healthy ferrets did not have a response to GnRH stimulation indicates that the LH-R protein found in healthy ferrets is not functional.

In the modified GnRH stimulation test, 5 of the 6 privately owned ferrets with hyperadrenocorticism responded to stimulation. The increase in plasma androstenedione concentrations cannot be ascribed to isoflurane anesthesia because the increase in androstenedione concentrations did not occur in the healthy ferrets. The non-elevated basal androstenedione concentrations in 3 diseased ferrets were in accordance with the findings of Rosenthal and Peterson,<sup>16</sup> who observed that basal plasma androstenedione concentrations are not increased in approximately 25% of ferrets with hyperadrenocorticism.

Based on our findings, it would appear that the neoplastic transformation of the adrenal cortex is associated with activation of pre-existent receptor protein. It is not clear whether this activation is a prerequisite for hyperplasia and/or neoplasia or a consequence of cell transformation. The positive correlation between disease incidence and time elapsed since castration<sup>18</sup> makes it tempting to speculate that a persistent elevation of plasma gonadotropin concentrations plays a crucial role in both receptor activation and cell multiplication.

During the breeding season, the 2 ferrets with hyperadrenocorticism had a significantly higher C/C ratio than the other neutered ferrets. Earlier Gould *et al.*<sup>4</sup> had found that the C/C ratio in ferrets with hyperadrenocorticism was significantly higher than the C/C ratio in healthy ferrets. In the non-breeding season, when gonadotropic hormones are low,<sup>6</sup> the urinary C/C ratio of the 2 control ferrets with hyperadrenocorticism was no longer elevated. This finding further supports the hypothesis that hyperadrenocorticism in ferrets arises under the influence of gonadotropic hormones.

The use of the urinary C/C ratio as tool to aid the diagnosis of hyperadrenocorticism in ferrets has been questioned by Rosenthal,<sup>15</sup> because cortisol is not considered to play an important role in the development of the signs and symptoms of hyperadrenocorticism in ferrets.<sup>16</sup> The 2 ferrets with hyperadrenocorticism in study II also did not have increased plasma cortisol concentrations, whereas the C/C ratio was increased. This may be because the cortisol assay measures other steroids in urine because the antibody used cross-reacts with other steroid hormones.<sup>21</sup> Although it is uncertain whether the increased C/C ratio was due to an increased secretion of cortisol, we found that urinary steroid hormone excretion is increased during the breeding season in ferrets with hyperadrenocorticism, an increase which coincides with the reported increase in plasma LH concentrations.<sup>6</sup>

LH-dependent Cushing's syndrome and LH-dependent adrenal androgen secreting tumors have been described in humans.<sup>7,8</sup> Patients with LH-dependent Cushing's syndrome have bilateral macronodular adrenal hyperplasia with high plasma concentrations of cortisol and suppressed concentrations of ACTH.<sup>7</sup> Patients with LH-dependent adrenal androgen secreting tumors may have unilateral adrenal neoplasia and unaltered cortisol and ACTH concentrations.<sup>8</sup>

The results of the present study support the hypothesis that gonadotropic hormones, and particularly LH, play a role in the pathogenesis of hyperadrenocorticism in ferrets. This disease is not a counterpart of LH-dependent Cushing's disease in humans because GnRH does not elicit cortisol secretion in ferrets. There are similarities between

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hyperadrenocorticism in ferrets and LH-dependent adrenal androgen-secreting tumors in humans, although the adrenal glands of diseased ferrets can also secrete estradiol.<sup>16</sup> Thus at this stage hyperadrenocorticism in ferrets is without a clear counter part in humans, and may probably be best defined as a disease resulting from LH-R expression by sex steroid-producing adrenocortical cells.

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