Use of a gonadotropin releasing hormone agonist implant as an alternative for surgical castration in male ferrets (Mustela putorius furo)

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Abstract

Surgical castration in ferrets has been implicated as an etiological factor in the development of hyperadrenocorticism in this species due to a castration-related increase in plasma gonadotropins. In search for a suitable alternative, the effect of treatment with the depot GnRH-agonist implant, deslorelin, on plasma testosterone concentrations and concurrent testes size, spermatogenesis, and the typical musky odor of intact male ferrets was investigated. Twenty-one male ferrets, equally divided into three groups, were either surgically castrated, received a slow-release deslorelin implant or received a placebo implant. Plasma FSH and testosterone concentrations, testes size and spermatogenesis were all suppressed after the use of the deslorelin implant. The musky odor in the ferrets which had received a deslorelin implant was less compared to the ferrets which were either surgically castrated or had received a placebo implant. These results indicate that the deslorelin implant effectively prevents reproduction and the musky odor of intact male ferrets and is therefore considered a suitable alternative for surgical castration in these animals.

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Keywords: Deslorelin; Gonadotropin; GnRH; Testosterone; Spermatogenesis

1. Introduction

Surgical castration of ferrets (Mustela putorius furo) is common practice in the USA and various European countries. Although in male pet ferrets (hobs) there is no medical need for castration, they are mainly castrated to prevent reproduction, to reduce interspecies aggression enabling them to be kept in groups, and to decrease the intensity of the musky odor produced by the sebaceous glands [1].

Hyperadrenocorticism is a common disease in neutered pet ferrets. The syndrome differs from hyperadrenocorticism in other species, such as humans and dogs, in that glucocorticoid excess is much more pronounced in
ferrets [2]. Instead, in ferrets the disease is characterized by excessive adrenal production of sex steroids, giving rise to vulvar swelling in neutered female ferrets (jills), recurrence of sexual behavior in neutered hobs, and alopecia [2-7].

It has been hypothesized that increased concentrations of gonadotropins, which occur after neutering due to the loss of negative feedback, persistently stimulate the adrenal cortex resulting in adrenocortical hyperplasia and tumor formation [6]. Strong support for this hypothesis may be found in the fact that the depot GnRH-agonist leuprolide acetate and deslorelin, can be used successfully to treat ferrets with hyperadrenocorticism [8,9], and that LH-receptors (LH-R) have been detected in the adrenal glands of ferrets with hyperadrenocorticism [10]. These receptors were considered to be functional because plasma concentrations of adrenal androgens increased after intravenous injection of a GnRH-agonist [10]. Based on these findings it has been proposed to search for alternatives for surgical castration in ferrets [11].

One of the possible alternatives for surgical castration is the continuous administration of a GnRH analogue. The results of the use of these analogues differ, however, among the different species. In dogs and caetahs continuous administration of a GnRH analogue suppresses spermatogenesis [12-14]. In the bull, however, continuous administration of a GnRH analogue leads to basal LH concentrations which are higher than in control animals, possibly explaining their concurrent increased plasma testosterone concentrations [15,16]. In addition, testis volume is also increased, and more round spermatids were found in the seminiferous tubules [17]. In marmoset monkeys and wallabies plasma testosterone concentrations remain within the normal range during the use of a long-acting GnRH agonist [18,19]. It is therefore not possible to predict the outcome of the use of long-acting GnRH analogues in ferrets.

The present study was designed to investigate whether a slow release depot GnRH-agonist implant containing 9.4 mg deslorelin (Peptidech Animal Health, North Ryde, New South Wales, Australia) would reduce plasma FSH and testosterone concentrations in combination with concurrent testes volume, spermatogenesis, and the typical musky odor of intact hobs. Unfortunately, the previously used and for ferrets validated heterologous antisera was no longer available and no suitable alternative was found to reliably measure plasma LH concentrations in ferrets.

2. Materials and methods

2.1. Animals

For this study, which was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University, 21 male ferrets between the age of 1 and 2 years were used. At the start of the study the mean (±S.D.) weight of the ferrets was 1.4 ± 0.2 kg (range: 1.0-1.7 kg). They were individually housed in outdoor suspended cages with a night box at Utrecht University with the following GPS—coordinates: 52°05’04’’N; 5°10’55’’E. No artificial lighting was provided. Pellets (FerRet®, Hope Farm, Woerden, The Netherlands) and water were available ad libitum. The ferrets were randomly divided into three groups of 7 ferrets each. The ferrets in Group 1 were surgically castrated [mean (±S.D.) weight: 1.4 ± 0.2 kg; range: 1.0-1.6 kg], the ferrets in Group 2 received a GnRH implant containing 9.4 mg deslorelin [mean (±S.D.) weight: 1.5 ± 0.1 kg; range: 1.3-1.7 kg], and the ferrets in Group 3 received a similar implant without the deslorelin (placebo implant) [mean (±S.D.) weight: 1.5 ± 0.1 kg; range: 1.3-1.7 kg].

2.2. Procedures

2.2.1. Castration and placement of implants

The implants were placed subcutaneously in the scruff of the neck in the ferrets from Groups 2 and 3, 1 week after the first blood collection (17 March 2005). On the same day the other ferrets were surgically castrated. Prior to surgery all ferrets received 4 mg/kg carprofen (Rimadyl®, Pfizer Animal Health, Capelle a/d IJssel, The Netherlands) IM. The ferrets were premedicated with an IM injection of 100 µg/kg medetomidine (Domitor®, Pfizer Animal Health, Capelle a/d IJssel, The Netherlands), followed after 10 min with a mask induction of 4% isoflurane (IsoFlo™, Abbot Animal Health, Hoofddorp, The Netherlands) in 100% oxygen. Anesthesia was maintained at 2% isoflurane, and castration was performed in a routine manner. The left testis was surgically removed from the ferrets in Groups 2 and 3, 15 weeks after the first blood collection (23 June 2005) for histological evaluation. The weight of the removed left testes was recorded and compared between both groups.

2.2.2. Blood collection and tests measurement

Blood samples were taken during the breeding season, from 10 March until 1 September 2005. Initially blood samples were taken weekly for 10 weeks, followed by once every fortnight for a period of 16
weeks. One year later, on 6 July 2006, blood samples were again taken from the same ferrets. At that time one ferret out of the placebo group had died from a thromboendoocarditis and one surgically castrated ferret had died during insulinoma surgery. During each sampling session the size of the right testis (width and length) was measured with a digital slide caliper. The testis volume was calculated using the following formula: \((\text{width}^3 \times \text{length} \times 0.524)\) (in cm), as previously used in tests measurements of black footed ferrets [20]. Blood was collected from the Vena cava cranialis while the ferret was anesthetized with isoflurane. Blood samples were divided into pre-chilled EDTA and heparinized tubes. All tubes were centrifuged for 10 min at 3000 rpm at 4°C, after which plasma from each tube was collected and stored in polystyrene tubes at -20°C, pending analysis.

2.2.3. Hormone analysis

Testosterone measurements were performed after diethylether extraction using a solid-phase radioimmunoassay (RIA) Coat-A-Count® (Diagnostic Products Corporation, Los Angeles, USA). In short 0.25 mL plasma was extracted with 1 mL diethylether by end-over-end rotation for 15 min followed by centrifugation for 5 min at 3000 \(\times\) g. After freezing of the lower aqueous phase the organic phase was decanted and evaporated to dryness. The extracts were reconstituted in 0.125 mL phosphate-buffered saline containing 0.5% (w/v) bovine serum albumin. Ferret plasma extracts were diluted 1, 2, 4 and 6 times and were parallel to the standard curve \((r^2 = 0.98)\). Spiking of plasma samples containing an endogenous concentration of 1.4 with 15 nmol/L testosterone showed a spiking recovery of 95 ± 7%. The detection limit amounted to 0.2 nmol/L. The intra- and inter-assay coefficients of variation were 5.1 and 8.4%, respectively.

 Follicle stimulating hormone concentrations were measured by RIA as described previously [21]. This RIA has been validated for use in ferrets [22]. Rat FSH-RP2 (NIAMD, Bethesda, MD) was used as a standard. The lower limit of detection was 0.8 pg/L and the intra-assay coefficient of variation was 7.2%.

2.2.4. Histological evaluation of the testes

Directly after excision, the testes were fixed by immersion in Bouin's solution for 15–24 h. They were then suspended in ethanol 70% for 3 days, after which they were embedded in paraffin. From each testis, five sections of 5 \(\mu\)m were taken with an interval of 30 \(\mu\)m. These sections were stained with the periodic acid Schiff (PAS) reaction.

One hundred round tubular cross-sections were studied per section at 400\(\times\) magnification, resulting in a total of 500 cross sections per testis. The spermatogenesis in the seminiferous tubules was scored using a modification of the Johnsen method, as previously described [23,24]. Each tubular cross-section was given a score of 1–10, according to the presence or absence of the different spermatogenic cell types (Table 1). A mean score was calculated for each animal and for each group.

2.3. Odor study

Cotton cloths were placed in the right boxes of all hobs \((n = 21)\) 14 weeks after castration and placement of the implants. After two nights these cloths were collected and individually placed in a vacuum sealed bag. Each bag was randomly given a number. A test panel consisting of students, coworkers, and other volunteers \((n = 83)\), scored the smell from each cloth after opening the bag, smelling the cloth and resealing the bag. The bags were opened in random order and given a score between 1 and 5, whereby a score of 1 indicated no odor and a score of 5 indicated a very strong odor.

2.4. Statistics

Statistical analysis was performed using the statistical package SPSS for Windows (Version 12.0) and R (Version 2.2.0).

Table 1

<table>
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<th>Johnsen score Criteria</th>
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<td>No cells in tubular cross-section</td>
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<td>No germ cells but only sertoli cells present</td>
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<td>Spermatogonia are the only germ cells present</td>
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<td>Only few spermatocytes (&lt;3) and no spermatids or spermatozoa present</td>
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<td>No spermatozoa, no spermatids but several or many spermatocytes present</td>
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<td>No spermatozoa and only few spermatids (&lt;3–5) present</td>
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<td>No spermatozoa but many spermatids present</td>
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<td>Only few spermatozoa (&lt;5–10) present in a tubular cross-section</td>
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<td>Many spermatozoa present but germinal epithelium disorganized, with marked sloughing or obliteration of the lumen</td>
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<tr>
<td>Complete spermatogenesis with many spermatozoa. Germinal epithelium organized in a regular thickness leaving an open lumen, or stage V of the seminiferous cycle, with sufficient round spermatids</td>
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The correlation coefficient between the testis volume and plasma testosterone concentration was corrected for time and group.

Testis volumes and FSH concentrations were log transformed to better conform to a normal distribution and analyzed using a linear mixed-effect model. The fixed part of the model contained a group effect, a time effect and their interaction.

A random intercept was fitted for each individual together with an AR(1) correlation structure to model the dependence in time. For testis volume a random slope for time and different variance estimates for each of the three groups were fitted, and for FSH variance was allowed to differ for each group and for the first 5 time points, to capture the extra variation at the start of the experiment.

Since plasma testosterone concentrations were too often below the detection limit, transformation to conformity with the normal distribution was not possible. The nonparametric Kruskal–Wallis test was used at selected time points (10 March, 7 April, 14 April, 5 May, 23 June, 1 September, and 6 July 2006), where significant followed by Dunn post hoc tests to determine which groups differed from each other [25].

Differences in body odor between the three groups were tested by use of a $\chi^2$ analysis. In all instances significance was assumed at $P < 0.05$.

3. Results

Based on plasma testosterone concentrations, testis size and histological evaluation of the left testis (an arrest of spermatogenesis at the level of meioisis) we diagnosed one ferret from the placebo group with hypogonadism unrelated to this study. Based on these findings we excluded its data from the results.

3.1. Plasma testosterone and FSH concentrations

On 10 March, there were no significant differences in plasma testosterone concentrations between the three groups. On 7 April, control ferrets had significantly higher testosterone levels than castrated ferrets ($P < 0.01$), and ferrets from the deslorelin group ($P < 0.05$). From 14 April onwards both surgically and chemically castrated ferrets had significantly less testosterone than ferrets from the placebo group ($P < 0.01$). Surgically and chemically castrated ferrets did not differ significantly at any time (Fig. 1). On 6 July 2006 the surgically and chemically castrated ferrets still had significantly less testosterone (all concentrations: $<0.05$ nmol/L) than the ferrets which had received a placebo implant ($P < 0.01$; $n = 5$; range: 9–73 nmol/L; mean ± S.D.: 41 ± 29 nmol/L). The testosterone concentrations between the surgically and chemically castrated ferrets did not differ significantly from each other.

On 10 March, plasma FSH concentrations were similar among the groups. From 31 March until 1 September, chemically castrated ferrets had lower plasma FSH concentrations than those which had received a placebo implant. From 24 March until 1 September, surgically castrated ferrets had higher plasma FSH concentrations than those which had received a placebo implant (Fig. 2).

3.2. Testis volume

On 10 March, the testis volumes were similar for all groups. The testis volume from the chemically castrated ferrets were significantly smaller compared to those from the placebo group from 21 April onwards ($P < 0.01$; Fig. 3). On 6 July 2006 the testis volume in the deslorelin group ($n = 7$; range: 0.08–0.15 cm$^3$; mean ± S.D.: 0.10 ± 0.03 cm$^3$) was still significantly
smaller compared to the testis volume in the placebo group \((n = 5); \) range: 0.94–1.38 cm\(^3\); mean ± S.D.: 1.18 ± 0.18 cm\(^3\) \((P < 0.01)\).

The weight of the left testes from the ferrets in the placebo group \((n = 6); \) range: 1.3–2.4 g; mean ± S.D.: 2.0 ± 0.4 g) was significantly higher compared to the weight of the testes of the ferrets in the deslorelin group \((n = 7); \) range 0.2–0.5 g; mean ± S.D.: 0.3 ± 0.1 g).

A significant correlation was found between the volume of the testes and the plasma testosterone concentration \((P < 0.05)\).

### 3.3. Tissue histology

Since all stages of spermatogenesis were present in the testis of the ferrets from the placebo group we considered these testes to be normal (Fig. 4A and B). The mean (±S.E.M.) Johnsen score for this group is 8.8 ± 0.3. The diameter of the seminiferous tubules in the testes from the deslorelin group was much smaller than that from the placebo group (Fig. 4). The number of sertoli cells was comparable between groups, but in the deslorelin group no normal germ cells (spermatogonia and spermatocytes) could be found. The Johnsen score for the testes in the deslorelin group therefore resulted in a consistent 2.

### 3.4. Odor

The strongest odor was found on the cloths which had been placed in the night boxes of the ferrets from the placebo group (mean ± S.E.M.: 3.8 ± 0.2). This was followed by the odor on the cloths from the surgically castrated ferrets (mean ± S.E.M.: 3.1 ± 0.1) and those from the ferrets from the deslorelin group (mean ± S.E.M.: 2.7 ± 0.1). The differences between the deslorelin group, the placebo group and the surgically castrated group were all significant \(\chi^2 = 230.1; \) d.f. = 4 and \(\chi^2 = 31.6; \) d.f. = 4.

### 4. Discussion

The present data indicate that plasma testosterone concentrations, testis volume, spermatogenesis, and body odor in ferrets which received a deslorelin implant decreased to a level equal to, or even below those of surgically castrated ferrets.

Plasma testosterone concentrations and testis volume started to increase in April, which is approximately 1 month later than previously reported [26]. The decrease in testosterone concentrations and testis size started in July, which is also approximately 1 month later than previously reported. During both studies the ferrets were housed outdoors on approximately the same latitude. They should therefore have come into season at the same time. Different weather conditions may have contributed to this discrepancy between both studies. No conclusive evidence, however, can be given for this.

The positive correlation between testis volume and testosterone, as seen in our study, is in accordance with previous findings [26,27]. This correlation will be of practical use in the future when the implants will be used in a clinical setting. Owners of privately owned ferrets will know when it is time to replace the implant, once the testes size starts to increase.

Although no reliable plasma LH concentrations could be measured, plasma FSH concentrations were significantly lower in the deslorelin group compared to the surgically castrated and placebo groups. The combination of decreased FSH and testosterone concentrations in ferrets which had received deslorelin indicates that this depot GnRH agonist is capable of suppressing gonadotropin concentrations in ferrets. If hyperadrenocorticism is indeed caused by the increase of plasma LH concentrations after castration, the deslorelin implant may prevent the incidence of hyperadrenocorticism in ferrets.

The plasma concentrations of FSH in the castrated ferrets increased directly after castration and remained significantly higher than those of the intact ferrets and those with a deslorelin implant. This is similar to what has previously been described for plasma LH concentrations in castrated male ferrets [28]. The previous study, however, lasted only for 20 days while our study lasted 173 days. It is therefore likely that the increased concentrations of gonadotropins are not a temporary effect of castration.

Histological evaluation of the testes from the ferrets in the deslorelin group revealed that no normal germ
cells were found in any of the seminiferous tubules. Therefore these ferrets may be regarded as infertile. These findings are superior to what was previously reported for the use of continuous administration of GnRH agonists in mice and dogs [14,29]. In the latter reports, either still some spermatogenesis was seen, or sperm precursors were seen.

During the odor study, the smell of the ferrets from the deslorelin group was judged as the least intense. A possible explanation for the less amount of smell compared to the surgically castrated ferrets may be found in the fact that the adrenal androgen androstenedione, a known pheromone [30], in the latter group may be slightly higher due to stimulation by their elevated plasma gonadotropins. Measurement of androstenedione in the ferrets at different time points of this study (data not shown) was not able to support this hypothesis.

We conclude that the deslorelin implant effectively prevents reproduction and the musky odor of intact male ferrets and is therefore considered a suitable alternative for surgical castration in these animals. Further studies are necessary to determine the duration of efficacy of the deslorelin implants in ferrets when used as an alternative for surgical castration. It can be expected that due to the fact that plasma gonadotropin concentrations are decreased also the incidence of hyperadrenocorticism will be reduced. Long-term follow-up of deslorelin-implanted animals is necessary to confirm this.

Acknowledgments

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References


Case report

Luteinizing hormone-dependent Cushing’s syndrome in a pet ferret (Mustela putorius furo)

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Abstract

Hyperadrenocorticism in ferrets is associated with increased circulating concentrations of adrenal androgens, whereas plasma concentrations of cortisol and ACTH are usually not affected. Here, we report on a 5-year-old castrated male pet ferret (Mustela putorius furo) in which the major presenting signs were polyuria and polyphagia. Routine biochemistry values were within their reference ranges. The urinary corticoid:creatinine ratio (UCCR) was increased and the plasma ACTH concentration was suppressed. Abdominal ultrasonography revealed an enlarged right adrenal gland and atrophy of the left adrenal gland. Administration of hCG resulted in an increase of plasma cortisol and androstenedione concentrations. Based on these findings LH/hCG-dependent hypercortisolism and hyperandrogenism were suspected and treatment was started with a depot GnRH-agonist implant containing 9.4 mg deslorelin. Within 3 weeks after placement of the implant all clinical signs had disappeared. Three months later the endocrine parameters had normalized, while abdominal ultrasonography revealed that the right adrenal gland had diminished in size and the left adrenal gland was considered of normal size. No recurrences of clinical signs were seen within 2 years after placement of the deslorelin implant. At that time urinary corticoid and plasma hormone concentrations were within their reference ranges, and no further change in the size of the adrenal glands was seen. In conclusion, this is the first confirmed case of LH-dependent hypercortisolism in a ferret that was treated successfully with a depot GnRH-agonist.

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Keywords: Deslorelin; Hypercortisolism; Hyperandrogenism; ACTH; hCG stimulation test

1. Introduction

The adrenal cortex secretes mineralocorticoids, glucocorticoids, and androgens. Thus, in principle three distinct syndromes may arise in adrenocortical hyperfunction, i.e., hyperadrenocorticism: hyperaldosteronism, hypercortisolism, and hyperandrogenism.

Primary hyperaldosteronism or Conn’s syndrome was initially considered a rare condition. Recent studies, however, have shown that it is a common form of hyperadrenocorticism in cats [1,2]. The disease is also well-known in humans [3].

Hypercortisolism or Cushing’s syndrome is the most common form of hyperadrenocorticism in dogs [4] and also occurs frequently in humans [5,6]. In these species, hypercortisolism most frequently results from excessive secretion of adrenocorticotropic hormone (ACTH) by a pituitary adenoma [4–6]. Hypercortisolism may also
be ACTH-independent and in these cases is often due to excessive secretion of glucocorticoids by a benign or malignant adrenocortical tumor. However, ACTH-independent hypercortisolism may also occur as a result of expression of aberrant or overactive eutopic hormone receptors. In humans, various membrane-bound receptors, functionally coupled to steroidogenesis, have been reported, including gastric inhibitory polypeptide, catecholamine, vasopressin, serotonin and luteinizing hormone (LH) receptors [7,8].

LH-dependent hypercortisolism has been reported in several women [9,10]. In addition, LH-dependent hypercortisolism was suspected in a few women who developed Cushing’s syndrome during pregnancy with complete resolution of symptoms after parturition [11,12]. In all these cases there was ACTH-independent bilateral macronodular adrenal hyperplasia. In addition to LH-dependent hypercortisolism, virilizing and feminizing LH-dependent adrenal tumors have been reported in humans [13–15].

In neutered pet ferrets (Mustela putorius furo) hyperandrogenism is the most common form of hyperadrenocorticism. In this species, hyperandrogenism is characterized by unilateral or bilateral neoplasia or hyperplasia of adrenocortical tissue that expresses LH receptors [16]. These LH receptors are found in adrenal glands of healthy ferrets in the zona glomerulosa and zona fasciculata and are predominantly present in clear cells in the adrenal glands of ferrets with hyperadrenocorticism [16]. The LH receptors were considered to be only functional in the adrenal glands of hyperadrenocorticoic ferrets, because plasma concentrations of adrenal androgens only increase after intravenous injection of a gonadotropin releasing hormone (GnRH) agonist in the latter ferrets, while this was not the case in healthy ferrets [16]. Continuous stimulation of GnRH receptors leads to their desensitisation which subsequently results in a decrease in gonadotropin secretion [17]. The exact mechanism of the desensitisation is still not clear [18]. The decreased gonadotropin secretion explains why treatment of ferrets with hyperandrogenism with depot GnRH-agonists, such as leuprolide acetate or deslorelin, has been reported to be effective [19,20]. Stimulation of the adrenocortical LH receptors by the elevated circulating LH levels in neutered ferrets is associated with excessive secretion of steroids (androstenedione, 17α-hydroxyprogesterone, and/or estriol), leading to symmetrical alopecia, vulvar swelling in neutered female ferrets, and recurrence of sexual behavior after neutering in male ferrets [21–25]. This report represents the first case description of a ferret with LH-dependent hypercortisolism.

2. Materials and methods

2.1. Blood sampling and hCG stimulation test

Blood samples were collected under isoflurane anaesthesia from the cranial vena cava, immediately before and 60 min after an intramuscular injection of 100 IU of the LH-receptor agonist human chorionic gonadotropin (hCG) (Pregnyl®, Organon, Oss, The Netherlands). The animals were allowed to wake up in between blood collections. Blood was transferred to EDTA-coated tubes (ice-chilled for ACTH measurement). Plasma was separated by centrifugation at 4°C for 10 min and was then stored at −20°C pending analysis.

To be able to evaluate the results in the diseased ferret, a control group of healthy neutered ferrets was used. These ferrets were considered healthy on the basis of their medical history and physical examination. In eight healthy neutered ferrets, five 8-month-old females, one 3-year-old female, and two 3-year-old males, the effect of hCG stimulation on the plasma androstenedione concentration was studied. In seven healthy castrated male ferrets, 1–2 years of age, the basal plasma ACTH concentration and the effect of hCG stimulation on the plasma cortisol concentration were studied. The studies were approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University.

2.2. Hormone determination

Plasma androstenedione concentrations were measured by radioimmunoassay (RIA) as described previously [26]. 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 1.4, 4.8, and 11.8 nmol/l, respectively.

Plasma 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%.

Plasma ACTH concentrations were measured by a commercially available immunoradiometric assay (Nichols Institute, Wijchen, The Netherlands). The lower limit of detection was 1 ng/l and the interassay coefficient of variation was 7.8%.

Urinary corticoid concentrations were measured by RIA as described previously [27]. The lower limit of detection was 1 nmol/l and the intra- and interassay coefficients of variation were 6 and 8%, respectively. The urinary corticoid:creatinine ratio (UCCR) was calculated by dividing the urinary corticoid concentration by the urinary creatinine concentration.
2.3. Diagnostic imaging

Ultrasonography was performed, in the anesthetized ferret, with a high definition ultrasound system (HDI 3000, Advanced Technology Laboratories/Philips, Eindhoven, The Netherlands) equipped with a 38 mm long, 10–5 MHz broadband linear array transducer. The adrenal glands were visualized following a previously described protocol [28].

2.4. Statistical analysis

The Wilcoxon Signed Ranks Test was used to determine whether plasma cortisol concentrations increased in healthy ferrets after administration of hCG. Significance was assumed at \( P < 0.05 \).

3. Case report

A 5-year-old castrated male ferret (Mustela putorius furo), weighing 1030 g, was presented to the Division of Avian and Exotic Animal Medicine of the Department of Clinical Sciences of Companion Animals, Utrecht University because of polyuria, polydipsia, polyphagia, and exercise intolerance of 8 months duration. Except for some alopecia on the head, no abnormalities were found at physical examination. Plasma concentrations of urea, calcium, phosphate, glucose, total protein and the complete blood count were all within reference ranges.

Based upon the results of physical and biochemical examination, hypercortisolism was considered as possible diagnosis and thus the urinary corticoid excretion was investigated. The UCCR was clearly elevated (7.5 \( \times \) 10\(^{-6} \)), reference range in castrated ferrets < 2.1 \( \times \) 10\(^{-6} \) [29]). The plasma ACTH concentration was very low (<1 ng/l, reference range 13–100 ng/l [30]), indicating ACTH-independent hypercortisolism. Abdominal ultrasonography revealed a unilateral (right-sided) enlargement of the adrenal gland (dimensions: 10 mm \( \times \) 8 mm \( \times \) 8 mm), while the left adrenal gland could not be detected. Based on these results, the possibility of LH-dependent hypercortisolism due to expression of functional adrenocortical LH receptors was explored.

Administration of hCG resulted in an increase of the plasma cortisol concentration from 22 to 24 nmol/l was detected after hCG administration.

The basal plasma androstenedione concentration in the ferret with hypercortisolism was clearly increased (2.9 nmol/l, reference range 0.1–0.4 nmol/l [16]). Administration of hCG resulted in a plasma androstenedione concentration of 14 nmol/l. In the control ferrets, the plasma androstenedione concentrations were below the detection limit of the assay (0.1 nmol/l) both before and after hCG administration.

The LH/hCG-dependent hypercortisolism and hyperandrogenism were treated with a depot GnRH-agonist implant containing 9.4 mg deslorelin (Peptech Animal Health, North Ryde, New South Wales, Australia). After 2 months, the owner reported that the condition of the ferret had improved dramatically. The polydipsia, polyuria, and polyphagia had disappeared within 3 weeks after the implant had been placed, and the entire coat of the ferret had fully regrown and no alopecic areas were present anymore. Four months after placement of the deslorelin implant the ferret returned to our clinic with complete resolution of the previous clinical signs. The UCCR (0.6 \( \times \) 10\(^{-6} \)) was within its reference range, while the plasma ACTH concentration had increased considerably (358 ng/l). The plasma androstenedione concentration prior to hCG administration (<0.2 nmol/l) was also within its reference range. Plasma concentrations of cortisol and androstenedione still increased slightly after the administration of 100 IU of hCG (<1–12 and <0.2–0.45 nmol/l, respectively). Ultrasonography revealed that the right adrenal gland had diminished in size (dimensions: 10 mm \( \times \) 5 mm \( \times \) 6 mm), whereas the left adrenal gland could now be visualized and was considered of normal size (6 mm \( \times \) 3 mm \( \times \) 4 mm). Twenty months after placement of the deslorelin implant the ferret was still doing fine. The plasma androstenedione concentration prior to and after hCG administration were 0.4 nmol/l and 0.5 nmol/l, respectively. Ultrasonography revealed that both adrenal glands had hardly changed in size since the evaluation 16 months earlier (dimension right adrenal gland: 9 mm \( \times \) 5 mm \( \times \) 5 mm; left adrenal gland: 6 mm \( \times \) 3 mm \( \times \) 4 mm).

Two years after placement of the deslorelin implant, the owner suspected recurrence of hypercortisolism because the ferret seemed to drink a little bit more. To investigate whether the deslorelin implant was still active, the owner was asked to measure water intake of her ferret. In addition, urine was collected for urine analysis and determination of the UCCR, and blood was collected for measurement of plasma hormone determination and biochemistry. The ferret weighed 1030 g and consumed a total of 70–80 ml water per day. The
specific gravity of the urine was 1.021, indicating that the kidneys were able to concentrate the urine appropriately. UCCRs, measured on 2 consecutive days, were \(0.7 \times 10^{-6}\) and \(0.5 \times 10^{-6}\). Plasma concentrations of urea, calcium, phosphate, glucose, total protein and the complete blood count were all within their reference ranges. The plasma androstenedione concentration was \(<0.2\) nmol/l and the plasma ACTH concentration was 44 ng/l.

4. Discussion

This case report documents the occurrence of LH-dependent hypercortisolism in a ferret. Elevated cortisol secretion has been reported in a minority of ferrets with hyperandrogenism [23], but this is the first case in which it is shown that the hypercortisolism is LH-dependent. The diagnosis of LH-dependent hypercortisolism in our ferret was based on: (1) the clinical signs; (2) an increased UCCR, that returned to within reference values during treatment with a deslorelin implant; (3) enlargement of one adrenal gland and atrophy of the contralateral adrenal gland, which returned to normal size during treatment with a deslorelin implant; (4) an increase of the plasma cortisol concentration after administration of hCG, which was absent in healthy control ferrets; and (5) a suppressed plasma ACTH concentration which became non-suppressed during treatment with a deslorelin implant.

Although polyuria is sometimes observed in ferrets with hyperandrogenism, polyphagia and lethargy are not typical signs of a ferret with hyperandrogenism [31]. The combination of polyuria, polydipsia, polyphagia, exercise intolerance, and alopecia in our ferret is very similar to the clinical picture of dogs with hypercortisolism [4]. For this reason, hypercortisolism was suspected in our ferret.

Determination of urinary cortisol excretion is an important diagnostic tool in diagnosing hypercortisolism in many species [5,27,32]. Since the UCCR is a reflection of cortisol excretion [29], the elevated UCCR in our ferret pointed to hypercortisolism. The UCCR returned to within reference values during treatment with the deslorelin implant, consistent with the supposition that the increased cortisol secretion in our ferret was under control of gonadotropins.

Ultrasonographic examination of the adrenal glands in our ferret demonstrated unilateral enlargement of the right adrenal gland whereas the contralateral adrenal gland could not be detected. This was a striking finding, because in 85% of ferrets with hyperandrogenism it is a unilateral disorder in which there is no atrophy of the contralateral adrenal gland [22,24]. Although previous reports state that adrenal glands may be missed with ultrasonography [33], using a standard protocol we were able to detect all left adrenal glands in healthy ferrets and ferrets with hyperandrogenism [28]. It is therefore likely that the left adrenal gland was indeed atrophic.

The small size of the left adrenal gland can be explained by the LH-induced hypersecretion of glucocorticoids by the right adrenal cortex. Subsequently, the elevated circulating cortisol concentration resulted in suppression of pituitary ACTH secretion. The low circulating level of this tropic hormone resulted in atrophy of unaffected adrenocortical tissue. Plasma ACTH concentrations below the detection limit of the assay have also been reported in women with LH-dependent Cushing’s syndrome [9,10]. Plasma ACTH concentrations of ferrets with hyperandrogenism are usually similar to those of healthy ferrets [30]. However, some ferrets with hyperandrogenism do have plasma ACTH concentrations below the detection limit [30], suggesting that the co-occurrence of hypercortisolism and hyperandrogenism may occur more often in this species.

LH-dependent hypercortisolism has only been associated with bilateral macronodular adrenal hyperplasia in humans [9,10]. In contrast, we diagnosed a LH-dependent hypercortisolemia in a ferret due to a unilateral adrenal neoplasm. Although there may seem a discrepancy between the presentation in humans compared to our ferret, unilateral virilizing and feminizing LH-dependent adrenal tumors have also been reported in humans [13–15], while in ferrets unilateral LH-dependent hyperandrogenism is very common [16]. Why LH only results in unilateral adrenal growth in so many ferrets still remains to be elucidated. Since recurrence of hyperandrogenism and growth of the contralateral adrenal gland is seen frequently in hyperadrenocorticoid ferrets after unilateral adrenalectomy [24], it must be concluded that stimulation by LH may result in only a unilateral adrenal neoplasm as seen in the ferret with LH-dependent hypercortisolism.

Treatment with deslorelin resulted in a significant increase of the plasma ACTH concentration to a value above the reference range. Because ACTH is released in a pulsatile fashion, this high ACTH value may be explained by blood sampling at the time of an ACTH pulse. It may also be that the recovery from the long-term suppression of the hypothalamic-pituitary-adrenocortical axis by the LH-induced hypersecretion of cortisol was still incomplete. After disappearance of the hypersecretion of cortisol, first the hypothalamic-pituitary axis recovers and finally the glucocorticoid-producing capability of the adrenal
cortex comes back. If the normal adrenocortical tissue is still unable to produce enough glucocorticoids, the low circulating glucocorticoid level will result in increased ACTH release by the already normally functioning hypothalamic-pituitary axis. Indeed, the basal plasma cortisol concentration and UCCR were low at this time point. On the other hand, the left adrenal gland could be visualized again, suggesting that the tropic effect of ACTH had already resulted in cell growth in the zona fasciculata and zona reticularis. Two years after the start of treatment the plasma ACTH concentration was within the reference range.

Decrease of size of the affected adrenal gland during treatment with a GnRH-agonist has not been reported previously. In a woman with LH-dependent hypercortisolism who was treated with the depot GnRH-agonist leuprolide acetate, both adrenal glands remained enlarged while clinical signs disappeared [9]. In ferrets with hyperandrogenism, no publications are available on the ultrasonographic evaluation of the adrenal glands during treatment with a GnRH-agonist. Wagner et al. [20], however, reported that in one-third of the ferrets treated with the GnRH-agonist deslorelin large tumors could be palpated after the disease had relapsed, suggesting continued growth of the tumor during treatment [20].

In neutered ferrets, LH-dependent hyperandrogenism is the most common form of hyperadrenocorticism [16,23]. Also in our ferret hypercortisolism was accompanied by hyperandrogenism. Basal plasma androstenedione concentrations are commonly increased in ferrets with hyperandrogenism [23]. Moreover, stimulation with hCG results in a further increase of the plasma androstenedione concentration in ferrets with hyperandrogenism [16]. Also in our ferret, plasma androstenedione concentration was increased prior to and after hCG administration, whereas this was not the case in healthy ferrets.

Administration of hCG resulted not only in an increase of the plasma androstenedione concentration but also in the plasma cortisol concentration in our ferret, indicating LH-dependent hypercortisolism. As may be expected, hCG did not result in stimulation of cortisol secretion in the healthy control ferrets. An increase of the plasma cortisol concentration after administration of hCG has also been reported in women with LH-dependent Cushing’s syndrome [9,10]. In these women, however, plasma androstenedione concentrations were not measured.

Treatment of ferrets with LH-dependent hyperandrogenism with the depot GnRH-agonists leuprolide acetate and/or deslorelin has been shown to be very effective [19,20]. Clinical signs in the latter ferrets return after the efficacy of the agonist has worn out [19,20], demonstrating that in these ferrets the disease is indeed LH-dependent. Also, in our ferret the deslorelin implant resulted in disappearance of the hypercortisolism and the hyperandrogenism. The longevity of the activity of the deslorelin implant in our case, however, did not allow us to confirm that the clinical signs would indeed reappear after the activity of the implant had worn out. The longevity of the implant, however, was chosen based on the only published report on the use of deslorelin for the treatment of hyperandrogenism in ferrets. In this report the implant contained 3 mg deslorelin, and was found to be effective for a period ranging from 8 to 20 months [20]. It has been shown that increasing the amount of deslorelin in the implant, increases the longevity of its activity [34]. In order to provide an effective treatment period as long as possible, we therefore decided to use an implant with 9.4 mg deslorelin in our ferret. Indeed, signs of hyperadrenocorticism were absent for more than 2 years.

In conclusion, this is the first confirmed case of LH-dependent hypercortisolism in a ferret that was treated successfully with a depot GnRH-agonist.

Acknowledgment

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References


Caring for your ferret

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Caring for your ferret

GENERAL INFORMATION
The popularity of ferrets as household pets is growing rapidly. They are intelligent and curious animals that can sleep for a large period of the day. However, when they are awake, they tend to be very active and need stimulation to occupy their time. A ferret can live for an average of eight to 10 years so will require a high level of commitment and care.

HOUSING A FERRET
A ferret requires a secure area where it can sleep and exercise. This is normally referred to as a court or a cub. A court is a larger enclosure with a private area where the ferret (or ferrets) will sleep, as well an extended area for exercise. The exercise area should incorporate tubes and platforms that your ferret can climb and explore. A cub is a smaller secure enclosure which provides your ferret with a sleeping area and limited exercise space. A cub is acceptable if you plan on taking your ferret out to exercise in a separate area such as your house or garden.

Whichever you choose, you will need to ensure your ferret has a secure, weatherproof and dark area where it can sleep as this is what it will do for most of the day. Commercial bedding is available from various sources; however, it is not advisable to bed your ferret on shredded paper or straw, as this offers little insulation and can lead to health problems.
Caring for your ferret

FEEDING YOUR FERRET
Ferrets will eat on demand so a supply of food should be readily available in your ferret’s enclosure. The ideal means of doing this is with a complete biscuit food from a trusted supplier. Your ferret can also be fed a supplement of raw or cooked meat such as chicken or rabbit. However, this may not always be suitable in warmer weather when fresh meat can go off quickly. Treats such as egg yolk, cod liver oil and commercial ferret snacks can be given to your ferret in small amounts, but not as an alternative to a balanced diet.

A ferret should also have a continuous supply of fresh water. Ferrets will drink throughout the day, and during periods of play will be seen returning for water at various intervals.

‘FERRET-PROOFING’ YOUR HOME AND GARDEN
As ferrets can live both indoors and outdoors, it is wise to ensure the area that they will be playing in is free from hazards and also secure to stop them escaping. A ferret is a naturally curious animal; if there is a hole then it will want to get into it, if there is a shelf then it will want to know what is on it!

Around the home there are lots of things to take into account when preparing for your ferret’s playtime. Cables and breakable items should all be moved away from where your
Caring for your ferret

Your ferret is going to play. Any gaps in furniture or flooring should be blocked or sealed, no matter how small. You may think ‘a ferret will never get in there’ but if you have thought about it then so has your ferret, and it will find a way.

Your ferret will like to dig and burrow so ensure your garden is extremely secure. You can always create a smaller pen or area to exercise your ferret while in the garden; however, ensure that it is not in direct sunlight and has adequate shade.

TOILET TRAINING

When your ferret is in its court or cub you will notice that it will probably go to the toilet in the same area – ferrets are creatures of habit. This behaviour can be used to your benefit if you allow your ferret to roam around your home. Placing a litter tray or cleanable surface in this area will make tidying up after your ferret as simple as possible. Should your ferret take to going to the toilet in different places, it would be advisable to allow it to go to the toilet before taking it from the enclosure and also to return it regularly to give it opportunities to use the litter.

While your ferret is in its court or cub, it is fine to put wood shavings in the toilet area to absorb moisture and ensure that the area smells fresh. It is not advisable to use
sawdust in the sleeping area as this can be very dusty and may also cause irritation to a ferret’s mouth and nose when asleep.

PLAYTIME
When you interact with your ferret you will find it to be a great addition to your family. It’s likely to race around looking to involve you (plus other ferrets) with games of hide-and-seek. It will chase you as well as stopping and expecting to be chased in return. Ferrets benefit greatly from having areas to investigate and hide in so the addition of tubes and cloth tunnels all give a ferret the means to exercise as well as areas where it can curl up and have a sleep if it all gets a bit too much.

BREEDING AND NEUTERING
A female ferret (jill) will come into season in the springtime and again later in the year, normally around late summer or into the autumn. Jills physically change by displaying a swelling around the genital area and will remain in this advanced state until they are either mated with by a male (hob) or brought out of season by a hormone supressant called a ‘jill-jab’.

The actual action of mating between ferrets is a very physical process and normally results in the hob using its teeth to grip the jill around the back of the neck.
This can result in bleeding and trauma and there is the possibility this may cause life-threatening injuries to the jill. Those who are new to breeding are advised to seek close supervision of an experienced person or vet.

If you don’t intend to breed from your ferrets, get them neutered as soon as possible. A ferret (either male or female) can be neutered from six months old. Removing the testes of the hob will help to calm its temperament, as well as removing the majority of the musky smell that hobs can produce. Neutering in jills is more important as they can suffer from anaemia or other potential infections due to the genital swelling, if allowed to remain in season.

**HEALTHCARE**

As ferrets are very active animals (when they are awake) they can injure themselves while they play. It is always wise to supervise the playtime of your ferrets so that you can closely monitor their wellbeing. Should you be worried about your ferret’s health then it is important that you seek advice from your vet.

You can also make healthcare part of the daily or weekly routine for your ferret. Keep a log of its size, condition and weight. Keep this information somewhere safe so in
the event that you do need to take your ferret to the vet then you have information about your ferret which may help the vet in caring for your animal.

Ferrets are very clean animals and will spend a good portion of their time awake cleaning themselves. As ferrets are very susceptible to ear mites, it may be necessary for you to assist them in regularly checking their ears and cleaning them with a piece of cottonwool dipped in warm water. Never insert or poke anything into the ear of your ferret; do not use cotton buds or anything that goes into the ear canal. You will also need to regularly clip your ferret’s claws. With some experience the clipping of claws is an easy action to master. However, for the first few attempts your vet will be able to help you and show you what is needed.

Veterinary check-ups

It is advisable to take your ferret for a routine check-up each year. Your vet will be familiar with what a healthy ferret looks like and they will be able to give the best care to your animal, as well as keeping your mind at rest.
Caring for your ferret

Common ferret questions

Q Do ferrets bite?
A Every animal has the ability to bite but as long as ferrets are well cared for and handled properly then they have no reason to bite.

Q Do ferrets live better as a group?
A Some ferrets prefer to live as individuals and these ferrets will require greater interaction with their owners to keep them occupied and stimulated. Ferrets which are kept together have the advantage of keeping each other company and having another ferret to play with.

Q Are ferrets expensive and difficult to keep?
A Keeping a ferret is no more complicated or costly than keeping a rabbit or a guinea pig.

Q I have heard of people taking ferrets for walks. Is this true?
A Ferrets love exercise and taking a ferret for a walk on a lead and a harness is very good for it. Ferrets are at risk from Canine Distemper so if you do plan on taking your ferret for a walk get it vaccinated beforehand.
Comparative vaginal cytology of the estrous cycle of black-footed ferrets (*Mustela nigripes*), Siberian polecats (*M. eversmanni*), and domestic ferrets (*M. putorius furo*)

Elizabeth S. Williams, E. Tom Thorne, Donald R. Kwiatkowski, Kim Lutz, Sandy L. Anderson

**Abstract.** Vaginal cytology and vulva size were used to characterize the reproductive cycle of female black-footed ferrets (*Mustela nigripes*), Siberian polecats (*M. eversmanni*), and domestic ferrets (*M. putorius furo*). Emphasis was on black-footed ferrets because of the need to breed these critically endangered animals and on Siberian polecats because of the close taxonomic relationship to black-footed ferrets. Vaginal cytology of the 3 species of ferret is similar. Proestrus was characterized by an increasing percentage of superficial epithelial cells and enlargement of the vulva. During estrus, superficial cells were usually ≥ 90% of epithelial cells in the vaginal lavage and after several days were fully keratinized. Neutrophils were more common during all stages of the estrous cycle in domestic ferrets than they were in the other species. Following copulation, percentage of superficial cells in the vagina declined and vulva swelling subsided. Large cells, probably of uterine symplasma origin, were observed in vaginal lavages following whelping or pseudopregnancy. Vaginal cytology is extremely useful in the reproductive management of black-footed ferrets and Siberian polecats. Knowledge of normal vaginal cytology could be applied to the diagnosis of female reproductive abnormalities in all 3 species.

The subgenus *Putorius* or polecat group of the family Mustelidae includes black-footed ferrets (*Mustela nigripes*), the Siberian or steppe polecat (*M. eversmanni*), and the European polecat (*M. putorius*), from which the domestic ferret (*M. putorius furo*) was probably derived. Black-footed ferrets and Siberian polecats are closely related and interbreed in captivity (unpublished observations).

Black-footed ferrets are among the most endangered mammalian species, and they are not known to exist in the wild. A colony was established for captive propagation in 1985-1986 at the Wyoming Game and Fish Department’s Sybille Wildlife Research and Conservation Education Unit near Laramie, Wyoming. Siberian polecats are used in fur production, and some Asian subspecies are considered endangered. We used Siberian polecats and domestic ferrets to develop safe and effective reproductive management techniques for black-footed ferrets. Domestic ferrets are commonly used in biomedical research and are popular household pets. Veterinary practitioners and diagnostic laboratories should be aware of the normal biology of this species and of ways to assess the reproductive status of these animals.

Ovulation in domestic ferrets, polecats, and black-footed ferrets occurs following copulation; these animals are considered induced ovulators. Following copulation and ovulation, ferrets and polecats either become pregnant or experience pseudopregnancy. Some animals cycle out of estrus without ovulation and thus do not experience true metestrus or diestrus as part of the estrous cycle. Domestic ferret females that are not bred remain in heat for prolonged periods of time, which may lead to estrogenic bone marrow depression.

Vaginal cytology is commonly used to detect estrus in some domestic species, especially dogs, and has been described in domestic ferrets and mink (*M. vison*). Vaginal cytology has been partially described in Siberian polecats and black-footed ferrets. The purpose of this paper is to describe normal vaginal cytology in 3 species of ferrets, with emphasis on the use of this technique in the management of an endangered species, the black-footed ferret.

**Materials and methods**

**Animals**

Reproductive cycles of 10 young (≤ 1 year of age) and 16 adult (> 1 year of age) female black-footed ferrets, 5 young (≤ 1 year of age) and 16 adult-to-aged female Siberian polecats, and 7 adult female domestic ferrets were studied. Black-footed ferrets were studied from December 1986 to June 1988, Siberian polecats were studied from June 1986 to June 1988, and domestic ferrets were studied from August 1987 to April 1988.
Vaginal cytology of ferrets

1988, and domestic ferrets were studied from June 1986 to May 1987. Husbandry of black-footed ferrets has been described (Thorne ET: 1987, Res Proj Segment Job NASWSM2550, pp. 58-77, Wyo Game Fish Dep, Cheyenne, WY; Thorne ET, Kwiatkowski DR: 1988, Res Proj Segment Job NASWSM2550, pp. 65-72, Wyo Game Fish Dep, Cheyenne WY). Siberian polecats were housed in painted wooden cages with vinyl-clad woven-wire fronts and tops and were fed commercial mink food, dry cat food, and canned cat food supplemented weekly with whole mice or hamsters. Fluorescent lighting was adjusted to the ambient natural photoperiod approximately weekly. Domestic ferrets were housed in stainless steel cages and were fed dry cat food, and fluorescent lighting was adjusted to 16 hr light: 8 hr dark. Domestic ferrets were not bred but were treated with hCG (100 IU) to induce ovulation after prolonged estrus as part of another study.

Cytology

Black-footed ferrets resisted manual restraint; therefore, to minimize stress they were handled in small tubular cages (Thorne ET: 1987, Res Proj Segment Job NASWSM2550, pp. 58-77, Wyo Game Fish Dep, Cheyenne WY). Siberian polecats and domestic ferrets were tractable and sampling was usually accomplished by 1 person with manual restraint.

Vaginal lavages were obtained using sterile plastic 1-ml syringes and plastic 100-μl pipette tips that had tip edges slightly rounded by heat and the hub cut to fit on the syringe. The tip was gently inserted approximately 1.0-1.5 cm into the vagina until it met slight resistance, and 0.05-0.1 ml sterile physiologic saline was flushed and aspirated several times. Contents of the syringe were expelled onto a clean glass slide, sprayed with cytologic fixative, and allowed to air dry. Papanicolaou-stained vaginal lavages were viewed by light microscopy at 200x magnification. A few lavages in 1986 were stained using Wright’s stain. Two hundred epithelial cells from each lavage were categorized and counted, fields to count were chosen in areas where cells were abundant and evenly distributed. Vaginal epithelial cells were categorized as parabasal, intermediate, superficial intermediate, or superficial cells (Fig. 1). Subjective assessment of numbers of neutrophils, erythrocytes, and bacteria (numerous = 4, many = 3, few = 2, rare = 1, none = 0) was made for each lavage. Vulval measurements were taken using a plastic ruler. Maximum length and width of the labia were
addition for analysis. Vaginal lavages and vulva measurements were conducted periodically during anestrus and postbreeding and weekly to daily during proestrus and estrus.

Anestrus was defined as the period of reproductive quiescence when percentage of superficial cells in the vaginal lavage was generally minimum and copulations did not occur. Proestrus was defined as the period of progressive increase in percentage of superficial cells in vaginal lavages and increase in size and intumescence of the vulva, and copulations did not occur. Estrus was defined as the period of ≥90% superficial cells in most vaginal lavages and maximum vulva size and corresponded to the time of breeding.

**Results**

**Black-footed ferrets**

Vaginal epithelial cells were morphologically similar to those described in dogs17 (Fig. 1a-1c). Parabasal cells were rarely observed. Occasionally, parabasal and intermediate cells were vacuolated and/or contained neutrophils within the cytoplasm (Fig. 1a). With Papanicolaou stain, superficial intermediate cells were basophilic (blue, green) or acidophilic (pale pink to red). The superficial cells were large with angular cytoplasmic margins and degenerative nuclear changes consisting of pyknosis or rhexis, or absence of nuclei (Fig. 1c). During anestrus and proestrus, superficial cells were either basophilic or acidophilic and were rarely orangeophilic (indicating keratinization). Staining of superficial cells changed as estrus progressed. For the first 3-5 days of estrus, superficial cells showed mixed staining properties (basophilic, acidophilic, and orangeophilic) and contained degenerated nuclei. After 4-6 days, lavages contained highly keratinized, generally anucleate cells. Papanicolaou stain demonstrated these changes much more clearly than did Wright’s stain.

Changes in vaginal cell types and vulva measurements are shown in Table 1 and Figs. 2 and 3. Most vaginal epithelial cells from black-footed ferrets in anestrus were intermediate and superficial intermediate cells. Fluctuation in percentage of superficial cells during anestrus occurred in most adult black-footed ferrets as they approached proestrus, especially in 1987 (Fig. 2). Fluctuations were not as pronounced in young anestrus females as compared with adults (Fig. 2). Neutrophils were often present in vaginal lavages but seldom in large numbers. Bacteria were rare in vaginal lavages from anestrus females.

A mixture of epithelial cell types was present in vaginal lavages collected during proestrus, including all cell types observed during anestrus. In most individuals, neutrophils increased slightly during proestrus. Changes

<table>
<thead>
<tr>
<th>Species</th>
<th>Anestrus % superficial cells, range</th>
<th>Vulva size (mm), range</th>
<th>Proestrus % superficial cells, range</th>
<th>Vulva size (mm), range</th>
<th>Estrus % superficial cells, range</th>
<th>Vulva size (mm), range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black-footed ferrets</td>
<td>≤40*, 1-81</td>
<td>3-13</td>
<td>3-88</td>
<td>5-19</td>
<td>≥90‡, 75-100</td>
<td>15-23</td>
</tr>
<tr>
<td>Siberian polecat</td>
<td>≥30‡, 0-57</td>
<td>2-16</td>
<td>1-86</td>
<td>6-22</td>
<td>≥90*, 53-100</td>
<td>11-39</td>
</tr>
<tr>
<td>Domestic ferrets</td>
<td>≤30‡, 1-36</td>
<td>5-16</td>
<td>15-87</td>
<td>11-18</td>
<td>≥90‡, 71-100</td>
<td>17-33</td>
</tr>
</tbody>
</table>

* 83% of lavages <40%.
‡ 96% of lavages <30%.
§ 98% of lavages <30%.
‖ 93% of lavages ≥90%.
¶ 68% of lavages ≥90%.
† 76% of lavages ≥90%.

Figure 2. Mean percentage of superficial epithelial cells in vaginal lavages from adult and young black-footed ferrets in 1987. Day 0 indicates day of copulation.
in percentage of parabasal, intermediate, and superficial intermediate cells were not useful for determining the start of proestrus. Bacteria (large bacilli) increased slightly during proestrus and were always closely associated with superficial cells (Fig. 1c). Vulva size increased during proestrus. Duration of proestrus was approximately 2-3 weeks.  

Percentage of superficial cells in vaginal lavages during estrus was usually ≥90%; occasional lavages of <90% superficial cells contained numerous highly keratinized superficial cells and scattered intermediate cells. Because of the highly keratinized nature of the superficial cells, these samples were easily differentiated from those of proestrus. Neutrophils were present in variable numbers during estrus. Erythrocytes were not observed in any lavage from an estrous female. Large bacilli were often associated with superficial cells. Vulva size and turgidity of the lips was maximum during estrus. Estrus in a few unbred females was approximately 30-40 days.**

Percentage of superficial cells in vaginal lavages declined below 70% 4-10 days following copulation and induced ovulation. Cells in postbreeding samples were highly keratinized for several days until there was abrupt appearance of nonkeratinized superficial, superficial intermediate, and intermediate epithelial cells (Figs. 2, 3). Within several days, superficial cells declined in number to anestrus levels. Vulvas decreased in size and turgidity 3-9 days following first copulation. Vaginal lavages taken 45-50 days postbreeding often contained scattered large (100-150 µm) epithelial cells with basophilic cytoplasm and large nuclei (50-75 µm) with clumped chromatin (Fig. 1d).

**Siberian polecats**

Morphology and dynamics of vaginal epithelial cells in Siberian polecats were similar to those in black-footed ferrets. Changes in cell types in vaginal lavages and vulval measurements are shown in Table 1 and Figure 4. Anestrous lavages generally contained <30% superficial cells, except those from aged females, which occasionally contained 40-50% superficial cells. Neutrophils were numerous, but bacteria were rare during anestrus and proestrus. Periods of proestrus varied from 7 to 35 days with most from 2 to 3 weeks in duration. Date of onset and duration of estrus was most irregular.
in old females. Mean duration of estrus in unbred females was 41 ± 27 days (range 4->97 days, n = 9). Bacteria were observed in most lavages from estrous females and were especially prominent in some females in prolonged estrus. Erythrocytes were only observed in lavages from 1 female; she subsequently died of uterine neoplasia. Large epithelial cells, similar to those observed in black-footed ferrets, were also observed in Siberian polecats following pseudopregnancy or pregnancy.

Vulval swelling increased during proestrus and was maximal during estrus. Following copulation and induced ovulation, the swelling of the vulva decreased within 3-7 days.

**Domestic ferrets**

Morphology and dynamics of epithelial cells in vaginal lavages were similar to those in the other species of ferrets (Table 1, Fig. 5). One female cycled out of estrus after 56 days. Others experienced estrus >120 days: all ovulated following hCG treatment. Neutrophils were very common in vaginal lavages during all stages of the estrous cycle. Bacteria were uncommon except during estrus and then were associated only with superficial cells. Numerous bacteria, neutrophils, cellular debris, and some erythrocytes were observed in a few females that experienced prolonged estrus.

**Discussion**

Vaginal cytology was successfully used to monitor the reproductive status of females in 3 species of ferrets. Use of this technique allowed successful breeding of black-footed ferrets in captivity and could be applied to other endangered polecat species or domestic ferrets. Although swelling of the vulva during estrus is an easily observed, noninvasive technique for detection of estrus in ferrets, cytology allows greater precision in the management of breeding. Extrapolation of these techniques should be done with care because cytology may not be reliable in some species of mustelids.

Fluctuations in percentage of superficial cells in the vagina during anestrus was more pronounced in black-footed ferrets than in the other polecat species and in older black-footed ferrets and polecats than in younger animals. These fluctuations may be associated with periods of follicular development prior to onset of the breeding season. The occurrence of these fluctuations could be confusing during the early phases of the breeding season and indicate the need for repeated vaginal lavages over time. Duration of proestrus was approx-
Vaginal cytology of ferrets

Figure 5. Mean percentage of superficial epithelial cells, neutrophils, and bacteria in vaginal lavages and mean vulval measurements from domestic ferrets in 1986-1987. Numbers of bacteria and neutrophils were judged subjectively as numerous = 4, many = 3, few = 2, rare = 1, none = 0. Domestic ferrets were not bred. Day 0 indicates first day of estrus.

Imately 2-3 weeks in all species. Superficial cells in vaginal lavages from animals in estrus was usually ≥90%, which is consistent with that observed in domestic dogs. More variability in percentage of superficial cells in lavages during estrus was apparent in domestic ferrets and Siberian polecats, possibly because of prolonged estrous periods with fluctuation in estrogen production.

Swelling of the vulva during proestrus and estrus occurred in all species. Vulval swelling was much greater in Siberian polecats and domestic ferrets as compared with black-footed ferrets, as had been previously observed. The reason for this difference is not known.

All ferrets may have a prolonged estrous period if not bred. Duration of estrus in unbred black-footed ferrets and Siberian polecats is similar and lasts approximately 30-40 days. Domestic ferrets that are not bred or treated with hCG may experience estrogenic bone marrow depression as a result of months of estrus. Bone marrow depression has not been recognized in black-footed ferrets or Siberian polecats.

Neutrophils were generally more common during all phases of the estrous cycle in domestic ferrets and polecats as compared with black-footed ferrets. The cause for this difference among the species is not known. Numbers of neutrophils were not directly correlated to the presence or abundance of bacteria. The presence of neutrophils in vaginal lavages is normal in all species of ferret and, in the absence of bacteria within neutrophils and other signs of inflammation, does not indicate vaginitis. Bacteria were rare except during estrus and were associated with superficial cells rather than neutrophils. These large bacilli are common during estrus in other species. Erythrocytes were rarely observed in vaginal lavages from any of the animals. A uterine neoplasm accounted for the presence of erythrocytes in an aged Siberian polecat. Erythrocytes have been described from vaginal smears from black-footed ferrets in estrus; the presence of these cells may have resulted from the use of manual restraint and swabs to obtain vaginal cells, which may be more traumatic to edematous mucosa than is lavage.

The large epithelial cells present in vaginal lavages several days postwhelping or postpseudopregnancy could be confused with neoplastic cells. These cells are probably derived from the uterine symplasma formed during pregnancy or pseudopregnancy in ferrets. The characteristics of these cells and the recent reproductive history of the ferret should allow for differentiation from neoplasia.

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Sources and manufacturers

a. Provided by US Fish and Wildlife Service; the Birmingham, Brookfield, Minnesota, Lincoln Park, and Moscow zoos; or bred in our facilities.
c. National Complete Mink Food Pellets, New Holstein, WI.
d. Purina ProPlan, Ralston Purina Co., St. Louis, MO.
e. Kal Kan Liver and Beef Dinner, Kal Kan Foods, Vernon, CA.
f. Sigma Chemical Co., St. Louis, MO.
g. Eppendorf, Brinkmann Instruments, Westbury, NY.
h. Spray-cyte, Clay Adams, Becton-Dickinson and Co., Parsippany, NJ.

References

26. Wyoming Game and Fish Department: 1987, A strategic plan for the management of black-footed ferrets in Wyoming, pp. 4-59. Wyoming Game and Fish Department, Cheyenne, WY.
La dysendocrinie la plus fréquente du Furet domestique castré est la "maladie surrénaïlienne" (ou AAE = Adrenal Associated Endocrinopathy). Cette dénomination doit désormais remplacer l'expression "syndrome de Cushing du Furet", car il ne s'agit pas d'une hyperproduction de glucocorticoïdes, mais d'autres stéroïdes sexuels par les surrénales des furets castrés.

La maladie surrénaïlienne du Furet

La prévalence de la maladie surrénaïlienne varie considérablement avec les continents : 0,55 % en Europe, contre 20 à 25 % aux États-Unis. En effet, en Europe, les furets sont encore majoritairement castrés à quelques mois, alors qu'aux États-Unis, la moyenne d'âge de la castration est de 6 semaines. Les furets atteints sont les sujets castrés, mâles autant que femelles, avec un intervalle moyen de 3,5 ans entre la castration et l'apparition des signes cliniques. La maladie surrénaîlienne est une maladie de l'animal âgé.

Trois signes cliniques dominent

Les trois signes cliniques les plus fréquents sont une alopecie bilatérale symétrique (65 % des cas), une léthargie (65 %), une atrophie musculaire (65 %), l'ensemble pouvant s'accompagner d'un prurit (< 30 %).

L'alopécie débute par la queue, prenant un aspect de "queue de rat" (PHOTO 1), s'étend aux lombes (PHOTO 2), peut régresser et réapparaître. Ces lésions ressemblent à l'alopécie saisonnière du Furet entier.

Chez la femelle castrée, il peut exister (dans 50 % des cas environ) une hypertrichose vulvaire, plus ou moins accompagnée de pertes mucöuses (PHOTO 3). Si la furette est jeune, on peut confondre ces signes avec un hyperestrogénisme (femelle entière) ou une rémanence ovarienne (femelle castrée).
Chez le mâle castré, on peut observer un comportement sexuel (15% des cas), une agressivité soudaine (10%) et/ou des signes urinaires (5% de dysurie ou strangurie) [1].

Photo 3. Hypertrophie vulvaire chez une furettes castrée atteinte d'une maladie surrenaliennne.

Photo 2. Alopécie de la queue et du tronc dans un cas plus évolué.

Une pathogénie complexe, encore mal élucidée

L'hyperproduction de stéroïdes surrenaliens est due à la sensibilité de cette glande aux gonadotrophines (LH = lutéotropine hormone et FSH = folliculostimuline hormone), qui sont aussi anormalement sécrétées pour au moins trois raisons [2] (FIGURE 1):

- Une castration précoce qui empêche la répression hypothalamo-hypophysaire naturelle de la sécrétion des gonadotrophines ;
- Une augmentation de la durée d'éclairement quotidienne due à la domestication (par rapport à la vie sauvage) ;
- Une forte sécrétion de leptine par le tissu adipeux (en raison d'un apport énergétique trop abondant). La leptine est un stimulant de la sécrétion de GnRH (gonadotrophin releasing hormone) par l'hypothalamus.

Ces trois facteurs seraient à l'origine d'une augmentation de la synthèse de GnRH, et donc de LH et de FSH.

Sous l'influence des gonadotrophines, les cellules surrenaliennes se multiplient et synthétisent divers stéroïdes sexuels en excès, dont la 17-hydroxyprogestérone et l'androsténédione. L'androsténédione est aromatisée en estrone au sein du tissu adipeux et du foie, puis réduite par le foie ou les surrenales en estradiol.

La toxicité des stéroïdes est variable.

- La 17-hydroxyprogestérone, comme tous les progestagènes, est particulièrement hétérophoxique.
- Les estrogènes perturbent l'apoptose et altèrent le système cardiovasculaire entraînant, à terme, une action délétère sur de nombreux organes (foie, rein, systèmes nerveux et immunitaire).

Leurs effets conjugués, associés à ceux de l'androsténédione, sont particulièrement visibles sur la sphère génitale avec une tuméfaction vulvaire (mais aussi éventuellement mammaire), une hyperplasie utérine et, chez le mâle une hyperplasie prostatique à l'origine de troubles urinaires. Le risque carcinogène (toujours organes) est considérablement accru.
Mise en évidence par échographie de la suspicion clinique

Une glande surrenale est anormale si elle est d'une épaisseur supérieure à 4 mm, arrondie ou présentant des bosses ou des points de minéralisation [3] (PHOTO 4).

L'examen de l'appareil reproducteur, tout particulièrement le moignon utérin ou la prostate, peut aussi être intéressant.

Les anomalies visibles ne sont bilatérales que dans 20 % des cas (la glande gauche est la plus fréquemment atteinte).

Il s'agit le plus souvent d'adénome et d'hyperplasie nodulaire (les adénocarcinomes sont plus rares).

La certitude : le diagnostic biologique

Pour pouvoir confirmer facilement une maladie surrenaliennne chez un furet castré, il faut mettre en évidence l'hyperscrétion de stéroïdes sexuels : estradiol, 17-hydroxyprogestérone et / ou androstènedione.

En l'absence d'études publiées, les valeurs de référence sont souvent celles proposées par l'Université du Tennessee. Mais les valeurs peuvent varier avec les techniques et, comme pour toute hormone, sont susceptibles d'osciller de façon importante tout à fait physiologiquement [6].

Tableau 1. Valeurs de référence des stéroïdes chez le Furet sain (données LDH).

<table>
<thead>
<tr>
<th>Stéroïde dosé</th>
<th>Furet &lt; 2 ans</th>
<th>Furet &gt; 2 ans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol basal ou post ACTH en pmol/L</td>
<td>&lt; 60</td>
<td>&lt; 65</td>
</tr>
<tr>
<td>17-OHprogestérone basal en nmol/L</td>
<td>&lt; 4</td>
<td></td>
</tr>
<tr>
<td>17-OHprogestérone post ACTH en nmol/L</td>
<td>&lt; 8</td>
<td></td>
</tr>
<tr>
<td>Androstènedione basale (nmol/L)</td>
<td>&lt; 10</td>
<td>&lt; 12</td>
</tr>
<tr>
<td>Androstènedione post ACTH (nmol/L)</td>
<td>&lt; 15</td>
<td>&lt; 17</td>
</tr>
<tr>
<td>Cortisol basale (nmol/L)</td>
<td>&lt; 300</td>
<td>&lt; 400</td>
</tr>
<tr>
<td>Cortisol post ACTH (nmol/L)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On remarquera que, même si les animaux sont apparemment sains, la production de stéroïdes augmente progressivement avec l'âge.

C'est pourquoi, une étude a été entreprise par le Laboratoire des Dosages Hormonaux de l'ENVN (LDH), pour déterminer les valeurs physiologiques et pathologiques des stéroïdes surrenaliens utilisables en France en routine (TABLEAU 1), au cours d'un test de stimulation à l'ACTH (Synacthène ® [HI] SC, 0,08 mg/animal).

L'ACTH présente l'avantage de stimuler la production de tous les stéroïdes par les surrenales, qu'ils soient glucocorticoïdes ou sexuels, puisqu'ils font partie de la même voie métabolique (FIGURE 2).

En pratique, le test le plus sensible et le plus spécifique de la maladie surrenaliennne est un prélèvement unique après stimulation par l'ACTH (75 à 90 minutes...
plus tard), avec dosages simultanés de l’estriodiol, de la 17-hydroxyprogésterone et de l’androsténédione.

Le prélèvement sanguin peut être réalisé sous anesthésie flash pour obtenir au moins 1,5 ml de sang à chaque fois (PHOTO 5).

Chez les animaux sains, la production de stéroïdes augmente progressivement avec l’âge [7].

La sévérité de cette maladie peut-être appréciable par dosage des stéroïdes sexuels : l’estriodiolémie basale est souvent précocement augmentée, mais pour un bilan correct, il est préférable de réaliser une stimulation par l’ACTH et de mesurer en plus la 17-hydroxyprogésterone et l’androsténédione.

PHOTO 5. Ponction à la veine cave crâniale chez un furet anesthésié à l’Isoflurane.

**Bien choisir le traitement**

La surrénaléctomie est à préférer en cas de tumeur

La technique de surrénaléctomie unilatérale (PHOTO 6), lors d’anomalie anatomoique surrénalienne avérée, est proche de celle pratiquée chez le Chien [4]. Il est important de surveiller l’équilibre hydrométral en post-opératoire, afin d’évaluer l’intérêt de l’administration de corticoïdes, qui doit être le plus faible possible.

La résection complète de la glande droite est délicate en raison de sa proximité avec la veine cave caudale.

La cryochirurgie est possible sur des nodules tumoraux de diamètre inférieur à 2 cm.

La régression des symptômes se ferait en un mois dans trois quarts des cas.

La surrénaléctomie bilatérale est déconseillée en raison de la lourdeur du traitement post-opératoire.

Les traitements médicaux sont intéressants en cas d’hyperplasie

En cas d’hyperplasie bilatérale, ils peuvent être efficaces, mais il n’existe pas de protocoles validés (ENCADRE 1).

Il existe deux types de molécules : celles qui inhibent la sécrétion de gonadotrophines comme des analogues de la GnRHa [5], de la mélatonine ou des androgènes, et celles qui inhibent directement l’activité surrénalienne :

- **Les analogues de la GnRHa comme l’acétate de leuprolide, (Lupron ® [H]), mais son prix est prohibitif ;**

- **L’acétate de deslorelin**, sous forme d’implant contraceptif pour Chien, possède une AMM européenne (Suprelora ®). Il est également possible d’utiliser le pamoate de triptoreline forme retard (Decapeptyl ® [H]), mais qui reste très coûteux (PHOTOS 7A, 7B, 7C) ;

- **La testostérone peut être employée sous forme retard (Androtardyl ® [H]). Elle présente l’avantage d’avoir à moyen terme un effet positif sur la peau, mais elle risque aussi d’exacerber la libido des mâles ;**

- **Le mélatonine devrait être administrée quotidiennement pour être efficace (la dose serait de 0,5 mg/animal).**

PHOTOS 7A, 7B, 7C. Traitement par le Decapeptyl LP ® [H]. Avant traitement (a), après 1 mois de traitement (b) et guérison après 4 mois de traitement (c).
Encadré 1. Propositions de traitement médical de la maladie surrénaliennne du Furet

- **Hypersécrétion de gonadotrophines**
  - Jeune animal (male ou femelle de moins de 3 ans) :
    - Androtardyl® [H] (éthanoate de testostérone) : 0,2 mg/100 g SC - 2 fois à 3 semaines d’intervalle - (utiliser des seringues à insuline). À renouveler tous les trimestres si efficacité du traitement.
  - Animal de tout âge :
    - Decapeptyl LP® [H] 3 mg (pamoate de triptortéline) : 0,2 mg (0,1 mL)/furet à aliquer et conglérer après reconstitution - injection mensuelle SC - (utiliser des seringues à insuline).

- **Adénome ou adénocarcinome**

  Protocoles non dépourvus de risques - À entreprendre que si les signes cliniques sont sérieux - (essai de traitement pendant 4 semaines avec contrôle de la cortisoolémie et de l’activité ALT avant l’instauration du traitement).
  - Triostane : Votoryl® : 2 mg/kg/j (gélules de 10 mg, à reconditionner). Risque d’hypocortisémie certain s’il n’y a pas d’hypercortisémie.

- **Les antistéroïdiques majeurs** comme le triostane (Votoryl®), médicament vétérinaire, pourraient être particulièrement intéressants en cas de tumeur surrénaliennne avérée, mais présentent le risque d’entraîner un hypocorticotisme, puisqu’il n’existe généralement pas d’hypercortisémie ;

- **Les antistéroïdiques spécifiques** (comme le tamoxifène) semblent sans intérêt, puisque plusieurs stéroïdes peuvent être anormalement produits en même temps.

### POINTS FORTS

- Les conditions de vie du Furet domestique (castration, augmentation du temps d’activité et excès de nourriture) conduisent à provoquer une sécrétion forte et permanente de gonadotrophines qui stimulent anormalement la corticosurrénale.
- Les surrénales des furets malades produisent surtout des hormones sexuelles (17-OHprogestérone et androsténédione, et estradiol) qui sont très élevées.
- L’hypersécrétion d’estriadiol permanente est toxique pour la plupart des organismes et affecte l’état général en quelques mois.
- La moitié des femelles atteintes présente des signes gonadiques d’activité sexuelle.
- Mâles et femelles présentent ces déplications qui débutent sur la queue et se poursuivent sur le dos.
- L’échographie des surrénales est très utile.
- Le dosage de 17-OHprogestérone, androsténédione et estradiol permet le diagnostic.
- La surrénalectomie est le traitement de choix en cas de tumeur avérée.
- Un traitement par un analogue de la GnRH peut donner d’excellents résultats.
- Il est recommandé de proposer aux furets “un nid” qui leur permet de se protéger de l’éclairage et de contrôler leur alimentation pour qu’ils restent sveltes.

### À LIRE...


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Case report

Luteinizing hormone-dependent Cushing’s syndrome in a pet ferret (Mustela putorius furo)

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Abstract

Hyperadrenocorticism in ferrets is associated with increased circulating concentrations of adrenal androgens, whereas plasma concentrations of cortisol and ACTH are usually not affected. Here, we report on a 5-year-old castrated male pet ferret (Mustela putorius furo) in which the major presenting signs were polyuria and polyphagia. Routine biochemistry values were within their reference ranges. The urinary corticoid:creatinine ratio (UCCR) was increased and the plasma ACTH concentration was suppressed. Abdominal ultrasonography revealed an enlarged right adrenal gland and atrophy of the left adrenal gland. Administration of hCG resulted in an increase of plasma cortisol and androstenedione concentrations. Based on these findings LH/hCG-dependent hypercortisolism and hyperandrogenism were suspected and treatment was started with a depot GnRH-agonist implant containing 9.4 mg deslorelin. Within 3 weeks after placement of the implant all clinical signs had disappeared. Three months later the endocrine parameters had normalized, while abdominal ultrasonography revealed that the right adrenal gland had diminished in size and the left adrenal gland was considered of normal size. No recurrences of clinical signs were seen within 2 years after placement of the deslorelin implant. At that time urinary corticoid and plasma hormone concentrations were within their reference ranges, and no further change in the size of the adrenal glands was seen. In conclusion, this is the first confirmed case of LH-dependent hypercortisolism in a ferret that was treated successfully with a depot GnRH-agonist.

1. Introduction

The adrenal cortex secretes mineralocorticoids, glucocorticoids, and androgens. Thus, in principle three distinct syndromes may arise in adrenocortical hyperfunction, i.e., hyperadrenocorticism: hyperaldosteronism, hypercortisolism, and hyperandrogenism. Primary hyperaldosteronism or Conn’s syndrome was initially considered a rare condition. Recent studies, however, have shown that it is a common form of hyperadrenocorticism in cats [1,2]. The disease is also well-known in humans [3]. Hypercortisolism or Cushing’s syndrome is the most common form of hyperadrenocorticism in dogs [4] and also occurs frequently in humans [5,6]. In these species, hypercortisolism most frequently results from excessive secretion of adrenocorticotropic hormone (ACTH) by a pituitary adenoma [4–6]. Hypercortisolism may also

Keywords: Deslorelin; Hypercortisolism; Hyperandrogenism; ACTH; hCG stimulation test
be ACTH-independent and in these cases is often due to excessive secretion of glucocorticoids by a benign or malignant adrenocortical tumor. However, ACTH-independent hypercortisolism may also occur as a result of expression of aberrant or overactive eutopic hormone receptors. In humans, various membrane-bound receptors, functionally coupled to steroidogenesis, have been reported, including gastric inhibitory polypeptide, catecholamine, vasopressin, serotonin and luteinizing hormone (LH) receptors [7,8].

LH-dependent hypercortisolism has been reported in several women [9,10]. In addition, LH-dependent hypercortisolism was suspected in a few women who developed Cushing’s syndrome during pregnancy with complete resolution of symptoms after parturition [11,12]. In all these cases there was ACTH-independent bilateral macronodular adrenal hyperplasia. In addition to LH-dependent hypercortisolism, virilizing and feminizing LH-dependent adrenal tumors have been reported in humans [13–15].

In neutered pet ferrets (Mustela putorius furo) hyperandrogenism is the most common form of hyperadrenocorticism. In this species, hyperandrogenism is characterized by unilateral or bilateral neoplasia or hyperplasia of adrenocortical tissue that expresses LH receptors [16]. These LH receptors are found in adrenal glands of healthy ferrets in the zona glomerulosa and zona fasciculata and are predominantly present in clear cells in the adrenal glands of ferrets with hyperadrenocorticism [16]. The LH receptors were considered to be only functional in the adrenal glands of hyperadrenocorticotoid ferrets, because plasma concentrations of adrenal androgens only increase after intravenous injection of a gonadotropin releasing hormone (GnRH) agonist in the latter ferrets, while this was not the case in healthy ferrets [16]. Continuous stimulation of GnRH receptors leads to their desensitisation which subsequently results in a decrease in gonadotropin secretion [17]. The exact mechanism of the desensitisation is still not clear [18]. The decreased gonadotropin secretion explains why treatment of ferrets with hyperandrogenism with depot GnRH-agonists, such as leuprolide acetate or deslorelin, has been reported to be effective [19,20]. Stimulation of the adrenocortical LH receptors by the elevated circulating LH levels in neutered ferrets is associated with excessive secretion of steroids (androstenedione, 17α-hydroxyprogesterone, and/or estradiol), leading to symmetrical alopecia, vulvar swelling in neutered female ferrets, and recurrence of sexual behavior after neutering in male ferrets [21–25]. This report represents the first case description of a ferret with LH-dependent hypercortisolism.

2. Materials and methods

2.1. Blood sampling and hCG stimulation test

Blood samples were collected under isoflurane anaesthesia from the cranial vena cava, immediately before and 60 min after an intramuscular injection of 100 IU of the LH-receptor agonist human chorionic gonadotropin (hCG) (Pregnyl®, Organon, Oss, The Netherlands). The animals were allowed to wake up in between blood collections. Blood was transferred to EDTA-coated tubes (ice-chilled for ACTH measurement). Plasma was separated by centrifugation at 4°C for 10 min and was then stored at −20°C pending analysis.

To be able to evaluate the results in the diseased ferret, a control group of healthy neutered ferrets was used. These ferrets were considered healthy on the basis of their medical history and physical examination. In eight healthy neutered ferrets, five 8-month-old females, one 3-year-old female, and two 3-year-old males, the effect of hCG stimulation on the plasma androstenedione concentration was studied. In seven healthy castrated male ferrets, 1–2 years of age, the basal plasma ACTH concentration and the effect of hCG stimulation on the plasma cortisol concentration were studied. The studies were approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University.

2.2. Hormone determination

Plasma androstenedione concentrations were measured by radioimmunoassay (RIA) as described previously [26]. 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 1.4, 4.8, and 11.8 nmol/l, respectively. Plasma 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%.

Plasma ACTH concentrations were measured by a commercially available immunoradiometric assay (Nichols Institute, Wijchen, The Netherlands). The lower limit of detection was 1 ng/l and the interassay coefficient of variation was 7.8%.

Urinary corticoid concentrations were measured by RIA as described previously [27]. The lower limit of detection was 1 nmol/l and the intra- and interassay coefficients of variation were 6 and 8%, respectively. The urinary corticoid:creatinine ratio (UCCR) was calculated by dividing the urinary corticoid concentration by the urinary creatinine concentration.
2.3. Diagnostic imaging

Ultrasonography was performed, in the anesthetized ferret, with a high definition ultrasound system (HDI 3000, Advanced Technology Laboratories/Philips, Eindhoven, The Netherlands) equipped with a 38 mm long, 10–5 MHz broadband linear array transducer. The adrenal glands were visualized following a previously described protocol [28].

2.4. Statistical analysis

The Wilcoxon Signed Ranks Test was used to determine whether plasma cortisol concentrations increased in healthy ferrets after administration of hCG. Significance was assumed at P < 0.05.

3. Case report

A 5-year-old castrated male ferret (Mustela putorius furo), weighing 1030 g, was presented to the Division of Avian and Exotic Animal Medicine of the Department of Clinical Sciences of Companion Animals, Utrecht University because of polyuria, polydipsia, polyphagia, and exercise intolerance of 8 months duration. Except for some alopecia on the head, no abnormalities were found at physical examination. Plasma concentrations of urea, calcium, phosphate, glucose, total protein and the complete blood count were all within reference ranges.

Based upon the results of physical and biochemical examination, hypercortisolism was considered as possible diagnosis and thus the urinary corticoid excretion was investigated. The UCCR was clearly elevated (7.5 × 10⁻⁶, reference range in castrated ferrets < 2.1 × 10⁻⁶ [29]). The plasma ACTH concentration was very low (<1 ng/l, reference range 13–100 ng/l [30]), indicating ACTH-independent hypercortisolism. Abdominal ultrasonography revealed a unilateral (right-sided) enlargement of the adrenal gland (dimensions: 10 mm × 8 mm × 8 mm), while the left adrenal gland could not be detected. Based on these results, the possibility of LH-dependent hypercortisolism due to expression of functional adrenocortical LH receptors was explored.

Administration of hCG resulted in an increase of the plasma cortisol concentration (from 22 to 24 nmol/l) was detected after hCG administration.

The basal plasma androstenedione concentration in the ferret with hypercortisolism was clearly increased (2.9 nmol/l, reference range 0.1–0.4 nmol/l [16]). Administration of hCG resulted in a plasma androstenedione concentration of 14 nmol/l. In the control ferrets, the plasma androstenedione concentrations were below the detection limit of the assay (0.1 nmol/l) both before and after hCG administration.

The LH/hCG-dependent hypercortisolism and hyperandrogenism were treated with a depot GnRH-agonist implant containing 9.4 mg deslorelin (Peptech Animal Health, North Ryde, New South Wales, Australia). After 2 months, the owner reported that the condition of the ferret had improved dramatically. The polydipsia, polyuria, and polyphagia had disappeared within 3 weeks after the implant had been placed, and the entire coat of the ferret had fully regrown and no alopecic areas were present anymore. Four months after placement of the deslorelin implant the ferret returned to our clinic with complete resolution of the previous clinical signs. The UCCR (0.6 × 10⁻⁶) was within its reference range, while the plasma ACTH concentration had increased considerably (358 ng/l). The plasma androstenedione concentration prior to hCG administration (<0.2 nmol/l) was also within its reference range. Plasma concentrations of cortisol and androstenedione still increased slightly after the administration of 100 IU of hCG (<1–12 and <0.2–0.45 nmol/l, respectively). Ultrasonography revealed that the right adrenal gland had diminished in size (dimensions: 10 mm × 5 mm × 6 mm), whereas the left adrenal gland could now be visualized and was considered of normal size (6 mm × 3 mm × 4 mm). Twenty months after placement of the deslorelin implant the ferret was still doing fine. The plasma androstenedione concentration prior to and after hCG administration were 0.4 nmol/l and 0.5 nmol/l, respectively. Ultrasonography revealed that both adrenal glands had hardly changed in size since the evaluation 16 months earlier (dimension right adrenal gland: 9 mm × 5 mm × 5 mm; left adrenal gland: 6 mm × 3 mm × 4 mm).

Two years after placement of the deslorelin implant, the owner suspected recurrence of hypercortisolism because the ferret seemed to drink a little bit more. To investigate whether the deslorelin implant was still active, the owner was asked to measure water intake of her ferret. In addition, urine was collected for urine analysis and determination of the UCCR, and blood was collected for measurement of plasma hormone determination and biochemistry. The ferret weighed 1030 g and consumed a total of 70–80 ml water per day. The
specific gravity of the urine was 1.021, indicating that the kidneys were able to concentrate the urine appropriately. UCCRs, measured on 2 consecutive days, were $0.7 \times 10^{-6}$ and $0.5 \times 10^{-6}$. Plasma concentrations of urea, calcium, phosphate, glucose, total protein and the complete blood count were all within their reference ranges. The plasma androstenedione concentration was $<0.2$ nmol/l and the plasma ACTH concentration was 44 ng/l.

4. Discussion

This case report documents the occurrence of LH-dependent hypercortisolism in a ferret. Elevated cortisol secretion has been reported in a minority of ferrets with hyperandrogenism [23], but this is the first case in which it is shown that the hypercortisolism is LH-dependent. The diagnosis of LH-dependent hypercortisolism in our ferret was based on: (1) the clinical signs; (2) an increased UCCR, that returned to within reference values during treatment with a deslorelin implant; (3) enlargement of one adrenal gland and atrophy of the contralateral adrenal gland, which returned to normal size during treatment with a deslorelin implant; (4) an increase of the plasma cortisol concentration after administration of hCG, which was absent in healthy control ferrets; and (5) a suppressed plasma ACTH concentration which became non-suppressed during treatment with a deslorelin implant.

Although polyuria is sometimes observed in ferrets with hyperandrogenism, polyphagia and lethargy are not typical signs of a ferret with hyperandrogenism [31]. The combination of polyuria, polydipsia, polyphagia, exercise intolerance, and alopecia in our ferret is very similar to the clinical picture of dogs with hypercortisolism [4]. For this reason, hypercortisolism was suspected in our ferret.

Determination of urinary cortisol excretion is an important diagnostic tool in diagnosing hypercortisolism in many species [5,27,32]. Since the UCCR is a reflection of cortisol excretion [29], the elevated UCCR in our ferret pointed to hypercortisolism. The UCCR returned to within reference values during treatment with the deslorelin implant, consistent with the supposition that the increased cortisol secretion in our ferret was under control of gonadotropins.

Ultrasonographic examination of the adrenal glands in our ferret demonstrated unilateral enlargement of the right adrenal gland whereas the contralateral adrenal gland could not be detected. This was a striking finding, because in 85% of ferrets with hyperandrogenism it is a unilateral disorder in which there is no atrophy of the contralateral adrenal gland [22,24]. Although previous reports state that adrenal glands may be missed with ultrasonography [33], using a standard protocol we were able to detect all left adrenal glands in healthy ferrets and ferrets with hyperandrogenism [28]. It is therefore likely that the left adrenal gland was indeed atrophic.

The small size of the left adrenal gland can be explained by the LH-induced hypersecretion of glucocorticoids by the right adrenal cortex. Subsequently, the elevated circulating cortisol concentration resulted in suppression of pituitary ACTH secretion. The low circulating level of this tropic hormone resulted in atrophy of unaffected adrenocortical tissue. Plasma ACTH concentrations below the detection limit of the assay have also been reported in women with LH-dependent Cushing’s syndrome [9,10]. Plasma ACTH concentrations of ferrets with hyperandrogenism are usually similar to those of healthy ferrets [30]. However, some ferrets with hyperandrogenism do have plasma ACTH concentrations below the detection limit [30], suggesting that the co-occurrence of hypercortisolism and hyperandrogenism may occur more often in this species.

LH-dependent hypercortisolism has only been associated with bilateral macronodular adrenal hyperplasia in humans [9,10]. In contrast, we diagnosed a LH-dependent hypercortisolism in a ferret due to a unilateral adrenal neoplasm. Although there may seem a discrepancy between the presentation in humans compared to our ferret, unilateral virilizing and feminizing LH-dependent adrenal tumors have also been reported in humans [13–15], while in ferrets unilateral LH-dependent hyperandrogenism is very common [16]. Why LH only results in unilateral adrenal growth in so many ferrets still remains to be elucidated. Since recurrence of hyperandrogenism and growth of the contralateral adrenal gland is seen frequently in hyperadrenocorticoid ferrets after unilateral adrenalectomy [24], it must be concluded that stimulation by LH may result in only a unilateral adrenal neoplasm as seen in the ferret with LH-dependent hypercortisolism.

Treatment with deslorelin resulted in a significant increase of the plasma ACTH concentration to a value above the reference range. Because ACTH is released in a pulsatile fashion, this high ACTH value may be explained by blood sampling at the time of an ACTH pulse. It may also be that the recovery from the long-term suppression of the hypothalamic-pituitary-adrenocortical axis by the LH-induced hypersecretion of cortisol was still incomplete. After disappearance of the hypersecretion of cortisol, first the hypothalamic-pituitary axis recovers and finally the glucocorticoid-producing capability of the adrenal
cortex comes back. If the normal adrenocortical tissue is still unable to produce enough glucocorticoids, the low circulating glucocorticoid level will result in increased ACTH release by the already normally functioning hypothalamic-pituitary axis. Indeed, the basal plasma cortisol concentration and UCCR were low at this time point. On the other hand, the left adrenal gland could be visualized again, suggesting that the tropic effect of ACTH had already resulted in cell growth in the zona fasciculata and zona reticularis. Two years after the start of treatment the plasma ACTH concentration was within the reference range.

Decrease of size of the affected adrenal gland during treatment with a GnRH-agonist has not been reported previously. In a woman with LH-dependent hypercortisolism who was treated with the depot GnRH-agonist leuprolide acetate, both adrenal glands remained enlarged while clinical signs disappeared [9]. In ferrets with hyperandrogenism, no publications are available on the ultrasonographic evaluation of the adrenal glands during treatment with a GnRH-agonist. Wagner et al. [20], however, reported that in one-third of the ferrets treated with the GnRH-agonist deslorelin large tumors could be palpated after the disease had relapsed, suggesting continued growth of the tumor during treatment [20].

In neutered ferrets, LH-dependent hyperandrogenism is the most common form of hyperadrenocorticism [16,23]. Also in our ferret hypercortisolism was accompanied by hyperandrogenism. Basal plasma androstenedione concentrations are commonly increased in ferrets with hyperandrogenism [23]. Moreover, stimulation with hCG results in a further increase of the plasma androstenedione concentration in ferrets with hyperandrogenism [16]. Also in our ferret, plasma androstenedione concentration was increased prior to and after hCG administration, whereas this was not the case in healthy ferrets.

Administration of hCG resulted not only in an increase of the plasma androstenedione concentration but also in the plasma cortisol concentration in our ferret, indicating LH-dependent hypercortisolism. As may be expected, hCG did not result in stimulation of cortisol secretion in the healthy control ferrets. An increase of the plasma cortisol concentration after administration of hCG has also been reported in women with LH-dependent Cushings syndrome [9,10]. In these women, however, plasma androstenedione concentrations were not measured.

Treatment of ferrets with LH-dependent hyperandrogenism with the depot GnRH-agonists leuprolide acetate and/or deslorelin has been shown to be very effective [19,20]. Clinical signs in the latter ferrets return after the efficacy of the agonist has worn out [19,20], demonstrating that in these ferrets the disease is indeed LH-dependent. Also, in our ferret the deslorelin implant resulted in disappearance of the hypercortisolism and the hyperandrogenism. The longevity of the activity of the deslorelin implant in our case, however, did not allow us to confirm that the clinical signs would indeed reappear after the activity of the implant had worn out. The longevity of the implant, however, was chosen based on the only published report on the use of deslorelin for the treatment of hyperandrogenism in ferrets. In this report the implant contained 3 mg deslorelin, and was found to be effective for a period ranging from 8 to 20 months [20]. It has been shown that increasing the amount of deslorelin in the implant, increases the longevity of its activity [34]. In order to provide an effective treatment period as long as possible, we therefore decided to use an implant with 9.4 mg deslorelin in our ferret. Indeed, signs of hyperadrenocorticism were absent for more than 2 years.

In conclusion, this is the first confirmed case of LH-dependent hypercortisolism in a ferret that was treated successfully with a depot GnRH-agonist.

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References


Adrenal disease can refer to changes to the adrenal cortex and/or to the adrenal medulla. The most common form of adrenal medulla pathology is a pheochromocytoma. These rare tumors are usually much larger than tumors of the adrenal cortex and can remain unnoticed for a long time. Although cases have been reported where pheochromocytomas have been diagnosed based on histologic characteristics of the adrenal tumor, measurement of urinary metanefrine is necessary to confirm the diagnosis. To the author’s knowledge this type of confirmation has not yet been performed in ferrets. The most common form of adrenal disease in ferrets is hyperadrenocorticism, also referred to as adrenocortical disease, in which the adrenal cortex is affected. The outermost layer of the adrenal cortex is the zona glomerulosa, which produces mineralocorticoids (primarily aldosterone). The zona fasciculata consists of an outer and inner part, and produces glucocorticoids (cortisol and corticosterone) and androgens. The most interior zone is the zona reticularis, which is extremely variable in its prominence and cellular composition. This zone contains the smallest cells of the adrenal cortex and produces primarily androgens. Thus, in principle, three distinct syndromes may arise in adrenocortical hyperfunction: hyperaldosteronism, hypercortisolism, and hyperandrogenism.

Primary hyperaldosteronism or Conn’s syndrome is the most common form of hyperadrenocorticism in cats, usually due to excessive secretion of mineralocorticoids by an adrenocortical neoplasia or bilateral adrenocortical hyperplasia. The exact pathogenesis of primary hyperaldosteronism remains to be elucidated.

Hypercortisolism or Cushing’s syndrome is the most common form of hyperadrenocorticism in dogs, and also occurs frequently in humans. In these species, hypercortisolism most frequently results from excessive secretion of adrenocorticotropic hormone (ACTH) by a pituitary adenoma. ACTH-independent hypercortisolism may be due to excessive secretion of glucocorticoids by a benign or malignant adrenocortical tumor. However, ACTH-independent hypercortisolism may also occur as a result of expression of aberrant or overactive eutopic hormone receptors. In humans, various membrane-bound receptors, functionally coupled to steroidogenesis, have been reported, including gastric inhibitory polypeptide, catecholamine, vasopressin, serotonin, and luteinizing hormone (LH) receptors. LH-dependent hypercortisolism has been reported in several women. In addition to LH-dependent hypercortisolism, virilizing and feminizing LH-dependent adrenal tumors have been reported in humans.

HYPERADRENOCORTICISM

In neutered pet ferrets hyperandrogenism is the most common form of hyperadrenocorticism. In ferrets, plasma androstenedione, 17-hydroxyprogesterone and estradiol concentrations are increased. It has been reported that approximately 85% of ferrets with hyperadrenocorticism have enlargement of one adrenal gland without atrophy of the contralateral adrenal gland. In the other 15% of cases bilateral enlargement is present. After surgical removal of a unilateral adrenal tumor, the disease commonly recurs due to involvement of the contralateral adrenal gland. The adrenal glands have been histologically classified as (nodular) hyperplasia, adenoma and adenocarcinoma. The histologic diagnosis, however, does not provide information on functionality of the tumor, nor does it provide any prognostic information. No relationship has been found between pituitary and adrenal tumors in ferrets. At this stage, pituitary tumors should be regarded as incidental findings.

Different etiologies have been suggested for the high occurrence of hyperadrenocorticism in ferrets. These include (early) neutering of ferrets, housing ferrets indoors, and genetic background.

In recent years, evidence has been gathered that increased concentrations of gonadotropins, which occur after neutering (due to the loss of negative feedback), stimulate the adrenal cortex, eventually leading to an adrenocortical neoplasm.

- First, the initial signs of hyperadrenocorticism occur only during the breeding season, when plasma concentrations of gonadotrophic hormones are high.
- Second, in the US, where the neutering of ferrets is common practice, hyperadrenocorticism is common, whereas hyperadrenocorticism is seldom diagnosed in the United Kingdom, where ferrets are usually not surgically castrated.
- Third, a significant correlation has been found between the age at neutering and age at onset of hyperadrenocorticism.
- Fourth, the depot gonadotropin-releasing hormone (GnRH)-agonists leuprolide acetate and deslorelin have been used successfully in the treatment of this disease. 
- Finally, luteinizing hormone (LH) receptors have been detected in the adrenal cortex of ferrets. These receptors are considered to be functional, because plasma concentrations of adrenal androgens increase after intravenous injection of a GnRH agonist.

There remains debate, however, if the neutering has to take place at an early age for this disease to occur. In the US, ferrets are commonly neutered at an age of 6 weeks. In the Netherlands, however, most pet ferrets are neutered between 6 and 12 months of age. Since the prevalence of hyperadrenocorticism in Dutch ferrets is approximately 0.55% (95% confidence interval: 0.2–1.1%), it is likely that this disease is just as common in the Netherlands as it is in the US. The age at which...
ferrets are neutered is therefore not likely to have an influence in the development of these tumors.

The hypothesis that ferrets that are being kept indoors have a higher chance of developing hyperadrenocorticism compared with ferrets housed outdoors is in line with the above mentioned hypothesis. Ferrets that are kept indoors will be more under the influence of light—and thus gonadotropins—than ferrets that are housed outdoors. This applies to neutered as well as intact ferrets. The fact that adrenal gland disease is less common in the United Kingdom can therefore be explained by the fact that many ferrets are still being kept outdoors without being neutered.

A genetic background can play a role in the etiology of this disease as well. In the US, a specific breeding facility, which provides an estimated 80% of all American ferrets, has been blamed for the high occurrence of hyperadrenocorticism in American ferrets. If this claim would be accurate, than why is the prevalence of hyperadrenocorticism so high in the Netherlands, where ferrets do not have the same genetic background as ferrets from this facility? Although the breeding facility cannot be blamed for the high incidence of hyperadrenocorticism in ferrets, this does not mean that a genetic background for the disease is not possible. In humans three different hereditary syndromes have been recognized in which multiple endocrine neoplasms are seen (MEN1, MEN2a and MEN2b). Since insulinomas and adrenal gland tumors are frequently seen simultaneously in ferrets, a condition similar to MEN in humans may exist. Research at the University of California, Davis is in progress to determine if this is indeed the case.

Clinical signs of hyperadrenocorticism in ferrets include symmetrical alopecia, vulvar swelling in neutered female ferrets, recurrence of sexual behavior after neutering, urinary blockage in males (due to peri-prostatic or peri-urethral cysts), occasional mammary gland enlargement in female ferrets, and pruritus. The skin is usually not affected, although some excoriations may be seen. Alopecia usually begins in spring, which coincides with the start of the breeding season, and may disappear without treatment. The next year the alopecia usually returns after which it usually does not resolve spontaneously at the end of the breeding season. Polyuria and polydipsia are reported in ferrets with hyperadrenocorticism. It is not clear, however, whether adrenal hormone production is responsible for these signs, or if these (elderly) ferrets have concurrent kidney disease. A case of LH-dependent hypercortisolism (Cushing’s disease) has been diagnosed by the author. The major complaint in this ferret was severe PU/PD. Only a minimal amount of alopecia was found in this ferret. The diagnosis in this case was confirmed by a plasma ACTH-concentration which was below the detection limit, an increased urinary corticoid creatinine ratio, and a rise of the plasma cortisol concentration after the administration of human chorionic gonadotropin (hCG). During ultrasonographic examination an enlarged right adrenal gland was detected while the left adrenal gland could not be located. Polyuria and polydipsia resolved within 3 weeks after the administration of a deslorelin implant (see later). Plasma and urinary hormones had returned to normal 3 months after the initial diagnosis. It was striking to find that the right adrenal gland had diminished in size, while the left adrenal gland now had a normal appearance, suggesting that the left adrenal gland was initially atrophic. Two years after diagnosis the ferret is still doing fine without recurrence of symptoms.

When considering predisposing factors, age appears to be an important factor, which is in its turn linked to age at neutering (as mentioned earlier). In the US, diagnosis of hyperadrenocorticism in ferrets is already possible at an age of 2 years. In the Netherlands, however, most cases of hyperadrenocorticism are seen in ferrets older than 3 years of age. Although initial reports suggested that the majority of ferrets with adrenocortical disease were females, a Dutch study could not confirm this sex predilection. In the author’s practice there is actually a tendency of seeing more male than female ferrets with hyperadrenocorticism.

The most important differential diagnoses for a ferret with signs of hyperadrenocorticism are a non-ovariectomized female or a ferret with active remnant ovaries. Severe alopecia and pruritus in a ferret, however, has also been seen due to a food allergy. Hormone analysis in blood and urine, as well as an abdominal ultrasound could not confirm the presence of a hyperfunctioning adrenal gland in this case. All signs in this ferret resolved after it had been converted to a different brand of ferret food.

Although many advanced techniques can be used in diagnosing hyperadrenocorticism in ferrets, the clinical signs remain the most important. Further confirmation can sometimes be obtained by palpating a (tiny) firm mass cranio-medial to the cranial pole of the kidneys, representing the enlarged adrenal gland(s). The right adrenal gland is more difficult to palpate due to the overlying right caudate process of the caudate liver lobe. Hormones that are commonly elevated are androstenedione, estradiol, and 17-hydroxy-progesterone. Blood can be sent to the University of Tennessee for analysis of these hormones. Dehydroepiandrosterone sulfate used to be included in this panel, but is currently no longer incorporated. Elevation of one or more of these hormones is considered to be diagnostic for hyperadrenocorticism. However, plasma concentrations of androstenedione, estradiol, and 17-hydroxyprogesterone in intact female ferrets are identical to those in hyperadrenocorticoid ferrets. It is therefore likely that this hormone panel does not aid in differentiating between a ferret with hyperadrenocorticism and one with an active ovarian remnant. The author therefore does not routinely measure these hormones in the diagnosis of this disease. He does, however, measure plasma concentrations of androstenedione (the only androgen in the panel and precursor of estradiol) for the evaluation of hormonal treatment.

ACTH stimulation tests and dexamethasone suppression tests—as commonly used in dogs with...
Cushing’s syndrome—are not considered diagnostic in ferrets. In addition, plasma concentrations of ACTH and α-MSH in hyperadrenocorticoid ferrets were found to be identical to those from healthy neutered ferrets. It was concluded that these hormones, therefore, could not aid in diagnosing hyperadrenocorticism in ferrets.

Plasma cortisol concentrations have, just as in dogs, been found to be of no use for the diagnosis of hyperadrenocorticism in ferrets. In dogs, it has become standard to measure the urinary corticoid-creatinine ratio (UCCR), in combination with a high dose dexamethasone suppression test (HDDST). An increased UCCR has also been found in ferrets with adrenocortical disease. The HDDST demonstrated that the hyperadrenocorticism is of adrenal and not pituitary origin. This is in agreement with the fact that no functional pituitary tumors have been found in hyperadrenocorticoid ferrets. Although the UCCR is elevated in ferrets with adrenocortical tumors, the UCCR is considered to be of no diagnostic value because this ratio is also increased in intact ferrets during the breeding season, and in ferrets with an active ovarian remnant.

The most useful tool in diagnosing hyperadrenocorticism in ferrets is abdominal ultrasonography. One has to remember, however, that with this technique only the size of abdominal organs is visualized. This technique does not provide any information on hormone release. It is therefore possible that only one adrenal gland is enlarged, while both adrenal glands contribute to the androgen release. Ultrasound is especially of great value prior to surgery, if you want to determine which adrenal gland is affected, or if an ovarian remnant is present. In this way the owner can be informed about the potential surgical risks that may be encountered. Another advantage of this technique is that other abdominal organs can be evaluated during the same procedure.

It has been reported that adrenal glands may remain undetected during an ultrasonographic exam. It is especially difficult to distinguish an adrenal gland from an abdominal lymph node. By using specific landmarks, however, the adrenal glands can fairly easily be detected in nearly 100% of the cases. The left adrenal gland is located lateral to the aorta, at the level of the origin of the cranial mesenteric and celiac arteries. The right adrenal gland is more difficult to locate. Since this adrenal gland lies adjacent to the caudate process of the caudate liver lobe, the liver may be used as an acoustic window. The three major vessels (aorta, portal vein and caudal vena cava) in that area are located. The vena cava is the most lateral and dorsal of the three. In addition, the portal vein has a wider diameter compared to the caudal vena cava. The right adrenal gland is attached to the dorsolateral surface of the caudal vena cava, at the level of and immediately cranial to the origin of the cranial mesenteric artery. The adrenal glands of ferrets with hyperadrenocorticism have a significantly increased thickness, have a rounded appearance, a heterogeneous structure, an increased echogenicity, and sometimes contain signs of mineralization.

When attempting to treat a ferret with hyperadrenocorticism, the most ideal treatment would probably be a combination of surgery and placement of an implant containing deslorelin (a depot GnRH analogue). Many different factors influence the eventual choice of treatment. An owner may decline surgery based on criteria such as the age of the ferret, presence of concurrent disease (cardiomyopathy), risk of surgery when the right or both adrenal glands are involved, and financial limitations. When an owner chooses for only surgery, gonadotropin release will persist, resulting in continued stimulation of the remaining adrenal gland. Disadvantage of hormonal therapy (use of a depot GnRH agonist such as leuprolide acetate) may be the price of this drug and the fact that it needs to be repeated on a regular basis. Once the deslorelin implants become registered for use in animals the latter disadvantage will be diminished. Autonomous production of steroids by the adrenal gland may result in loss of response to treatment with a depot GnRH agonist.

Surgical removal of the left adrenal gland is fairly easy. The adrenal gland is dissected out of the retroperitoneal fat and the Vena phrenicaobdominals is ligated. The location of the right adrenal gland makes it much more difficult to remove. The close proximity to the liver and the dorsolateral attachment to the caudal Vena cava would make a dorsal approach more logical. This is in fact the surgical approach to the adrenal glands in humans. In ferrets, however, an abdominal approach is most commonly used. During resection of the right adrenal gland, either a part of the adrenal needs to be left attached to the Vena cava, or part of the wall of the vein has to be removed. Ligation of the caudal Vena cava is only possible if this vein is already occluded for a major portion of its diameter and collateral veins have opened up. If this is not the case there is a great risk of hypertension distal to ligation which may lead to acute kidney failure. Although the author is not in favor of removing bilateral adrenocortical tumors, different surgical protocols have been proposed. Many advise to leave part of an adrenal gland behind, while others advise to remove both adrenal glands. It would seem likely that hypoadrenocorticism would occur after removing both glands, but this seems to occur only in a minority of cases. Accurate diagnosis of an Addisonian crisis, including an ACTH stimulation test to confirm the diagnosis, has not been published. It appears, however, that short-term treatment with cortisone and fludrocortisone seems to be sufficient in most cases.

The most effective drugs at this moment are the depot GnRH-agonists of which leuprolide acetate (Lupron Depot, TAP Pharmaceutical Products) is the most well known. Deslorelin is another pharmaceutical GnRH-analogue. This drug is commercially available as implant for chemical castration of male dogs in Australia (Suprelorin®, Peptech Animal Health, Australia). Advantages of these implants over leuprolide acetate are that the drug does not need to be reconstituted, lasts much longer than the depot injections, will be registered...
for use in animals, and will probably be cheaper. These implants have already been used in ferrets with hyperadrenocorticism and seem to be very effective. Once this drug becomes commercially available in Europe and the US, it is likely that this will become the drug of choice. Approximately 10% of ferrets seem to develop adrenal carcinomas after 1.5 to 2 years of treatment. More research will be necessary to determine why these tumors are seen, and how high the frequency actually is.

The deslorelin implants are commercially available leuprolide acetate provides a suitable alternative. The Lupron 30-day Depot formulation is given in a dose of 100 μg IM for ferrets less than 1 kg and 200 μg IM for ferrets over 1 kg. This drug will suppress adrenocortical hormone release for at least 1 month in ferrets and may even last up to 3 months. Some veterinarians use a 3-month formulation (which is 3 times as expensive), but this drug does not seem to work 3 times longer than the 30-day formulation.

It may seem strange that a depot GnRH-agonist is used in ferrets with hyperadrenocorticism, when the increased release of GnRH and gonadotropins, which occur after neutering, are responsible for the disease in the first place. To understand the mechanism behind this treatment, it is important to know that pituitary and hypothalamic hormones are released in a pulsatile fashion. Gonadotropins are only released when GnRH is secreted in pulses. The depot GnRH-agonist overrides the pulsatile release, thereby blocking the release of gonadotropins. The administration of a depot GnRH agonist therefore results in an initial single release of gonadotropins followed by baseline concentrations.

Melatonin has also been proposed as a therapeutic option for hyperadrenocorticaloic ferrets. Mink which receive such an implant develop appealing thick furs. This has also been reported in ferrets. Melatonin supposedly suppresses the release of GnRH. Researchers showed in the early eighties of the last century that ferrets, which were kept under 8 h light : 16 h darkness (8L : 16D), would come into estrus only 7 weeks later than ferrets exposed to long photoperiods (14L : 10D). It is therefore debatable if melatonin is indeed capable of suppressing the release of gonadotropins. Clinical improvement, however, is seen in hyperadrenocortical ferrets either receiving 0.5 mg melatonin daily PO or an implant containing 5.4 mg melatonin. In the study in which melatonin was given orally, however, hormone concentrations, in general, rose and the tumors continued to grow. This treatment may therefore pose a risk to the ferrets as their condition deteriorates, which remains unnoticed by the owner. Another point to consider is that melatonin can be purchased in drugstores in the US. Home-medication with melatonin may therefore delay the initial presentation of ferrets with hyperadrenocorticism to veterinarians.

As described above, the most common medical treatment option for ferrets with hyperadrenocorticism is the use of a depot GnRH agonist. Ketoconazole and mitotane (o,p’-DDD) are well known drugs for treating hypercortisolism in dogs and humans. These drugs have also been tried in ferrets, but both were not considered very effective and should be considered obsolete.

In recent years Triostane (Vetoryl®, Arnolds Veterinary Products/Dechra Veterinary Products), a 3β-hydroxysteroid dehydrogenase (3β-HSD) blocker, has become an important drug for treating pituitary-dependent hyperadrenocorticism in dogs. Since 3β-HSD is necessary for the synthesis of androstenedione and 17-hydroxyprogesterone it is tempting to speculate that this drug would be very effective in treating ferrets with hyperadrenocorticism. In a pilot study 5 mg trilostane was given orally once daily to a ferret with hyperadrenocorticism. Within a month the owner complained that the alopecia and vulvar swelling in the ferret increased. Plasma hormone analysis showed a decreased 17-hydroxyprogesterone concentration, but increased concentrations of androstenedione, estradiol, and dehydroepiandrosterone sulfate. These results can be explained by the fact that a decrease of 3β-HSD may lead to an activation of 17,20-lyase, and thus the androgen pathway. In another hyperadrenocorticoid ferret in which the depot GnRH agonist did not seem to work anymore, no improvement was seen after a month of treatment with trilostane. The hormone concentrations in this ferret did not decrease or increase in this ferret during the treatment with trilostane. More research is necessary before this drug can be safely used in ferrets.

REFERENCES


ADDITIONAL READING

Chapter 10

Current and Future Options for Non-Surgical Neutering of Ferrets (Mustela putorius furo)

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Summary

Female ferrets (jills) are commonly ovariectomized to prevent estrus-induced bone marrow suppression, and male ferrets (hobs) are castrated to reduce intraspecies aggression and skin odor. There is increasing evidence, however, that castration may precipitate the development of hyperadrenocorticism in ferrets. The detection of luteinizing hormone receptors in the adrenal cortex of ferrets with hyperadrenocorticism has strengthened this notion. Since surgical castration is a major risk factor for the development of hyperadrenocorticism in ferrets, this paper concentrates on the current and possible future options for neutering ferrets. Some of the alternatives, such as progestagen administration, seem very practical in jills but the effect in hobs is uncertain. Possible future alternatives may be the use of slow-release gonadotropin-releasing hormone (GnRH) implants, GnRH antagonists, or immunization against GnRH.
Introduction

Surgical castration of ferrets (Mustela putorius furo) is common practice in the USA and various European countries. Female ferrets (jills) are induced ovulators and therefore remain in estrus until they are mated, or for as long as daylight lasts longer than 12 hours. In the early 1980s several publications appeared concerning estrogen-induced bone marrow suppression in jills with prolonged oestrus. Since then, preventive ovari(ohyster)ectomy of jills has been advised. In male pet ferrets (hobs) there is no medical need for castration. The main reason to castrate hobs is to reduce aggression so that they can be kept in groups, and to decrease the intensity of the musky odor produced by the sebaceous glands. In the USA it is common practice to castrate ferrets at 6 weeks of age, before their delivery to pet shops.

Hyperadrenocorticism is a common disease among pet ferrets and is characterized by signs of excessive production of sex steroids (androstenedione, 17α-hydroxyprogesterone, dehydroepiandrosterone sulfate and/or oestradiol), i.e., symmetrical alopecia, vulvar swelling in neutered jills, and recurrence of sexual behavior in neutered ferrets. In recent years, evidence has accumulated that hyperadrenocorticism in ferrets is mediated by an increased secretion of gonadotropic hormones after castration. First, the initial signs of hyperadrenocorticism occur only during the breeding season, when plasma concentrations of gonadotropic hormones are high. Second, in the USA and in The Netherlands, where the neutering of ferrets is common practice, hyperadrenocorticism is a common condition. In contrast, hyperadrenocorticism is seldom diagnosed in the United Kingdom, where ferrets often remain entire. Third, a significant correlation has been found between the age at neutering and age at onset of hyperadrenocorticism. Fourth, the gonadotropin-releasing hormone (GnRH)-analogue leuprolide acetate has recently been reported to have beneficial effects in the treatment of this disease. Finally, luteinizing hormone (LH) receptors have been detected in the adrenal cortex of ferrets.

Because several lines of evidence point to surgical castration as a major risk factor in the development of hyperadrenocorticism in ferrets, this paper concentrates on current and future options for the neutering of ferrets.

Reproductive physiology

Gonadal activity is seasonal in both male and female ferrets, and more than 12 hours of light per day promotes reproductive activity. The pineal hormone melatonin plays a central role in the regulation of these changes, and plasma and pineal gland concentrations of melatonin are significantly higher during the dark phase of the day (scotophase) than in the light phase (photophase). Plasma concentrations of follicle-stimulating hormone (FSH) increased within a few days after male hamsters were transferred from a short to a long photoperiod. Plasma LH concentrations, however, increased only after exposure to a female hamster. The different regulation of LH and FSH secretion can be explained by the fact that there are at least two types of GnRH receptors and several GnRH isotypes, some of which may have specific FSH-releasing activity. Anand et al. also found that both melatonin and a GnRH-antagonist (antide) could prevent the release of LH in male hamsters exposed to a female.
These findings indicate that melatonin suppresses the release of GnRH in seasonal breeders.

During the breeding season, GnRH stimulates the production of the gonadotropic hormones LH and FSH, which stimulate the gonads to produce either oestradiol or testosterone. The latter two hormones exert a negative feedback on the hypothalamus and pituitary gland, thereby preventing excessive secretion of GnRH, LH, and FSH.

**Definitions**

In its classical definition, castration denotes the removal of gonads. Thus the term covers both the removal of the ovaries (ovariectomy or spaying) in females and the removal of the testes (orchietomy) in males. In recent years, this definition has been extended by the introduction of new methods to create non-functional gonads, e.g., chemical castration and immunological castration. The term castration is controversial since it is sometimes used to indicate the removal of the gonads in male individuals only. A term that encompasses all means of eliminating gonadal function in both males and females is neutering, and this term is used in this article.

There are methods of contraception that do not affect gonadal function. For example, fertilization of oocytes can be prevented by vaccination against zona pellucida proteins or vaccination against sperm proteins. Another option to prevent oocyte fertilization is surgical sterilization, by means of tubal ligation. Immunization of female rats and monkeys with riboflavin carrier protein caused termination of pregnancy around the pre-implantation stage, while immunization of male rats and monkeys resulted in a reduced fertilizing potential of their spermatozoa. Since these methods do not eliminate gonadal function and therefore do not prevent the resulting detrimental effects in ferrets, they are not discussed here.

**Progestagens**

*Principle:* Although not fully understood, the probable mode of action of progestagens is suppression of the secretion of gonadotropic hormones, thereby preventing ovarian cyclicity.

*Method:* Several progestagens are used in veterinary medicine: medroxyprogesterone acetate, megestrol acetate, and proligestone. Megestrol acetate (Ovaban; Schering Plough) can be given orally, medroxyprogesterone acetate (Depo-provera; Pharmacia) can be given orally or by injection and proligestone (14α, 17α-propylienedioxy progesterone) (Delvosteron; Intervet) is given by depot injection. The latter is recommended in the United Kingdom for the prevention of estrus in ferrets at a dose of 0.5 ml (100 mg/ml SC) just prior to the breeding season. Proligestone can also be used in jills in estrus.

*Effect:* Return of estrus was reported in approximately 8% of ferrets 2 – 5 months after the initial dose of proligestone. In these cases, a second dose suppressed estrus for the rest of the breeding season. Megestrol acetate has been used in ferrets to prevent estrus but is not recommended because of the assumed risk of pyometra. However, this restriction is probably not justified because pyometra has not been described in ferrets after the use of megestrol acetate.
Remarks: Reported side-effects associated with the use of progestagens in either dogs or cats are the development of cystic endometrial hyperplasia (CEH), prolonged pregnancy, hypersecretion of growth hormone (GH), diabetes mellitus, and increased risk of neoplastic transformation of mammary tissue. Of these side-effects, only prolonged pregnancy (gestation 51 days, normal 38 – 44 days) has been reported after the use of proligestone. Proligestone had been given to these two ferrets when they were in estrus and had been mated.

There is no information on the use of progestagens in hobs. In other species, including humans, progestagens have been used to suppress libido and fertility in males. Progestagens are rarely used for contraception of human males since they cause loss of libido and incomplete suppression of spermatogenesis. For this reason the combination of progestagens and androgens is often used: it provides a better contraceptive effect than progestagens alone and the libido is maintained. This combination would not be an option in ferrets, because libido is an undesirable characteristic in hobs. Delmadinone acetate (Tardak; Pfizer) is used to suppress libido in dogs. A recent study of Beagles, however, has revealed that this progestagen does not suppress plasma testosterone concentrations. Thus delmadinone acetate, when used in ferrets, may not suppress the musky odor produced by the sebaceous glands. In humans, cyproterone acetate (Androcur; Schering) and medroxyprogesterone acetate have been used to suppress libido in sex offenders. Both drugs suppress plasma testosterone concentrations.

Studies with progestagens are needed to determine whether these drugs can be used to control libido and odor in hobs. In addition, the effect of progestagen administration on GH release should be studied in jills, because progestin-induced expression of the mammary GH gene has now been demonstrated in dogs, cats, and humans.

“Sham” mating

Principle: Ferrets, rabbits, and cats are all induced ovulators. When ovulation is achieved without fertilization, pseudopregnancy will occur.

Method: In rabbit does, the proximity of an intact male, mechanical stimulation of the vagina, or mounting by a female rabbit can induce ovulation. In cats, stimulation of the vagina will result in ovulation. In ferrets, both vaginocervical stimulation and neck-gripping are necessary to induce ovulation. Because of this elaborate procedure, it is not practical for owners to try to induce ovulation in jills. As an alternative, vasectomized hobs are used in the UK to induce ovulation. One mating leads to cessation of estrus in about 75% of ferrets and two matings to cessation of estrus in 85% of ferrets.

Effect: Termination of estrus followed by pseudopregnancy lasting about 42 days.

Remarks: During pseudopregnancy, jills may display nesting behavior and enlargement of the abdomen and mammary glands. The nesting behavior, which includes dragging cage mates around the cage and increased aggression towards the owners, does not make this an attractive option.
Vasectomized hobs remain aggressive and have a musky odor similar to that of intact hobs.

### hCG or GnRH administration

**Principle:** After mating there is a preovulatory LH surge that may last to up to 12 hours. This LH surge can be mimicked by the administration of either human chorionic gonadotropin (hCG) or indirectly by stimulating endogenous LH release with the hypothalamic releasing hormone GnRH.

**Method:** Ten days after the onset of estrus, 20 µg GnRH or 100 IU hCG is given intramuscularly.

**Effect:** Approximately 35 hours after injection the ferrets ovulate, resulting in the formation of corpora lutea in 95% of the cases. Vulvar swelling will start to decrease within 1 week of injection. Anoestrus (pseudopregnancy) will last for 40 – 60 days. Fox et al. found that multiple injections of GnRH may sensitize the ferret to the drug, resulting in anaphylactic reactions shortly after administration. Antihistamine administration ameliorates these reactions within minutes. The consequences of pseudopregnancy have been mentioned in the previous section and neutering ferrets with hCG or GnRH injections only applies to jills.

### Manipulation of photoperiod and administration of melatonin

**Principle:** As described above, the ferret’s reproductive season starts when there is more than 12 hours of light per day. During the scotophase, melatonin concentrations in plasma are high. It has been speculated that keeping ferrets either under conditions with short photoperiods or giving them melatonin would suppress the pituitary-gonadal axis.

**Method:** Provision of a maximum of 8 hours of light per day, or daily administration of 1 mg melatonin 8 hours after the onset of light.

**Effect:** Ferrets kept under 8h light : 16 h darkness (8L : 16D) come into estrus only 7 weeks later than ferrets exposed to long photoperiods (14L : 10D). When ferrets kept under long photoperiods (14L : 10D) received melatonin (1 mg/day) 8 hours after the onset of light, they come into estrus only 7 weeks later than ferrets kept under similar conditions and receiving oil injections. Herbert et al. found that in the first year after ferrets were blinded they came into estrus at the expected time, but thereafter estrus synchrony was lost. Estrus periods in blinded ferrets lasted from just a few weeks to up to 60 weeks. A limited light regimen and administration of melatonin are not effective in inhibiting the hypothalamus-pituitary-gonadal axis in ferrets.

### Immunization against GnRH

**Principle:** There are several reports on the use of GnRH vaccines in mammals. Depending on the species, these vaccines aim at contraception, the prevention of boar taint, or the control of hormone-dependent cancers, including breast and prostate cancers. In ferrets, use of a GnRH vaccine would serve two goals: inactive gonads in both sexes in combination with low plasma concentrations of gonadotropin hormones.
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Method: Depending on the study inoculations were performed intramuscularly, subcutaneously or intranasally.

Effect: Decreased plasma gonadotropic hormone concentrations have been reported in male rats after immunization against GnRH. Testosterone concentrations decreased in bulls, boars, male rats and dogs after immunisation, while in female mice a sterilizing effect was seen. Reduced testes size has also been reported in boars and rats after immunization against GnRH.

Remarks: In a pilot study conducted at Utrecht University, eight out of twelve ferrets immunized against GnRH had to be euthanased (Schoemaker and others, in preparation). Post mortem examination disclosed aspecific lympho-plasmacellular infiltrations in multiple organs (liver, kidney, lung and intestines) suggesting an aspecific immune reaction. The background of these reactions has to be unraveled before GnRH immunization can be employed in ferrets.

Immunization with LH

Principle: Inhibition of LH secretion by immunization with heterologous LH.

Method: Heterologous LH is injected intramuscularly or subcutaneously.

Effect: Injection of bovine LH causes a 90% reduction in the weight of rabbit testes, and genital atrophy in female rabbits and loss of receptiveness to males. In another study with male rabbits, LH and testosterone plasma concentrations decreased significantly after immunization against LH; however, FSH concentrations increased significantly. Similar, but less consistent, effects of LH immunization were seen in dogs. In ewes estrus and pregnancy were prevented for 2 years after immunization against LH, although plasma LH concentrations were not lower than in control ewes. In these ewes FSH concentrations were also increased.

Remarks: So far, there are no reports of LH vaccination in ferrets. Since not only LH but also FSH may influence the development of hyperadrenocorticism in ferrets, there is reason for caution with LH immunization in ferrets.

Immunization with LH receptor

Principle: Induction of LH receptor dysfunction by immunization with heterologous LH receptor.

Method: Immunization of bitches with 0.5 mg bovine LH receptor (bLH-R) encapsulated in a silastic subdermal implant, followed by intramuscular booster injections.

Effect: In bitches immunization with bLH-R suppresses serum progesterone concentrations for approximately 1 year, while serum concentrations of oestradiol and LH are not affected. Although stimulation with GnRH in immunized dogs leads to a LH surge, serum progesterone concentrations do not increase. Thus bLH-R immunized bitches do not ovulate or do not produce active corpora lutea.

Remarks: The main drawback of this approach is that ferrets may fail to ovulate. Prolonged estrus can be expected, which may result in bone-marrow suppression. In male mice, immunization against LH-R caused reduces androgen production. In male ferrets, this might result in reduced aggressive behavior and decreased intensity of their musky
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odor. Again some caution is warranted because high LH-R antibody titers in mice had an agonistic effect, resulting in hypertestosteronemia.  

**Depot GnRH agonist**

*Principle:* Depot GnRH agonists increase the levels of gonadotropic hormones, followed by a desensitization of gonadotroph receptors, resulting in decreased LH and FSH plasma concentrations. The exact mechanism of the desensitization is still not clear.

*Method:* Of the available formulations, leuprolide acetate (Lupron Depot 3.75 mg, TAP Pharmaceuticals Inc) is used to treat hyperadrenocorticism in ferrets. Ferrets weighing less than 1 kg receive an intramuscular dose of 100 µg at monthly intervals and ferrets heavier than 1 kg receive 200 µg per month. A similar treatment protocol might also be effective for contraceptive purposes. Slow-release implants have been described in humans and dogs.

*Effect:* When leuprolide acetate is given to jills in estrus, ovulation will probably occur due to the initial LH surge seen after injection. Therefore leuprolide acetate should be administered before the breeding season, otherwise it has no advantage over regular GnRH. In dogs, however, leuprolide acetate induces estrus. It is therefore uncertain whether this drug will be useful in neutering ferrets.

*Remarks:* Slow-release implants suppress reproductive function in dogs. GnRH implants might therefore be an option for use in ferrets.

**GnRH receptor antagonist**

*Principle:* Competitive GnRH receptor occupancy with GnRH receptor antagonists results in a decreased release of gonadotropic hormones by the pituitary gland.

*Method:* The available GnRH receptor antagonists have to be injected, but orally active non-peptide GnRH antagonists are currently being developed for use in humans.

*Effect:* The initial increase in gonadotropic hormones, seen with GnRH agonists, are not seen with GnRH receptor antagonists. The use of these receptor antagonists will therefore result in an immediate decrease in gonadotropic hormone concentrations.

*Remarks:* Until now, only a few GnRH receptor antagonists have been registered for use in humans. New and longer-acting drugs are being developed. Degarelix (FE200486, Ferring) currently seems to be the most promising of these GnRH receptor antagonists. While older GnRH antagonists caused increased histamine release after injection, the newer drugs do not have this side-effect. Nevertheless, local reactions at the site of injection are still common. These drugs seem to be promising for future use in ferrets.

**Conclusion**

There are several potential ways to influence reproductive function in ferrets, other than surgical castration. Progestagens seem practical for use in jills but need to be studied for use in hobs. Possible future alternatives may be the use of slow-release GnRH implants, GnRH antagonists, or immunization against GnRH. Detailed studies are needed before these techniques can be recommended for neutering ferrets.
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The role of luteinizing hormone in the pathogenesis of hyperadrenocorticism in neutered ferrets

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Abstract

Four studies were performed to test the hypothesis that gonadotrophin 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 (LH-R); (II) gonadotrophin-releasing hormone (GnRH) stimulation tests were performed in 10 neutered ferrets, with measurement of androstenedione, 17α-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 (C/C) 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 hyperadrenocortical ferrets stained positive with the LH-R antibody. Plasma androstenedione and 17α-hydroxyprogesterone concentrations increased after stimulation with GnRH in 7 out of 8 hyperadrenocortical ferrets but in only 1 out of 7 healthy ferrets. Hyperadrenocortical ferrets had elevated urinary C/C ratios during the breeding season. The observations support the hypothesis that gonadotrophic 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-R on sex steroid-producing adrenocortical cells.

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1. 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 (Rosenthal, 1997). There is no sex predilection (Schoemaker et al., 2000). The diagnosis is based upon increased plasma concentrations of androstenedione, 17α-hydroxyprogesterone, dehydroepiandrosterone sulfate, and/or estradiol. In contrast plasma concentrations of cortisol are increased in a minority of cases (Rosenthal and Peterson, 1996). Measurement of urinary corticoid/creatinine (C/C) ratios (Gould et al., 1995) and ultrasonography of the adrenals (Rosenthal, 1997) 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 (Rosenthal et al., 1993; Weiss and Scott, 1997). After unilateral adrenalectomy there may be recurrence of the disease due to enlargement of the contralateral adrenal gland (Weiss and Scott, 1997). The histologic changes of the adrenals range from

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(nodular) hyperplasia to adenoma and adenocarcinoma (Rosenthal et al., 1993; Weiss and Scott, 1997).

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 (Fekete et al., 1941; Murthy et al., 1970; Sharawy et al., 1980). 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 (Rosenthal, 1997; Schoemaker et al., 2000), whereas in the UK the condition is seldom diagnosed. This difference in incidence may be ascribed to the fact that ferrets are usually not neutered in the UK (Lloyd, 1999), 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 (Schoemaker et al., 2000). The observation that initially signs of hyperadrenocorticism occur only during the breeding season (Rosenthal, 1997), when plasma concentrations of gonadotrophic hormones are high (Jallageas et al., 1994), and the recently reported beneficial effects of treatment with leuprolide acetate (Wagner et al., 2001) have led to the hypothesis that hyperadrenocorticism in ferrets is mediated by gonadotrophic influences, for which castration may play a precipitating role (Lipman et al., 1993; Rosenthal et al., 1993; Schoemaker et al., 2000).

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 gonadotrophin-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 C/C ratios were measured at 2-week intervals for 1 year in the ferrets of study II to investigate possible seasonal fluctuations.

2. Materials and methods

2.1. Immunohistochemical staining for the presence of LH-R

2.1.1. Tissues

Adrenal glands of 6 neutered ferrets with signs of hyperadrenocorticism, and histologically diagnosed hyperplasia or adenoma, were examined for the presence of LH-R. 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 used as positive controls.

2.1.2. Antibody and immunohistochemical staining

The murine LH-R monoclonal antibody (P1B4) was a gift from Dr Wimalasena (Department Obstetrics and Gynecology, University of Tennessee, Knoxville, TN). The antibody had been raised against purified rat LH-R,
as described by Indrapichate et al. (1992). The antibody binds specifically to LH-R in various tissues of different species (Bukovsky et al., 1993; Peters et al., 2001).

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-µm sections. One section was routinely stained with hematoxylin and eosin. For immunohistochemical staining, sections were deparaffinized and endogenous peroxidase was blocked with 1% H2O2 in methanol for 30 min. The slides were washed in 0.01 M Tris–buffered saline (TBS, pH 7.4), incubated with 0.75% glycerine 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 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 (0.05 M, 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% H2O2 was added. Sections were incubated with the DAB solution for approximately 50 s and counterstained with Mayer’s hematoxylin.

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

2.2. GnRH stimulation test in catheterized ferrets

2.2.1. 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 2 ferrets elevated basal plasma androstenedione and 17α-hydroxyprogesterone concentrations, as well as unilaterally enlarged adrenal glands visualized on ultrasonographic examination, indicated the presence of hyperadrenocorticism.

2.2.2. 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 Tubing [602-155], Down Corning Co., Midland, MI) had an inner diameter of 0.025 in. (0.64 mm) and an outer diameter of 0.047 in. (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 (5000 IU/ml)) to keep it patent.

2.2.3. 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 IV injection of 10 µg 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).

2.3. 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 min. Blood samples were placed in pre-chilled EDTA-coated tubes and centrifuged. Plasma was stored at −20 °C pending analysis.

2.3.1. 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.

2.3.2. 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).

2.3.3. Hormone determinations

Androstenedione concentrations were measured by radioimmunoassay (RIA) as described previously (van Landeghem et al., 1981). 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 11-hydroxy-androstenedione, 2.2% for adrenosterone (11-keto-androstenedione), 5.5% for 5α-androstenediol, and 2.2% for 5α-stanediol.

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[1,2,6,7-3H]-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, LA). The lower limit of detection was 1 nmol/l and the interassay coefficient of variation was between 4.0 and 6.4%.

2.4. Serial measurements of urinary C/C ratios

2.4.1. 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.

2.4.2. 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 (Pastoor et al., 1990). The litter boxes were underneath the cages from 17:00 to 8:00 h. Urine was transferred to tubes and stored at 4 ºC pending analysis, which was performed within 5 days after collection of the sample.

2.4.3. Hormone determination

Urinary corticoid concentrations were measured by RIA for cortisol as described previously (Rijnberk et al., 1988). 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 (1%), cortisone (2%), 11-deoxycortisol (1.3%), deoxycorticosterone (1.3%), and 17α-hydroxyprogesterone (0.1%) (Thijssen et al., 1980). The urinary corticoid concentration was related to the urinary creatinine concentration (Jaffe kinetic method, initial rate reaction) by calculation of its quotient (× 10^-6) (Stolp et al., 1983).

3. Statistics

The increase in plasma androstenedione concentrations in the GnRH stimulation test in the two hyperadrenocortical ferrets was compared with the (mean ± 2 S.D.) 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 hyperadrenocortical ferrets was higher than the mean increase ±2 S.D. in the 8 other ferrets.

The reference values for plasma androstenedione and 17α-hydroxyprogesterone concentrations were established in percentiles (Elveback and Taylor, 1969). The inner limits of the percentiles P2.5 and P97.5 are presented with a probability of 95%.

Student’s t-test was used to compare the mean C/C 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.
4. Results

4.1. Immunohistochemical staining for the presence of LH-R

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-R were seen in the hyperplastic and neoplastic adrenal glands of the ferrets with hyperadrenocorticism. These cells were mostly clear cells (Fig. 2).

4.2. 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 17α-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. 3).

4.3. GnRH stimulation test with blood collection under anesthesia

4.3.1. 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; P2.5–P97.5: 0.1–0.4 nmol/l). Basal plasma 17α-hydroxyprogesterone concentrations in the same ferrets ranged from 0.3 to 1.2 nmol/l (median 0.4 nmol/l; P2.5–P97.5: 0.3–0.7 nmol/l).

4.3.2. Ferrets used in study II

Basal plasma androstenedione and 17α-hydroxyprogesterone concentrations of the 2 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 17α-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 17α-
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).

4.3.3. 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 three ferrets (Table 1).

4.4. Serial measurements of urinary C/C ratios

During the breeding season (March–August) and the non-breeding season (September–February), the urinary C/C ratio was measured on 12 occasions (Fig. 4). During the breeding season the mean (±S.D.) C/C ratio of the 2 ferrets with hyperadrenocorticism (both 3.5 ± 1.1 × 10^{-6}) was significantly higher than that of the neutered control animals (1.4 ± 0.5 × 10^{-6}); however, during the non-breeding season the mean C/C ratio of only 1 ferret was significantly higher (3.0 ± 1.3 × 10^{-6} and 1.8 ± 0.3 × 10^{-6} versus 1.8 ± 0.7 × 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 ± 1.1 × 10^{-6} versus 1.9 ± 0.7 × 10^{-6}).
5. Discussion

The detection of LH-R 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 17α-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 (Indrapichate et al., 1992), 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 gonadotrophin (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 (Leinonen et al., 1991), and in a woman with LH-dependent Cushing’s syndrome plasma cortisol levels increased after hCG (Lacroix et al., 1999). However, we used GnRH as stimulating hormone, because it is not known which gonadotrophic hormone stimulates the adrenal cortex in ferrets. Only the 2 ferrets (study II) with high basal androgen levels responded to stimulation with GnRH. Plasma concentrations of androstenedione and 17α-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 three diseased ferrets were in accordance with the findings of Rosenthal and Peterson (1996), 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 (Schoemaker et al., 2000) makes it tempting

<table>
<thead>
<tr>
<th>Ferret number</th>
<th>Androstenedione</th>
<th>17 OH prog</th>
</tr>
</thead>
<tbody>
<tr>
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<td>t = 30</td>
</tr>
<tr>
<td>1</td>
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<td>0.2</td>
</tr>
<tr>
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</tr>
<tr>
<td>C5</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>C6</td>
<td>0.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

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.

Table 1

Plasma concentrations of androstenedione and 17α-hydroxyprogesterone (17 OH prog) (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 and intravenous injection with 10 μg Fertagyl® (GnRH analogue).

Fig. 4. The mean (±S.D.) urinary C/C ratio (× 10⁻⁴) of 7 healthy neutered control ferrets (●) and the urinary C/C ratios of 2 neutered ferrets (■, ×) with hyperadrenocorticism. During the breeding season (March–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).
to speculate that a persistent elevation of plasma gonadotrophin 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. (1995) 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 gonadotrophic hormones are low (Jallageas et al., 1994), the urinary C/C ratio of the two control ferrets with hyperadrenocorticism was no longer elevated. This finding further supports the hypothesis that hyperadrenocorticism in ferrets arises under the influence of gonadotrophic hormones.

The use of the urinary C/C ratio as tool to aid the diagnosis of hyperadrenocorticism in ferrets has been questioned by Rosenthal (1997), because cortisol is not considered to play an important role in the development of the signs and symptoms of hyperadrenocorticism in ferrets (Rosenthal and Peterson, 1996). 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 (Thijssen et al., 1980). 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 (Jallageas et al., 1994).

LH-dependent Cushing’s syndrome and LH-dependent adrenal androgen secreting tumors have been described in humans (Lacroix et al., 1999; Leinonen et al., 1991). Patients with LH-dependent Cushing’s syndrome have bilateral macronodular adrenal hyperplasia with high plasma concentrations of cortisol and suppressed concentrations of ACTH (Lacroix et al., 1999). Patients with LH-dependent adrenal androgen secreting tumors may have unilateral adrenal neoplasia and unaltered cortisol and ACTH concentrations (Leinonen et al., 1991).

The results of the present study support the hypothesis that gonadotrophic 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 hyperadrenocorticism in ferrets and LH-dependent adrenal androgen-secreting tumors in humans, although the adrenal glands of diseased ferrets can also secrete estradiol (Rosenthal and Peterson, 1996). Thus at this stage hyperadrenocorticism in ferrets is without a clear counterpart 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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hydroxysteroid dehydrogenase and vimentin to characterize different types of testicular tumour in dogs. Reproduction 121, 287–296.


Use of a gonadotropin releasing hormone agonist implant as an alternative for surgical castration in male ferrets

(Mustela putorius furo)

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Abstract

Surgical castration in ferrets has been implicated as an etiological factor in the development of hyperadrenocorticism in this species due to a castration-related increase in plasma gonadotropins. In search for a suitable alternative, the effect of treatment with the depot GnRH-agonist implant, deslorelin, on plasma testosterone concentrations and concurrent testes size, spermatogenesis, and the typical musky odor of intact male ferrets was investigated. Twenty-one male ferrets, equally divided into three groups, were either surgically castrated, received a slow release deslorelin implant or received a placebo implant. Plasma FSH and testosterone concentrations, testis size and spermatogenesis were all suppressed after the use of the deslorelin implant. The musky odor in the ferrets which had received a deslorelin implant was less compared to the ferrets which were either surgically castrated or had received a placebo implant. These results indicate that the deslorelin implant effectively prevents reproduction and the musky odor of intact male ferrets and is therefore considered a suitable alternative for surgical castration in these animals.

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Keywords: Deslorelin; Gonadotropin; GnRH; Testosterone; Spermatogenesis

1. Introduction

Surgical castration of ferrets (Mustela putorius furo) is common practice in the USA and various European countries. Although in male pet ferrets (hobs) there is no medical need for castration, they are mainly castrated to prevent reproduction, to reduce interspecies aggression enabling them to be kept in groups, and to decrease the intensity of the musky odor produced by the sebaceous glands [1].

Hyperadrenocorticism is a common disease in neutered pet ferrets. The syndrome differs from hyperadrenocorticism in other species, such as humans and dogs, in that glucocorticoid excess is much less pronounced in
ferrets [2]. Instead, in ferrets the disease is characterized by excessive adrenal production of sex steroids, giving rise to vulvar swelling in neutered female ferrets (jills), recurrence of sexual behavior in neutered hobs, and alopecia [2–7].

It has been hypothesized that increased concentrations of gonadotropins, which occur after neutering due to the loss of negative feedback, persistently stimulate the adrenal cortex resulting in adrenocortical hyperplasia and tumor formation [6]. Strong support for this hypothesis may be found in the fact that the depot GnRH-agonists, leuprolide acetate and deslorelin, can be used successfully to treat ferrets with hyperadrenocorticism [8,9], and that LH-receptors (LH-R) have been detected in the adrenal glands of ferrets with hyperadrenocorticism [10]. These receptors were considered to be functional because plasma concentrations of adrenal androgens increased after intravenous injection of a GnRH-agonist [10]. Based on these findings it has been proposed to search for alternatives for surgical castration in ferrets [11].

One of the possible alternatives for surgical castration is the continuous administration of a GnRH analogue. The results of the use of these analogues differ, however, among the different species. In dogs and cheetahs continuous administration of a GnRH analogue suppresses spermatogenesis [12–14]. In the bull, however, continuous administration of a GnRH analogue leads to basal LH concentrations which are higher than in control animals, possibly explaining their concurrent increased plasma testosterone concentrations [15,16]. In addition, testis volume is also increased, and more round spermatids were found in the seminiferous tubules [17]. In marmoset monkeys and wallabies plasma testosterone concentrations remain within the normal range during the use of a long-acting GnRH agonist [18,19]. It is therefore not possible to predict the outcome of the use of long-acting GnRH analogues in ferrets.

The present study was designed to investigate whether a slow release depot GnRH-agonist implant containing 9.4 mg deslorelin (Peptech Animal Health, North Ryde, New South Wales, Australia) would reduce plasma FSH and testosterone concentrations in combination with concurrent testes volume, spermatogenesis, and the typical musky odor of intact hobs. Unfortunately, the previously used and for ferrets validated heterologous antiserum raised against ovine LH (GDN 15) is no longer available and no suitable alternative was found to reliably measure plasma LH concentrations in ferrets.

2. Materials and methods

2.1. Animals

For this study, which was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University, 21 male ferrets between the age of 1 and 2 years were used. At the start of the study the mean (±S.D.) weight of the ferrets was 1.4 ± 0.2 kg (range: 1.0–1.7 kg). They were individually housed in outdoor suspended cages with a night box at Utrecht University with the following GPS — coordinates: 52°05′04″N; 5°10′55″E. No artificial lighting was provided. Pellets (FerRet®, Hope Farm, Woerden, The Netherlands) and water were available ad libitum. The ferrets were randomly divided into three groups of 7 ferrets each. The ferrets in Group 1 were surgically castrated [mean (±S.D.) weight: 1.4 ± 0.2 kg; range: 1.0–1.6 kg], the ferrets in Group 2 received a GnRH implant containing 9.4 mg deslorelin [mean (±S.D.) weight: 1.5 ± 0.1 kg; range: 1.3–1.7 kg], and the ferrets in Group 3 received a similar implant without the deslorelin (placebo implant) [mean (±S.D.) weight: 1.5 ± 0.1 kg; range: 1.3–1.7 kg].

2.2. Procedures

2.2.1. Castration and placement of implants

The implants were placed subcutaneously in the scruff of the neck in the ferrets from Groups 2 and 3, 1 week after the first blood collection (17 March 2005). On the same day the other ferrets were surgically castrated. Prior to surgery, all ferrets received 4 mg/kg carprofen (Rimadyl®, Pfizer Animal Health, Capelle a/d IJssel, The Netherlands) IM. The ferrets were premedicated with an IM injection of 100 µg/kg medetomidine (Domitor®, Pfizer Animal Health, Capelle a/d IJssel, The Netherlands), followed after 10 min with a mask induction of 4% isoflurane (IsoFlo™, Abbot Animal Health, Hoofddorp, The Netherlands) in 100% oxygen. Anesthesia was maintained at 2% isoflurane, and castration was performed in a routine manner. The left testis was surgically removed from the ferrets in Groups 2 and 3, 15 weeks after the first blood collection (23 June 2005) for histological evaluation. The weight of the removed left testes was recorded and compared between both groups.

2.2.2. Blood collection and testis measurement

Blood samples were taken during the breeding season, from 10 March until 1 September 2005. Initially blood samples were taken weekly for 10 weeks, followed by once every fortnight for a period of 16
weeks. One year later, on 6 July 2006, blood samples were again taken from the same ferrets. At that time one ferret out of the placebo group had died from a thromboendocarditis and one surgically castrated ferret had died during insulinoma surgery. During each sampling session the size of the right testis (width and length) was measured with a digital slide calliper. The testis volume was calculated using the following formula: \((\text{width})^2 \times \text{length} \times 0.524\) (in cm), as previously used in testis measurements of black footed ferrets [20]. Blood was collected from the \textit{Vena cava cranialis} while the ferret was anaesthetized with isoflurane. Blood samples were divided into pre-chilled EDTA and heparinized tubes. All tubes were centrifuged for 10 min at 3000 rpm at 4 °C, after which plasma from each tube was collected and stored in polystyrene tubes at −20 °C, pending analysis.

2.2.3. Hormone analysis

Testosterone measurements were performed after diethylether extraction using a solid-phase radioimmunoassay (RIA) Coat-A-Count® (Diagnostic Products Corporation, Los Angeles, USA). In short 0.25 mL plasma was extracted with 1 mL diethylether by end-over-end rotation for 15 min followed by centrifugation for 5 min at 3000 × g. After freezing of the lower aqueous phase the organic phase was decanted and evaporated to dryness. The extracts were reconstituted in 0.125 mL phosphate-buffered saline containing 0.5% (w/v) bovine serum albumin. Ferret plasma extracts were diluted 1, 2, 4 and 6 times and were parallel to the standard curve \((r^2 = 0.98)\). Spiking of plasma samples containing an endogenous concentration of 1.4 with 15 nmol/L testosterone showed a spiking recovery of 95.7%. The detection limit amounted to 0.2 nmol/L. The intra- and inter-assay coefficients of variation were 5.1 and 8.4%, respectively.

Follicle stimulating hormone concentrations were measured by RIA as described previously [21]. This RIA has been validated for use in ferrets [22]. Rat FSH-RP2 (NIAMD, Bethesda, MD) was used as a standard. The lower limit of detection was 0.8 μg/L and the intra-assay coefficient of variation was 7.2%.

2.2.4. Histological evaluation of the testes

Directly after excision, the testes were fixed by immersion in Bouin’s solution for 15–24 h. They were then suspended in ethanol 70% for 3 days, after which they were embedded in paraffin. From each testis, five sections of 5 μm were taken with an interspace of 30 μm. These sections were stained with the periodic acid-Schiff (PAS) reaction.

One hundred round tubular cross-sections were studied per section at 400× magnification, resulting in a total of 500 cross sections per testis. The spermatogenesis in the seminiferous tubules was scored using a modification of the Johnsen method, as previously described [23,24]. Each tubular cross-section was given a score of 1–10, according to the presence or absence of the different spermatogenic cell types (Table 1). A mean score was calculated for each animal and for each group.

2.3. Odor study

Cotton cloths were placed in the night boxes of all hobs \((n = 21)\) 14 weeks after castration and placement of the implants. After two nights these cloths were collected and individually placed in a vacuum sealed bag. Each bag was randomly given a number. A test panel consisting of students, coworkers, and other volunteers \((n = 83)\), scored the smell from each cloth after opening the bag, smelling the cloth and resealing the bag. The bags were opened in random order and given a score between 1 and 5, whereby a score of 1 indicated no odor and a score of 5 indicated a very strong odor.

2.4. Statistics

Statistical analysis was performed using the statistical package SPSS for Windows (Version 12.0) and R (Version 2.2.0).

Table 1

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<td>1</td>
<td>No cells in tubular cross-section</td>
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<tr>
<td>2</td>
<td>No germ cells but only sertoli cells present</td>
</tr>
<tr>
<td>3</td>
<td>Spermatogonia are the only germ cells present</td>
</tr>
<tr>
<td>4</td>
<td>Only few spermatocytes (&lt;5) and no spermatids or spermatooza present</td>
</tr>
<tr>
<td>5</td>
<td>No spermatozoa, no spermatids but several or many spermatocytes present</td>
</tr>
<tr>
<td>6</td>
<td>No spermatozoa and only few spermatids (&lt;5–10) present</td>
</tr>
<tr>
<td>7</td>
<td>No spermatozoa but many spermatids present</td>
</tr>
<tr>
<td>8</td>
<td>Only few spermatozoa (&lt;5–10) present in a tubular cross-section</td>
</tr>
<tr>
<td>9</td>
<td>Many spermatozoa present but germinal epithelium disorganized, with marked sloughing or obliteration of the lumen</td>
</tr>
<tr>
<td>10</td>
<td>Complete spermatogenesis with many spermatozoa. Germinal epithelium organized in a regular thickness leaving an open lumen, or stage V of the seminiferous cycle, with sufficient round spermatids</td>
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</table>
The correlation coefficient between the testis volume and plasma testosterone concentration was corrected for time and group.

Testis volumes and FSH concentrations were log transformed to better conform to a normal distribution and analyzed using a linear mixed-effect model. The fixed part of the model contained a group effect, a time effect and their interaction.

A random intercept was fitted for each individual together with an AR(1) correlation structure to model the dependence in time. For testis volume a random slope for time and different variance estimates for each of the three groups were fitted, and for FSH variance was allowed to differ for each group and for the first 5 time points, to capture the extra variation at the start of the experiment.

Since plasma testosterone concentrations were too often below the detection limit, transformation to conformity with the normal distribution was not possible. The nonparametric Kruskal–Wallis test was used at selected time points (10 March, 7 April, 14 April, 5 May, 23 June, 1 September, and 6 July 2006), where significant followed by Dunn post hoc tests to determine which groups differed from each other [25]. Differences in body odor between the three groups were tested by use of a $\chi^2$ analysis. In all instances significance was assumed at $P < 0.05$.

3. Results

Based on plasma testosterone concentrations, testis size and histological evaluation of the left testis (an arrest of spermatogenesis at the level of meiosis) we diagnosed one ferret from the placebo group with hypogonadism unrelated to this study. Based on these findings we excluded its data from the results.

3.1. Plasma testosterone and FSH concentrations

On 10 March, there were no significant differences in plasma testosterone concentrations between the three groups. On 7 April, control ferrets had significantly higher testosterone levels than castrated ferrets ($P < 0.01$), and ferrets from the deslorelin group ($P < 0.05$). From 14 April onwards both surgically and chemically castrated ferrets had significantly less testosterone than ferrets from the placebo group ($P < 0.01$). Surgically and chemically castrated ferrets did not differ significantly from each other.

On 10 March, plasma FSH concentrations were similar among the groups. From 31 March until 1 September, chemically castrated ferrets had lower plasma FSH concentrations than those which had received a placebo implant. From 24 March until 1 September, surgically castrated ferrets had higher plasma FSH concentrations than those which had received a placebo implant (Fig. 2).

3.2. Testis volume

On 10 March, the testis volumes were similar for all groups. The testis volume from the chemically castrated ferrets were significantly smaller compared to those from the placebo group from 21 April onwards ($P < 0.01$; Fig. 3). On 6 July 2006 the testis volume in the deslorelin group ($n = 7$; range: 0.08–0.15 cm$^3$; mean ± S.D.: 0.10 ± 0.03 cm$^3$) was still significantly lower than that of ferrets which had received a placebo implant ($P < 0.01$; $n = 5$; range: 9–73 nmol/L; mean ± S.D.: 41 ± 29 nmol/L). The testosterone concentrations between the surgically and chemically castrated ferrets did not differ significantly from each other.
smaller compared to the testis volume in the placebo group (n = 5; range: 0.94–1.38 cm$^3$; mean ± S.D.: 1.18 ± 0.18 cm$^3$) (P < 0.01).

The weight of the left testes from the ferrets in the placebo group (n = 6; range: 1.3–2.4 g; mean ± S.D.: 2.0 ± 0.4 g) was significantly higher compared to the weight of the testis of the ferrets in the deslorelin group (n = 7; range 0.2–0.5 g; mean ± S.D.: 0.3 ± 0.1 g).

A significant correlation was found between the volume of the testis and the plasma testosterone concentration (P < 0.05).

3.3. Testis histology

Since all stages of spermatogenesis were present in the testes of the ferrets from the placebo group we considered these testes to be normal (Fig. 4A and B). The mean (±S.E.M.) Johnsen score for this group is 8.8 ± 0.3. The diameter of the seminiferous tubules in the testes from the deslorelin group was much smaller than that from the placebo group (Fig. 4). The number of sertoli cells was comparable between groups, but in the deslorelin group no normal germ cells (spermato- gonia and spermatocytes) could be found. The Johnsen score for the testes in the deslorelin group therefore resulted in a consistent 2.

3.4. Odor

The strongest odor was found on the cloths which had been placed in the night boxes of the ferrets from the placebo group (mean ± S.E.M.: 3.8 ± 0.2). This was followed by the odor on the cloths from the surgically castrated ferrets (mean ± S.E.M.: 3.1 ± 0.1) and those from the ferrets out of the deslorelin group (mean ± S.E.M.: 2.7 ± 0.1). The differences between the deslorelin group, the placebo group and the surgically castrated group were all significant $\chi^2 = 239.1$; d.f. = 4 and $\chi^2 = 31.6$; d.f. = 4.

4. Discussion

The present data indicate that plasma testosterone concentrations, testis volume, spermatogenesis, and body odor in ferrets which received a deslorelin implant decreased to a level equal to, or even below those of surgically castrated ferrets.

Plasma testosterone concentrations and testis volume started to increase in April, which is approximately 1 month later than previously reported [26]. The decrease in testosterone concentrations and testis size started in July, which is also approximately 1 month later than previously reported. During both studies the ferrets were housed outdoors on approximately the same latitude. They should therefore have come into season at the same time. Different weather conditions may have attributed to this discrepancy between both studies. No conclusive evidence, however, can be given for this.

The positive correlation between testis volume and testosterone, as seen in our study, is in accordance with previous findings [26,27]. This correlation will be of practical use in the future when the implants will be used in a clinical setting. Owners of privately owned ferrets will know when it is time to replace the implant, once the testes size starts to increase.

Although no reliable plasma LH concentrations could be measured, plasma FSH concentrations were significantly lower in the deslorelin group compared to the surgically castrated and placebo groups. The combination of decreased FSH and testosterone concentrations in ferrets which had received deslorelin indicates that this depot GnRH agonist is capable of suppressing gonadotropin concentrations in ferrets. If hyperadrenocorticism is indeed caused by the increase of plasma LH concentrations after castration, the deslorelin implant may prevent the incidence of hyperadrenocorticism in ferrets.

The plasma concentrations of FSH in the castrated ferrets increased directly after castration and remained significantly higher than those of the intact ferrets and those with a deslorelin implant. This is similar to what has previously been described for plasma LH concentrations in castrated male ferrets [28]. The previous study, however, lasted only for 20 days while our study lasted 173 days. It is therefore likely that the increased concentrations of gonadotropins are not a temporary effect of castration.

Histological evaluation of the testes from the ferrets in the deslorelin group revealed that no normal germ
cells were found in any of the seminiferous tubules. Therefore these ferrets may be regarded as infertile. These findings are superior to what was previously reported for the use of continuous administration of GnRH agonists in mice and dogs [14,29]. In the latter reports, either still some spermatogenesis was seen, or sperm precursors were seen.

During the odor study, the smell of the ferrets from the deslorelin group was judged as the least intense. A possible explanation for the less amount of smell compared to the surgically castrated ferrets may be found in the fact that the adrenal androgen androstenedione, a known pheromone [30], in the latter group may be slightly higher due to stimulation by their elevated plasma gonadotropins. Measurement of androstenedione in the ferrets at different time points of this study (data not shown) was not able to support this hypothesis.

We conclude that the deslorelin implant effectively prevents reproduction and the musky odor of intact male ferrets and is therefore considered a suitable alternative for surgical castration. It can be expected that due to the fact that plasma gonadotropin concentrations are decreased also the incidence of hyperadrenocorticism will be reduced. Long-term follow-up of deslorelin-implanted animals is necessary to confirm this.

Acknowledgments

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The authors are grateful to Ms. W. Bergmann, C.H.A. van Blankers, Ms. R.J.A. Oostendorp, Ms. A. Slob, Ms. A. Strookappe and Ms. M. Timmerman for their skillful technical assistance.

References


Use of a gonadotropin releasing hormone agonist implant as an alternative for surgical castration in male ferrets

(Mustela putorius furo)

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Abstract

Surgical castration in ferrets has been implicated as an etiological factor in the development of hyperadrenocorticism in this species due to a castration-related increase in plasma gonadotropins. In search for a suitable alternative, the effect of treatment with the depot GnRH-agonist implant, deslorelin, on plasma testosterone concentrations and concurrent testes size, spermatogenesis, and the typical musky odor of intact male ferrets was investigated. Twenty-one male ferrets, equally divided into three groups, were either surgically castrated, received a slow release deslorelin implant or received a placebo implant. Plasma FSH and testosterone concentrations, testis size and spermatogenesis were all suppressed after the use of the deslorelin implant. The musky odor in the ferrets which had received a deslorelin implant was less compared to the ferrets which were either surgically castrated or had received a placebo implant. These results indicate that the deslorelin implant effectively prevents reproduction and the musky odor of intact male ferrets and is therefore considered a suitable alternative for surgical castration in these animals.

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Keywords: Deslorelin; Gonadotropin; GnRH; Testosterone; Spermatogenesis

1. Introduction

Surgical castration of ferrets (Mustela putorius furo) is common practice in the USA and various European countries. Although in male pet ferrets (hobs) there is no medical need for castration, they are mainly castrated to prevent reproduction, to reduce interspecies aggression enabling them to be kept in groups, and to decrease the intensity of the musky odor produced by the sebaceous glands [1].

Hyperadrenocorticism is a common disease in neutered pet ferrets. The syndrome differs from hyperadrenocorticism in other species, such as humans and dogs, in that glucocorticoid excess is much less pronounced in...
ferrets [2]. Instead, in ferrets the disease is characterized by excessive adrenal production of sex steroids, giving rise to vulvar swelling in neutered female ferrets (jills), recurrence of sexual behavior in neutered hobs, and alopecia [2–7].

It has been hypothesized that increased concentrations of gonadotropins, which occur after neutering due to the loss of negative feedback, persistently stimulate the adrenal cortex resulting in adrenocortical hyperplasia and tumor formation [6]. Strong support for this hypothesis may be found in the fact that the depot GnRH-agonists, leuprolide acetate and deslorelin, can be used successfully to treat ferrets with hyperadrenocorticism [8,9], and that LH-receptors (LH-R) have been detected in the adrenal glands of ferrets with hyperadrenocorticism [10]. These receptors were considered to be functional because plasma concentrations of adrenal androgens increased after intravenous injection of a GnRH-agonist [10]. Based on these findings it has been proposed to search for alternatives for surgical castration in ferrets [11].

One of the possible alternatives for surgical castration is the continuous administration of a GnRH analogue. The results of the use of these analogues differ, however, among the different species. In dogs and cheetahs continuous administration of a GnRH analogue suppresses spermatogenesis [12–14]. In the bull, however, continuous administration of a GnRH analogue leads to basal LH concentrations which are higher than in control animals, possibly explaining their concurrent increased plasma testosterone concentrations [15,16]. In addition, testis volume is also increased, and more round spermatids were found in the seminiferous tubules [17]. In marmoset monkeys and wallabies plasma testosterone concentrations remain within the normal range during the use of a long-acting GnRH agonist [18,19]. It is therefore not possible to predict the outcome of the use of long-acting GnRH analogues in ferrets.

The present study was designed to investigate whether a slow release depot GnRH-agonist implant containing 9.4 mg deslorelin (Peptech Animal Health, North Ryde, New South Wales, Australia) would reduce plasma FSH and testosterone concentrations in combination with concurrent testes volume, spermatogenesis, and the typical musky odor of intact hobs. Unfortunately, the previously used and for ferrets validated heterologous antiserum raised against ovine LH (GDN 15) is no longer available and no suitable alternative was found to reliably measure plasma LH concentrations in ferrets.

2. Materials and methods

2.1. Animals

For this study, which was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University, 21 male ferrets between the age of 1 and 2 years were used. At the start of the study the mean (±S.D.) weight of the ferrets was 1.4 ± 0.2 kg (range: 1.0–1.7 kg). They were individually housed in outdoor suspended cages with a night box at Utrecht University with the following GPS—coordinates: 52°05’04”N; 5°10’55”E. No artificial lighting was provided. Pellets (FerRet®, Hope Farm, Woerden, The Netherlands) and water were available ad libitum. The ferrets were randomly divided into three groups of 7 ferrets each. The ferrets in Group 1 were surgically castrated [mean (±S.D.) weight: 1.4 ± 0.2 kg; range: 1.0–1.6 kg], the ferrets in Group 2 received a GnRH implant containing 9.4 mg deslorelin [mean (±S.D.) weight: 1.5 ± 0.1 kg; range: 1.3–1.7 kg], and the ferrets in Group 3 received a similar implant without the deslorelin (placebo implant) [mean (±S.D.) weight: 1.5 ± 0.1 kg; range: 1.3–1.7 kg].

2.2. Procedures

2.2.1. Castration and placement of implants

The implants were placed subcutaneously in the scruff of the neck in the ferrets from Groups 2 and 3, 1 week after the first blood collection (17 March 2005). On the same day the other ferrets were surgically castrated. Prior to surgery, all ferrets received 4 mg/kg carprofen (Rimadyl®, Pfizer Animal Health, Capelle a/d IJssel, The Netherlands) IM. The ferrets were premedicated with an IM injection of 100 µg/kg medetomidine (Domitor®, Pfizer Animal Health, Capelle a/d IJssel, The Netherlands), followed after 10 min with a mask induction of 4% isoflurane (IsoFlo™, Abbot Animal Health, Hoofddorp, The Netherlands) in 100% oxygen. Anesthesia was maintained at 2% isoflurane, and castration was performed in a routine manner. The left testis was surgically removed from the ferrets in Groups 2 and 3, 15 weeks after the first blood collection (23 June 2005) for histological evaluation. The weight of the removed left testes was recorded and compared between both groups.

2.2.2. Blood collection and testis measurement

Blood samples were taken during the breeding season, from 10 March until 1 September 2005. Initially blood samples were taken weekly for 10 weeks, followed by once every fortnight for a period of 16
weeks. One year later, on 6 July 2006, blood samples were again taken from the same ferrets. At that time one ferret out of the placebo group had died from a thromboendoocarditis and one surgically castrated ferret had died during insulinoma surgery. During each sampling session the size of the right testis (width and length) was measured with a digital slide calliper. The testis volume was calculated using the following formula: (width)² × length × 0.524 (in cm), as previously used in testis measurements of black footed ferrets [20]. Blood was collected from the Vena cava cranialis while the ferret was anaesthetized with isoflurane. Blood samples were divided into pre-chilled EDTA and heparinized tubes. All tubes were centrifuged for 10 min at 3000 rpm at 4 °C, after which plasma from each tube was collected and stored in polystyrene tubes at −20 °C, pending analysis.

2.2.3. Hormone analysis

Testosterone measurements were performed after diethylether extraction using a solid-phase radioimmunoassay (RIA) Coat-A-Count® (Diagnostic Products Corporation, Los Angeles, USA). In short 0.25 mL plasma was extracted with 1 mL diethylether by end-over-end rotation for 15 min followed by centrifugation for 5 min at 3000 × g. After freezing of the lower aqueous phase the organic phase was decanted and evaporated to dryness. The extracts were reconstituted in 0.125 mL phosphate-buffered saline containing 0.5% (w/v) bovine serum albumin. Ferret plasma extracts were diluted 1, 2, 4 and 6 times and were parallel to the standard curve (r² = 0.98). Spiking of plasma samples containing an endogenous concentration of 1.4 with 15 nmol/L testosterone showed a spiking recovery of 95 ± 7%. The detection limit amounted to 0.2 nmol/L. The intra- and inter-assay coefficients of variation were 5.1 and 8.4%, respectively.

Follicle stimulating hormone concentrations were measured by RIA as described previously [21]. This RIA has been validated for use in ferrets [22]. Rat FSH-RP2 (NIAMD, Bethesda, MD) was used as a standard. The lower limit of detection was 0.8 μg/L and the intra-assay coefficient of variation was 7.2%.

2.2.4. Histological evaluation of the testes

Directly after excision, the testes were fixed by immersion in Bouin’s solution for 15–24 h. They were then suspended in ethanol 70% for 3 days, after which they were embedded in paraffin. From each testis, five sections of 5 μm were taken with an interspace of 30 μm. These sections were stained with the periodic acid-Schiff (PAS) reaction.

One hundred round tubular cross-sections were studied per section at 400 × magnification, resulting in a total of 500 cross sections per testis. The spermatogenesis in the seminiferous tubules was scored using a modification of the Johnsen method, as previously described [23,24]. Each tubular cross-section was given a score of 1–10, according to the presence or absence of the different spermatogenic cell types (Table 1). A mean score was calculated for each animal and for each group.

2.3. Odor study

Cotton cloths were placed in the night boxes of all hobs (n = 21) 14 weeks after castration and placement of the implants. After two nights these cloths were collected and individually placed in a vacuum sealed bag. Each bag was randomly given a number. A test panel consisting of students, coworkers, and other volunteers (n = 83), scored the smell from each cloth after opening the bag, smelling the cloth and rescaling the bag. The bags were opened in random order and given a score between 1 and 5, whereby a score of 1 indicated no odor and a score of 5 indicated a very strong odor.

2.4. Statistics

Statistical analysis was performed using the statistical package SPSS for Windows (Version 12.0) and R (Version 2.2.0).

Table 1
The Johnsen method: criteria to quantify the level of spermatogenesis in humans [23], modified for use in dogs [24]

<table>
<thead>
<tr>
<th>Johnsen score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No cells in tubular cross-section</td>
</tr>
<tr>
<td>2</td>
<td>No germ cells but only sertoli cells present</td>
</tr>
<tr>
<td>3</td>
<td>Spermatogonia are the only germ cells present</td>
</tr>
<tr>
<td>4</td>
<td>Only few spermatocytes (&lt;5) and no spermatids or spermatooza present</td>
</tr>
<tr>
<td>5</td>
<td>No spermatooza, no spermatids but several or many spermatocytes present</td>
</tr>
<tr>
<td>6</td>
<td>No spermatooza and only few spermatids (&lt;5–10) present</td>
</tr>
<tr>
<td>7</td>
<td>No spermatooza but many spermatids present</td>
</tr>
<tr>
<td>8</td>
<td>Only few spermatooza (&lt;5–10) present in a tubular cross-section</td>
</tr>
<tr>
<td>9</td>
<td>Many spermatooza present but germinal epithelium disorganized, with marked sloughing or obliteration of the lumen</td>
</tr>
<tr>
<td>10</td>
<td>Complete spermatogenesis with many spermatooza. Germinal epithelium organized in a regular thickness leaving an open lumen, or stage V of the seminiferous cycle, with sufficient round spermatids</td>
</tr>
</tbody>
</table>

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The correlation coefficient between the testis volume and plasma testosterone concentration was corrected for time and group.

Testis volumes and FSH concentrations were log transformed to better conform to a normal distribution and analyzed using a linear mixed-effect model. The fixed part of the model contained a group effect, a time effect and their interaction.

A random intercept was fitted for each individual together with an AR(1) correlation structure to model the dependence in time. For testis volume a random slope for time and different variance estimates for each of the three groups were fitted, and for FSH variance was allowed to differ for each group and for the first 5 time points, to capture the extra variation at the start of the experiment.

Since plasma testosterone concentrations were too often below the detection limit, transformation to conformity with the normal distribution was not possible. The nonparametric Kruskal–Wallis test was used at selected time points (10 March, 7 April, 14 April, 5 May, 23 June, 1 September, and 6 July 2006), where significant followed by Dunn post hoc tests to determine which groups differed from each other [25].

Differences in body odor between the three groups were tested by use of a $\chi^2$ analysis. In all instances significance was assumed at $P < 0.05$.

3. Results

Based on plasma testosterone concentrations, testis size and histological evaluation of the left testis (an arrest of spermatogenesis at the level of meiosis) we diagnosed one ferret from the placebo group with hypogonadism unrelated to this study. Based on these findings we excluded its data from the results.

3.1. Plasma testosterone and FSH concentrations

On 10 March, there were no significant differences in plasma testosterone concentrations between the three groups. On 7 April, control ferrets had significantly higher testosterone levels than castrated ferrets ($P < 0.01$), and ferrets from the deslorelin group ($P < 0.05$). From 14 April onwards both surgically and chemically castrated ferrets had significantly less testosterone than ferrets from the placebo group ($P < 0.01$). Surgically and chemically castrated ferrets did not differ significantly from each other.

On 10 March, plasma FSH concentrations were similar among the groups. From 31 March until 1 September, chemically castrated ferrets had lower plasma FSH concentrations than those which had received a placebo implant. From 24 March until 1 September, surgically castrated ferrets had higher plasma FSH concentrations than those which had received a placebo implant (Fig. 2).

3.2. Testis volume

On 10 March, the testis volumes were similar for all groups. The testis volume from the chemically castrated ferrets were significantly smaller compared to those from the placebo group (mean ± S.D.: 0.10 ± 0.03 cm$^3$) was still significantly
smaller compared to the testis volume in the placebo group \((n = 5);\) range: 0.94–1.38 cm\(^3\); mean \(\pm\) S.D.: 1.18 ± 0.18 cm\(^3\) \((P < 0.01)\).

The weight of the left testes from the ferrets in the placebo group \((n = 6);\) range: 1.3–2.4 g; mean \(\pm\) S.D.: 2.0 ± 0.4 g) was significantly higher compared to the weight of the testis of the ferrets in the deslorelin group \((n = 7);\) range 0.2–0.5 g; mean \(\pm\) S.D.: 0.3 ± 0.1 g).

A significant correlation was found between the volume of the testis and the plasma testosterone concentration \((P < 0.05)\).

### 3.3. Testis histology

Since all stages of spermatogenesis were present in the testis of the ferrets from the placebo group we considered these testes to be normal (Fig. 4A and B). The mean \(\pm\) S.E.M. Johnsen score for this group is 8.8 ± 0.3. The diameter of the seminiferous tubules in the testes from the deslorelin group was much smaller than that from the placebo group (Fig. 4). The number of sertoli cells was comparable between groups, but in the deslorelin group no normal germ cells (spermatogonia and spermatocytes) could be found. The Johnsen score for the testes in the deslorelin group therefore resulted in a consistent 2.

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The strongest odor was found on the cloths which had been placed in the night boxes of the ferrets from the placebo group (mean \(\pm\) S.E.M.: 3.8 ± 0.2). This was followed by the odor on the cloths from the surgically castrated ferrets (mean \(\pm\) S.E.M.: 3.1 ± 0.1) and those from the ferrets out of the deslorelin group (mean \(\pm\) S.E.M.: 2.7 ± 0.1). The differences between the deslorelin group, the placebo group and the surgically castrated group were all significant \(\chi^2 = 239.1;\) d.f. = 4 and \(\chi^2 = 31.6;\) d.f. = 4.

### 4. Discussion

The present data indicate that plasma testosterone concentrations, testis volume, spermatogenesis, and body odor in ferrets which received a deslorelin implant decreased to a level equal to, or even below those of surgically castrated ferrets.

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