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**Postgraduate School of Veterinary Science**

**Sexual endocrine diagnostic and treatment methods  
in the domestic ferret (*Mustela putorius furo*)**

**Theses of Ph.D. dissertation**

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Az értekezés a Dr. Huszenicza Gyula témavezető elnökletével Budapesten 2009. augusztus 28. án tartott munkahelyi vita nyomán nyerte el végleges formáját.

Készült 8 példányban. Ez a \_\_\_\_ sz. példány.

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dr. Proháczik Angella

## INTRODUCTION

In the last decades, due to its aesthetic attractiveness and comical personality the domestic ferret (*Mustela putorius furo*) became worldwide more and more popular as an exotic pet animal. Although ferret breeding has been under human control for several centuries, in the past, the overwhelming part of these animals were kept in outdoor kennels under the influence of natural photoperiod and changes of temperature, which determined their life circumstances (semidomesticated conditions). The more abrupt changes caused by full domestication began to appear just recently, in the last few decades, when the species (as an exotic companion animal) became isolated from the habitat. The indoor keeping year round under artificial lighting, the permanent temperature and the abundant (but not always adequate) feeding extended the breeding season for almost the whole year, and lengthened the lifespan of individuals. These housing conditions are frequently accompanied with complete sexual isolation of individuals, increasing the demand for surgical removal of gonads (ovariectomy, castration) and/or the non-invasive medical alternatives of these operative interventions. All these factors result in relatively high incidence of several endocrine diseases, such as various forms of hyperoestrogenism (including the side effects of neutering at young age), furthermore hormone-producing tumors of adrenocortex and pancreatic  $\beta$ -cells. Small animal practitioners have more and more ferrets as patients, and require wide range of information on biology, pathology, and diseases of this new exotic companion animal species.

## AIMS OF THE STUDIES

The author wishes to contribute to this developing subdiscipline of veterinary science with her recent work covering the following subjects:

- (a) Biological validation of a newly adapted assay system for quantification of progesterone metabolite (P<sub>4</sub>-met) content in serially collected fecal samples, which may be suitable for following up the ovarian cyclicity (e.g. ovulation with subsequent development of luteal tissue) or proving the ovarian quiescent (*Exp. 1a, 1b and 1c*).
- (b) Using the fecal P<sub>4</sub>-met determinations, the description of ovarian characteristics in postpartum female ferrets suckling and nursing their kittens for various periods after delivery (*Exp. 2a and 2b*). We wished to verify or refute whether the atypical phenomenon of elevated fecal P<sub>4</sub>-met during lactation and out of breeding season – which was recently postulated to occur in certain reflex ovulator Carnivores (*Felidae*: lynxes) – is present. If so, we wanted to prove (i) the ovarian origin of this progesterone (P<sub>4</sub>), (ii) which ovarian structures may produce it, and (iii) how this tendency in fecal P<sub>4</sub>-met interferes with returning to oestrus of these females after a regular or early weaning, compared to non-lactating post-partum individuals.
- (c) Using fecal P<sub>4</sub>-met profiles completed with classic clinical parameters as diagnostic tools, comparison of different endocrine treatment procedures used for reversible control of ovulation and ovarian cyclicity (*Exp. 3*).
- (d) Categorization of main forms of hyperoestrogenism in ferrets, and estimating the efficacy of a new form of their therapy (*Exp. 4*).
- (e) Introduction of certain endocrine characteristics of pregnancy toxemia in ferrets (*Exp. 5*).

## STUDIES

### *Experiment 1a, 1b, and 1c*

#### Design and results

The *Exp. 1a* was conducted on jills (n=6) undergone ovariectomy. At their preceding oestrus – e.g. about 9 to 21 days before the surgical intervention – in all females the ovulation was induced with 100 IU of hCG (Choriogonin inj.<sup>1</sup>). At the ovariectomy (in ketamine-xylazine anaesthesia<sup>2</sup>), blood and fecal samples were collected; then the ovaries were removed with a traditional surgical method. At that time, 3 to 12 CLs were visualized in all females. The plasma P<sub>4</sub> (measured by MEIA (microparticle enzyme immunoassay<sup>3</sup>)) and fecal P<sub>4</sub>-met (measured by ELISA (enzyme-linked immune assay<sup>4</sup>)) concentrations ranged between 7 and 33 nmol/L, and 600 and 2500 ng/g, respectively, and showed positive correlation (r=0.817; P< 0.05). In the second fecal samples taken 7 to 9 days after ovariectomy, low (< 500 ng/g) P<sub>4</sub>-met levels were measured by ELISA. However, (due to its more P<sub>4</sub>-specific, non-cross-reactive antibody) MEIA was not suitable for detection of any tendencies in the extracts of the fecal sample pairs.

In *Exp. 1b*, three formerly ovariectomised females were sampled once a week for six weeks in late March and April. Their fecal P<sub>4</sub>-met concentration remained low (< 500 ng/g; measured by ELISA) at all times.

In *Exp. 1c*, several months after gonadectomy, two ovariectomised females, and a castrated male were treated intramuscularly with 12.5 mg of progesterone (Luteosan inj.<sup>5</sup>). Fecal samples were repeatedly collected every 12 hours for 11 days, starting 60 hours before treatment. In all samples taken before treatment, fecal P<sub>4</sub>-met concentration was low (< 500 ng/g), and peaked to 3400 to 4000 ng/g 12 hours after treatment, and then gradually declined to baseline levels (< 500 ng/g) within 60 to 84 hours, remaining low until the end of the sampling process (all samples were assayed by ELISA).

#### Discussion and conclusions

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<sup>1</sup> Richter Gedeon, Budapest, Hungary

<sup>2</sup> Intramuscular combination of ketamine (2.5 mg/100 g body weight; SBH-Ketamine inj., Produlab Pharma BV.) and xylazine (0.2 mg/100 g body weight; Rompun inj., Bayer AG).

<sup>3</sup> highly specific antibody for P<sub>4</sub> (cross-reactivity of antibody with gestagen metabolites: 5 $\alpha$ -pregnan-3,20-dione: 6.3%; 5 $\beta$ -pregnan-3,20-dione: 3.2%; 5 $\alpha$ -pregnan-3 $\alpha$ -ol-20-ne: 1.7%; pregnenolone: 1.5%)

<sup>4</sup> anti-P<sub>4</sub> monoclonal antibody (5D4) cross-reacting with a wide range of gestagen metabolites (5 $\beta$ -pregnan-3,20-dione: 100%; 11 $\alpha$ -OH-progesterone: 20%; 5 $\alpha$ -progesterone -3,20-dione: 15.6%; 17 $\alpha$ -OH-progesterone: 3.6%; pregnenolone: 1.8%; 11 $\beta$ -OH-progesterone: 1.6%)

<sup>5</sup> Wefft-Chemie, Vienna, Austria

In the pseudopregnant jills of *Exp. 1a*, the circulating concentration of P<sub>4</sub> (measured by MEIA) was in strong positive correlation with the fecal P<sub>4</sub>-met content (measured by ELISA), and both methods detected elevated gestagen levels proving the presence of CL. Taking together the experiences of *Exp. 1a*, *1b* and *1c*, the fecal P<sub>4</sub>-met content of  $\geq 500$  ng/g (measured by ELISA) was considered as evidence of luteal activity in the ferret. So, the ELISA determination of fecal P<sub>4</sub>-met content (with this threshold level indicating simultaneous luteal function) was used also in our further studies (*Exp. 2* and *3*). Data of *Exp. 1a* proves that P<sub>4</sub> is excreted via the bile into the feces in form of its metabolites (5 $\beta$ -pregnan-3,20-dione and others), rather than in unmetabolised form. This finding fully agrees with the earlier studies (*Möstl et al., 1993; Larter et al., 1994; Schwarzenberger et al., 1996a*) reporting that unmetabolised P<sub>4</sub> is barely present, if at all, in fecal samples of *Carnivores*. So, the highly P<sub>4</sub>-specific MEIA technology is suitable for measuring P<sub>4</sub> in blood (plasma or serum), but it can not be used for quantification of fecal P<sub>4</sub>-met content.

## ***Experiment 2a and 2b***

### **Design and results**

Both in *Exp. 2a* and *2b*, only healthy jills (age:  $1.5 \pm 1.0$  years, range: from 0.7 to 3.7 years; body weight:  $873 \pm 197$ g, range: from 600 to 1250g) were enrolled from a private breeding kennel in Veszprém (Hungary), before the beginning of the breeding season (in mid February).

In *Exp. 2a*, eighteen jills producing 23 pregnancies were studied. Their clinical data on reproduction were collected and furthermore, ovarian activity was monitored by individual P<sub>4</sub>-met profiles. For this purpose, fecal samples were taken from each jill twice a week from mid February until the end of October (from those showing oestrus also in October and November: for about further 40 days) regularly, including also the period of gestation / pseudopregnancy and lactation. To increase the number of cases in which the period of late pregnancy, lactation, and post-weaning oestrus could be studied, further eleven healthy jills of the farm, conceived in March - early April were involved. These complementary animals were examined and sampled on the same way as the others, but only for a limited period (from their positive pregnancy detection until the time of post-weaning oestrus). Hence, altogether 34 pregnant cases were followed up. Based on the length of lactation, jills were allocated into one of the three following

groups: *normal-length lactation (NormL)* jills (n=21): nursed their kittens for > 5 weeks; *medium-length lactation (ML)* jills (n=9) could nurse their kittens only for 15 to 30 days, due to *agalactia* (n=8) or *mastitis* (n=1); *short-length lactation (SL)* jills (n=4) lost their litters due to some technical problems of farming on 4 to 6 days of lactation (n=3), or due to metritis and concomitant fever (n=1) kittens were weaned on day 6 postpartum. These jills delivered  $5\pm 3$ ,  $7\pm 2$  and  $4\pm 3$  kittens after  $41\pm 2$ ,  $41\pm 2$  and  $40\pm 2$  day long pregnancies, and lactated for  $44\pm 5$ ,  $23\pm 6$  and  $5\pm 1$  days in the NormL, ML and SL groups, respectively. Two jills conceiving in March, and nursing 2 and 5 kittens in the NormL group showed **lactational oestrus** on days 11 and 5 post-partum, respectively. This oestrus stopped spontaneously in both cases. Further experiences throughout the breeding season were that (1) at oestruses observed in early and late summer, or in the autumn period the pregnancy rate declined, whereas the incidence of pseudopregnancy, and the rate of females not returning to oestrus in the same breeding season showed the opposite tendency, (2) regardless of the season of oestrus / mating, the length of gestation and normal lactations, furthermore the litter size at birth did not differ; however, **the life span of CLs developed after the autumn mating was significantly shorter** (~ 3 weeks), than that one in the other seasons, (3) **lactational oestrus** was observed only after spring pregnancies, whereas **copulations not followed by formation of luteal tissue** were detected only in jills mated in autumn, e.g. between 15<sup>th</sup> of September and 7<sup>th</sup> of November and (4) **luteinization without copulation** occurred in the early summer period (in June).

In *Exp. 2b*, seventeen jills were randomly allocated into three groups: (i) non-lactating (NL) females weaned at delivery (n=4); (ii) jills lactating for 12 to 14 days (L1) (n=4); whereas those in the L2 group lactated for 16 to 22 days (n=9). NL jills were ovariohysterectomised 5 to 11 days after delivery, whereas those in L1 on day 12 to 14 and those in L2 group on day 16 to 22 post-delivery. Kittens were separated from their mothers 24 hours after delivery (NL group), or just before the operation (L1 and L2 females). The surgically removed ovaries were used for histological examination (light- and electron microscopy). To monitor their ovarian activity, fecal samples were taken for assaying P<sub>4</sub>-met content 3 times a week from oestrus / mating to the ovariectomy. From five jills in the group L2, additional fecal samples were taken daily for assaying P<sub>4</sub>-met content on days 3, 4 and 5 post-ovariohysterectomy. These jills delivered  $6\pm 2$  and  $5\pm 2$  kittens after  $43\pm 1$  and  $42\pm 1$  day long pregnancy in the NL and the two lactating (L1, L2) groups, respectively. All 4 jills weaned at delivery showed oestrus  $5\pm 1$

days postpartum, while vulval swelling was detected in none of the further 13 females until the end of the experiment.

In both experiments, fecal P<sub>4</sub>-met concentrations were low before and at the time of mating, started to elevate within 3-4 days after copulation, remained high during pregnancy ( $\geq 500$ -800 ng/g, up to about 2500 ng/g representing the upper detection limit of our P<sub>4</sub>-met ELISA), and dropped to baseline ( $< 500$  ng/g) at the time of, or within 2-4 days after delivery. Five to seven days after delivery, however, a sharp increase of P<sub>4</sub>-met concentrations ( $\geq 500$ -800 ng/g) was observed in all lactating females (n=47) of *Exp. 2a* and *2b*, while it remained baseline in females weaned at delivery (n=4). In *Exp. 2b*, **in fecal samples of lactating jills (L1 and L2 jills; n=13) elevated P<sub>4</sub>-met concentrations was detected** until the ovariectomy, whereas baseline level ( $< 500$  ng/g) P<sub>4</sub>-met concentrations could be measured in the fecal samples collected 3, 4 and 5 days post- ovariectomy from 5 jills in the group L2.

During their lactation, jills in the *Exp. 2a* excreted elevated P<sub>4</sub>-met content for  $37\pm 12$  (n=21),  $21\pm 9$  (n=9) and  $8\pm 11$  (n=4) days (calculated as the period between delivery and the last day with  $\geq 500$  ng/g P<sub>4</sub>-met concentration) in the NormL, ML and SL groups, respectively. After weaning, jills showed oestrus again on days  $13\pm 6$  (n=12/21),  $8\pm 8$  (n=7/9) and  $11\pm 6$  (n=3/4) (neither the rate nor the time returning to oestrus showed significant differences). In lactating jills (*Exp. 2b*), the histology of the ovaries proved the presence of follicles representing different, mainly less advanced stages of follicular development, and many luteinized cells. The follicular development appeared to be stopped at the late pre-antral/early antral phase. In such follicles, **granulosa cells had undergone luteinisation and** they were much larger than normal granulosa cells. Sections also presented **cells with more pronounced form of luteinization, resembling CL cells**. Such luteinized cells were studied also with electron microscopy. These cells had large cytoplasm filled with endoplasmatic vesicles, and had round or oval mitochondria with tubular structure. In the lateral deflection of the endoplasmatic reticulum, more electron dense secretory granula could be seen. In ovaries of non-lactating females (*Exp. 2b*), many growing tertiary (developing) follicles – together with some antral, but already atretic follicles – were the predominant structures. Luteinized cells were present in much lower rate. They were presumably derived from the hypertrophied theca cells developed in the atretic follicles or from the former CL still existing as remnants of the



earlier pregnancy. Moreover, many growing and developing follicles together with atretic follicles were observed.

### **Discussion and conclusions**

The aim of *Exp. 2a* was to describe the atypical phenomenon (elevated post-partum P<sub>4</sub>-met concentrations) in ferrets postulated to occur during lactation in the lynx. Both species are carnivores with induced ovulation. Our finding was in full agreement with that described in the post-partum Eurasian and Iberian lynx (*Pelican et al., 2006; Dehnhard et al., 2008; Göritz et al., 2009*). **During lactation, elevated fecal P<sub>4</sub>-met concentrations were measured, moreover the length of the period with elevated P<sub>4</sub>-met concentrations (37±12, 21±9 and 8±11 days) tended towards the duration of lactation (44±5, 23±6 and 5±1 days), respectively.** This post-partum elevation of fecal P<sub>4</sub>-met content seems to be one of the species-based characteristics in nursing females of certain carnivores (ferret, Eurasian and Iberian lynx). One aim of *Exp. 2b* was to describe which organ (the ovary or the adrenal gland) is responsible for the P<sub>4</sub> production during lactation. The fecal P<sub>4</sub>-met concentrations were high in all lactating jills while they dropped to the baseline value (< 500 ng/g) immediately after ovariohysterectomy in the 5 jills of *Exp. 2b* sampled also after the operation, proving the **ovarian origin of P<sub>4</sub>-met in the feces**. Similar phenomenon was not observed in some related species, like domestic cat (*Tsutsui and Stabenfeldt, 1993*) and otters (*Bateman et al., 2009*).

It is suggested that the suckling stimulus has primary importance to maintain lactation since reduction in the number of young being suckled to less than five may result in return to oestrus 2 or 3 weeks post-partum (lactational oestrus) if the photoperiod is adequate. Lactational oestrus was seen in *Exp. 2a*, when females (n=2) nursed 2 and 5 kittens. Interestingly, other females (n=8/21 in the normal-length lactation group) with less than 5 kittens did not show lactational oestrus. Post-weaning return to oestrus generally occurs within 2 weeks after weaning if female is exposed to stimulatory photoperiod (*Fox and Bell, 1998*). This was also shown in *Exp. 2a* without any difference between the L1 and L2 groups, which demonstrated that the length of lactation did not influence directly the time window between weaning and the first returning to oestrus. Interestingly, however, after normal lactation, jills needed longer time (13±6 days) to return oestrus than jills weaned at delivery (5±1 days; P< 0.05).

In the final, orienting part of this study (*Exp. 2b*) histological (light- and electron-microscopic) examinations of ovaries from lactating and non-lactating females after de-

livery were conducted, with the aim of looking after some morphological differences between ovaries of NL, L1 and L2 females, furthermore providing more evidences for the potential intraovarian origin of the elevated P<sub>4</sub> and P<sub>4</sub>-met production in nursing jills. In the ovary of lactating females, the development of growing follicles seemed to have been ceased in the pre-antral, early antral stage. Many luteinized cells in several presentations (follicles with luteinized granulosa cells, atretic follicles with luteinized theca cells and luteinized cells presumably coming from CL) were seen. Several hypotheses rose to explain which cells could be responsible for the P<sub>4</sub> production and how could develop such an ovarian pictures. Luteinized theca cells may originate from the still functional CL of pregnancy (stimulated by prolactin to produce P<sub>4</sub>), or maybe can come from post-partum ovulation. However, in lactating females, the essential requirement of LH for the final maturation of pre-ovulatory follicles is presumably missing. Moreover, ferrets are reflex ovulators. Follicles with luteinized granulosa cells in ferrets may be perhaps comparable with LUFs described in the unmated mink at the end of the breeding season (*Douglas et al., 1994*), when low frequency of LH pulses occurred due to the gonado-inhibitory photoperiod (*Jallageas et al., 1994a, 1994b*), in guinea pigs receiving hCG prior to the spontaneous ovulation (*Westfahl, 1993*) and in rats when the pre-ovulatory LH surge was artificially delayed (*Mattheij and Swarts, 1995*). In many mammals, the lactation delay and/or decrease the LH level and/or the frequency of LH pulses. Probably, this may happen also in ferrets generating the development of such tissue. Moreover, if prolactin has luteotropic property in ferrets (*Agu et al., 1986; McKibbin et al., 1984*), it can also distribute to this process (if prolactin is elevated during lactation in ferrets). This hypothesis may be supported with the fact that in rats, suppression of prolactin reduced LUFs' formation (*Mattheij and Swarts, 1995*). One case of LUF was observed also in one of our non-lactating jills, and as a related phenomenon, partial luteinisation with short-lived CL-s was detected in females copulated at late autumn oestruses. Nevertheless, references in mink describe that LUFs have the ability to produce P<sub>4</sub>; the real intraovarian origin of the elevated P<sub>4</sub>-met in lactating ferrets remained unexplained and hypothetic. To confirm the exact cell-level source and the physiological regulatory mechanisms of increased P<sub>4</sub> production, further studies (for example to prove the presence of 3 $\beta$ -hydroxysteroid dehydrogenase or prolactin receptors etc.) would be necessary.

In conclusion, our findings showed early recruitment of follicular growth in non-lactating post-partum female ferrets, while the cyclic ovarian activity is blocked in the lactating ones (however occasionally lactational oestrus may occur). Coinciding with the results described in the lynx, elevated fecal P<sub>4</sub>-met concentrations (likely with ovarian origin) in the lactating ferret could be detected. Moreover, as the length of the elevated fecal P<sub>4</sub>-met concentrations in the lactating ferret tended towards the duration of lactation, it can be supposed that this phenomenon together with suckling and other hormonal effects contribute to prevention of the early returning to oestrus in nursing female ferrets.

### ***Experiment 3***

#### **Design and results**

Twenty-five jills (age 1.5±0.9 years and body weight 760±115 g; mean ±SD) were randomly allocated to five groups (n=5 per group). Before the breeding season, in mid-February 2002, jills in three of these groups received one of the following cycle-suppressing treatments: subcutaneous administration of (i) 15 mg MPA<sup>6</sup>; (ii) 40 mg PROL<sup>7</sup>; or (iii) a srGnRH<sup>8</sup>. Implants were inserted subcutaneously in the scruff of the neck under short anaesthesia<sup>9</sup>. The other ten jills were left untreated in mid-February 2002. Later, when they showed oestrus in spring, usually in March, (iv) half of them were treated with 100 IU of hCG<sup>10</sup>, (v) whereas the other 5 jills were left untreated and naturally mated when showing signs of oestrus in spring and in summer (untreated controls). All females were assessed for vulval intumescence proving oestrus twice daily for 10 months, and were mated at any detected oestrus until their first pregnancy followed by regular delivery or abortion. In those not conceiving in 2002 (only srGnRH group), this process was continued and repeated regularly (twice a week) in the two subsequent years. Date of oestruses, copulations, deliveries, and numbers of the kittens were recorded. The ovarian response was monitored by individual fecal P<sub>4</sub>-met profiles (fecal samples were collected from each jill twice per week). Evaluation of the safety of treatments was based on fur quality (normal or alopecia) and on body condition changes.

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<sup>6</sup> Depo-Promone inj.®, Pharmacia & Upjohn, MPA group

<sup>7</sup> Covinan inj.®, Intervet International B.V.; PROL group

<sup>8</sup> 4.7 mg per jill; Deslorelin implant®, Peptech Animal Health; srGnRH group

<sup>9</sup> intramuscular combination of ketamine (5mg/kg body weight; SBH-Ketamine inj.; Produlab Pharma BV.) and medetomidine (0.08mg/kg body weight; Domitor inj.; Orion Pharma)

<sup>10</sup> (Choriogonin inj.®, Richter Gedeon; hCG group)

Examinations were repeated twice a week until the first post-treatment delivery, abortion or culling.

While safety was assessed on all of the included females (n=25), efficacy and reversibility could be evaluated on 22 jills. During the breeding season, untreated **control females** produced 8 pregnancies, 1 pseudopregnancy and 1 post-mating anovulation. In the **hCG group** (n=5), the treatment triggered ovulation followed by luteinisation and pseudopregnancy. Four jills returned to oestrus again in May or June,  $53\pm 9$  days after hCG administration: 3 of them conceived (75%) and delivered kittens and 1 of them became pseudopregnant. **Gestagen-treated jills** returned to oestrus after an ovarian quiescence of  $94\pm 18$  and  $99\pm 40$  days in the MPA and PROL groups, respectively. After mating, either pseudopregnancy (one jill of each group), or pregnancy (3 jills of each group; of them one MPA-treated female aborted four premature kittens on day 37) or anovulation was observed (one jill in the PROL group). In the control, hCG, MPA and PROL groups, the  $P_4$ -met concentrations were representative of the physiological status. All **srGnRH-treated jills** (n=5) displayed intensive oestrus signs with vulval swelling within 4 days after implant insertion. Two weeks after treatment, oestrus stopped spontaneously and the  $P_4$ -met concentrations remained low ( $< 500$  ng/g), thus proving lack of ovulation. Four jills showed oestrus 17 to 27 months ( $698\pm 122$  days) post-treatment. All 4 jills were mated at their first post-treatment oestrus, but none of them became pregnant. Three of them were mated again at their second post-treatment oestrus and all of them became pregnant and delivered 5 to 6 kittens.

Both gestagen treatments caused **progressive hormonal alopecia** in one case of each group. Four out of the 5 srGnRH treated females showed light alopecia on the tail from 166 days post-treatment for about 6 weeks. No side effect was recorded in the hCG treatment group; furthermore, srGnRH implants were well tolerated by the animals.

### **Discussion and conclusions**

The two long-acting forms of gestagens (15 mg of MPA and 40 mg of PROL per jill, s.c.) proved efficient **to prevent cyclic ovarian activity** for the same duration, 3 to 5 months ( $94\pm 18$  and  $99\pm 40$  days, respectively). The duration of efficacy of hCG treatment was shorter than that of the two gestagens. Jills resumed ovarian activity  $53\pm 9$  days post-treatment. This treatment is not suitable for suppression of ovarian activity for a longer period because ovarian follicular growth is not blocked. The longest follicle-suppressive effect ( $23\pm 4$  months) was detected in the srGnRH group. It can be supposed

that if jills (kept under natural photoperiod) receive this treatment at their oestrus in spring, ovarian activity for two entire breeding seasons could be suppressed. **Fertility** of the hCG, MPA and PROL treated females at the first oestrus after treatment was not different from the fertility of control jills. Reversibility of fertility after srGnRH treatment was however not achieved at the first oestrus post-treatment (each jill became pseudo-pregnant); three of them were mated at the second post-treatment oestrus (26±1 months after treatment) and all (100%) conceived and delivered. As regards **safety** of treatment, the Deslorelin implant and hCG were the best and MPA the worst.

## ***Experiment 4***

### **Design and results**

The study was conducted on domestic ferrets (*Mustela putorius furo*) ( $n_{\text{control}}=14$  and  $n_{\text{alopecic neutered}}=3$ ; age: 9 months to 4 years; body weight: 800 to 1000g). Each neutered ferret ( $n=2$  females and  $n=1$  male) showed pathognostic signs of endocrine alopecia. From each included ferret, blood samples were taken to determine  $17\beta$ -oestradiol ( $E_2$ ) concentrations ( $^{125}\text{I}$ -RIA; radio immuno assay). The alopecic ferrets were subcutaneously treated with 4.7 mg srGnRH<sup>11</sup>. Blood samples for  $E_2$  determination were again collected approximately one month after the insertion of the implant. Before Deslorelin implant insertion, two ferrets with suspected **hyperadrenocorticism** showed **high  $E_2$  concentrations** in plasma. These values (*Emmy*: 139.9 pmol/L and *Peti*: 99.45 pmol/L) were similar to healthy, untreated, control female  $E_2$  concentrations in oestrus (61.6 to 123.02 pmol/L,  $n=5$ ) ( $p=0.229$ ). In the third, unsuccessfully bled female (*Pipi*), hyperoestrogenism was supposed due to a swollen vulva together with clinical signs (alopecia). Some weeks after the Deslorelin implant insertion, all previously alopecic ferrets ( $n=3$ ) had recovered. Hair growth had resumed, good appetite had returned, and body condition had shown improvement in each case. In the females, vulval swelling had disappeared. In plasma samples of the two bled ferrets,  $E_2$  concentrations significantly decreased compared to the pre-treatment values ( $p=0.035$ ).  $E_2$  concentrations reached the baseline values (*Emmy*: 12.89 pmol/L and *Peti*: 16.08 pmol/L) typical to females in luteal phase and in anoestrus (12.0 to 30.58 pmol/L,  $n=9$ ) ( $p=0.137$ ). All treated ferrets were re-examined **19 to 21 months after Deslorelin implant insertion and all of them had normal fur and were clinically healthy.**

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<sup>11</sup> Deslorelin implant<sup>®</sup> Peptech Animal Health, North Ryde, Australia

## Discussion and conclusions

In three neutered ferrets, based on the clinical signs (oestrus in females and hormonal alopecia in both genders), hyperoestrogenism was supposed and plasma E<sub>2</sub> concentrations were measured. Ovarian remnant syndrome in the females was excluded. The diagnosis therefore was hyperadrenocorticism in both females. In the castrated male, after excluding ectoparasite or fungal infections, the diagnosis was also hyperadrenocorticism. Recently, deslorelin was used as a long-lasting treatment of hyperadrenocorticism in ferrets (*Wagner et al., 2005*). Based on our results, which show the safety and efficacy of using srGnRH (4.7 mg) to suppress ovarian function in intact female ferrets for  $\geq 1.5$  years, the three ferrets with clinical signs of hyperadrenocorticism included in this study were subcutaneously treated with a Deslorelin implant. In summary, **decreasing E<sub>2</sub> levels and improving clinical signs after Deslorelin implant insertion** prove that E<sub>2</sub> was the cause of hair loss in neutered ferrets with hormonal alopecia. E<sub>2</sub> was probably produced by the adrenal gland. The secretion of **sex steroids in ferrets (particularly E<sub>2</sub> production) was efficiently suppressed by 4.7 mg deslorelin for >19 months.**

## Experiment 5

### Design and results

The study was conducted on female ferrets (n=18; age: 9 to 35 months; body weight: 800 to 1000g). Four animals showed pathognostic signs of **pregnancy toxemia** (severe lethargy, dehydration, hypothermia, hair loss, uterus full of foetuses) on days 40 to 42 of their first (n=2), second (n=1) and fourth (n=1) pregnancy. At the time of veterinary examination, they were in the final comatose stage of this disease, and died spontaneously within minutes, before preparation for cesarean section. Samples were taken just at dying, as the final event of the emergency situation. These animals were necropsied immediately. During necropsy, the urinary pH and urobilinogen-, bilirubin- and ketone contents were also measured<sup>12</sup>. **Healthy female ferrets** (n=14) were used as control animals. Blood samples from each female were taken for BHB- ( $\beta$ -hydroxybutyrate), glucose-, insulin-, T<sub>4</sub>- (thyroxine) and T<sub>3</sub> (3,3',5-triiodothyronine) measurements<sup>13</sup>. Necropsy confirmed the suspected diagnosis of pregnancy toxemia:

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<sup>12</sup> Medi-Test Combi-9 strips, Macherey-Nagel, Duren, Germany

<sup>13</sup> Glucose was measured using glucose oxidase-peroxidase reaction (Glucose kit, Cat. #40841, Diagnosztikum Co. Ltd., Budapest, Hungary), BHB was measured using BHB-dehydrogenase reaction (D-3-Hydroxybutyrate kit, Cat. #RB 1007, Randox Laboratories Ltd., Ardmore, UK), insulin was determined using <sup>125</sup>I-Insulin RIA CT kit (CIS Bio International Ltd, Gif-Sur-Yvette,

mild icterus and severe form of hepatic lipidosis, furthermore ketonuria, low pH and increased urobilinogen and bilirubin contents in the urine were observed in all the four cases. Two of these were primiparous females carrying 9 and 10 kittens. The third ferret was in its second pregnancy and was carrying 14 kittens and the fourth female was in its fourth pregnancy with 13 kittens. In all animals with pregnancy toxemia, glucose levels were lower and BHB concentrations were higher than those found in healthy controls ( $p=0.034$  and  $0.001$ , respectively). Significant differences were found also in the endocrine parameters. The insulin-,  $T_4$ - and  $T_3$  contents were lower in the four sick female ferrets than in the 14 controls ( $p=0.0001$ ,  $0.024$  and  $0.001$ , respectively).

### **Discussion and conclusions**

Plasma glucose-, BHB-, insulin-,  $T_4$ - and  $T_3$  levels in animals with pregnancy toxemia were compared to those in healthy ferrets. Using comparison with healthy controls, significant differences were found in all these parameters. However, due to low number of animals, the results should be treated with caution. Whereas hematological and clinical biochemical parameters (among others, hyperketonemia, hypoglycemia and ketonuria) in pregnancy toxemia are well described in the ferret (*Fox et al., 1998; Batchelder et al., 1999; Dalrymple, 2004; Lewington, 2007a*), the endocrine response to relative or real fasting during late pregnancy in female ferrets carrying large litters (changes in the insulin,  $T_4$  and  $T_3$  blood concentration) had not been studied to date. It can be concluded that pregnancy toxemia caused by a negative energy balance in ferrets resembles the metabolic disease of ketonemia in late pregnant ruminants and that similar endocrine changes may occur. Since the endocrine and metabolic background of pathophysiology changes has not yet been fully elucidated in ferret pregnancy toxemia, further investigations are needed to confirm our suggestion that there exists a similarity to ruminant ketosis.

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France),  $T_4$  was measured using  $^{125}\text{I}$ -  $T_4$  RIA-Spec MIS kit (Institute of Isotopes Co. Ltd., Budapest, Hungary) and  $T_3$  was measured using  $^{125}\text{I}$ - $T_3$  RIA MIS kit (Institute of Isotopes Co. Ltd., Budapest, Hungary).

## OVERVIEW OF RESULTS

The author hopes that the results of these experiments represent some contributions to the management of reproduction and breeding practice of the domestic ferret. The findings obtained from the studies above represent novelty that has only very little literature or has not yet been reported elsewhere. The results were categorized based on their importances.

### 1.1. Reproductive biology (oestrus, ovulation/CL formation, lactation and return to oestrus) of domestic ferret

- Female ferrets displaying **oestrus** during  $9\pm 4$  days are ready to mate. At that time, the vulva swelling reaches its maximal size, and the vulval lips slightly open and become unglazed. Females mated in spring at that time become very likely pregnant (*Exp. 2*).
- In female ferrets mated in late summer or in autumn, (1) the heat may sporadically stop after mating without CL formation (i.e. **anovulation after mating**), (2) the heat may occasionally stop without mating with luteal structure formation (i.e. **spontaneous luteinization of the mature follicles without mating**), furthermore (3) if oestrus stops and CL formation occurs after mating, the subsequent **CL phase is rarely shorter** (~ 3 weeks) **than the normal** (*Exp. 2a*). However, these phenomena are sporadic and likely requires particular, to date unknown/unpublished conditions, and may probable be one of the reasons wherefore fewer kittens born at the seasonally late heat/mating.
- In **lactating ferrets** – like in other induced ovulator Carnivore, the lynx (but unlike in otters) – elevated fecal P<sub>4</sub>-met concentrations can be detected. The source of the elevated fecal P<sub>4</sub>-met excretion is the ovary; the length of this fecal P<sub>4</sub>-met elevation tends towards the duration of lactation (*Exp.2b*). This phenomenon together with suckling and other hormonal effects presumably contribute to prevention of the early returning to oestrus in nursing female ferrets.
- In **non-lactating post-partum female** ferrets, early recruitment of cyclic ovarian function can be detected (< 1 week postdelivery/weaning), while the final follicular development is blocked in the lactating ones; however, occasionally lactational oestrus occurs. Such **lactational oestrus** has not by all means detri-



mental effect on lactation and it may stop spontaneously without additional mating or hormonal treatment (*Exp. 2a*).

- Jills with normal length lactation need longer time (~2 weeks) to **return to oestrus** than jills weaned (lost their kittens) at delivery (<1 week) (*Exp. 2a*).

### **1.2. Clinical endocrinology of ferret**

The endocrine background of **pregnancy toxemia** caused by a negative energy balance in late pregnant relative or real fastened female ferrets strongly resembles that of the ketonaemia in late pregnant small ruminants (*Exp. 5*).

### **1.3. Endocrine diagnostic importance**

An ELISA system was validated for quantitative determination of fecal P<sub>4</sub>-met content providing an available, non-invasive diagnostic tool for the ferret health- and reproduction management (*Exp.1*).

### **1.4. Therapeutic importance**

- Subcutaneous Deslorelin implant (4.7 mg deslorelin acetate per animal) suppresses oestrus and cyclic ovarian function for a long period (~ 23 months) (*Exp.3*). This method can also be used for treating hyperoestrogenism of adrenocortical origin in neutered ferrets: long lasting (>19 months) improvement of clinical signs (hormonal alopecia) and reduction of elevated E<sub>2</sub> concentrations can be achieved (*Exp. 4*).
- Both gestagen treatments (proligestone and medroxyprogesterone acetate) can be used for a mid term (3 to 5 months) suppression of cyclic ovarian function, but their possible adverse events (alopecia and pyometra) should be kept in mind. Using proligestone is safer than the other gestagen (medroxyprogesterone acetate).

## PUBLICATIONS RELATED TO THE PRESENT DISSERTATION

### Full-text papers published in peer-reviewed journals in English

*Proháczik A., Kulcsár M., Huszenicza G.* (2009): Metabolic and endocrine characteristics of pregnancy toxemia in the ferret. *Vet. Med. Cz.* 54, pp. 75-80. (IF: 0,659)

*Proháczik A., Kulcsár M., Huszenicza G.* (2009): Deslorelin treatment of hyperoestrogenism in neutered ferrets (*Mustela putorius furo*). *Vet. Med. Cz.* 54, pp. 89-95. (IF: 0,659)

*Proháczik A., Kulcsár M., Huszenicza G., Trigg T., Driancourt M.A.* (2009): Comparative study on suppression of ovarian activity in ferrets (*Mustela putorius furo*). *Vet. Rec.* accepted for publication. (IF: 1,240)

*Proháczik A., Kulcsár M., Huszenicza G., Glávits R.* (2009): Reproductive features and fecal progesterone metabolite profile in female ferrets. *Reprod. Domest. Anim.* ongoing publication (IF: 1,526)

### Full-text papers published in peer-reviewed journals in Hungarian

*Proháczik A., Fodor K., Kulcsár M., Huszenicza Gy.* (2004): A vadászgörény (*Mustela putorius furo*) nemi működése, valamint gyakoribb ivarszervi és hormonális megbetegedései. 1. A faj bemutatása, táplálása és ivari működésének élettana. Irodalmi áttekintés. *Magyar Állatorvosok Lapja.* 126, 353-363. (IF: 0,088)

*Proháczik A., Kulcsár M., Huszenicza Gy.* (2004): A vadászgörény (*Mustela putorius furo*) nemi működése, valamint gyakoribb ivarszervi és hormonális megbetegedései. 2. Ivarszervi működészavarok, megbetegedések. Irodalmi áttekintés. *Magyar Állatorvosok Lapja.* 126, 364-369. (IF: 0,088)

*Proháczik A., Kulcsár M., Juhász Cs., Huszenicza Gy.* (2004): A vadászgörény (*Mustela putorius furo*) nemi működése, valamint gyakoribb ivarszervi és hormonális megbetegedései. 3. A nem kívánt ivarzás megelőzésének lehetőségei és korlátai. Irodalmi áttekintés. *Magyar Állatorvosok Lapja.* 126, 419-423. (IF: 0,088)

*Proháczik A., Kulcsár M., Huszenicza Gy.* (2004): A vadászgörény (*Mustela putorius furo*) nemi működése, valamint gyakoribb ivarszervi és hormonális megbetegedései. 4. Endokrin eredetű bőrelváltozások, hormonális megbetegedések. Irodalmi áttekintés. *Magy. Áo. Lapja.* 126, 553-560. (IF: 0,088)

### Abstracts published in peer-reviewed journals in English

*Proháczik A., Kulcsár M., Huszenicza Gy.* (2003): Fecal progesterone metabolite profile of nursing ferrets (*Mustela putorius furo*). *Abstract. Reprod. Domest. Anim.* 38, 362.

*Proháczik A., Kulcsár M., Trigg T., Huszenicza Gy.* (2003): Treatments suppressing ovarian activity in ferret (*Mustela putorius furo*). *Abstract. Reprod. Domest. Anim.* 38, 331.

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Angella Proháczik