Szent István University
Postgraduate School of Veterinary Science

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

Ph.D. dissertation

Written by:

Dr. Angella Proháczik

Supervisor: Prof. Gyula Huszenicza

2009

Szent István Egyetem
Állatorvos-tudományi Doktori Iskola

Témavezető:

.....
Dr. Huszenicza Gyula egyetemi tanár, DSc
Szent István Egyetem Állatorvostudományi Kar
Szülészeti és Szaporodásbiológiai Tanszék és Klinika

Témabizottsági tagok:

.....
Dr. Hornung Erzsébet int. vez. egyetemi docens, CSc
Szent István Egyetem Állatorvos-tudományi Kar
Biológiai Intézet

.....
✝ Dr. Rudas Péter tv. egyetemi tanár, DSc
Szent István Egyetem Állatorvos-tudományi Kar
Élettani és Biokémiai Tanszék

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.

…….
dr. Proháczik Angella

List of content

Abbreviations and acronyms

ACTH	adrenocorticotropic hormone
ALT	alanine transferase
ANOVA	analysis of variance
ARC	tonic centre of hypothalamus
BHB	β-hydroxybutyrate
CL	corpus luteum / corpora lutea
E_2	17β-oestradiol
e.g.	for example ("exempli gratia")
ELISA	enzyme-linked immune assay
etc.	and the rest ("et cetera")
FFA	free fatty acid
FSH	follicle-stimulating hormone
GnRH	gonadotropin releasing hormone
hCG	human chorionic gonadotropin hormone
i.e.	that is ("id est")
i.m.	intramuscular
IU	international unit
L1	group of jills lactating for 12 to 14 days in *Exp. 2b*
L2	group of jills lactating for 16 to 22 days in *Exp. 2b*
LH	luteinizing hormone
LUF	luteinized unruptured follicle
MA	megestrol acetate
MEIA	microparticle enzyme immunoassay
mg	milligram
mL	milliliter
ML	medium-length lactation
mm	millimeter
MPA	medroxyprogesterone acetate
NL	group of the non lactating females in *Exp. 2b*
NormL	normal-length lactation
ORS	ovarian remnant syndrome
P_4	progesterone
P_4-met	progesterone metabolite
pm	picometer
pmol/L	picomol/litre
PROL	proligestone
RIA	radio-immune assay
s.c.	subcutaneous
SCN	suprachiasmatic nuclei located in the anterior hypothalamus
SD	standard deviation
SEM	standard error of the mean
SL	short-length lactation
$_{sr}$GnRH	slow-release formulation of deslorelin acetate (i.e. Deslorelin implant)
T_3	3,3',5-triiodothyronine
T_4	thyroxine
UK	United Kingdom
US	United States
χ^2	Chi-square

Summary

Reproductive and endocrine disorders in the domestic ferret (*Mustela putorius furo*) have been generated due to the involvement of the species in the modern human civilization. After reviewing the relevant literature, this work focuses on certain fields of ferret's reproductive management. The first item was the adaptation and biological validation of an assay system (used only in ruminants in our lab earlier), which is suitable for quantification of progesterone metabolite (P_4-met) content in serially collected fecal samples (*Exp. 1a, 1b* and *1c*). The successful biological validation was followed by series of studies (*Exp. 2a*, *2b*, *3*, *4*, and *5*) based partly on the use of this assay system.

In the *Exp. 2a* and *2b* with 51 animals (47 lactating jills and 4 controls) we tended to verify or refute whether the atypical phenomenon (i.e. elevated fecal P_4-met during lactation and out of breeding season), recently postulated to occur in certain reflex ovulator Carnivores (*Felidae*: lynxes), is present also in ferrets. In addition, ovarian pictures and post-weaning return to oestrus features were monitored. Our results indicates that (i) similarly to lynxes, elevated fecal P_4-met concentrations occurs in nursing ferrets, furthermore the length of that fecal P_4-met elevation tended towards the duration of lactation; (ii) in lactating females, the ovarian stroma is more active than that in non-lactating ones likely being partly or completely responsible for the elevated P_4-met content; (iii) early resumption of the entire ovarian activity (developed follicles and oestrus) occurs in non-lactating post-partum females, while final follicular development is blocked (follicles stalls at antral stage) in the lactating ones. We suppose that the elevated fecal P_4-met during lactation together with suckling and other hormonal effects may contribute to prevention of early returning to oestrus in nursing female ferrets.

The purpose of the *Exp. 3* was to compare four hormonal treatments used for suppression of ovarian activity in a total of 25 female ferrets (n=20 treated; e.g. 5 in each group, and 5 untreated controls). The females received either 15 mg medroxyprogesterone-acetate (MPA), or 40 mg proligestone (PROL), or a slow-release formulation of deslorelin acetate ($_{sr}$GnRH, i.e. Deslorelin implant) (4.7 mg) before the beginning of the breeding season, or were treated with 100 international units (IU) human chorionic gonadotropin hormone (hCG) at the spring oestrus, or left untreated. Fecal P_4-met profiles and post-treatment return to oestrus were monitored. Durations of treatment induced ovarian quiescence were 94±18, 99±40, 53±9 and 698±122 days in

groups MAP, PROL, hCG and $_{sr}$GnRH, respectively. As regards safety, hCG and $_{sr}$GnRH proved to be the best and MPA the worst. Both gestagen treatments caused alopecia; moreover MPA caused purulent like vaginal discharge. At the first post-treatment mating, fertilities in the PROL, MPA and hCG groups were similar to that in the control group. Furthermore, three $_{sr}$GnRH-treated jills conceived at the second post-treatment oestrus. In conclusion, clear differences were demonstrated in the ability of $_{sr}$GnRH versus the other three treatments to suppress ovarian function.

In the *Exp. 4* it was tested whether 4.7 mg $_{sr}$GnRH has any value in the therapy of hyperoestrogenism of adrenocortical origin. Alopecic ferrets with elevated E_2 (17β-oestradiol) levels (n=3, $E_{2\ pre\text{-}treatment}$: 99.45 to 139.9 pmol/L) and control ferrets (n=14) ($E_{2\text{-}oestrus}$: 61.6 to 123.02 pmol/L, $E_{2\text{-}anoestrus}$: 12.0 to 30.58 pmol/L) were involved in the study. Some weeks after treatment, in the previously alopecic animals, hair began to grow and E_2 concentrations decreased from 12.89 to 16.08 pmol/L. The effect of treatment was long-lasting (at least 19 months). We concluded that $_{sr}$GnRH can be useful in the therapy of hyperoestrogenism of adrenocortical origin in neutered ferrets.

The objective of the *Exp. 5* was to monitor endocrine changes, described in pregnancy toxemia of ewes, in ferrets (n=4 with pregnancy toxemia and n=14 control). Our results show that, in contrast to healthy controls, hypoglycemia, hyperketonemia, hypoinsulinemia and decreased thyroxine (T_4) and 3,3',5-triiodothyronine (T_3) levels occur in females with pregnancy toxemia. In conclusion, pregnancy toxemia caused by a negative energy balance in ferrets resembles the late-gestational hyperketonemia of twin/multiple-pregnant ewes, and moreover that similar endocrine changes may occur.

The results of our experiments hopefully represent some contributions to the management of ferret reproduction and breeding.

Összefoglalás

A háziasított vadászgörény (*Mustela putorius furo*) modern tartástechnológiája számos ivarszervi működészavar és hormonális megbetegedés megjelenéséhez vezetett. A faj szaporodásbiológiai jellemzőire vonatkozó irodalom áttekintését követően első feladatunk egy korábban már egyéb (kérődző) fajokban általunk is használt, a bélsár progeszteron-metabolit (P_4-met) tartalmának a meghatározására szolgáló analitikai módszernek az adaptálása, majd az eljárás vadászgörényen történő biológiai validálása volt (*1. kísérlet*). A továbbiakban – részben e módszer használatával – a faj néhány szaporodásbiológiai jellegzetességét, egyes, a petefészek-működésre ható gyógykezelési módszerek hatékonyságát, illetve a vemhességi toxikózis egyes endokrinológiai jellemzőit vizsgáltuk.

A *2a* és *2b kísérletben* 51 állatot (47 szoptató nőstényt és 4 kontrollt) vizsgáltunk, és arra kerestük a választ, hogy vajon a közelmúltban egyes provokált ovulációjú húsevőkben (*Felidae*: hiúz) leírt atipikus jellegzetességek (a bélsár szoptatás alatti és a tenyész-szezonon kívüli emelkedett P_4-met tartalma) megtalálhatóak-e görényben is. Az állatoktól gyűjtött bélsár-minták P_4-met tartalmának meghatározásán túl, néhány nőstény petefészekének szövettani képét vizsgáltuk, amíg a többi állatban a választás utáni ivarzást követtük nyomon. Eredményeink szerint, (i) hasonlóan a hiúzban tapasztaltakhoz, emelkedett P_4-met tartalom mérhető a szoptató görények bélsarában, továbbá e hormon emelkedett szintjének hossza közelít a szoptatás hosszához; (ii) a szoptató nőstényekben, a petefészek, amely valószínűleg részben vagy teljes mértékben felelős az emelkedett P_4-met szintért, aktívabb, mint a nemszoptató egyedekben; (iii) amíg a komplett petefészek-működés (fejlődő tüszők és ivarzás) korán megjelenik az ellett, de nem-szoptató nőstényekben, addig a végső tüsző-fejlődés gátolt a szoptató egyedekben. Feltételezzük, hogy a kölyöknevelés alatti emelkedett P_4 termelés, a szoptatással és egyéb hormonális hatásokkal együtt, hozzájárul a szoptató nőstények korai ciklusba lendülésének megakadályozásához.

A *3. kísérletben* 25 nőstény görényt vizsgálva (n=20 kezelt (5/csoport) és n=5 kontrol)), négy, az ivari működés elnyomására használt hormonális kezelés hatékonyságát és biztonságát hasonlítottunk össze. Az állatokat (1) a tenyész-szezon kezdete előtt kezeltük az alábbiak valamelyikével: 15 mg medroxiprogeszteron-acetát (MPA), 40 mg proligeszton (PROL), illetve 4,7 mg deslorelin ($_{sr}$GnRH); (2) a tavaszi első ivarzáskor kezeltük 100 NE humán choriongonadotropin hormonnal (hCG); (3)

vagy kezeletlenül hagytuk (kontrol). A kísérlet során, az állatoktól gyűjtött bélsár P_4-met tartalmának változását és a nőstények kezelés utáni ivarzásba lendülését követtük nyomon. Az alkalmazott MPA, PROL, hCG illetve, $_{sr}$GnRH kezelés 94±18, 99±40, 53±9 és 698±122 napon át késleltette a következő ivarzás kialakulását. Amíg a hCG és a $_{sr}$GnRH kezelés nem okozott mellékhatást, addig mindkét gesztagén kezelés után szőrhullás, továbbá a MPA kezelés után gennyes jellegű hüvelyfolyás volt tapasztalható. A hCG, MPA és PROL kezelt nőstények termékenysége a kezelést követő első fedeztetéskor hasonló volt a kontrolokéhoz, amíg a $_{sr}$GnRH kezelt állatok csak a kezelést követő második ivarzáskor vemhesültek. Eredményeink alapján, a $_{sr}$GnRH kezelés hosszabb időre képes elnyomni az ivarzást görényben, mint a többi három hormonális kezelés.

A *4. kísérlet* során 4,7 mg $_{sr}$GnRH kezelés hatását vizsgáltuk olyan hormonális szőrhullást mutató görényekben, amelyek vérében, nagy valószínűség szerint mellékvesekéreg eredetű, emelkedett ösztrogénszint (E_2) (n=3, $E_{2\ \text{kezelés előtt}}$: 99,5 - 139,9 pmol/L) volt mérhető. A kísérletben 14 kontrol nőstényt ($E_{2\text{-ivarzó}}$: 61,6 – 123,0 pmol/L, $E_{2\text{-nem ivarzó}}$: 12,0 - 30,6 pmol/L) használtunk. Néhány héttel a $_{sr}$GnRH kezelést követően a szőrhullást mutató egyedekben a szőr növekedése és alacsony E_2 szint (12,9 - 16,1 pmol/L) volt tapasztalható. A kezelés hatásossága legalább 19 hónap volt. Véleményünk szerint, a $_{sr}$GnRH kezelés sikeresen alkalmazható a görények azon mellékvese megbetegedésében, amelyben az E_2 fokozott termelődése tapasztalható.

Az *5. kísérletben* juhok vemhességi toxikózisában leírt endokrin változásokat követtünk nyomon és bizonyítottuk azokat vadászgörényben ($n_{\text{vemhességi toxikózis}}$ = 4 és n_{kontrol} = 14). A beteg anyákban csökkent vércukorértéket, szignifikánsan alacsonyabb inzulin-, tiroxin- és 3,3',5-trijódtironin-, valamint emelkedett βOH-vajsav szinteket mértünk. Megállapításunk szerint a negatív energia egyensúly alapján, a vemhesség késői időszakában kialakuló kórkép hasonló a más fajokban – leggyakrabban ikervemhes juhokban – kialakuló hyperketonaemiához, továbbá a kórkép hátterében mindkét fajban hasonló endokrin változások következnek be.

Reményeink szerint eredményeink hozzájárulnak a vadászgörény szaporodásbiológiai gondozására és tenyésztésére vonatkozó tudásanyaghoz.

1. Introduction and the aims of the studies

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 β-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.

After an extensive reviewing the relevant updated literature – as the aims of these series of 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_4-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_4-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_4-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_4), (ii) which ovarian structures may produce it, and (iii) how this tendency in fecal P_4-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_4-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*).

2. Review of literature

2.1. Taxonomy, domestication and current status of ferrets

The ferret (*Mustela putorius furo*) was named by Linnaeus in 1758, and is a domesticated subspecies of the species *Mustela putorius* (European polecat) belonging to the genus *Mustela*, the subfamily *Mustelinae*, the family *Mustelidae*, the suborder *Caniformia* (caniform carnivores) and the order *Carnivora* (carnivore mammals) (*Sato et al., 2003*). The number of mustelid species and genera is not standardized and several classifications are reported in the recent literature likely due to that many taxonomic schemes proposed for mustelids within the last century were based on morphology and classified genera into various numbers of subfamilies whose boundaries were largely determined by eco-morphological similarities. Presently, it is proposed that there are 59 mustelid species (including weasels, ferrets, minks, badgers, otters, wolverines) extant and they are classified in 22 genera and 5 subfamilies. These subfamilies are *Mustelinae* (weasels, martens, and their allies), *Lutrinae* (otters), *Melinae* (badgers), *Mellivorinae* (honey badger), and *Taxidinae* (American badger) (*Koepfli et al., 2008*).

Within the subfamily *Mustelinae* and genus *Mustela*, ferrets belong to the subgenus *Putorius*, in which there are only three extant species: *Mustela putorius*, the European polecat; *Mustela eversmanni*, the Siberian, or steppe polecat; and *Mustela nigripes*, the black-footed ferret (the "ferret group") (*Sato et al., 2003*). Based on phylo- and molecular genetic studies, the members of the "ferret group" has similar 2n chromosome number (2n=40) and they can be successfully hybridized, moreover their offspring are fertile. However, low levels of genetic divergence among European polecat, *Mustela putorius*, steppe polecat, *Mustela eversmannii*, and European mink, *Mustela lutreola*, suggest that all these species may be considered subspecies within a single species, *Mustela putorius* (*Marmi et al., 2004; Kurose et al., 2000*). Interestingly, the European polecat may also hibridise with the European mink (2n=38) and have fertile hybrids (*Ternovsky, 1977 cited by Lodé et al, 2005; Amstislavsky et al, 2004*).Mustelids are widely distributed geographically and occur throughout Eurasia, Africa, America and New Zealand. They have adapted to numerous climatic and biotic conditions and are found in habitats that range from the arctic tundra to tropical rain-forest and from deserts to inland waterways and even the open sea. In many ecosystem

of the Northern Hemisphere, mustelids are the most-common predator mammals (*Sato et al., 2003*).

The scientific names *Mustela putorius furo*, *Mustela putorius form. furo*, *Mustela furo, Putorius putorius furo*, and *Putorius furo* are alternative terms used to describe the same animal, the domestic ferret. The name *Mustela* is a Latin derivation of the term “mus” for mouse. Putorius is from the Latin “putor”, which means a stench referring to the musky odor of the ferret. Furo comes from the Latin “furonem” meaning "thief". So we have a "mouse-catching, smelly, thief"! The word ferret most likely comes from the Latin “furo” or the Italian “furone” with the same meaning of "thief".

The “domestic” ferret has been recorded since the fourth century BC (before Christ) when *Aristotle* mentioned it in one of his works (*Lewington, 2007d)*. Biogeographical analyses indicate that most of the extant diversity of mustelids originated in Eurasia and mustelids have colonized Africa, North America and South-America on multiple occasions (*Koepfli et al., 2008*). The ferret is generally thought to have been “domesticated” somewhere in the Mediterranean region, although Strabo explicitly indicated its African origin and Linnaeus (1758) named this taxon as being from Africa. Ferrets were introduced into Europe possibly by the Romans or the Normans during their invasions. Over the centuries, numerous references have been made to the use of ferrets in Europe, including for rodent control in homes, farms, and ships, and for hunting rabbits both for damage control and for human food. The domestic ferret was introduced into Australia from Europe in the 1800s to control the populations of European rabbits. Fortunately, enough other predators, such as foxes, dingoes preyed on the ferret so that feral population never developed. However, when they were introduced into New Zealand for the same reason in the late 1800s along with stoats and weasels, there were no predators to control their numbers. Feral populations of domestic ferrets therefore developed and are still present today. The domestic ferret was probably introduced into the United States (US) also from Europe by the shipping industry in the 1700s. They may have come as pets or as hunting companions (*Fox, 1998b; Church, 2007*).

It was not until the 1900s, when the ferret was first formally introduced as an animal model for biomedical research. Ferrets are tractable, share many anatomical, metabolic, and physiologic features with several mammals, including humans. Hence, they are used in several studies including cardio-pulmonary (heart hypertrophy and

failure), neurological (neurological changes associated with brain and spinal cord injury), and gastrointestinal (gastric infections and ulcerations) research. The ferret is also ideally suited for research in behavior, neuro-endocrinology and parasitological research. Among other diseases the influenza infection in ferrets closely resembles that in humans. The similarities are seen in clinical signs, pathogenesis and immunity. Hence, the ferret is an ideal model in studying the virulence of different influenza strains, age related susceptibility to infection, treatment and vaccination. The ferret has also been used as a model in pancreatic β-cell tumors, furthermore in studies of canine distemper, infectious bovine rhinotracheitits, feline parvovirus, pseudo-rabies and several other viral diseases (*Fox, 1998b*). Ferrets have also been used as a model for the demonstration of medical procedures (pediatric tracheal intubation) and for many basic research applications (non-rodent test system in pharmaceutical drug development) (*Gad, 2000*). A ferret model for evaluating the emetic potential of drugs is another use of this species in the field of drug development (*Crawford et al, 2002*).

The successful production of viable ferret progeny following somatic cell nuclear transfer also provides exciting new opportunities for basic research, investigating early embryogenesis and the propagation of endangered black-footed ferrets and European minks (*Li et al., 2006a, 2006b*). The domestic ferret is an ideal model animal also in the nature conversation programs. It can be used for developing new procedures in assisted reproduction (artificial insemination, *in vitro* fertilization, frozen storage of embryos and surrogate parenting) and for research of basic physiological features and reproductive biology of the endangered species (*Li et al., 2006b*).

Ferrets are also used as pelt producers. A coat made of ferret fur referred as fitch. Each year, approximately 15 000 ferrets are raised and slaughtered for their fur. The bulk of ferret fur farming takes place in Finland (11 000 a year), though a small number of these ranches exists in Sweden, New Zealand, Poland and in the US (*Roots, 2007*).

Due to their esthetical attractiveness, in the last few decades, the ferret was getting more and more popular as an exotic companion animal. The domestic ferret is about the 3rd most popular carnivore pet in the US (survey in 1998 showed that there were 10 million pet ferrets and 50 million dogs) and in various European countries. Among the large variety of house pets, wild carnivores, especially ferrets, have experienced increasing popularity, with an estimated at least 600 000 ferrets sold annually in the US. In the last 10 years, the same increase of pet ferret population was detectable also in

Hungary; however, we didn't have exact data about the number of animals. According to some estimation, there are about 100.000 individuals kept in Hungary (*personal communication*).

2.2. Reproduction in the domestic ferret

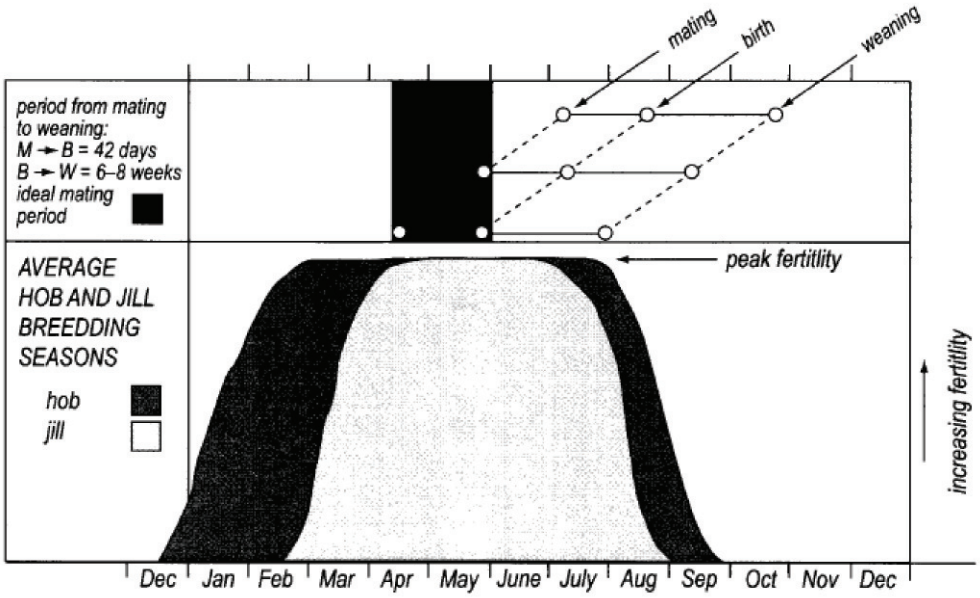
The domestic ferret is a typical seasonal breeder with induced (reflex) ovulation. Its reproductive pattern is quite close to that one of the domestic cat and several other *Felidae*, but there are also remarkable species-based characteristics (*Lewington, 2007b*).

2.2.1. Influence of photoperiod, puberty and breeding season

Generally, ferrets reach sexual maturity (**puberty**) at 8-12 months of age. However, since they are seasonal breeders, the season in which the kittens are born, influences the age at which puberty occurs. Kittens born in the autumn will reach puberty at an earlier age than those born earlier in the summer (*Fox and Bell, 1998*). Ferrets become sexually mature above a particular, critical body weight of 420 g (*Donovan, 1986*).

Both male and female reproductive functions are controlled by **photoperiod**. Female ferrets (**jills**) come in oestrus in response to lengthening days (more than 12 hours of light per day; *Bissonnette, 1932*) which occur in early spring (in March; approximately 2.5 months after the winter solstice, 21st of December in the northern hemisphere). This time of oestrus, to which the gestation period is added, allows delivery of the young an optimal season of year (kittens have best chance to survive in the nature). Under the current housing conditions, after weaning the litter, jills can return to oestrus again until late August - early September (or even longer if the artificial light exposure is intensive). Male ferrets (**hobs**) are in breeding condition more than one month before the first jills are in oestrus and their breeding season lasts from late January until late July - August - early September (reviewed by *Lewington, 2007b*; see also *Figure 1*).

Figure 1: Breeding chart for ferrets in the northern hemisphere (after *Porter* and *Brown*: The complete book of ferrets. Bedford, D&M Publications, 1997; cited by *Lewington, 2007b*) (M: mating, B: birth, W: weaning)



The link between ambient photoperiod and seasonal breeding in ferrets was first reported by *Bissonnette (1932)*. This early evidence states that the initiation of gonadal activity is dependent on the light-dark cycle, which stimulates or inhibits reproduction through transmission of information about the day length to the brain. Information about day length is transmitted from the retinal cells of the eye, via the optic nerves, to the suprachiasmatic nuclei (SCN), located in the anterior hypothalamus. SCN is the major circadian pacemaker in mammals. The output from the SCN is transmitted via the paraventricular nucleus and the superior cervical ganglia to the pineal gland (*Forsberg, 1992*).

In addition to exhibiting seasonal breeding as adults, female ferrets are responsive to the stimulatory effect of photoperiod before puberty occurs (*Thorpe, 1967; Ryan, 1984*). Female ferrets reared in short-day photoperiod (8 hours light / 16 hours dark) attain sexual maturity at about 35-50 week of age. If ferrets are moved at 15 week of age to a long-day photoperiod of 16 hours light / 8 hours dark, sexual maturation is accelerated to completion within 7-8 week of the onset of long days, when the animals are 22 week of age. It has been reported, however, that if developing ferrets are reared in long days from birth or are placed in long days any time before 15 week of age, sexual maturation is delayed for periods as long as 2 year (*Ryan, 1984; Ryan, unpublished observations*).

In ferrets (as in mammals, in birds and in reptailes) the timing of the circadian rhythm is set by the transition from dark to light at dawn and results in a photosensitive window that opens 12 or 13 hours later. If the animal is exposed to light during this window, the photoperiod seems to be long day; if there is no light during this window, a short day is perceived. Thus, animals can be "tricked" into perceiving a long day by two brief (15-min.-long) light pulses 13 hours apart. This endogenous rhythm in photosensitivity resides in the SCN, which controls a number of endogenous circadian rhythm in mammals. One main function of this system is to traduce this photic information into the endocrine signal that controls reproductive function. This is accomplished by **melatonin** secretion from the pineal gland. Melatonin patterns controls reproduction in many seasonal breeders. The duration of the nightly melatonin rise is the critical characteristics indicating the length of the day (e.g. if he melatonin rise is longer than 12 hours it is a short day) (recently reviewed by *Faigl et al., 2007; Chemineau et al., 2008*). *Baum et al.* (*1986*) documented that adult ferrets exhibit distinct rhythms of melatonin concentrations in the pineal gland that follow the light / dark cycle and coincide with a similar rhythm of this hormone in the plasma. Melatonin secretion in ferrets rises during the dark phase and falls with the onset of the light phase of the photoperiod (*Figure 2*).

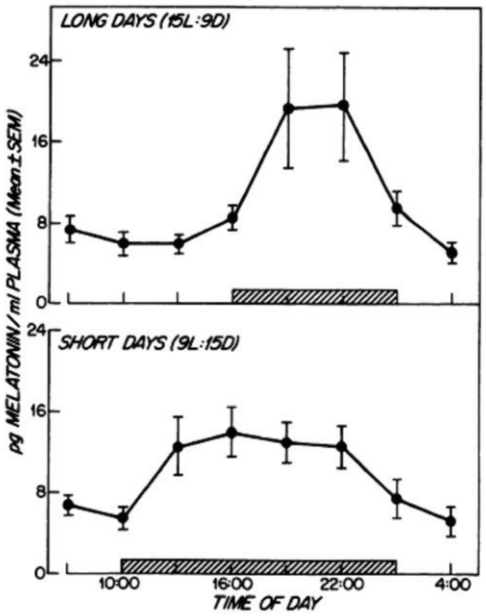


Figure 2: Plasma melatonin concentrations in ovariohysterectomized female ferrets (n=6 to 12) housed under either long or short days. The samples collected under long days at 16.00 h and under short days at 10.00 h were taken just before the colony lights went off. The samples collected at 1.00 h under both long and short days were taken just before the colony light went on. Hatched bar indicates period of darkness (*Baum et al, 1986*)

The pineal gland is an essential component of the neuro-endocrine system regulating **annual rhythms** in the reproductive activity of the ferret. Removal of this gland, or its autonomic nerve supply from the superior cervical ganglia, disrupts synchrony between breeding seasons and time of year. The same operations also prevent either stimulation or inhibition of gonadal function by appropriate artificial photoperiods (*Herbert, 1969 and 1972; Herbert et al., 1978*). Moreover, it was also shown that exogenous melatonin treatments can affect the onset and termination of oestrus depending on the timing of daily hormone administration (*Carter et al., 1982*).

Ferrets exposed to artificially changing long and short day light conditions (*long days*: 16 hours light and 8 hours darkness; *short days*: 8 hours light and 16 hours darkness) start showing oestrus about 3 weeks after the change from short days to long days (*Fox and Bell, 1998*). The repeated change from long days to short days after every six months causes one period of gonadal activity per year similar to a natural breeding season when the animals are exposed to natural outdoor light conditions. A change in light conditions every four or two months causes two or three periods of gonadal activity a year, respectively (*Herbert, 1989*). Through the use of reverse light cycles, ferrets can be induced to breed any time during the year (*Harvey and MacFarlane, 1958 cited by Lindeberg, 2008*).

2.2.2. Anoestrus, oestrus cycle and ovulation

Photoperiod controls the pulsatile release of gonadotropin-releasing hormone (**GnRH**) from the tonic centre of hypothalamus (ARC). GnRH is secreted episodically, and changes in GnRH pulse frequency are critical for seasonal breeding. In the non-breeding season, GnRH pulse frequency is low, ferrets are anoestrus and characterized by small ovaries without developing antral (tertiary) follicles and an absence of sexual behavior (*Hammond and Marshall, 1930*). During breeding season, GnRH pulse frequency is high, resulting in elevated luteinizing hormone (LH), follicle-stimulating hormone (FSH) concentrations, and active gonads.

Folliculogenesis is dependent on classical endocrine signaling within the hypothalamic-pituitary-gonadal axis (reviewed by *Senger, 2003*). In the stage "pro-oestrus", FSH from the pituitary induces ovarian follicle development. Up to our knowledge, there is only limited number of observations available on folliculogenesis of ferret in the literatures, and in addition these data are quite old. Hence, the mink

(*Mustela vison*) is taken as model to describe the possible folliculogenesis in ferrets. In mink, at the beginning of the breeding season, more growing follicles are recruited into the pool of follicles that are commencing the maturation process, resulting in a decrease in the number of growing follicles and an increase in the number of maturing antral follicles. Follicles are recruited from the small tertiary follicle classes into the 0.4-0.6 mm class. From the recruited group, a smaller cohort of follicles is selected and these follicles continue to grow and become the dominant follicles that will respond to the ovulatory stimulus (*Douglas et al., 1994*). Early papers describe that in ferret the development of primordial follicles to early antral stage takes approximately 21 days (*Robinson, 1918*), while the time required for a wave of antral follicles to develop prior to induced ovulation is approximately 6 days (*Murphy, 1989*).

A very early paper described some ovarian pictures in different stages in the ferret (*Robinson, 1918*):

- Jill in heat for 12 weeks: "*There were numerous large follicles and many smaller ones. No corpora lutea were observed, but there was one apparently collapsed follicle which was degenerate; it contained a persistent ovum. Interstitial tissue was abundant.*"
- Jill in heat for 18 weeks: "*The ovaries were entirely normal. Large protruding follicles could be seen on the surface with the naked eye. Sections showed that some of the follicles were mature and normal and there were other smaller ones at all stages. No recent corpora lutea were observed and no atrophic follicles. There was no evidence that follicles had become ripe in batches and subsequently degenerated. There was a great abundance of typical interstitial cells but no cells that could certainly be said to be old luteal cells.*"

The presence of degenerating pre-ovulatory follicles in oestrus ferrets provides indirect evidence that follicle turnover occurs. Moreover, the simultaneous occurrence of large follicles together with developing and atretic follicles further suggest that there are waves of follicular development before and during the oestrus. *Hammond and Marshall* (*1930*) described the size of the largest follicles in ferrets' ovaries at different stages (*Table 1*).

Table 1: Size of the larges follicles in ovaries of ferrets (after *Hammond and Marshall, 1930*)

Phase:	An-oestrus	Pro-oestrus		Oestrus			Anoestrus			
Stage:	Just before season began	Post PP (8^{th} week)	Post part., young removed at birth (8 ½ ws)	Just on	On short time	On long time	Just after birth of young (6 w)	Sucklin g young 17 days (8 ½ ws)	Just after seaso n over	Long time after seaso n over
Number of ferret:	4	12	15	20	3	1	19	14	8	9
Diameter of follicle (µ):	720	900	1232	1440	1224	1392	540	432	462	240

PP: pseudo-pregnancy, Post-part.: post-partum, w: week

Based on that, a mature, developed ferret follicle could be approximately 1200-1400 µm.

Vaginal cytology and vulval intumescence have been used to characterize stages of the oestrus cycle in ferrets (domestic ferrets, black-footed ferrets and Siberian polecats) (*Williams et al., 1992*). Vaginal cytology is commonly used to detect oestrus in some domestic carnivores, especially dogs (*Olson et al., 1984*), and the technique has also been described for mink (*Hansson, 1947, cited by Williams et al., 1992*):

- In **anoestrus** domestic ferrets, the vulva can be barely seen. Females refuse the presence and advance of sexually active males. The vaginal cytology is characterized by 1 to 36% superficial (epithel) cells (*Williams et al., 1992*).
- **Pro-oestrus** is a 2- to 3-week long period at the beginning of the breeding season (*Hammond and Marshall 1930*). It is characterized by an increasing percentage of superficial epithelial cells (15 to 87%) together with enlargement of the vulva (*Williams et al., 1992*). This is controlled by the increase of E_2 produced by the developing follicles. E_2 controls not only vulval swelling and changes in vaginal cells, but also uterine development, proceptive and receptive sexual behavior; however, at this stage, jills are not yet sexually receptive.
- **Oestrus** generally is defined by maximal swelling of the vulva labia (*Figure 3*) (*Thorpe, 1967; Hammond and Marshall 1930*). The surface of the vulva is moist and there may be wetness around it (*Lewington*, *2007b*).

Figure 3: Oestrus signs (swollen vulva) in a female ferret which is ready to mate (photo by *A. Proháczik*)

The vaginal smears contain ≥90% cornified epithel cells (*Mead and Neirinckx, 1990; Williams et al, 1992*). Usually > 90% of epithelial cells are superficial cell. After several days, these cells are fully keratinized. Neutrophils are very common in vaginal smear in all stages of the oestrus cycle. Bacteria are uncommon except during oestrus and then are associated only with superficial cells. Numerous bacteria, neutrophils, cellular debris, and some erythrocytes have been observed in a few females that experienced prolonged oestrus (*Williams et al., 1992*).

Young et al. (*2001*) suggested that the cumulative effect of circulating oestradiol (E_2), rather than single-day absolute concentrations is responsible for the gradual development of vulval intumescences and vaginal cornification. At this stage, jills become sexually receptive (i.e. they display behavioral oestrus).

To date, the exact time elapsing between the appearance of the oestrus sign and the breeding readiness is not documented in ferrets. Fox and Bell (*1998*) (after *Mead and Neirinckx, 1989*) suggested that the mating should occur 14 days after the onset of oestrus displayed phenotypically by vulval swelling. In experimental breeding colonies, black-footed ferret jills are bred when the vulva reaches its maximal size (and the vaginal smear contains > 90% cornified epithal cells for 3 consecutive days) (*Young et al., 2001*).

Furthermore, *Young et al*. (*2001*) reported that in the black footed ferret (*Mustela nigripes*) the fecal gestagen metabolite concentrations were minimal during the pro-oestrus and peri-copulatory periods and rose significantly above baseline 5 days after breeding (induced ovulation), similar to the circulating profiles observed in the domestic ferret (*Heap and Hammond, 1974; Blatchley and Donovan, 1976*).

There is some variability in the **duration and periodicity of oestrus** between species with induced ovulation (*Senger, 2003*). Most species with induced ovulation display well-defined periods of behavioral oestrus which last few days. In contrast to the well-known reflex ovulator female cats, where the oestrus cycle usually occurs repeatedly at an interval of 2-3 weeks per 1-2 months if mating does not occur, ferrets stay in **oestrus until the copulation** (*Robinson, 1918; Hammond and Marshall, 1930*). Hence, the oestrus signs are present continuously (i.e. when follicular growth is initiated, this process is continuous without resting phase and without resting period).

In contrast to spontaneous ovulators, induced ovulators generally do not show spontaneous E_2-induced GnRH/LH surges during their reproductive cycles (*Senger, 2003*). While, in spontaneous ovulators, the E_2, has a positive feedback effect on the hypothalamus (E_2 reaching certain threshold level causes surge in the release of GnRH, and subsequently of LH, resulting in pre-ovulatory LH peak), in the ferret, there is only evidence of a negative feedback action of E_2 (*Carroll et al., 1985*). Female ferrets in oestrus have high levels of circulating E_2 coupled with low or undetectable levels of LH (*Ryan et al., 1985*).

In females of induced ovulating species (such as rabbits, ferrets, cats and camelids), the pre-ovulatory release of GnRH, and the resultant pre-ovulatory LH surge, is triggered by the receipt of genital somatosensory stimuli during reception of an intromission from a male (**mating**), which activate the midbrain and brainstem noradrenergic neurons. These noradrenergic neurons project to the hypothalamus and, when activated, promote the release of GnRH from terminal axons in the median eminence. The immediate release of GnRH from the surge centre of the hypothalamus stimulates the pre-ovulatory LH surge (an immediate release of LH from the anterior pituitary). This will result in **ovulation** 30-40 hours after copulation (*Carroll et al. 1985*; *Bakker and Baum, 2000a* and *2000b; Bakker et al., 2001; Senger, 2003*).

The latency, duration and magnitude of increase in plasma LH measured in ferrets after mating are different from those previously have been found in other reflex

ovulators. In rabbits, as in some queens, LH is elevated 20 to 40-fold within 10 minutes of copulation, reaches a peak by 0.5 to 2 hours and is still elevated at 4 hours. By contrast, in ferrets, a single increase in plasma LH (5 to 9-fold) can not be seen until 2 hours after the onset of mating and plasma LH does not return to pre-intromission levels until 12 h after introduction of the male (*Carroll et al., 1985* and *1987*).

Moreover, while in the cat a single copulation induces ovulation in only about 50 % of cases (multiple copulations is needed to reach 100%; *Wildt et al., 1980*), in ferrets, a single copulation, with an intromission, is sufficient to induce ovulation in 100% of the time (*Carroll et al., 1985* and *1987*). Moreover, in the cat a more prolonged coital stimulation can assure ovulation. In the ferret, an intromission as short as 2 - 3 minutes is sufficient to induce a clear-cut rise in plasma LH (*Carroll et al., 1985* and *1987*).

Following copulation, significant reductions in jills' acceptance of neck gripping by the male (receptivity) and in proceptivity could be first observed 3 days after receipt of an intromission. *Villars et al.* (*1990*) reported a study where plasma concentrations of P_4 were significantly elevated beginning 5 days after, whereas plasma E_2 was significantly reduced beginning 4 days after receipt of an intromission. Daily subcutaneous (s.c.) administration of the P_4 receptor antagonist (mifepristone), significantly retarded the lengthening in females' approach latencies to a stimulus male, suggesting that post-coital elevations in circulating P_4 normally contribute to the expected decline in proceptive responsiveness. By contrast, post-coital reductions in acceptance quotients occurred at equivalent rates in females treated with mifepristone versus vehicle, leading the author to infer that post-coital reductions in oestrogenic stimulation may cause this decline in ferrets' receptive responsiveness.

In ferrets, both vagino-cervical stimulation and neck gripping are necessary to induce ovulation, i.e. the masculine coital behaviors that occur prior to an intromission (neck gripping, pelvic thrusting) are not sufficient to cause a significant rise in LH (*Carroll et al., 1985*), as opposed to queens where non-copulatory stimulation (stroke of a hand down the back, single stimulation of the vagina by a rod, visual, auditory, or olfactory cues from a nearby tomcat) can result in ovulation (*Lawler et al., 1993; Gudermuth et al., 1997*). Moreover, as opposed to rabbit does where the proximity of an intact male, mechanical stimulation of the vagina, or mounting by a female rabbit can induce ovulation (*Harcourt-Brown, 2002*).

Three or four days after mating, vulva starts regression and regains its normal size in 2-3 weeks. If the vulva does not recede, ovulation has probably not taken place and the female may need to be again mated. If mating does not occur, the developed follicles will undergo atresia, and a new cohort of follicles develops. Follicular development and atresia overlap and there is always a recent cohort of follicles available for ovulation whenever copulation might occur (*Murphy, 1989*).

2.3. Hyperoestrogenism in the domestic ferret

In the domestic ferret, the various forms of hyperoestrogenism are the most common endocrine malfunctions. Ferrets are oversensitive for long-lasting production of oestrogens (hyperoestrogenism), even if – as demonstrated earlier (see in 2.2.2. paragraph) – the follicular E_2 production by itself is not sufficient enough to induce pre-ovulatory-like GnRH / LH release, and the copulation-related mechanic insults (vagino-cervical stimuli and neck gripping) have obligatory supplemental role in triggering the endocrine interactions leading to ovulation (*Fox and Marini, 1998*; *Lewington, 2007b* and *2007c*).

In pet ferrets, hyperoestrogenism can result predominantly from (a) **prolonged oestrus** due to the lack of copulation. However, (b) technical failures of ovariectomy (**ovarian remnant syndrome**), (c) the **nodular hyperplasia of adrenocortex** in neutered ferrets (in both sexes), (d) some other – usually very rare, and only exceptionally diagnosed – diseases of **adrenocortex** (LH-dependent hypercortisolism, adrenal gland tumor secreting both sex hormones and aldosterone, bifunctional adrenal gland tumours), furthermore (e) **E_2 producing ovarian tumors,** and ovary-related **neoplastic changes in the ovarian remnant tissue, or at the site of an ovarian pedicle**) may also be the unusual causes of elevated sexual steroid (E_2 and some others) levels. Regardless of its origin, in the current case, the pathomechanism and clinical relevance of hyperoestrogenism are quite similar, well described in the literature (*Sherrill and Gorham, 1985; Bernard et al., 1983; Kociba and Caputo, 198; Fox et al., 1998; Lewington, 2007c; ect.*), and detailed in the session 2.3.1..

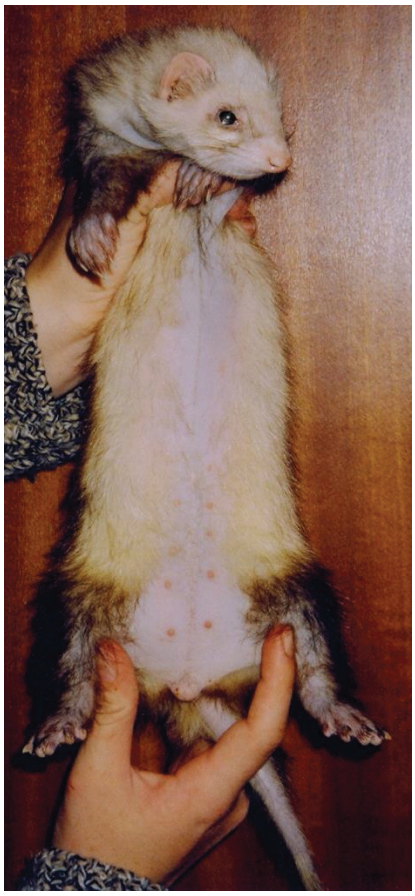
2.3.1. Prolonged oestrus

In female ferrets kept separated from male at oestrus, the absence of mating will result in continued follicular activity for long intervals. The pattern of follicular development during a prolonged oestrus is unknown, but it is assumed to be continuous (*Robinson, 1918*): the well-developed pre-ovulatory follicles eventually undergo atresia, but soon they will be followed by the development of another group of follicles. These waves of follicular development result in long periods of elevated plasma E_2, due to the continuous E_2-producion by follicles in the ovaries. Therefore, jills will usually remain in oestrus for the duration of the breeding season (under natural conditions generally until August/September and under artificial conditions – in the flat – even until death). Also in a breeding colony, the loss of 30% of oestrus females was reported during one breeding season (*Bernard et al., 1983*). Anyway, if a jill stays in heat longer than one month, it is at risk of developing hyperoestrogenism induced bone-marrow suppression and endocrine alopecia.

Ferrets are highly susceptible to E_2-induced toxicity of the haematopoetic tissue like dogs (*Hart, 1990*). Absolute or relative high doses of E_2 in the ferret cause bone marrow suppression of myeloid, erythroid, and megakaryocytic cell lines. Severe bone marrow suppression can be generated by administering high level of exogenous E_2 to ferrets. Such treatment generates suppression of all blood cell population, regardless of gender or ovariohysterectomy (*Bernard et al., 1983*). In the peripheral blood after the initial leucocytosis, thrombocytopenia, anemia and leucopenia occur (*Sherrill and Gorham, 1985*). Thrombocytopenia predisposes the ferret to bleeding disorders (subcutaneous ecchymoses, gastrointestinal hemorrhages, and hematomyelia), while neutropenia predisposes ferrets to secondary bacterial infections.

In advanced cases of prolonged oestrus, hyperoestrogenism can result in clinical signs such as swollen vulva, vulval discharge, depression, pale mucous membranes often flecked with petechia of the skin and the mucosa, inappetence, weakness, bilateral thinning or loss of hair on the hind-quarters or trunk (*Figure 4*), and in severe forms it can result in aplastic anemia and death.

Figure 4: Bilateral thinning / loss of hair in hyperoestrogenism in a female ferret (photo by *A. Proháczik*)



Hemorrhagic anemia due to thrombocytopenia is the most common cause of death and the mortality rate can be 40%. Ferrets generally are in oestrus for two months before death occurs (*Sherrill and Gorham, 1985; Sirivaidyapong and Swangchanuthai, 2003*). Gross pathologic changes include pale tissues, thin watery blood, pale fatty bone marrow, petechia or ecchymotic hemorrhages throughout the subcutis and gastrointestinal tract, hydrometra or pyometra, and occasionally pyogenic infections in other organs. The major histopathologic changes are hemorrhages, hypocellular bone marrow and decreased splenic extramedullary hematopoiesis (*Sherrill and Gorham, 1985*)

In general, treatment of severe bone marrow depression is difficult and almost uniformly unsuccessful, so a poor prognosis should be offered to any ferret that has been in oestrus for more than 8 weeks. The first objective in the treatment of this disease is to remove (hormonally or surgically) the source of endogenous E_2. If a jill has been in oestrus for at least 2 weeks and has not developed severe thrombocytopenia, it can be simply treated with an intramuscular (i.m.) injection of 100 IU of hCG or of 20 µg GnRH. If the size of the vulva has not decreased 7 to 10 days after treatment, a second

dose of either hormone may be given. Both treatments will trigger ovulation approximately 30-35 hours post-injection and jill will remain anoestrus for a pseudopregnancy of 40-42 days. Oestrus may reoccur after this period. It should be noted that several GnRH injections may sensitize the jills to the drug and therefore anaphylaxis may occur when repeated (*Fox et al., 1998*). Females can also be bred with a vasectomized male to induce ovulation with a subsequent period of pseudopregnancy.

If oestrus has not extended beyond 2 to 3 weeks and the female has normal complete blood count, ovariohysterectomy can be performed, while in cases of marked thrombocytopenia (several weeks in oestrus), jills may be in need to receive supportive care in addition to ovulatory manipulations. Use of whole blood transfusions (5 to 10 mL per animal), anabolic steroids, vitamins and antibiotics may also be necessary to save the females' life.

Prevention of E_2 toxicity caused by persistent oestrus is done by routine spaying of all jills not intended for breeding (e.g. in the US and United Kingdom (UK) such ferrets are neutered at the age of 4-6 weeks). However, due to the risks of early neutering (see in section 2.3.3.), nowadays, neutering at age of 5-6 months is advocated by American veterinarians (*Lewington, 2007c*). In countries where the spaying of young jills is not routine procedure, vets, breeders and dealers should inform the owners about the risk of this disease.

Hormonal stimulation of ovulation at oestrus with hCG, or GnRH (described above), can be used for females that are to be bred at a later date. hCG may be given at least 10 days after the onset of oestrus (*Pollock, 2004*). Moreover, oestrus can be terminated by the use of PROL (*Oxenham, 1990*) or by other gestagens bearing in the mind their secondary effects and risks of treatment (e.g. pyometra) (*Howard, 1979*) (see in section 2.8.1.). Another method for stimulating ovulation with or without fertilization is by mating the females with an intact or vasectomized male, respectively (*Hillyer, 2004*).

2.3.2. Ovarian remnant syndrome

Ovarian remnant syndrome (ORS) usually is due to failure in surgical technique of neutering, where some part of the ovaries or ovarian tissue has been left in the abdominal cavity. Signs of ORS due to E_2 production of the remained and re-activated ovarian tissue (vulval swelling and endocrine alopecia) are frequently seen in young (generally <2 year old) ovariectomized ferrets (*Purcell and Brown, 1999; de Wit et al., 2001; Ludwig and Aiken, 2004*) in the breeding season.

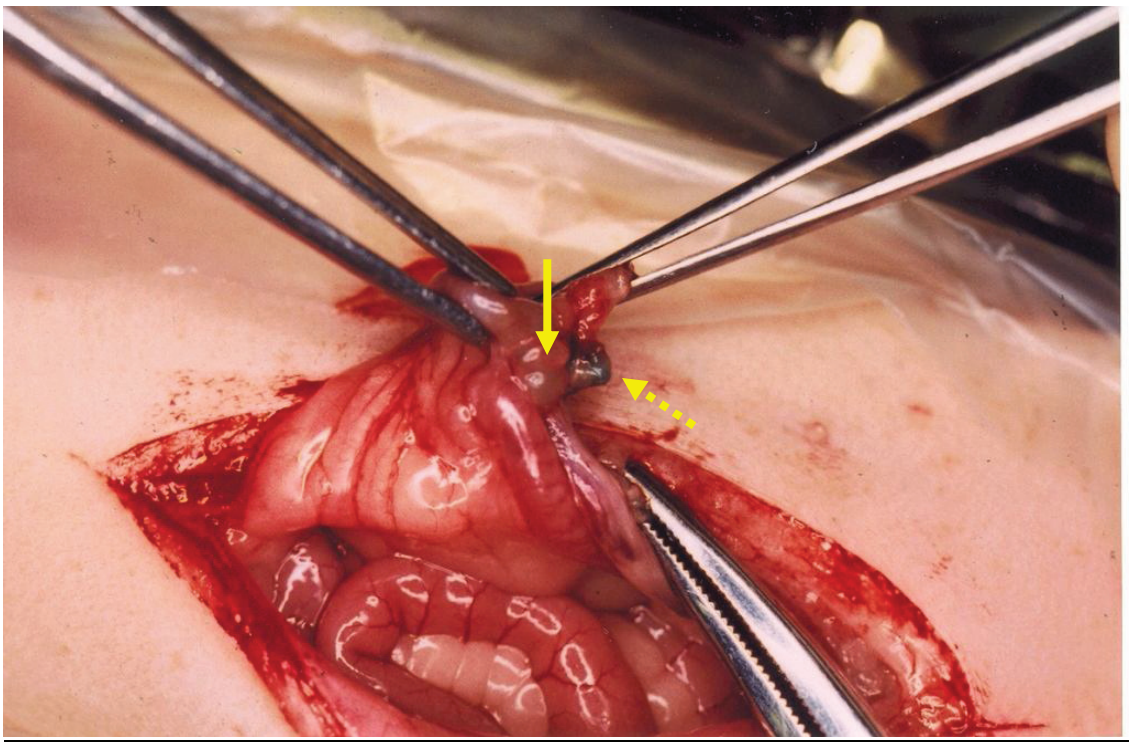
The pathophysiology of ORS is well described in cats (*DeNardo et al., 2001*). In the abdominal cavity remaining ovarian cells may implant on the abdominal wall, omentum or around the ligature. After revascularization, these cells may reactivate, become steroidogenic and behave as normal ovarian tissue (growing tertiary follicles) producing E_2. Histopathologic examination of such tissue in cats can show any combination of the following: primordial and mature follicles, corpora lutea, cysts, para-ovarian structures, and granulation tissue. While in cats, the recurrence of oestrus can vary from several weeks to over 5 years (*Heffelfinger, 2006*), in ferrets there is no information documented about the interval between the original ovariohysterectomy surgery and the first sign of ORS. Based on personal communications, it seems to be that jills with ORS commonly display oestrus in the next breeding season after their neutering.

The diagnosis of ORS in ferrets commonly based on the case history (neutering), appearance of clinical signs (oestrus and endocrine alopecia) and on a hormone stimulation test (hCG; 100 IU/ferret i.m. or GnRH; 20µg/ferret i.m.). The goal of the hormonal stimulation test is to cause ovulation of follicle(s), thus confirming the presence of functional ovarian tissue. If ovulation occurs, oestrus will stop in 7-10 days. Some ovarian remnant does not respond to a single hormonal treatment, therefore it may be repeated in 7-10 days if vulval swelling does not begin to decrease (*Purcell and Brown; 1999, Orcutt, 2003*).

In cats, an additional challenge assay is described, which could be likely adapted in ferrets as well. This tool consists measurements of P_4 (and E_2 if desired) in a pre- and a post-hCG / GnRH-treatment collected blood samples. After 5 to 7 days, the P_4 level in blood will increase and E_2 will decrease compared to the pre-treatment level, if ovulation has taken place.

Not all general treatments for ORS in cats can be used in ferrets. While a queen may live a healthy life with an ovarian remnant, as long as its owners can tolerate oestrus behavior, ferrets (if this condition lasts longer than some weeks) are at risk of developing typical endocrine alopecia, and other consequences of E_2 hyperstimulation (see above). Gestagens (e.g. PROL) or long-acting GnRH analogues (e.g. deslorelin acetate, in the following referred as deslorelin), generally used for oestrus suppression in several carnivore species, may be useful also in this case, however these techniques are not mentioned in the literature for ferrets. The recent recommended treatment is an exploratory laparotomy, ideally when the ferret is in oestrus (but in good health) or has been induced to ovulate, so that the increased vascularity makes the ovarian remnant more obvious. The entire exploration of the peritoneal cavity is essential, checking the caudal poles of kidneys, the omentum, the ligatures (*Figure 5*) and the peritoneal walls, as these are commonly affected sites (*Purcell and Brown, 1999*).

Figure 5: Intra-operative picture of a female ferret with ORS. This jill displayed oestrus in the next breeding season after the ovaryectomy. hCG treatment (100 IU, i.m.) was given to her and oestrus signs stopped in 10 days. Six weeks after hCG treatment oestrus signs reoccurred. At that time, an exploratory laparotomy was done and developed follicles (solid arrow) were found around the ligature (dashed arrow). After removing this tissue, jill stopped showing oestrus in one week (photo by *A. Proháczik*)



The excised tissue may be submitted for microscopic examination and postoperative blood P_4 levels may be assessed to evaluate the success of the surgery (if it was done at latest in 3 weeks after hormone challenge test) (LH induced pseudopregnancy lasts for approximately 6 weeks in ferrets with the most elevated P_4 levels at around 3 weeks post-ovulation).

In ferrets, differential diagnosis should be done between ORS and E_2 (and/or other sexual steroid) producing adrenal disease (hyperadrenocorticism). While in females with ORS, hormone(s) levels will change according to the ovulation (described above), in ferrets with hyperadrenocorticism, E_2 and/or P_4 levels 5-7 days after hormone challenge test will not change (i.e. hormones will stay on the same level in the pre- and post-hCG/GnRH samples), moreover vulval swelling will not disappear as well (*Rosenthal, 1997; Ludwig and Aiken, 2004*).

2.3.3. Nodular hyperplasia of adrenocortex

Hyperoestrogenism and its consequences commonly can be caused by adrenal-associated endocrinopathies (i.e. nodular hyperplasia of adrenocortex or hyperadrenocorticism). Adrenocortical disease has been recognized for almost 20 years as a common disease affecting pet ferrets in the US and in European countries (*Lipman et al., 1993; Rosenthal et al., 1993; Rosenthal, 1997; Weiss and Scott, 1997; Wheler and Kamieniecki, 1998; Weiss et al., 1999; Schoemaker et al., 2000, Wagner et al., 2001; Schoemaker et al., 2002; Schoemaker, 2003; Hillyer, 2004; Schoemaker et al., 2004; Wagner et al., 2005; Bielinska et al., 2006*). To date, the prevalence of this disease is only estimated, but considered high. In countries, where ferrets are neutered at approximately 6 weeks of age (in the US and in Japan), the disease is more common diagnosed (20 to 25%, *Weiss and Scott, 1997*; *Miwa et al, 2008*) than in European countries (0,55%, in the Netherlands, *Schoemaker , 2003*) where the ovario(hyster)ectomy/castration is generally performed around or after the sexual maturity. The day by day growing literure data prove however that also in Europe, the disease is recognized and diagnosed more and more frequently. Primarily, it can be seen in middle-age (>2 year old) **neutered** ferrets. Although, there is an equal incidence both in male and female animals, females may be presented more frequently for veterinarian examination than males because of the prominent appearance of the vulval swelling.

This disease is manifested by a pathological rise in blood levels of one or more oestrogen precursors: androstenedione, oestradiol and 17-hydroxyprogesterone (*Rosenthal and Peterson, 1996a; Desmarchelier et al, 2008*) and clinical sings include a range of cutaneous, reproductive, or behavioral symptoms, all related to the elevated concentrations of sexual steroids. Cutaneous signs are characterized by bilaterally symmetric alopecia (*Figure 6*).

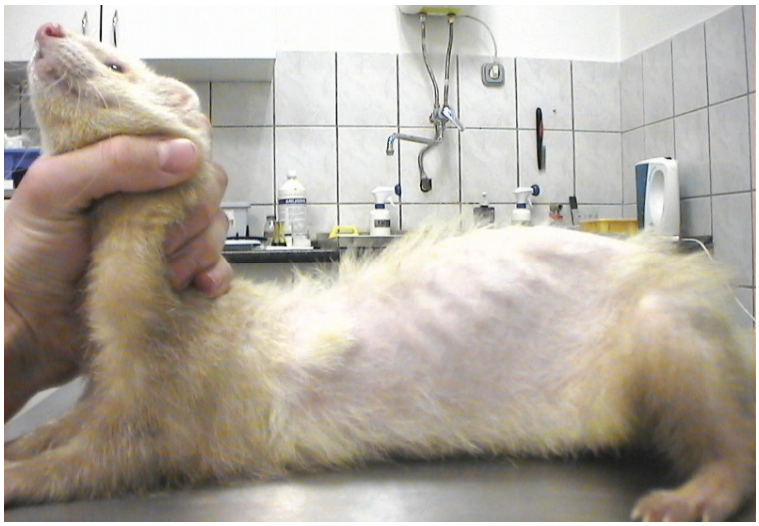


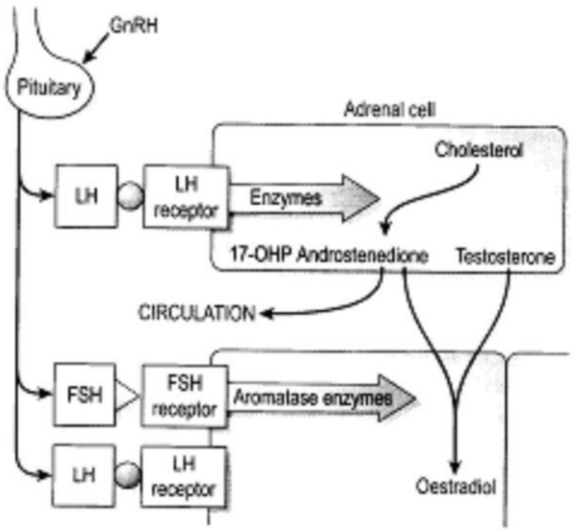
Figure 6: A female ferret (Prücsök) showing dermatological signs of nodular hyperplasia of adrenocortex (photo by C. *Horváth*)

It was shown that 95% of neutered ferrets with bilaterally symmetric alopecia have adrenal disease. Hair loss often begins in late winter or early spring. Hair initially may regrow later in the year, followed by progressive alopecia the following spring. Alopecia commonly begins in the tail region and progress cranially. Finally, in severe case, the ferret may become completely bald. In most cases, the skin has a normal appearance, although skin thickening. About 30% of affected ferrets are pruritic. Reproductive abnormalities (depending on which sex steroid is elevated) may include swelling of the vulva (oestrus) in females, and dysuria in males (*Rosenthal and Peterson, 1996b*). Behavioral abnormalities may include increased mounting behavior or aggression in both genders, and marking behavior in males. Longstanding cases may show mild anemia and petechiation (as a result of the suppressive effect of E_2 on the bone marrow), muscle wasting, and other non-specific signs such as lethargy and posterior paresis.

As pathomechanism, it has been supposed that competent cells in the ferret adrenal cortex undergo excessive cell proliferation and adopt features of gonadal steroidogenic cells in response to the hormonal changes that follow gonadectomy (i.e. gradual rise in plasma LH (*Ryan et al, 1985; Carroll and Baum, 1989*). These cells may

be committed gonadal progenitors that are ectopically located in the adrenal gland or multipotential progenitors capable of differentiation into either adrenocortical or gonadal-like steroidogenic cells, depending on the hormonal milieu and other environmental factors (*Patterson et al., 2003*). Given the similarities between neoplastic adrenocortical cells and normal gonadal somatic cells, it comes as no surprise that reproductive hormones along with their receptors and intracellular effectors play prominent roles in adrenocortical tumorgenesis in ferrets (*Bielinska et al., 2006*). Several lines of evidence

Figure 7: Hypothesized neuro-endocrine pathway for adrenal stimulation in neuterized ferrets (after *Lewington, 2007c*)



suggest that LH signaling plays a central role in adrenocortical tumorgenesis in neutered ferrets (*Schoemaker et al., 2000; Schoemaker, 2003; Bielinska et al., 2006*). Hypothesized neuro-endocrine pathway for adrenal stimulation in the ferret is presented in *Figure 7*.

In most cases, diagnosis is based on clinical signs, measurement of sexual hormones in blood, but ultrasonography of the adrenals (*Rosenthal, 1997*) as well may contribute to the diagnosis. Complete blood counts and chemistry panels are within normal limits in the vast majority of animals, except in longstanding cases in which anemia or decreased platelet numbers may be seen. As the elevated hormone in hyperadrenocorticism may be one or more sex hormones (*Rosenthal and Peterson, 1996a*), practitioners should be aware that serum cortisol is rarely elevated (if ever) and diagnostic testing for Cushing's disease based on cortisol levels will be of little or no diagnostic value (except in rare cases detailed in section 2.3.4.; *Schoemaker et al., 2008b*).

While in neutered males the elevated E_2 concentration is diagnostic, in females differential diagnosis has to be made between hyperadrenocorticism and ORS. The symmetrical alopecia (in both genders) has to be differentiated from seasonal flank alopecia, although it appears commonly in intact animals (in such cases, bilaterally alopecia begins at the tail base and progress throughout the breeding season (*Figure 8*), but it is not as extensive as in ferrets with adrenal disease).

Figure 8: Typical sign of the seasonal alopecia on the tail in intact male ferrets (photo by *Gy. Proháczik*)

Recently, two treatment modalities have been used to cure this disease: surgical removal of the affected gland(s) or medical treatment. In the last 10 years, adrenalectomy has been the preferred treatment for ferrets (*Lawrence et al., 1993; Rosenthal et al., 1993; Wagner and Dorn, 1994; Weiss and Scott, 1997*). Whilst the prognosis with this method can be good, in cases of unilateral adrenalectomy complications may occur due to the possible development of adrenal disease in the remaining adrenal gland. In recent years, medical management of the disease has come to the fore. However, some drugs (mitotane and ketokonazole) generally used in the therapy of Cushing's disease in other species prove ineffective for ferrets' hyperadrenocorticism (*Rosenthal, 1997*) and other drugs (androgen receptor blockers, aromatase inhibitors) are effective only in some, but not all ferrets (*Quesenberry and Rosenthal, 2004*), drugs controlling reproductive functions are promising treatment options. One of them, melatonin (5.4 mg melatonin implant, Prime-X registered for mink), has been administered with success to ferrets (n=100) with hyperadrenocorticism by *Murray* (*personal communication*). The supposed length of its efficacy was 3 to 4

months. *Ramer et al.* (*2006*) studied the oral administration of melatonin. Their results showed that it decreased clinical signs associated with adrenocortical disease in ferrets, but that daily treatment over the course of months did not decrease adrenal gland tumor growth. The GnRH agonists may be other therapeutic possibilities bearing in mind the background of the pathomechanism of this disease. GnRH agonists used as long-term therapy down-regulate GnRH receptors of gonadotrophs of the pituitary gland and inhibit elevated LH secretion. An injectable GnRH agonist, leuprolide acetate, was used in ferrets (n=20) in a single dose of 100 μg per animal by *Wagner et al.* (*2001*). They showed that this treatment reduced the elevated oestradiol, 17α-OH-progesterone, androstenedione, and dehydroepiandrosterone concentrations and eliminated the clinical signs of hyperadrenocorticism (vulval swelling, pruritus, alopecia and undesirable sexual behaviors and aggression). The treatment effects were temporary and typically after 3 months, clinical signs reoccurred in all ferrets. More recently another drug, deslorelin, was used as a long-lasting treatment. Deslorelin is used more and more commonly in the reproductive management and veterinary praxis. It was showed that it can reversibly suppress reproductive function in several species (e.g. male and female dogs, female eastern grey kangaroos, female cats and ferrets) for extended periods (*Munson et al., 2001; Gobello, 2006; Herbert et al., 2006; Schoemaker et al., 2008a*). Moreover, seeing that such treatment caused long-term reduction of circulating FSH and LH concentrations to very low or undetectable levels, it was successfully used in the treatment of urinary incontinence in neutered female dogs (*Reichler et al., 2003*). References of studies using deslorelin are also available in farm animals for ovulation induction, ovarian function suppression etc. (*Kraeling et al., 2000; Silvestre et al., 2009). Delbecchi and Lacasse* (*2006*) showed that such treatment could temporarily suppress the return of ovarian cycles in cows. This study described that deslorelin significantly reduced serum concentrations of E_2 and P_4 as compared with untreated cows.

Wagner et al. (*2005*) reported a study in which 15 ferrets with hyperadrenocorticism were subcutaneously treated with a single 3-mg implant of deslorelin. Compared with findings before treatment, vulval swelling and pruritus were reduced or eliminated completely within 2 weeks after implant insertion. Four to 6 weeks post-insertion, fur had re-grown. One month post-treatment, oestradiol, androstenedione, and 17-hydroxyprogesterone concentrations decreased and remained

low until clinical recidivism. The average time to reoccurrence of clinical signs was 13.7 ± 3.5 months (range: 8.5 to 20.5 months).

2.3.4. Unusual cause of the elevated sexual steroid levels in neutered ferrets

Rarely, hyperoestrogenism can be caused by some **E_2-producing tumors** in the reproductive system in intact (*Li and Fox, 1998*) and neutered ferrets (*Patterson et al., 2003; Jekl et al., 2006*).

Alopecia in neutered female ferrets were reported by *Patterson et al.* (*2003*) that were related to **neoplastic tissue found at the site of an ovarian pedicle**. Androstenedione- and 17-hydroxyprogesterone-, but not oestradiol, concentrations were high in these ferrets. Following surgical removal of the abnormal tissue, the high hormone values diminished rapidly and hair re-growth ensued. In both cases, histological examinations showed features consistent with classical sex cord-stromal (gonadostromal) tumors (prominent spindle cells, along with polyhedral epithelial cells and cells with vacuolated cytoplasm). Although similar cell types have been described in the adrenal glands of ferrets with adrenal-associated endocrinopathy, an ovarian origin for the current neoplasm was considered likely on the basis of their anatomic location (accessory adrenal tissue has only been described close to an adrenal gland or in the cranial perirenal fat of ferrets).

Jekl et al. (*2006*) diagnosed a 6 year old neutered female ferret with bilateral alopecia due to **neoplastic changes in the ovarian remnant tissue**. The ferret underwent ovariectomy at the age of 8 months and than had regularly displayed alopecia and signs of oestrus (swollen vulva and behavioral changes) during the 5 years. At each time, it had been treated on two or three occasions with hCG at a dose of 100 μg/kg and had been recovered. However, on the last recurrence of this problem, a triple application of hCG failed to have any effect. The ferret was in good body condition, but the alopecia was almost complete over the whole body, with only single hairs left on the head and thighs. Palpation revealed two oval masses located behind the right kidney. All the other physical examination findings were within normal limits. Abnormal haematology and biochemistry results included reductions in the haematocrit and haemoglobin and an elevation in blood urea nitrogen Urinalysis was unremarkable. High levels of E_2 and normal level of P_4 were detected in the blood serum. Based on these findings, an exploratory laparotomy was performed and the presence of a neoplastic mass at location of the right ovary, a massive enlargement of the uterus filled

with a clear fluid and a subcapsular cyst on the left kidney were found. After removing the neoplastic mass, E_2 level decreased and six weeks later, new fur growth was evident. Histopathologic examination of the mass identified it as ovarian leiomyoma. The final diagnosis was ovarian leiomyoma and hydrometra. The author of the recent work notes that leiomyomas are known as hormone dependent tumors rather than hormone producers. Oestrogen and progesterone appear to be promoters of the growth of genital tract leiomyomas (*Porter et al, 1995*).

Schoemaker et al. (*2008b*) reported a case of **LH-dependent hypercortisolism together with hyperandrogenism**. In a 5 year old castrated male pet ferret with clinical signs of polyuria and polyphonies, the urinary corticoid: creatinine ratio was increased and the plasma adrenocorticotropic hormone (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 done with an implant formulation of 9.4 mg deslorelin. Within 3 weeks after insertion 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 insertion 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.

Clinical findings with an **adrenal gland tumor secreting both sex hormones and aldosterone** were described in a 6 year old neutered female ferret by *Desmarchelier et al.* (*2008*). The clinical signs were lethargy, alopecia, pruritus, and an abdominal mass. At clinical examination, non-regenerative anaemia, mild azotemia, and a large left adrenal gland mass were found. However, worsening of the ferret's general condition prevented removal of the mass, and dyspnea, weakness, hypertension, and severe hypokalemia developed. Plasma aldosterone concentration was high confirming the diagnosis of hyperaldosteronism. High concentrations of oestradiol (245 pmol/L [reference range, 30 to 180 pmol/L]), 17α-OH-progesterone (2.4 nmol/L [reference range, 0 to 0.8 nmol/L]), and androstenedione (17.8 nmol/L [reference range, 0 to 15.0 nmol/L]) concentrations were also observed, but baseline cortisol concentration was

within reference limits. Treatment included oral administration of spironolactone, potassium gluconate, leuprolide acetate, amlodipine, and benazepril. Inhalation of albuterol proved effective in reducing the dyspnea. In the following weeks, serum potassium concentration returned to within reference limits and hypertension decreased, but dyspnea persisted. Two months after the first examination, the ferret became anorectic and was euthanized. Histological examination showed a large left adrenal gland adenoma, progressive chronic nephropathy, severe pulmonary oedema, and focal fibrosis in the left ventricle. Immunohistochemical staining of the adrenal gland mass proved the source of the elevated aldosterone concentrations in neoplastic adrenocortical cells.

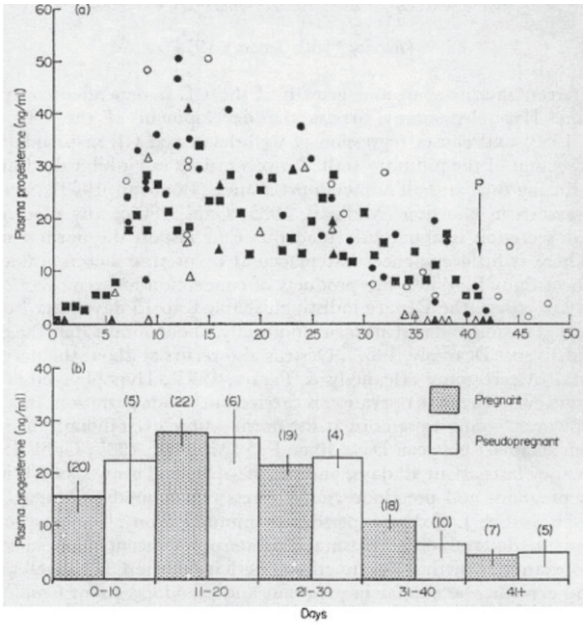
Bifunctional adrenal gland tumours have been reported to occur only rarely in the veterinary literature, although 2 cats with adrenal gland carcinomas secreting P_4 and aldosterone (*DeClue et al., 2005*) or cortisol and P_4 (*Rossmeisl et al., 2000*) and a dog with an adrenocortical tumour secreting aldosterone and corticosterone (*Behrend et al., 2005*) have been described.

2.4. Corpus luteum function and maintenance of pregnancy

During ovulation the pre-ovulatory follicle bursts, releasing the egg. The structures remaining in the ovary, the granulose and theca cells of the follicle wall will form the corpus luteum (CL). The presence or absence of embryos appears to have no effect on luteal life span (*Blatchley and Donovan, 1976*) and pregnancy and pseudopregnancy last for similar period (approximately for 42 days; *Hammond and Marshall, 1930*).

Ferret CL start secreting P_4 after ovulation. P_4 concentrations rise continuously from day 5 to peak at about day 12-14 during the period of implantation, and then decrease steadily after about day 15, stabilize by day 24 of pregnancy and decline continuously between day 24 and parturition at day 42 (*Figure 9*; *Heap and Hammond, 1974; Blatchley and Donovan, 1976; Daniel, 1976*).

Figure 9: Plasma P_4 concentrations in pregnant and pseudo-pregnant ferrets.
(a) values in pregnant (solid symbols) and pseudo-pregnant (open symbols) females measured by fluorimetry, competitive protein binding (■) and radioimmunoassay (▲, Δ),
(b) mean values ± S.E.M. of figures obtained by different assays. Figures in parentheses are the number of observations (after *Heap and Hammond 1974*)



Kintner and Mead (1983) studied the biochemistry of luteal steroid production both *in vivo* and *in vitro* in ferrets. They could demonstrate that on both days 6 and 8 of pregnancy CL primarily accumulate steroids of the Δ^4 pathway, with P_4, 17α-OH-P_4, androstenedione and testosterone being the most abundant products after *in vito* incubation. Thus, ferret CL appear to produce and metabolize steroids in a manner similar to that observed in rats, sows and mares.

Histological and ultrastructural characteristics of CL in ferrets were reported by *Joseph and Mead* (*1988*). Based on their findings it can be said that ferret CL consist of small (< 25 µm) and large (> 25 µm) luteal cells, with the smaller cells predominating on day 6. A shift toward larger sizes can be observed by days 13 and 24 of pregnancy. Ultrastructural features of both large and small luteal cells are similar in most respects on day 6 of pregnancy, at which time blood levels of P_4 are relatively low. Mitochondria of both large and small cells are pleomorphic on day 6. At this time, the mitochondria are oval to slightly elongate in shape and their tubular cristae are often less densely packed. In contrast, large luteal cells are more numerous and remarkably better differentiated by day 13, at which time plasma P_4 levels are at their peak. By day 13, large luteal cells can be more readily differentiated from small cells by the presence of their more electrondense, elongated mitochondria, which contains more tightly packed tubular cristae by their more extensively developed smooth endoplasmic reticulum,

which is now whorled, as well as by loss of heterochromatin from the nuclear membrane. Such changes have been correlated with increased P_4 production in another mustelid (mink) (*Moller, 1973*). By day 24 of pregnancy, at which time plasma P_4 levels have leveled off, the ultrastructure of both large and small luteal cells was essentially similar to that observed in large luteal cells on day 13; however, the smooth endoplasmic reticulum was quite dilated and was more extensively whorled in the large cells.

Size of the largest CL on the ovaries of ferrets at different stage was described by *Hammond and Marshall* (*1930*) (*Table 2)*. The authors noted that during suckling the CL do not remain large, but rapidly undergo atrophy, as occurs in rabbit.

Table 2: Size of the larges CL on the ovary of ferrets (after *Hammond and Marshall, 1930*)

Weeks after coitus:	2	3	5	5 ½	5 ½	6	7	8	8 ½	8 ½
Number of PP ferret	10	7	5	18	ND	6	2	12 on heat again	ND	ND
Diameter of CL (µ)	1932	2082	2010	1650	ND	1980	1110	870	ND	ND
Number of pregnant ferret	ND	17	11	13	16	19 just post-part.	ND	ND	14 young sucklin g	15 young remove d at birth
Diameter of CL (µ)	ND	1992	1962	1770	1572	1332	ND	ND	612	612

PP: pseudo-pregnancy, Post-part.: post-partum, ND: no data

CL in ferrets is maintained by a luteotrophic complex. Prolactin and LH are important component of this complex, but to date, the relative significance of LH and prolactin dependency in the ferret remained to be determined (*Agu et al., 1986*). While *Murphy* (*1979*) demonstrated that prolactin administration maintained plasma P_4 levels and resulted in implantation of embryos in ferrets hypophysectomized on day 5 or day 6 of pregnancy (suggesting that prolactin is the major luteotrophic hormone in this species), the role for LH has also been demonstrated (*Agu et al.*, *1986*). The role of the LH in maintenance of CL function was shown by the following evidences:

(a) passive immunization against LH reduced the circulating P_4 concentrations,

(b) administration of a monoclonal antibody against GnRH, which reduced LH but not prolactin, reduced circulating P_4,

(c) daily administration of hCG (shown to interfere with LH stimulation of luteal function in the rat by reducing LH receptors) was highly effective in reducing circulating concentrations of P_4 during the first half of pseudopregnancy in the ferret.

Further arguing for a role for LH is that 3',5'-cyclic adenosine monophosphate, the second messenger for LH in luteal tissue, induces P_4 secretion by ferret luteal cells in vitro (*Agu et al., 1986*).

2.5. Pseudopregnancy

Pseudopregnancy in ferrets is a normal physiological stage occurring in the lack of conception (hormonal induction of ovulation, breeding with vasectomized or intact, but infertile male or mating with intact, fertile male late in the breeding season when the light intensity is not appropriate for pregnancy). During pseudopregnancy, jills may display nesting behavior and enlargement of the abdomen and mammary glands (*Fox and Bell, 1998*). The nesting behavior, which includes dragging cage mates around the cage and increased aggression towards the owners, does not make this an attractive option.

Pseudopregnancy can be related to implantation failure due to a photoperiod effect or to reduced light intensity one month before mating (*Fox et al., 1998*). In some ferret kennels, the occurrence of pseudopregnancy can be even 35-70 % (*Lewington, 2007b*). Explanation of this phenomenon has not yet been described in the literature, but the reason likely can be that short days have inhibitory effect in prolactin secretion. In the ferret, prolactin is essential not only for maintenance of luteal function, but also plays a role in creating a favourable uterine environment during pregnancy. Blastocyst implantation fails to occur in ferrets that are hypophysectomized on day 5 or 6 of pregnancy, whereas ferrets that are hypophysectomized and receive exogenous prolactin develop blastocysts and exhibit implantation. Nevertheless, the physiological role of prolactin in the uterus is probably related to long-term maintenance of some epithelial function rather than constituting a specific requirement for blastocyst implantation only (*Rose et al., 1993*).

2.6. Gestation, parturition, lactation and re-breeding

2.6.1. Gestation

Ovulation occurs 24-36 hours after copulation. Female ferrets generally ovulate six to ten oocytes after mating with a hob (*Mead et al., 1988*). The relatively long time-span between copulation and ovulation is associated with prolonged survival of the sperm in the female reproductive tract: spermatozoa can survive and still have their fertilizing capacity 120 hours after copulation (*Amstislavsky and Terovskaya, 2000*).

By day 6 after mating, the embryos enter to the uterus as morulae and blastocysts (*Robinson, 1918*). Blastocysts in uterus expand from a size of ≈200 μm in diameter to more than 2 mm in diameter during their pre-implantation development (*Enders and Schlafke, 1972*). P_4 has been reported to support blastocyst expansion up to a diameter of 1 mm, but for further blastocyst expansion, additional ovarian factors are required (*McRae, 1992*). Between days 12 and 13 after mating, the embryos have become implanted in the endometrium (*Enders and Schlafke, 1972*). Neverthless, the pre-implantation period of the ferret is relatively long and the embrios growth slowly, embrionic diapauses – in contrast to the mink – does not exist in this mustelid species (*Daniel, 1970*).

Implantation in the ferret is central with rapid invasion of the uterine epithelium by the trophoblast over a broad area that eventually becomes a zonary band of endotheliochorial placenta (*Strahl and Ballman 1915*, cited by *Enders and Schlafke 1972*). The endotheliochorial placenta has three fetal (endothelium, connective tissue, trophoblast) but only two maternal layers (connective tissue, endothelium); since the maternal epithelium is lost. The fetal trophoblast invades the endometrial epithelium, but does not destroy the endothelium of the maternal capillaries.

Pregnancy can be detected by abdominal palpation as early as 2-3 weeks after fertilization. At that time, the fetuses should be about the size of small walnuts and the uterus should be enlarged. As jills generally have approximately 8-10 developing fetuses in the uterus, in the last half third of pregnancy, the reproductive tract relative enlarges and fills almost all the abdominal cavity (reducing the place to take large amount of food). This phenomenon should be taken in account when fed late-pregnant jills (*Lewington, 2007b*).

2.6.2. Pregnancy toxemia

Pregnancy toxemia caused by negative energy balance in late gestation is occasionally observed in ferrets (*Batchelder et al., 1999; Dalrymple, 2004; Lewington, 2007a*). The background of the disease is the result of fetal carbohydrate- or energy-demand exceeding maternal supply during the last trimester of pregnancy. In the ferret, pregnancy toxemia usually occurs between days 32 and 42 of gestation, especially just before the whelping date. It is more common in primiparous female ferrets carrying > 10 kittens (*Batchelder et al., 1999; Lewington, 2007a*) or among females carrying average litters and fed adequate diets, but where an accidental fast occurs during this period (*Bell, 1999*). The reason may be a change of diet (ferrets are food addictive animals), an inadequate food quality (in order to take sufficient energy and protein a large quantity of food should be consumed which is limited by the reduced abdominal space as a consequence of the enlarged uterus) or a limited ability of the water intake (jills stop to eat dry food without adequate water).

Even one overnight fast can induce toxemia in jills with a large litter. The body of the mother tries to overcome the hypoglycemia by gluconeogenesis with the jill taking more triglycerides from fat stores, which are hydrolyzed to free fatty acids (FFA; syn.: non-esterified fatty acids). The result is that the liver is stressed, as triglycerides are re-synthesized from the FFA, leading to severe hepatic lipidosis. With the increase of FFAs from triglycerides, and change in the hepatic metabolism from fat synthesis to fat oxidation, ketogenesis occurs. The result is a ketonuria and osmotic diuresis as the ketones move into the extracellular space and elude complete resorption to pass into the urine (*Dalrymple, 2004*).

In the affected jills, clinical signs include a sudden onset of lethargy, hypothermia, dehydration, sternal recumbency with decreased awareness, open glazed eyes, black tarry stools and doughy feeling to the skin (*Wallach and Boever, 1983; Batchelder et al., 1999; Lewington, 2007a*). At the veterinary examination, females are usually in a critical condition; coma and death often ensue (*Batchelder et al., 1999; Lewington, 2007a*). Laboratory testing often reveals anemia, azotemia, bilirubinemia, hypoglycemia, hypoproteinemia, high level of hepatocellular serum enzyme (ALT), hyperketonemia and ketonuria (*Batchelder et al., 1999; Lewington, 2007a*). Serum glucose is initially low (usually < 2.77 mmol/L), but at terminal stage it may rise above normal to 8.32 mmol/L or more. Urea nitrogen is often > 35.7 mmol/L. Despite clinical

dehydration, the haematocrit is usually below 30% (reference values for a pregnant ferrets are generally over 40%; *Fox, 1998a*). At necropsy, the uterus is full of a large number (> 10) of foetuses. The liver is enlarged, yellowish, and mild icterus is encountered. Histopathology reveals excessive hepatocellular lipid accumulation (*Batchelder et al., 1999; Lewington, 2007a*).

Treatment of this condition includes immediate supportive care (treatment with parenteral fluids containing electrolytes and glucose, with frequent feeding of a high energy, high protein diet). Medical management alone, however, is almost never adequate, and an emergency cesarean section is the treatment of choice.

As pregnancy toxemia in jills is almost always associated with inadequate nutrition, decreased feed intake, or fasting, owners should provide high-calorie nutritional supplements to jills that appear to be carrying large litters (*Bell, 1999*). Ferrets require a higher level and quality of protein, more fat, and less fiber than do most other carnivores (*Fox and McLain, 1998*). Rations suitable for pregnant jills must contain > 35% high quality animal-source protein, > 18% fat (including 7% to 15% of linoleic acid), and minimal grain or fiber ingredients. Without such a high quality ration, the demand for nutrients by the developing kittens exceeds the energy resources available to the jill from her diet, and body fat mobilization and hypoglycemia occur.

2.6.3. Parturition

Domestic ferrets living in the wild normally give birth between six and ten kittens per litter (*Amstislavsky and Terovskaya, 2000*). For primiparous jills, the gestation period can be shorter (≈ 41 days) than that for multiparous females (≈ 42 days). Mammary gland development occurs the last week of gestation, but lactation commonly begins just after the last kitten is born (*Fox and Bell, 1998*).

A litter of fewer than three kittens may generate inadequate hormonal stimulus to initiate parturition. After the 43rd day of gestation, the kittens die. Parturition can be induced on the 41st day of gestation with prostaglandin $F_{2\alpha}$ (0.5 mg per animal i.m.) and then oxytocin (6 IU) 1 to 4 hours later. Most jills deliver 2 to 12 hours after this treatment. If medical treatment is unsuccessful, a cesarean section is indicated (*Bell, 2004*).

Normal parturition in jills usually takes 2 to 3 hours. Kittens are born hairless, blind and weight 8-10 g. They try to nurse almost immediately. When prolonged

parturition occurs, it is advantageous to keep the kittens warm and separated from the mother until the delivery ends as jills will take care only for warm kittens and only after the end of the parturition (*Fox and Bell, 1998*).

2.6.4. Lactation and interaction between milk production and ovarian function

Ferret jills have strong maternal instincts, which make cross-fostering a simple procedure even when delivery dates are not coincident. Cross-fostering is especially useful given the difficulty of hand rearing neonates (*Ball, 2006*).

Well nourished jills taken care of with proper husbandry techniques can have average litter of 8 to 10 and lose a minimal number of kittens (≈ 1 kitten per litter). Good producing jill milks as well on a body weight basis as a Holstein cow; however ferret milk has a much higher fat content. Ferret milk has only about 8 to 10% fat at parturition, but by 3 weeks lactation the fat content may be 15 to 20%. Lactating jills loose weight even if fed well. Lactation in the ferret lasts 4-6 weeks generally depending on the number of young being suckled and the sucking stimulus is of prime importance in the maintenance of lactation (*Lewington, 2007b*).

Jills failing to produce enough milk to feed kittens in normal litters of 5 to 10, commonly have systemic diseases, chronic mastitis or genetic problem, or are managed and fed inappropriately (*Bell, 2004*). On the other hand females nursing fewer than 5 kittens may not produce milk because of kittens can not stimulate the mammary glands effectively enough to maintain the lactation. In the latter case, jills may return to oestrus when the litter is only 2 or 3 weeks old, especially if stimulatory photoperiod occurs simultaneously. Some authors propose to induce ovulation (with hormonal treatment or breeding) in such jills as soon after the 10^{th} day of oestrus even while nursing the litter (*Fox and Bell, 1998*). Interestingly, however, jills nursing 2 to 3 kittens may have normal lactation with or without lactational oestrus and such oestrus may stop spontaneously after some days (*personal experience*).

There are only very few – if any – data available in the literature on hormonal regulation and other factors responsible for maintenance of lactation in ferrets (or even in other Mustelids). In mink (*Mustela vison*) prolactin profiles of pregnant and lactating females had a biphasic pattern (*Tauson, 1997*). There was an increase during the mid and late gestation in April, a decline immediately before parturition, and peak values were recorded in early May, when females were in the first weeks of lactation. In non-

mated control females the plasma prolactin did not rise above the basal concentration. Based on the experience that ferret females with small litters may stop lactation (and return to oestrus), it seems likely that suckling stimulus play main role in stimulation of lactation (*Lewington, 2007b*). Prolactin, which is luteotrophic – responsible for maintenance CL function and for stimulating synthesis of implantation factor(s) – in this species *Murphy* (*1979*), may have another crucial function in mammary development and maintenance of lactation.

Most mammalian domestic animals adopt a strategy of being anoestrus to avoid becoming pregnant during lactation. In seasonal breeder mammals, follicular maturation is inhibited during lactation even if stimulating photoperiod occurs. The inhibitory effect of suckling on postpartum return to oestrus has been well documented in several species (dairy and beef cows, pigs, monkey, human, etc.). In species with spontaneous ovulation, the absence of ovulation during lactation is considered to be due to the inhibited pulse-like basal GnRH/LH secretion with coincidently depressed follicular growth and maturation, rather than a direct inhibition of pre-ovulatory-like release of GnRH/LH from the hypothalamic surge center / anterior pituitary (*Senger, 2003*).

Probably also in ferrets (as described in rats), during the first half of lactation the strong suckling stimulus (together with the β-endorphin) reduces serum LH directly or indirectly via high levels of serum prolactin, which in turn inhibit follicular maturation. During the second half of lactation, the suckling stimulus wanes, prolactin decline and serum LH recovers to basal cyclic values which are reflected in the initiation of follicular maturation (*Senger, 2003; Lewington, 2007b*). In certain wild Felids, such as the Eurasian lynx (*Lynx lynx*) and the Iberian lynx (*Lynx pardinus*), elevated P_4 levels suggestive of luteal activity were detected during the lactation, as well as after weaning the cups, until the onset of a new oestrus cycle (*Dehnhard et al., 2008; Göritz et al., 2009*). Although similar phenomenon was not observed in domestic cats (*Tsutsui and Stabenfeldt, 1993*) and in some otter species (such as North American river otter, *Lontra canadensis* and Asian small-clawed otter, *Amblonyx cinereus*; *Bateman et al*., 2009), we suppose that during nursing and lactation – beside the reduced LH pulsatility and increased prolactin levels – also the elevated P_4 concentration may be one of the factors inhibiting the follicular growth and maturation in certain species with induced ovulation.

2.6.5. Re-breeding after nursing, abortion or early weaning

In the wild, female ferrets mated in the early spring period usually become pregnant, whelp after 42 days and nurse their kittens for 6 weeks. It is quite exceptional, when after weaning some of them return to oestrus again within the same season. However, domestic ferret females exposed to stimulatory photoperiod usually return to oestrus in approximately 2 weeks after (a) loosing their kittens at delivery or during lactation, (b) after weaning of kittens following 4-6 week long normal lactation or (c) after the end of the pseudopregnancy. In most of the domestic female ferrets the early return to oestrus and re-conception is a characteristic trait (*Lewington, 2007b*). This change in the reproductive pattern of ferrets can be stated as a consequence of domestication.

Indoor kept ferrets are exposed to an artificial photoperiod. This situation can be mimicked with an experimental situation. *Mead* and *Neirinckx* (1990) run an experiment in this topic. Twenty polecats were allocated to 2 groups, each consisting of 4 males and 6 females, and subjected to either a natural photoperiod (controls) or alternating periods of short (8 hours light/16 hours dark for 8 to 9 weeks) and long days (16 hours light/8 hours dark for 16 to 20 weeks). The experimental photoperiod significantly accelerated sexual maturation in both sexes, with males developing maximal testis size within 57 days and females breeding after an average of 52 days exposure to long days. Males in the experimental group completed 2.5 testicular cycles and participated in mating during 3 successive breeding seasons during the 18 month period whereas males in the control group completed a single testicular cycle and only had an opportunity to mate during a single breeding season. Females in the experimental group produced 3 litters whereas females in the control group only gave birth to a single litter. Pseudopregnant females returned to oestrus within 12 days after the expected date of parturition, were bred, and gave birth to kittens. Death or removal of kittens within 8 days after birth resulted in 12/12 females returning to oestrus within 6 to 26 days. Eleven of these females were re-mated and gave birth to kittens.

2.7. Luteinisation without ovulation

The proximal event that initiates luteinisation is the pre-ovulatory gonadotropin surge that provokes ovulation. Ovulation is, however, not a *sine qua non* for luteinisation. There are numerous examples of luteinisation in unruptured follicles, including follicles of rodents (*Westfahl, 1993; Mattheij and Swarts, 1995*), humans (*Zaidi et al., 1995*), and subhuman primates (*D'Hooghe et al., 1996*). Disruption of ovulation does not affect luteinisation in the pig (*Hall et al., 1993*) and luteinisation appears to be the fate of all large, unovulated follicles in the mink (*Douglas et al., 1994*).

Follicles in different states of maturation respond differently to gonadotrophins. Studies describing the effect of gonadotropin treatment before ovulation in species with spontaneous ovulation describe, that administration of hCG prior to spontaneous ovulation induces the formation of luteinized unruptured follicles (LUFs) in guinea pigs. It was shown that LUFs and CL contained equivalent amounts of P_4/mg tissue, but LUFs were significantly smaller than CL. Cells from CL and LUFs responded to hCG in vitro with significant increases in P_4 release, but the response was greatest with LUFs (*Westfahl, 1993*). *Mattheij and Swarts* (*1995*) suggested that deficient LH secretion in the period before ovulation may be involved in the formation of LUF. They performed a study in rats where they studied the formation of LUF after injection of a different small dose of LH with or without subsequent injection of GnRH. In addition, the effect of suppression of prolactin on LUF formation was studied. Luteinisation without ovulation occurred in virtually all Graafian follicles, if low dose (0.5 to 1.0 μg) of LH was injected some hours before the presumed endogenous LH surge (suppressed by Nembutal). With increasing doses of LH progressively increasing numbers of ovulations were observed. Interestingly, if in early pro-oestrus 1 μg of GnRH was given 4 hours after 1 μg of LH, formation of LUF was partly prevented, but if the interval between LH and GnRH applications was 8 hours or more, the great majority of Graafian follicles developed into LUF. If early in pro-oestrus 1 μg of LH was given and 8 hours later 0.1 μg of a potent GnRH analogue, about 50% of the follicles became LUF; in similarly treated rats, suppression of prolactin reduced, but did not prevent LUF formation.

One other pathomechanism of LUF formation (disruption of ovulation) was described in human and monkey. Administration of general cyclooxygenase inhibitors

around the time of administration of the ovulatory dose of gonadotrophin can cause the development of LUF (*Duffy and Stouffer, 2002*).

In species with induced ovulation, LUF formation is also documented. A very early reference by *Robinson* (*1918*) describes that in ferrets, even after copulation, some pre-ovulatory follicles may persist in the ovaries and become luteinized without ovulation. In cats, pre-ovulatory follicles can be transformed into luteal-like tissue if mating does not occur during oestrus (*Dawson and Friedgood, 1940*). In llama, regressing follicles may also luteinise if follicles are exposed to an LH surge (mating). In llamas and alpacas, the pre-ovulatory release of LH depends on ovarian follicles size. Copulation of females with regressing follicles can provoke of LH similar to that observed in animals with growing mature follicles, but luteinisation, not ovulation, occur. It appears that granulose cells in the regressing follicle yet have lost the capacity to secrete factors that are important for rupture of the follicle. The luteal structure formed after luteinisation of a regressing follicle had a life span of 5 days, which is shorter than the 10 days (minimum) observed in animals that formed normal corpora lutea (*Bravo et al., 1991*).

LUF formation can be also the fate of all large, unovulated follicles in mink. These large degenerating LUFs are considered to represent the demise of large follicles that failed to receive an ovulatory stimulus (*Douglas et al., 1994*). This experiment reveled that follicle in the earliest stage of the luteinisation-degeneration process had diameters of approximately the same size as that of the largest follicles observed in mink during the breeding season. *Elofson et al.* (*1989*) examined ovaries from females in the breeding season. The ovaries mainly contained follicles characterized as active, but occasionally single atretic or luteinized follicles were observed. In a few cases, luteinized follicles were accompanied by a rise in concentration of plasma P_4. *Lagerkvist et al.* (*1992*) found the same pictures in unmated females. The active follicles dominated but, the number of degenerating follicles had increased markedly and luteinisation had frequently started in the atretic follicles. The luteinized follicles were generally smaller than corpora lutea of mated females. In addition, in such females increased concentrations of plasma P_4 could be measured. This implies that unmated females may enter a luteal phase in the absence of ovulation.

2.8. Current possibilities for controlling reproduction in ferrets

2.8.1. Progestins

Long-acting forms of progestins may be used to control the breeding season of pet ferrets. These hormones have been previously used for prevention and suppression of oestrus in several carnivores and in zoo animals with success. Numerous reproduction studies on P_4 and other progestins demonstrate that their administration can have actions that can be classified as progestational, anti-oestrogenic, anti-androgenic, anti-gonadotrophic, and contraceptive. The anti-gonadotrophic action is by far and large the most important clinical effect in small animal reproduction. P_4 and other synthetic progestins act in the same manner as endogenous P_4. They appear to have their main contraceptive effect by preventing an increase in gonadotropin secretion that would otherwise occur in the normal course of events. This in turn prevents full follicle maturation. The direct effect is primarily if not entirely at the level of the hypothalamus, and perhaps at the level of opioidergic neurons, causing a decrease in the frequency of GnRH pulse secretion, which in turn decreases LH and FSH pulsatility. Progestins can also acutely inhibit the pre-ovulatory surge in LH (an effect that can involve actions both on hypothalamic GnRH secretion), and on pituitary responses to oestrogen if administered before oestrogen reaches high concentrations (only in species with spontaneous ovulation) (*Romagnoli and Concannon, 2003; Lewington, 2007b*).

Apart from the reproductive effects, progestins display a range of metabolic and non-reproductive endocrine actions. One of these non-reproductive effects is the potential depression of pituitary ACTH secretion and adrenocortical function, with reduced cortisol levels (clinically reported, at pharmacological doses) (*Romagnoli and Concannon, 2003*). Cats were given megestrol acetate (MA) (5 mg once daily for 14 days) or subcutaneous PROL (100 mg on two occasion one week apart) developed suppression of basal and ACTH-stimulated cortisol concentration during treatment. Effects on cortisol persisted for two weeks after MA and for 14 to 22 weeks after PROL dosage ceased (*Waston et al., 1989*). Owing to their structural resemblance to the glucocorticoids, many progestins have an intrinsic glucocorticoid-like action. In humans, prolonged treatment can even result in an iatrogenic Cushing's syndrome or adrenocortical insufficiency.

MPA was one of the first progestin developed for human use and has therefore been extensively tested in dogs and cats, particularly at high-multiples of the human dose. Depot-MPA is typically not marketed with an indication for use in ferrets.

PROL is a third generation progestin developed in the hopes of achieving inhibitory effects on the hypothalamic- anterior pituitary-gonadal axis without causing side effects on the uterus or mammary gland. However, studies performed show that PROL treatment can also result in many of the same side effects caused by MPA. Nevertheless, some clinicians have suggested that effective contraceptive doses of PROL do appear to provide a weaker stimulus on the endometrium and on the mammary epithelium, compared to presumably comparable doses of other progestins (*Romagnoli and Concannon, 2003*). PROL containing injection, such as Covinan® (i.e. Delvosteron®) is recommended in the UK for prevention of oestrus in ferrets at a dose of 0.5 mL (100 mg/mL s.c.) just prior to the breeding season (*Oxenham, 1990; Kluth, 1993*). PROL can also be used in jills in oestrus. Return of oestrus was reported in approximately 8% of ferrets 2 – 5 months after the initial dose. In these cases, a second dose of PROL suppressed oestrus for the rest of the breeding season (*Oxenham, 1990*).

Both MPA and PROL, apart from their progestational activity, act as glucocorticoid agonist and are less specific than P_4. The have high affinity to glucocorticoid receptors which explains their glucocorticoid actions: (a) suppression of the hypothalamus-anterior pituitary-adrenal gland axis resulting in a decreased production of endogenous cortisol and ACTH concentration, (b) excess of glucocorticoid action and effects similar to the iatrogenic Cushing's syndrome resulting in an impaired glucose tolerance and histological changes in the adrenal cortex, the pancreas and the liver (*Selman et al., 1997*).

MA has been used in ferrets to prevent oestrus, but is not recommended because of the assumed risk of pyometra (*Fox et al., 1998*; *Ryland and Lipinski, 1994*). However, this restriction is probably not justified because pyometra has not been described in ferrets after the use of MA (*Evans and Sutton, 1992 cited by Schoemaker, 2003; Howard, 1979*). MA (e.g. Ovarid®) was used devoid of safety problem by *Lewington* (*2007c*) given orally at dose of 0.05 mg per jill.

2.8.2. hCG and GnRH

Pre-ovulatory LH surge can be mimicked by administration of hCG or indirectly stimulated with GnRH.

Mead et al. (*1988*) performed an interesting study to determine the optimal dose of **hCG** to induce ovulation. They compared the ovulatory response of 119 mated ferrets (controls) with that of estrous females induced to ovulate with five different dosages (50, 75, 100, 150 and 300IU) of hCG. Copulation induced formation of 13±5 corpora lutea (CL) in all 119 females and resulted in a 91% conception rate as evidenced by finding approximately eight blastocysts per female in the uteri of 108 ferrets. All doses of hCG tested induced ovulation; however, the lower doses (50 and 75 IU) resulted in a lesser percentage of females ovulating. The highest doses of hCG (150 and 300 IU) resulted in fewer CL/female being formed. The optimal dose of hCG for simulating copulation induced ovulation was 100 IU. Therefore, ten days after the onset of oestrus, 100 IU hCG is advised to give to jills. Approximately 30 to 35 hours post-treatment females ovulate, resulting in the formation of corpora lutea (*Fox and Bell, 1998*). Vulval swelling starts to decrease within 1 week post-treatment. Anoestrus will last for 40 to 60 days (*Bernard et al., 1983*).

Fox and Bell (*1998*) give the dose for **GnRH** at 20 µg per jill, but they describe that multiple injections of GnRH may sensitize the ferret to the drug, resulting in anaphylactic reactions shortly after administration.

Treatment with hCG (or GnRH) at oestrus is safe, but has one disadvantage. It is only a temporary solution, the jills usually return to oestrus after the end of pseudo-pregnancy (if they are exposed to stimulatory photoperiod), and thus the treatment needs to be repeated several times during the breeding season.

2.8.3. Long-acting GnRH agonists

GnRH has long been recognized as a potential target for the control and management of fertility in animals. One of the ways that GnRH can be employed to inhibit reproduction by direct suppression of the pituitary-gonadal axis at the level of the gonadotrope is the chronic stimulation of GnRH agonist causing down regulation of GnRH receptors and desensitization of pituitary gonadotrophs (*Herbert and Trigg, 2005*). At gonadotroph cells, GnRH binds to membrane-bound surface receptors that undergo micro-aggregation and internalisation. This activates second messenger signal

transduction pathways and culminates in release of stored LH and FSH and *de novo* synthesis of both gonadotrophins. Internalisation of GnRH receptors after binding by GnRH induces a transient insensitivity in gonadotroph cells to stimulation by GnRH. Under normal circumstances, GnRH receptors are deplenished within several hours and this restores responsiveness of gonadotroph cells to GnRH. Numerous agonists of GnRH have been synthesized that characteristically have a longer half-life in circulation relative to natural sequence GnRH, and increased binding affinity for the GnRH receptor.

The response of animal to long-term treatment with GnRH agonists involves an acute phase and a chronic phase. During the **acute phase**, which can last for several days, the secretion of LH and FSH are increased. The **chronic phase** is characterized by a reduction in GnRH receptors on gonadotroph cells, uncoupling of signal transduction mechanisms, insensitivity to GnRH, depletion in pituitary content of LH and FSH, lack of pulsative secretion of FSH and LH, the absence of pre-ovulatory surge releases of LH (*D'Occhio et al., 2002*).

In several species, **continuous administration of high doses of a GnRH agonist** induces (after an initial gonadotropin hypersecretion) pituitary desensibilization, and decreased gonadotropin release. However, there are clear species differences in susceptibility to the down-regulatory effects of GnRH analogue. For example, whereas continuous GnRH analogue administration to men (with prostate cancer) or dogs leads to a marked drop in circulating testosterone concentration and libido, continuous administration to bulls or red deer stags results in an increase in both plasma testosterone concentration and aggressive behavior (*Thomson, 2000*). Nevertheless, GnRH analogue have been proven effective for contraception in wild dog and cat species (*Bertschinger et al., 2001, 2002*), for preventing a premature LH surge in women undergoing ovarian stimulation (*Felberbaum and Diedrich, 1999*) and in arresting steroid-dependent disease such as prostate cancer in men (*Talwar, 1997*).

The *GnRH agonist* **deslorelin**, as a subcutaneous implant, was initially developed as an ovulation-inducing agent in mares. Its uses, for the suppression of reproduction in the domestic dog, cat and cow and in other species, including humans, have been developed subsequently. Such implants have been used as a contraceptive modality in a variety of wild carnivores, both males and females. However, deslorelin treatments were varying in success for the different species, no adverse effects were

observed on social behavior, general health or body weight. Use of deslorelin in the control of hyperadrenocorticism in ferrets was primary documented in the literature (*Wagner et al., 2005; Schoemaker et al., 2008b*), but later its effect on reproduction of hobs was described as well. *Schoemaker et al.* (*2008a*) reported the effect of a slow release, subcutaneous implant containing 9.4 mg deslorelin (Deslorelin implant) on plasma testosterone concentrations and concurrent testes size, spermatogenesis, and the typical musky odor of intact male ferrets. Twenty-one male ferrets (divided into three groups) were either surgically castrated, received a 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 ferrets which had received a Deslorelin implant was less compared to ferrets which were either surgically castrated or had received a placebo implant. The authors concluded that 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. *Vinke et al.* (*2008*) described a study about the effects of Deslorelin implant and surgical castration on the occurrence of inter-male aggression, sexual behavior and play behavior in male ferrets. Male domestic ferrets received either an implant containing deslorelin (n=7), a placebo implant (n=7), or were surgically castrated (n=7). Their data showed that (a) chemical castration with the GnRH agonist deslorelin results in a decrease in the occurrence of aggressive behavior between male ferrets both in presence and absence of a receptive female (in addition, their data showed that a deslorelin castration had more obvious effect on the reduction of aggression than surgical castration); (b) sexually motivated behavioral patterns were reduced in the deslorelin and surgical castrated groups in the male–female confrontation; (c) the deslorelin group, and to a lesser extent the surgically castrated group, had a higher incidence of play behavior in comparison to intact males in the inter-male confrontation tests.

The another commercially available GnRH agonist **leuprolide acetate** (Lupron Depot 3.75 mg, TAP Pharmaceutical Products) is primary used to treat hyperadrenocorticism in ferrets (*Wagner et al., 2001*). Ferrets weighing < 1 kg receive an intramuscular dose of 100 µg at monthly intervals, and ferrets > 1 kg receive 200 µg per month. A similar treatment protocol might also be effective for contraceptive purposes.

2.8.4. Spaying

Spaying is a common and efficient method to control the reproduction of most companion animals. While in the US, neutering is performed routinely in ferrets at the age of 6 to 8 weeks, in Europe, most of the ferrets are neutered when they are several months old. Female ferrets are commonly neutered to prevent oestrus-induced bone marrow suppression and unwanted pregnancy (in colony), and male ferrets are castrated to reduce skin odor and inter-individual aggressiveness. Unfortunately, in the ferret, neutering in both genders brings certain risks (*Lewington, 2000b* and *2007c*).

In female ferrets, the removal of all the ovarian tissue is not always successful. The ovaries may be difficult to localize due to its small size, and the fact that it is hard to differentiate them from surrounding adipose tissue on palpation, thus it is not uncommon the spayed jills will suffer from ORS (described in section 2.3.2.). There are different opinions whether ovariectomy (removal of the ovaries) or ovariohysterectomy (removal of the ovaries together with the uterus) is best technique in ferrets. Generally, ovariohysterectomy is technically more complicated, time consuming, and is probably associated with greater morbidity (larger incision, more intraoperative trauma, increased discomfort) compared with ovariectomy. While in dogs, no significant differences between techniques were observed for incidence of long-term urogenital problems, including endometritis/pyometra and urinary incontinence making ovariectomy the preferred method of gonadectomy in the healthy bitch (*Van Goethem et al., 2006*), in ferrets certain authors advice to prefer ovariohysterectomy. One explanation is that pyometra and hydrometra may be associated with ovarian tumours especially frequent after incomplete ovariectomy (*Jekl et al., 2006*), other reason could likely be that females with well-done ovariohysterectomy (no opportunity for the development of stump pyometra) has less chance to suffer from pyometra/stump pyometra when having hyperadrenocorticism post-neutering. Neutering in both genders leads to an increased risk of adrenocortical diseases (described in section 2.3.3.).

2.9. Fecal P_4 monitoring in mustelids

Several endocrine techniques are used to monitor CL function. The traditional methods quantify P_4 concentrations in blood, milk or saliva samples. However, repeated blood sampling is not easy in zoo and wild animals and exotic pets with small body weight. So assaying the urinary and fecal P_4-met content is an attractive option. Non-invasive fecal P_4-met determinations are well established approaches for monitoring reproductive function in variety of mammalian species. These methods are based on the phenomenon that certain P_4-met (20-oxo-, 20α-OH-, 20β-OH-and 5α- or 5β-pregnane) are excreted with the bile through the gastrointestinal tract and exhibit a similar pattern to those in plasma, but have a lag time. Unmetabolised P_4 is barely present in the fecal samples (*Möstl et al., 1993; Larter et al., 1994; Schwarzenberger et al., 1996a*).

The huge quantity of P_4-met present in the feces explains why several assay system can be used for their measurement. In general, antibodies for the analysis of fecal P_4-met identify a certain C20 position (20-oxo, 20*α*-OH, and 20β-OH) of the pregnane molecule (i.e. P_4-met antibodies cross-react with 20-oxo-pregnanes). Since different extraction methods and assays with different cross-reactivity were used, values between the studies are difficult to compare. Cross-reactivity with pregnanes could give indications about comparability of results and therefore should be published in any study on fecal P_4-met analysis. *Schwarzenberger et al.* (*1996a* and *1996b*) compared 3 enzyme-immunoassays with different degrees of specificity and the results indicated that the most suitable assay(s) show considerable cross-reactivity with several 20-oxo-pregnanes. The authors strongly suggest that antibodies for fecal steroid analysis should be raised again 5*α*- or 5*β*-pregnane immunogens conjugated at the 3-position. These antibodies have high cross-reactivity with pregnanes sharing a similar C20 group. For this reason the assays are termed group-specific.

Siberian polecats were utilized as a model species to document steroid excretion in ferrets. Polecats were treated with ^{14}C-labeled oestradiol, P_4, or testosterone. Feces- and urine samples were collected every 12 hours for 96 hours and radioactivity measured. Results indicate that 93±2.4% of each of the steroids examined were excreted into the feces. Following enzyme hydrolysis, extracted steroid increased indicating an ability to almost completely hydrolyse steroid conjugates *Gross, 1992 cited by Young,*

1998). These results demonstrate that the major route of steroid excretion in ferrets is through the bile and feces. Studies on the excretion of radioactively labeled steroids in Siberian polecat showed that the administered P_4 hormone excreted in the feces in 93%; however this ratio was in the North American river otter 89% (*Gross, 1992 cited by Schwarzenberger et al., 1996a*). Several further studies were run with this non-invasive method to monitor reproduction in mustelids (e.g. in the European mink: *Möstl et al., 1993; Amstislavsky et al. 2009*; in the black-footed ferret: *Brown, 1997; Young et al., 2001;* etc.).

3. Materials and methods

Animal experimentation was performed in compliance with the Hungarian regulations, and was permitted and controlled by the competent units of State Veterinary Service. Owners gave their permission for the veterinary manipulations (ovariectomy, sampling and/or treatment procedures) in each case. Laboratory procedures and other methods generally used throughout the studies are described here in this chapter. The study design, clinical procedures and statistical methods employed only in one experiment are detailed where that trial is introduced.

3.1. Collection, preparation, and storage of samples

In *Exp. 1a*, *1b*, *1c*, *2a*, *2b* and *3,* the ovarian activity of jills was monitored by P_4-met profiles. For this purpose, fecal samples were collected from each jill (as a part of the daily husbandry routine). Approximately 3 g of fecal samples were collected on each occasion. Samples were stored at about –18 ^{0}C in polypropylene bags until steroid extraction.

In some studies also blood samples were collected. Jills showing pathognostic clinical signs of hyperoestrogenism (*Exp. 4*) or pregnancy toxaemia (*Exp. 5*) were sampled simultaneously with the diagnostic procedures.

In *Exp. 1a*, *4* and *5,* the blood samples of non-lactating healthy (control) jills were taken at the beginning of an operative manipulation (ovariectomy, ovariohysterectomy; at the evaluation these samples are considered and cited as healthy controls), after 6 to 8 hour long food deprivation, using intramuscular combination of ketamine and xylazine for immobilization and anesthesia (for details, see below: section 3.3.).

Blood samples (2 mL) from each female were taken from the jugular vein into fluoride-containing (for determination of plasma glucose levels in *Exp. 5* only) and heparinized tubes (for assaying any other metabolites and hormones in *Exp. 1a*, *4* and *5*). Samples were cooled and centrifuged within 3 hours. Plasma was harvested immediately, and stored at either +4 ^{o}C (≤48h) until β-hydroxybutyrate (BHB) and glucose determinations or at -20 ^{o}C until assaying for P_4 (*Exp. 1a*), E_2 (*Exp. 4*), furthermore for insulin and thyroid hormones (T_4 and T_3; *Exp. 5*).

3.2. Oestrus detection and mating system

In *Exp. 2a*, *2b* and *3,* female ferrets were checked for oestrus by visual examination. Beginning of oestrus was noted when the vulval lips began to swell. After 4 to 17 (9±4) days of the beginning of heat, females were palced in the cage of a male for one or two (consecutive) nights. At that time, the vulva reached its maximal size, and the vulval lips slightly opened and became unglazed.

3.3. Operative interventions in genitals (Exp. 1a, 2b, 4 and 5)

In *Exp. 1a*, *4* and *5,* blood (control) samples of non-lactating healthy females were collected for assaying them for various hormones and metabolites. All these jills were pet animals, ovariectomized or ovariohysterectomized by the request of their owners, who gave their preceding permission for this extra sampling.

Lactating jills of *Exp. 2b* were ovariohysterectomized, for histological examination (light- and electron microscopy) of surgically removed ovaries, on days 12 to 22 (lactating jills nursing their kittens; n=8) and on days 7 to 12 post-partum (non-lactating females weaned at delivery; n=4). Kittens were weaned just before the operation, given immediately to fostering jills and later weaned at 6 weeks old devoid of problem.

The operative manipulation (ovariectomy, ovariohysterectomy) was conducted after 6 to 8 hour long food deprivation, using intramuscular combination of ketamine (2.5 mg/100 g body weight; SBH-Ketamine inj., Produlab Pharma BV, Raamsdonksveer, The Netherlands) and xylazine (0.2 mg/100 g body weight; Rompun inj., Bayer AG, Leverkusesen, Germany) for immobilization and anesthesia, in full accordance with the suggested standard surgical methods (*Bennett and Pye, 2007*). All females recovered rapidly, without any complications. Jills of *Exp. 2b* ovariohysterectomized during their lactation were sold as pet animals later.

3.4. Laboratory procedures

3.4.1. Fecal steroid extraction and P_4-met determination (Exp. 1a, 1b, 1c, 2a, 2b and 3)

For assaying P_4-met, steroids were extracted from feces according to a slight modification of the method described by *Palme et al.* (*1996 and 1999*). Briefly, 0.50 g of feces was dispersed in 0.5 mL double distilled water in thick-walled glass tubes

suitable for centrifugation. After adding 4 mL methanol (80 %), samples were shaken for 20 minutes with a multitube vortex. Thereafter, 3 mL petroleum ether (40-70 ^{o}C) was added to remove lipids. Then, samples were mixed at high speed for 10 seconds by hand-vortex. After centrifugation (3600 g, 15 min., + 4 ^{o}C), samples were put at -70 ^{o}C for 25-30 minutes to separate the three phases of petroleum ether (above), methanol (in middle) and the frozen water with the extracted feces (below). Half to one mL of the methanol phase was pipetted into clean tubes, and 1:50 or 1:20 working solutions were diluted with PBS buffer (pH: 7.4) when high or low concentrations of P_4-met were expected, respectively. Generally, the methanolic extract of samples collected within 7 weeks after oestrus (e.g. during the suspected pregnancy) were diluted to 1:50; other wise 1:20 dilution was used.

The P_4-met concentrations were quantified in triplicate 20 µl aliquots of fecal extracts with a microplate ELISA (*Nagy et al., 1998*) based on the use of an anti-P_4 monoclonal antibody (5D4) cross-reacting with a wide range of gestagen metabolites (5β-pregnan-3,20-dione: 100%; 11α-OH-progesterone: 20%; 5α-progesterone -3,20-dione: 15.6%; 17α-OH-progesterone: 3.6%; pregnenolone: 1.8%; 11β-OH-progesterone: 1.6%) (*Siklódi et al., 1995*). This ELISA was developed for assaying P_4 in equine plasma (*Nagy et al., 1998*), and was validated for quantifying P_4-met in methanolic extracts of ovine fecal samples (*Kulcsár et al., 2006a*). The estimated sensitivity of the assay was 10ng/g. The intra- and inter-assays coefficients of variation were ≤ 8.7 and ≤ 12.9%, respectively. The binding pattern of serially diluted fecal extracts from pregnant jills was parallel to that of the standard curves. The recovery rates of added known quantities of hormones were the following: (i) for P_4 and 5β-pregnan-3,20-dione (125, 250 and 500 nmol/g of each; both from Steraloids Inc., Newport, USA) added to methanolic extract of fecal samples taken from oestrus jills (n=3): recovery between 93 and 102%; (ii) for 5β-pregnan-3,20-dione (125, 250 and 500 nmol/g) added to the same samples (n=3) before extraction: recovery rate between 78 and 87%.

3.4.2. Measurements of P_4 in plasma (Exp. 1a)

The P_4 contents in plasma (150 µl) and in the above-detailed (3.4.1.) fecal extract (in 20, 50, 100 and 150 µl, completed to the volume of 150 µl with adding assay buffer) were quantified with a commercially available microparticle enzyme immunoassay

(MEIA) technology (AxSYM system, Abbot Diagnostics, Tokyo - Japan / Weisbaden, Germany), based on using a computer-guided automatic counter and a compatible human kit, which is declared as highly specific for P_4 (cross-reactivity of antibody with gestagen metabolites: 5α-pregnan-3,20-dione: 6.3%; 5β-pregnan-3,20-dione: 3.2%; 5α-pregnan-3α-ol-20-ne: 1.7%; pregnenolone: 1.5%; further 32 metabolites: <1.0%, mostly not detectable; for further details see the manual of the kit). For assaying plasma samples the sensitivity of MEIA was 0.6 nmol/l, with intra- and inter-assays coefficients of variation < 3 % and < 5 %, respectively. The recovery rates of known added quantity of P_4 to plasma samples with pre-determined P_4 content, as well as to fecal extracts varied between 98 and 102%. The binding pattern of serially diluted ferret plasma with known high P_4 content was parallel to that of the standard curves. However, due to the highly specific character of the antibody, neither added known quantities of P_4 metabolites (5β-pregnan-3,20-dione, Steraloids Inc., Newport, USA), nor naturally excreted P_4-met were detectable with MEIA.

3.4.3. Assaying E_2 in plasma (Exp. 4)

The E_2 content in 0.5 mL of plasma was extracted with 5 mL of diethyl-ether (Reanal, Budapest, Hungary). After evaporation and resolving in assay buffer the E_2 content was determined by a commercial ^{125}I-RIA (radio immuno assay) kit (3rd Generation Estradiol RIA, DSL-39100, Diagnostic Systems Laboratories, Inc., Webster, Texas, USA). However, before assaying the current samples, the analytical procedure was validated for ferret plasma: the binding pattern of two serially diluted sample pools was parallel to that of the standard curves, and the recovery rates of added known quantity of E_2 were between 86 and 101% (added before extraction), and between 96 and 104% (added into the extract). The sensitivity and intra- and interassay CV of procedures were within the acceptable range (2.2 pmol/L, < 7.5% and < 16.4%).

3.4.4. Measurements of other hormones and metabolites in plasma (Exp. 5)

All metabolites and hormones were determined with commercial kits adapted for assaying bovine and ovine plasma samples in our lab (*Meikle et al., 2004; Kulcsár et al., 2006a; Balogh et al., 2008; Cavestany et al., 2009*). 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 ^{125}I-Insulin RIA CT kit (CIS Bio International Ltd, Gif-Sur-Yvette, France), T_4 was measured using ^{125}I- T_4 RIA-Spec MIS kit (Institute of Isotopes Co. Ltd., Budapest, Hungary) and T_3 was measured using ^{125}I-T_3 RIA MIS kit (Institute of Isotopes Co. Ltd., Budapest, Hungary). However, before assaying the current samples, all these endocrine analytical procedures were validated for ferret plasma: the binding pattern of two serially diluted sample pools was parallel to that of the standard curves, and the recovery rates of added known quantity of hormones were between 85 and 106%. The sensitivity and intra- and interassay CV of procedures were within the acceptable range (insulin: 7.75 μIU/mL, 5.5 - 8.4% and ≤ 8.8%; T_4: 1.46 nmol/L, 6.6 - 8.5% and ≤ 7.7%; T_3: 0.18 nmol/L, 6.2 - 8.8% and ≤ 6.7%, respectively).

3.4.5. Histology of surgically removed ovaries (Exp. 2b)

Lactating jills of *Exp. 2b* were ovariohysterectomized, removing the ovaries for histological examinations (light and electron microscopy). For this purpose, one half of each ovary was fixed in 4% phosphate-buffered formaldehyde solution (for light microscopy), while the other half was cut into small pieces and fixed in 2.5 (v/v) % sodium-cacodilate-buffered glutaraldehyde and were post-fixed in 1 (v/v) % osmium tetroxide (for electron microscopy). For light microscopy, ovaries were embedded in paraffin wax. Sections (4 μm and 10 μm) were cut and stained with hematoxylin and eosin and together with periodic acid-Schiff, respectively. For electron microscopic examinations, ovaries were embedded in Durcupan wax and were cut at 1 μm. Such sections were stained with toluidine blue. Before examinations focusing on the luteinized cells of ovaries, the ultra-thin sections were contrasted with uranyl acetate and lead citrate. They were examined under 11.000 and 36.000 x scale. with a Philips 2085 transmission electron microscope.

3.5. Statistics

In general, for presentation of results the group means and their standard deviations (SD) were calculated. The results were usually subjected to chi-square (χ^2) test (distributions), Student's t test (pair-wise comparison of group means), or a single trait analysis of variance (ANOVA; for comparison of 3 or more group means in a particular stage). If ANOVA proved significant difference, the least significant differences were calculated at 5% ($LSD_{P<0.05}$) for further comparison. Interrelations were estimated by using linear regression. These methods were employed and

conducted, as suggested by *Juvancz and Paksy* (*1982*), *Snedecor and Cochran* (*1982*) and *Petrie* and *Watson* (*1999*). Analyses were done using the Microsoft® Office Excel 2003 program. Further statistical methods are detailed at the description of current studies.

4. Studies

4.1. Biological validation of fecal P_4-met ELISA in ferrets (*Exp. 1a*, *1b* and *1c*)

After the adaptation of our P_4-met ELISA used in ewes earlier (*Kulcsár et al., 2006a*) for analyzing fecal samples of ferrets, our first responsibility was to check and improve the biological validity of findings provided by this laboratory procedure. For this purpose, three preliminary studies (*Exp. 1a*, *1b* and *1c*) were conducted. Within the framework of these studies we wished to reproduce some expected tendencies known from the literature.

4.1.1. Design and results

The *Exp. 1a* was conducted on jills (n=6) undergone ovariectomy upon the request of their owners. 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.®, Richter Gedeon, Budapest, Hungary). At the ovariectomy (in ketamine-xylazine anesthesia[1]), 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_4 (measured by MEIA) and fecal P_4-met (measured by ELISA) concentrations ranged between 7 and 33 nmol/L, and 600 and 2500 ng/g, respectively, and shoved positive correlation ($r=0.817$; $P< 0.05$). After the operation, all jills recovered without any complications. In the second fecal samples taken 7 to 9 days after ovariectomy, low (< 500 ng/g) P_4-met levels were measured by ELISA. However, (due to its more P_4-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_4-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.®, Wefft-Chemie, Vienna, Austria). Fecal samples were repeatedly collected every

[1] 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).

12 hours for 11 days, starting 60 hours before treatment. In all samples taken before treatment, fecal P_4-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).

4.1.2. Discussion and conclusions

In the pseudopregnant jills of *Exp. 1a,* the circulating concentration of P_4 (measured by MEIA) was in strong positive correlation with the fecal P_4-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_4-met content of ≥ 500 ng/g (measured by ELISA) was considered as evidence of luteal activity in the ferret. So, the ELISA determination of fecal P_4-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_4 is excreted via the bile into the feces in form of its metabolites (5β-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_4 is barely present, if at all, in fecal samples of *Carnivores*. So, the highly P_4-specific MEIA technology is suitable for measuring P_4 in blood (plasma or serum), but it can not be used for quantification of fecal P_4-met content.

4.2. Fecal P_4-met pattern and reproductive features of jills kept under kennel conditions (*Exp. 2a* and *2b*)

In the recent few years, determination of fecal P_4-met content has been extensively used, as a non-invasive technique for monitoring reproduction in various species of *Carnivores* including *Mustelids* (e.g. North American river otters: *Gross, 1992 cited by Schwarzenberger et al., 1996a*; *Bateman et al., 2009*; European minks: *Möstl et al., 1993; Amstislavsky et al. 2009*; black-footed ferrets: *Brown, 1997; Young et al., 2001*; domestic ferrets: *Möstl et al., 1993*; and several other studies). However, most of these trials were restricted to detection of luteal activity within a pre-selected time window, rather than used for following up the ovarian activity for a longer period, and none of these studies were extended to the period of lactation.

Up to our knowledge, there are no studies published, in which complete P_4-met profiles are compared with reproductive performance in females of the domestic ferret throughout the breeding season. So, concerning the real life span of CL-s and intensity of luteal function in pseudopregnant individuals compared to those found in pregnants, our current knowledge (*Lewington, 2007b*) is rather limited and inconsistent, and is based on relatively old observations (*Hammond and Marshall, 1930; Heap and Hammond 1974*). Luteinisation of unruptured follicles (LUF) is quite common in mink, which failed to receive an ovulatory stimulus at copulation (*Elofson et al., 1989; Lagerkvist et al., 1992; Douglas et al., 1994*). Although there are related observations also in ferrets published long time ago (*Robinson, 1918*), the most recent reviews usually do not improve the clinical relevance of this phenomenon in this species (*Lewington, 2007b*).

As discussed earlier (section 2.6.4.), if stimulatory photoperiod occurs simultaneously, jills nursing fewer than 5 kittens may return to oestrus when the litter is only 2 to 3 week old, which may justify hormonal induction of ovulation with hCG or GnRH (*Fox and Bell, 1998*). Based on our observation, however, jills nursing 2 to 3 kittens may have normal lactation with or without lactational oestrus and such oestrus may stop spontaneously after some days (*Prohászik*, *unpublished data*). In some studies (*Pelican et al., 2006; Dehnhard et al., 2008; Göritz et al., 2009; Jewgenow et al., 2009*), non cat-like ovarian function with elevated P_4-met content in feces and urine was reported to occur in postpartum lactating lynxes (in both the Eurasian and Iberian

species). *Bateman et al*. (2009), however, did not find this phenomenon in otters (North American river otter, *Lontra canadensis* and Asian small-clawed otter, *Amblonyx cinereus*). In the light of these inconsistent observations, we supposed that the contradictory experiences with lactational oestrus in ferrets may interact with individual differences in the fecal P_4-met profiles. However, the postpartum fecal P_4-met pattern has never been studied yet in this species of *Mustelids*.

All these data would be especially useful in better understanding the reproductive characteristics of breeding jills kept under modern kennel conditions, helping us to develop more effective reproductive technology and management for these animals. Using the fecal P_4-met determination, as a diagnostic tool, the main purpose of this trial (*Exp. 2a* and *2b*) was to study whether the atypical phenomenon of elevated fecal P_4-met during lactation may occur also in domestic ferrets; if so, it has ovarian or perhaps adrenocortical origin, which ovarian structures may produce this P_4, and how this supposed tendency in fecal P_4-met interferes with returning to oestrus of these jills after a regular or early weaning, compared to non-lactating post-partum females. The study was expected to provide further information about the presence or absence of LUF, as well as on the luteal characteristics of pseudopregnancy.

4.2.1. Materials and methods

The trial lasting for the breeding season of three years (*Exp. 2a*: 2000 and 2001; *Exp. 2b*: 2004) was conducted on female ferrets of a private breeding kennel in Veszprém (Hungary). All animals of the farm were housed individually in outside facilities under natural lighting, were fed *ad libitum* with cat chow for kittens (Purina® Cat Chow Kitten), and were regularly immunized against distemper and leptospirosis (Caniffa®, Merial SAS) and treated against ecto- and endoparasites (Stronghold®, Pfizer). Water was continuously *ad libitum* available from open drinking bowls. Hobs were kept within the same kennel (allowing the pheromone-based between-gender stimulation), but were physically separated from the jills. As a regular daily routine, all females were assessed for vulval swelling proving oestrus twice daily from the beginning of the breeding season (mid February) till the first oestrus with observed copulation, and again twice daily from the expected time of delivery (i) to the next post-weaning oestrus and mating, (ii) until mid November (e.g. until the end of breeding season; females of *Exp. 2a*, and any other jills of the farm), (iii) or until the day of ovariohysterectomy performed during the pre-determined stage of lactation in *Exp. 2b*.

On day 4 to 17 of the observed oestrus, the jills were always put into the cage of a hob for natural mating (with exception of females showing oestrus during the lactation). Pregnancy was checked by careful transabdominal palpation of foetuses at day 18 to 21 post-mating. As part of regular farm management, the health condition of females, dates and duration of oestrus, dates of matings, dates and course of delivery, number of kittens (delivered, nursed and weaned), lengths of lactation and the intervals between mating and weaning to the subsequent returning to oestrus were regularly recorded.

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

In *Exp. 2a,* seven and eleven jills were studied in 2000 and 2001, respectively; four of them in both years. Their clinical data on reproduction were collected and furthermore ovarian activity was monitored by individual P_4-met profiles throughout the extended breeding season. For this purpose, fecal samples were taken from each jill twice a week from mid February till 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.

The course of delivery, number of kittens and the health status of the mothers were recorded also in *Exp. 2b*. These 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_4-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_4-met content on days 3, 4 and 5 post-ovariohysterectomy.

Females suffering from mastitis / endometritis in *Exp. 2a*, or undergoing ovario-hysterectomy (in *Exp. 2b*) received appropriate antibiotic and supportive treatments and recovered rapidly without complications. In accordance with the usual farm practice, kittens of *Exp. 2a* and *2b* separated from their mothers earlier than normal (e.g. before 5th week of age) were given routinely to fostering jills within the breeding facility and then weaned regularly at the age of 6 weeks. The ovariohysterectomised jills of *Exp. 2b* were sold later as pet animals.

4.2.2. Results

4.2.2.1. Fecal P_4-met pattern and oestrus in lactating and weaned jills (Exp 2a and 2b)

In *Exp. 2a* the 18 females followed up throughout the breeding season produced 23 pregnancies, and their relevant data were completed with those of the 11 supplementary pregnant jills. So, the course of delivery, health status of females, number of kittens nursed, lengths of lactation, peri-parturient and lactational P_4-met profiles, data on expression of oestrus during lactation or after weaning, and the intervals between mating and weaning to the subsequent returning to oestrus were available in altogether 34 cases. 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 fiver (n=1) kittens were weaned on day 6 postpartum.

In *Exp. 2a*, jills delivered 5±3, 7±2 and 4±3 kittens after 41±2, 41±2 and 40±2 day long pregnancies, and lactated for 44±5, 23±6 and 5±1 days in the NormL, ML and SL groups, respectively. In *Exp. 2b*, jills delivered 6±2 and 5±2 kittens after 43±1 and 42±1 day long pregnancy in the NL and the two lactating (L1, L2) groups, respectively. All 4 jills weaned at delivery showed oestrus 5±1 days postpartum, while vulval swelling was detected in none of the further 13 females until the end of the experiment.

Two jills of *Exp. 2a* 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.

In both experiments, **fecal P_4-met** concentrations were low before and at the time of mating, started to elevate within 3-4 days after copulation, remained high during pregnancy (≥ 500-800 ng/g, up to about 2500 ng/g representing the upper detection limit of our P_4-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_4-met concentrations (≥ 500-800 ng/g) was observed in all lactating females (n=47) of *Exp.2a* and *2b* (*Figure 10*), while it remained baseline in females weaned at delivery (n=4) *(Figure 11)*. In *Exp. 2b*, in fecal samples of lactating jills (L1 and L2 jills; n=13) elevated P_4-met concentrations was detected until the ovariohysterectomy, whereas baseline level (< 500 ng/g) P_4-met concentrations could be measured in the fecal samples collected 3, 4 and 5 days post- ovariohysterectomy from 5 jills in the group L2 (*Figure 12*).

Figure 10: Representative fecal P_4-met profile in a lactating female ferret: P_4-met concentration dropped to the baseline level (< 500 ng/g) within 2-4 days post-delivery, but on days 5-7 after delivery, it increased again (≥ 500-800 ng/g). (*Exp. 2b*)

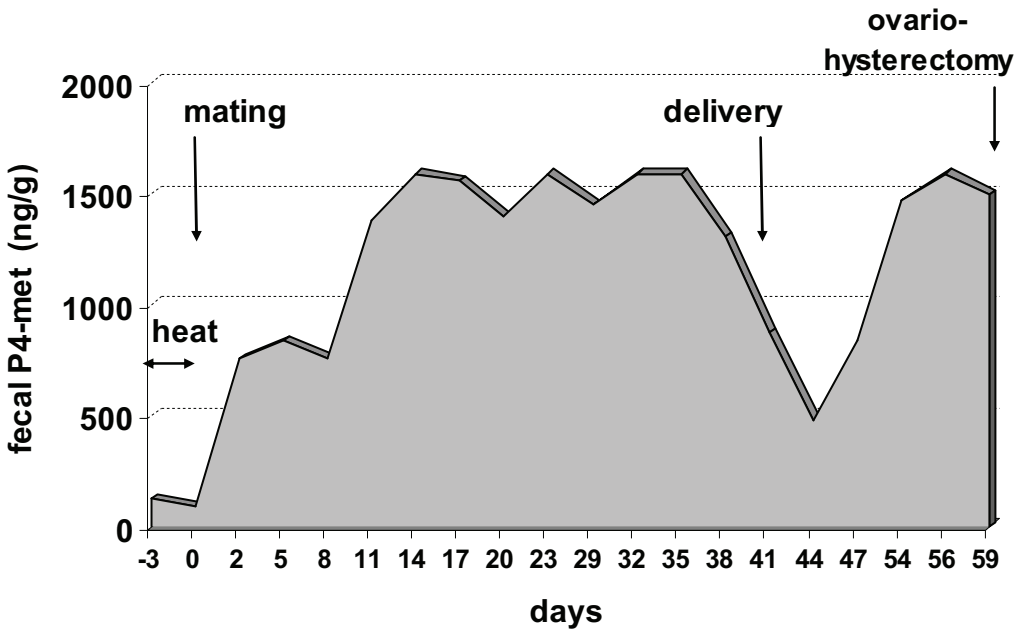


Figure 11: Representative fecal P_4-met profile in a non-lactating female ferret: P_4-met concentration remained at the baseline level (< 500 ng/g) after parturition. This jill displayed oestrus 5 to 7 days after delivery (*Exp. 2b*)

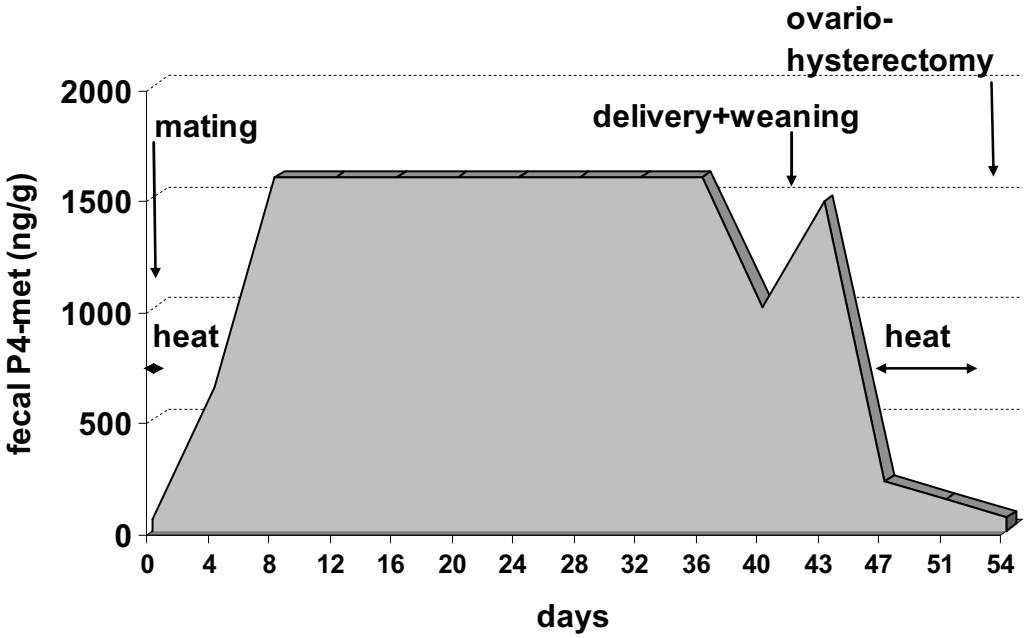
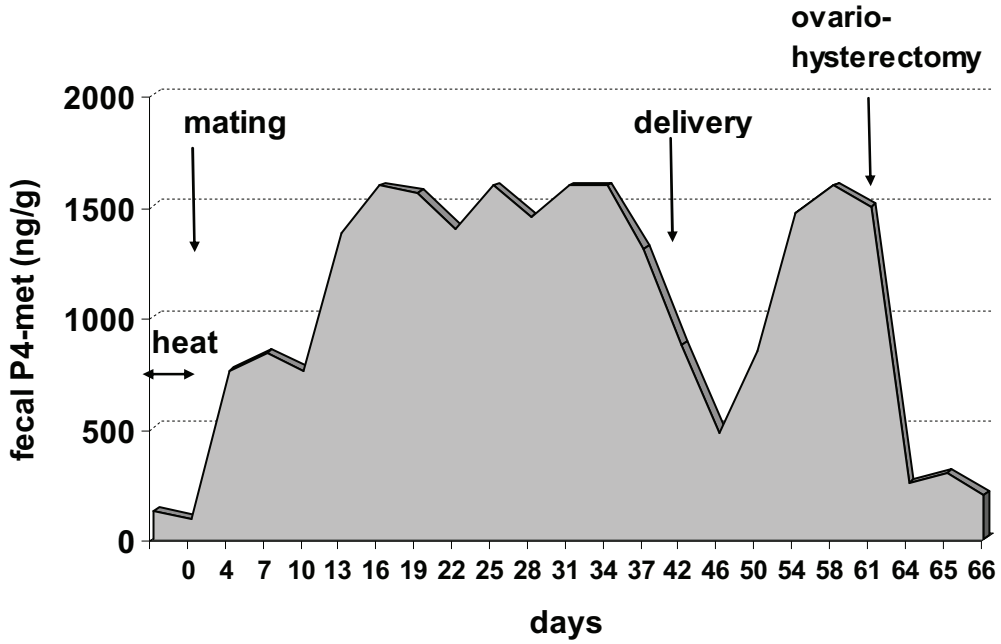


Figure 12: Representative fecal P_4-met profile in a lactating female ferret: P_4-met concentration dropped to the baseline level (< 500 ng/g) within 2-4 days post-delivery, but on days 5-7 after delivery, it increased again (≥ 500-800 ng/g). Three days after ovariohysterectomy, P_4-met concentration dropped to the baseline level (< 500 ng/g) (*Exp. 2b*)



In the two females showing lactational oestrus on days 5 and 11 after delivery, this postpartum rise of fecal P_4-met content was less rapid: elevated P_4-met levels were measured only in samples collected after the spontaneous cessation of oestrus signs.

During their lactation, jills in the *Exp. 2a* excreted elevated P_4-met content for 37±12 (n=21), 21±9 (n=9) and 8±11 (n=4) days (calculated as the period between delivery and the last day with ≥ 500 ng/g P_4-met concentration) in the NormL, ML and SL groups, respectively ($P< 0.05$; $F=21.40$; $LSD_{5\%}=9$). In general, in the NormL group, the P_4-met content tends to decline, and sometimes it may approach its nadir in the last few days of lactation, whereas ML and SL jills drops to the baseline values only at or after weaning. After weaning, jills showed oestrus again on days 13±6 (n=12/21), 8±8 (n=7/9) and 11±6 (n=3/4) (neither the rate nor the time returning to oestrus showed significant differences).

4.2.2.2. Ovarian histology in lactating and non-lactating postpartum jills (Exp. 2b)

In lactating jills, the histology of the ovaries proved the presence of follicles representing different, mainly less advanced stages of follicular development (*Figure 13*), and many luteinized cells (*Figure 14*). The follicular development appeared to be stopped at the late pre-antral/early antral phase. In such follicles, granulosa cells had undergone luteinisation and were much larger than normal granulosa cells. There were not too many luteinized theca cells present. Oocytes could also be seen (*Figure 14*). Atretic follicles could be also observed in lactating females. In such follicles, some granulosa cells were present and theca cells were hypertrophized. Sections also presented cells with more pronounced form of luteinization, resembling CL cells (*Figure 14*).

.

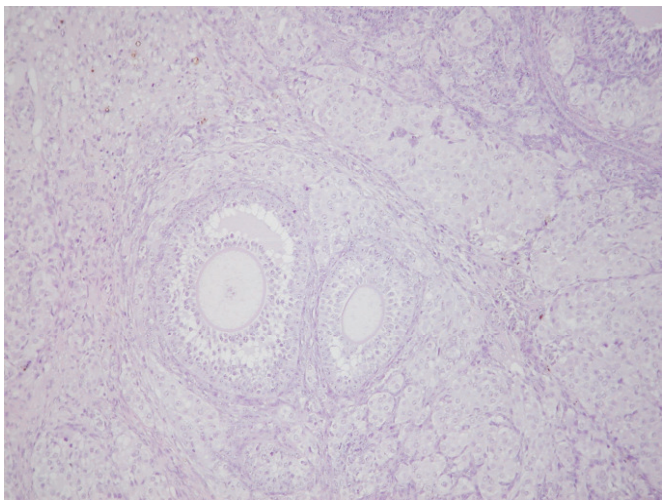
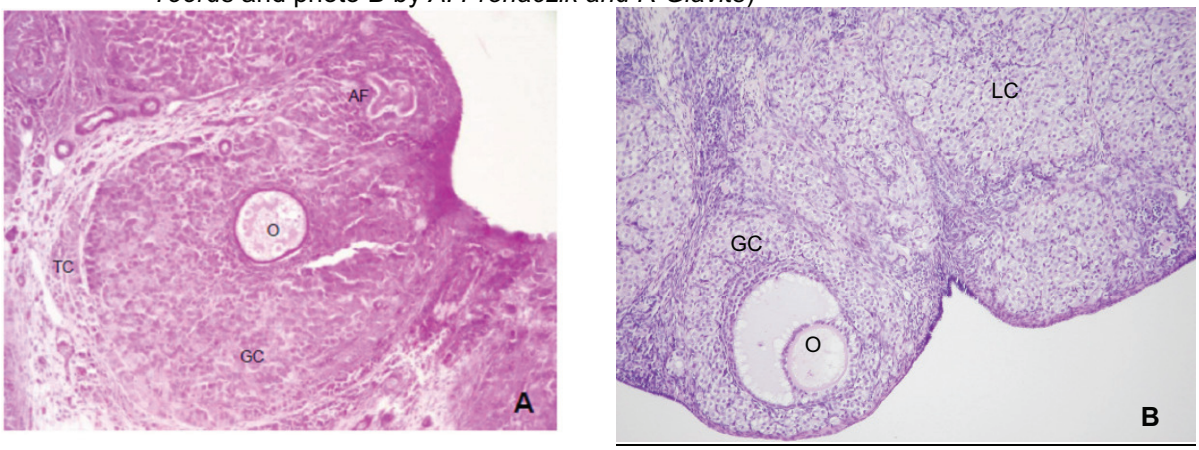


Figure 13: Representative ovarian picture in a lactating jill (12 days post-partum). One early antral (left) and one secondary follicles (right) (hema-toxylin and eosin) (100x) (*Exp. 2b*) (photo by *A. Proháczik and M. A. Driancourt*)

Figure 14: Representative ovarian picture in a lactating jill (14 days post partum)
A: Follicle where granulosa cells (GC) have undergone luteinisation and are much larger than normal granulosa cells. There are not many theca cells (TC) present. Oocyta (O) is also present. AF: old atretic follicle
B: Antral follicle with luteinized granulosa cells (GC), oocyta (O) and luteinized cells (CL like structures) (LC) (hematoxylin and eosin) (100x) (*Exp. 2b*) (photo A by *K. Teerds* and photo B by *A. Proháczik and R Glávits*)



Such luteinized cells were studied also with electron microscopy. These cells had large cytoplasm filled with endoplasmatic reticulum, 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 (*Figures 15* and *16*).

Figure 15: Typical transmission electromicroscopic appearance of luteinized cell in a lactating ferret. These cells had large cytoplasm filled with endoplasmatic vesicles, and had round or oval mitochondria (M) with tubular contents. In the lateral deflection of the endoplasmatic reticulum more electron dense secretum granulum (S) could be seen. N: nucleus of the lutein cell, B: basal membrane. (11.000 x) (*Exp. 2b*) (photo by *A. Proháczik and R. Glávits*)

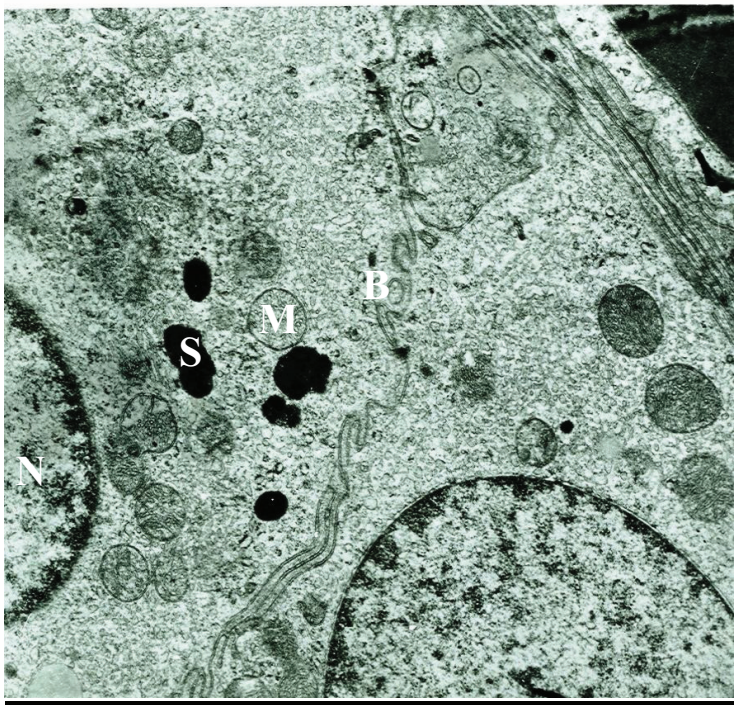
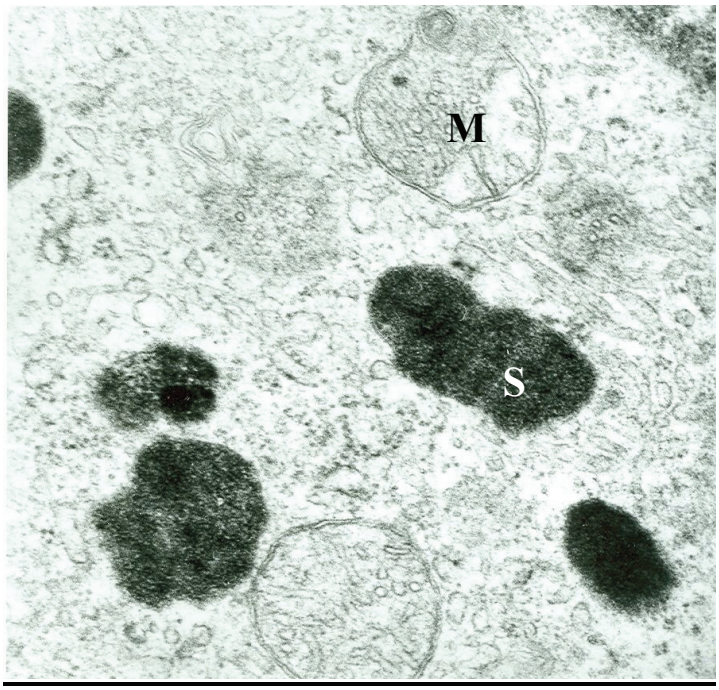
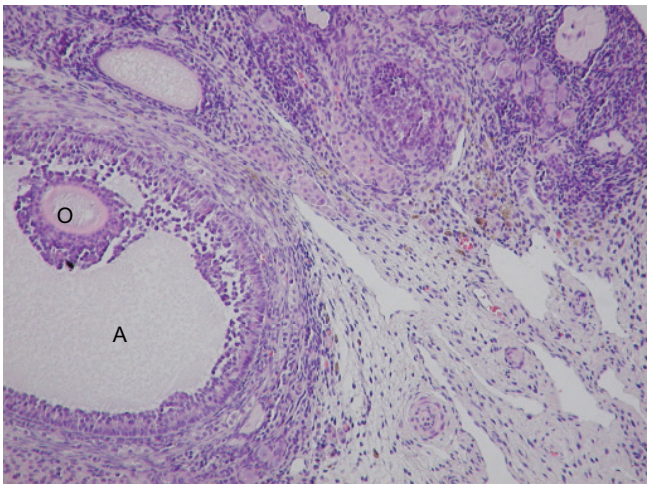


Figure 16: Typical transmission electromicroscopic appearance of luteinized cell in a lactating ferret. These cells had large cytoplasm filled with endoplasmatic vesicles, and had round or oval mitochondria (M) with tubular contents. In the lateral deflection of the endoplasmatic reticulum more electron dense secretum granulum (S) could be seen. (36.000 x) (*Exp. 2b*) (photo by *A. Proháczik and R. Glávits*)



In ovaries of non-lactating females, many growing tertiary (developing) follicles – together with some antral, but already atretic follicles – were the predominant structures (*Figure 17*). 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.

Figure 17: Representative ovarian picture of a non-lactating jill (5 days post-partum and post-weaning at delivery). Mature tercier follicle (Graafian follicle). A: large, fluid-filled antrum, O: oocyte (100x) (*Exp. 2b*) (photo by *A. Proháczik and M. A. Driancourt*)

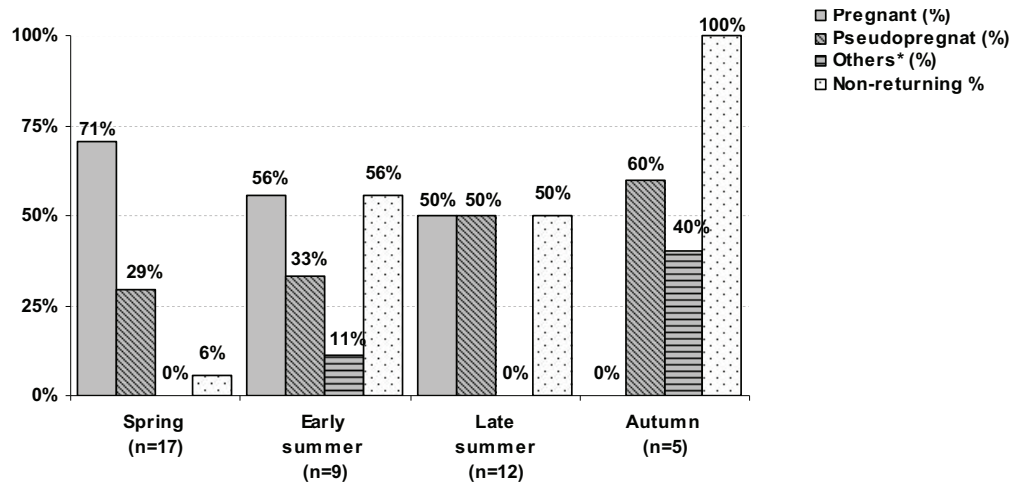


4.2.2.3 Further experiences produced by the continuous monitoring of individual P_4-met pattern throughout the breeding season

In *Exp. 2a,* all data for the whole extended breeding season were available for evaluation in 7 and 11 jills in 2000 and 2001, respectively. In mid February, prior to breeding season, all animals had very low P_4-met content in the feces. At first time, vulval swelling proving the beginning of oestrus was generally observed between 1st of March and 10th of April (e.g. around the time of the vernal equinox; n=17), and the females become sexually receptive and were mated 9±4 days later. The only exception was a young female born on 1st of August of the preceding year (body weight: 800 g), which started her first oestrus on 16th of May.

Most of the jills showing oestrus and mated in the spring period conceived resulting in pregnancy with subsequent delivery and lactation, or became pseudopregnant (with elevated P_4-met content in the feces during the gestation, lactation and pseudopregnancy), and returned to oestrus and were mated again after a shorter or longer period (11±7 days). With the exception of two young females (one of them was the jill showing the first oestrus only on 16th of May), all the other jills returned to oestrus again 1 to 3 times within the extended breeding season (the last one started on 7th of November). 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 (*Figure 18*).

Figure 18: Rate of jills conceived and became *pregnant*, not conceived and showed *pseudopregnancy*, and of those *not returning to oestrus again* within the breeding season after the spring (1st of March – 10th of April), early summer (1st of May – 15th of June), late summer (5th of July – 25th of August) and autumn (15th of September – 7th of November) oestruses
* Spontaneous heat cessation without mating (spring) and anovulation after mating (autumn)



Only limited cases of metritis (n=1), hepatic lipidosis with icterus (n=1), mastitis (n=1) and hypo- and agalactia (n=7) were diagnosed. Depending on the season of oestrus / mating, the incidence of normal lactations varied a bit [9/12, 2/5 and 4/6 after the spring (1st of March – 10th of April), early summer (1st of May – 15th of June) and late summer (5th of July – 25th of August) conceptions, respectively], with a disadvantage of lactations during the hot summer period. However, the low number of animals did not allow realistic statistical comparison. 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**, than that one in the other seasons (*Table 3*). **Lactational oestrus** was observed only after spring pregnancies (2 of 17 lactations), whereas **copulations not followed by formation of luteal tissue** (e.g. after copulation fecal P_4-met content remained < 500 ng/g continuously) were detected only in jills mated in autumn, e.g. between 15th of September and 7th of November (3 of the 6 cases). In the early summer period (in June), in one jill, the vulval swelling proved the beginning of oestrus. However, the animal did not become sexually receptive, were not mated, and subsequently the elevated fecal P_4-met content proved the presence of luteal tissue for about 3 weeks (**luteinization without copulation**, LUFs).

Table 3: The length of gestation and normal lactation, litter size, and the length of the fecal P_4-met elevations in pseudopregnants (data of 18 jills, examined and sampled throughout the breeding season in *Exp. 2a*)

Oestrus, mating	Pregnant animals			Pseudopregnant animals	Others
	Length of gestation (day)	Litter size born	Length of normal lactations (day)	Pseudo-pregnancy, length (days)	
Spring (n=17)[a]	41.0 ± 2.3 n = 12	5.8 ± 2.9 n = 12	48.4 ± 5.9 n = 9	41.8 ± 6.1 n = 5	n=1 Spontateous cessation of oestrus without mating
Early summer (n=9)[b]	40.5 ± 1.3 n = 5	6.4 ± 2.1 n = 5	53.5 ± 10.6 n = 2	41.3 ± 6.7 n = 3	n = 0
Late summer (n=12)[c]	41.0 ± 0.6 n = 6	5.5 ± 2.9 n = 6	45.8 ± 3.8 n = 4	45.2 ± 7.8 n = 6	n = 0
Autumn (n=5)[d]	n = 0	n = 0	n = 0	21.0 ± 4.0 n = 3	n=2 Anovulation after mating
	No significant differences			p>0.05 F=9.53/LSD=10.0	

Onset of oestruses: a: 1st of March – 10th of April, b: 1st of May – 15th of June, c: 5th of July – 25th of August, d: 15th of September – 7th of November

4.2.3. Discussion and conclusions

In this study, changes in fecal P_4-met concentrations and return to oestrus in post-partum lactating and non-lactating female ferrets were monitored. The aim of Exp. 2a was to describe the atypical phenomenon (elevated post-partum P_4-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_4-met concentrations were measured, moreover the length of the period with elevated P_4-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_4-met content seems to be one of the species-based characteristics in nursing females of certain carnivores (ferret, Eurasian and Iberian lynx). The fecal P_4-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_4-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*).

Normal lactation in ferrets lasts for about 5-6 weeks, but the natural weaning is gradual process and beginnings about 3 weeks post-partum, when the kittens begin to eat solid food (*Fox and Bell, 1998*). After this date kittens consume more and more quantities of a soft diet and less milk (suckling frequency decreases). 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 results 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; $P< 0.05$).

Ovarian follicular development in ferrets has limited references (*Robinson, 1918*; *Hammond and Marshall, 1930*). Pivotal studies of ovarian activity before, after and during the breeding season (during prolonged oestrus, lactation, post-weaning) would be beneficial to increase knowledge of reproductive characteristics of this species.

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 delivery 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_4 and P_4-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_4 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_4), 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 luteotrophic 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_4; the real intraovarian origin of the elevated P_4-met in lactating ferrets remained unexplained and hypothetic. To confirm the exact cell-level source and the physiological regulatrory mechanisms of increased P_4 production, further studies (for example to prove the presence of 3b-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_4-met concentrations (likely with ovarian origin) in the lactating ferret could be detected. Moreover, as the length of the elevated fecal P_4-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.

4.3. Comparative study on suppression of ovarian activity in ferrets (*Exp. 3*)

Female ferrets are reflex ovulators which when kept as pets or as experimental animals separately from males cannot ovulate at oestrus due to the lack of copulation (*Carrol et al., 1985; Villars et al., 1990; Amstislavsky and Ternovskaya, 2000; Bakker and Baum, 2000a, 2000b*). Therefore, their E_2-producing follicles may persist for up to several weeks. In addition, within the breeding season following follicle turn over, a new wave of follicular development is likely to occur. Thus, the prolonged elevation of plasma E_2 results in not only the continuous expression of oestrus-like symptoms (swollen vulva), but also other consequences of advanced hyperoestrogenism (bilateral symmetric thinning or loss of the hair coat on the hindquarters or on the trunk, suppression of bone marrow function followed by aplastic anemia and death in the most severe cases) (*Fox and Bell, 1998; Fox et al., 1998*).

The purpose of the current study was to compare the following four hormonal treatments for suppression of ovarian activity: two synthetic gestagens (MPA, PROL); a slow-release formulation of a GnRH agonist ($_{sr}$GnRH); and hCG-induced ovulation and luteinisation. The treatments were compared based on (1) their efficacy (duration of ovarian quiescence after treatment), (2) reversibility (fertility at the first oestrus after the treatment), and (3) safety (occurrence of alopecia and body condition changes) in female ferrets.

4.3.1. Material and methods

4.3.1.1. Animals

Twenty-five females (age 1.5±0.9 years and body weight 760±115 g; mean ±SD) were involved in the study and were individually housed in outside facilities with natural lighting at a private breeding farm (Veszprém Zoo, Hungary). All jills were fed a daily diet consisting of cat chow for kittens (Purina® Cat Chow Kitten). Each animal received water ad libitum from opened drinking bowls.

At the beginning of the study (mid-January 2002), body weights of the jills were measured, and their fur condition was evaluated. Only healthy females with good fur quality and a body weight of at least 600 g were included in the experiment. All animals

were immunized against distemper and leptospirosis (Caniffa®, Merial.) and were treated against ecto- and endoparasites (Stronghold®, Pfizer).

4.3.1.2. Design and treatments

Jills 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 (Depo-Promone inj.®, Pharmacia & Upjohn, MPA group); (ii) 40 mg PROL (Covinan inj.®, Intervet International B.V.; PROL group); or (iii) a srGnRH (4.7 mg per jill; Deslorelin implant®, Peptech Animal Health; $_{sr}$GnRH group). Implants were inserted subcutaneously in the scruff of the neck under short anesthesia using 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).

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 (Choriogonin inj.®, Richter Gedeon; hCG group), (v) whereas the other 5 jills were left untreated and naturally mated when showing signs of oestrus in spring and in summer (untreated controls).

4.3.1.3. Assessment of reproductive and clinical response

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_4-met profiles. For this purpose, from mid-January to end of September 2002, 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. These parameters were detected by physiological examination of fur and body shape (always by the same person).

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

4.3.1.4. Statistics and evaluation of the results

General changes in P_4-met profiles, fertility, length of pregnancies, and numbers of kittens born after the first- and second- observed post-treatment oestruses, occurrence of alopecia, and changes in body condition (i.e. safety) were described in each treatment group. Statistical analysis was performed using the statistical software Sigma®Stat 3.5.

Efficacy (i.e. days elapsed between treatment and the first day of the subsequent oestrus) of the four hormonal treatments was expressed as group means and their SDs. Results were compared between each group by one way ANOVA.

Reversibility (fertility in PROL, MPA, hCG and $_{sr}$GnRH treated females at the first oestrus after treatment and fertility in control animals in spring and summer) was expressed as the percentage of jills delivering versus the total number of females mated. Moreover, the length of pregnancies (in days) and the number of kittens born were expressed as group means and their SDs. Fertility was compared between each group by χ^2 -test, while length of pregnancy and numbers of the kittens born were compared between each group by one way ANOVA. In all calculations, significance was set at $P < 0.05$.

4.3.2. Results

While safety was assessed on all of the included females (n=25), efficacy and reversibility could be evaluated on 22 jills as some females developed pathologies interfering with treatment evaluation (see in the last paragraph of this section).

All untreated control females (n=5) displayed oestrus starting in March to April, and were mated at that time, and all of them delivered 6 to 8 kittens after a normal pregnancy of 41 to 45 days. All jills (n=5) returned to oestrus after weaning and were mated again in June and July: three re-conceived and delivered 5 to 8 kittens again after a normal pregnancy of 42 to 43 days, whereas one became pseudopregnant and one failed to ovulate (however her vulval swelling disappeared post-mating) (*Table 4*, *5* and *6*). Regardless of season, before and at oestrus, the P_4-met concentrations were low (< 500 ng/g), started to increase after mating, remained elevated (≥ 500-800 ng/g) until the end of the pregnancy (n=5 in spring and n=3 in summer) or pseudopregnancy (n=1 in summer), and then dropped to baseline values (< 500 ng/g). The P_4-met content of the fifth jill (in which vulval swelling disappeared after mating) in the summer period

remained low (< 500 ng/g) from weaning till the end of the sampling process (in late September) indicating anovulation.

Table 4: Reproductive performance in the control jills (n=5, 660±55 g of body weight and 2.1±1.2 years of age (mean±SD) in January 2002) and in the srGnRH treated female ferrets (n=4) at the second post-treatment oestrus (*Exp. 3*)

Group	Date of oestrus after treatment	Post-mating events: Number of jills showing oestrus and mated	Number of jill showing anovulation	Number of jills with pseudo-pregnancy	Number of jills with pregnancy (FERTILITY %)	Length of pregnancy (days; mean±SD)	Number of kittens born (mean±SD)
controls in spring 2002	March-April 2002	5/5 (100%)	0/5 (0%)	0/5 (0%)	5/5 (100%)	42±1	7±1
controls in summer 2002	June-July 2002	5/5 (100%)	1/5 (20%)	1/5 (20%)	3/5 (60%)	41±1	6±2
srGnRH treated jills in 2004	Apr-May 2004	3/4 (75%)*	0/3 (0%)	0/3 (0%)	3/3 (100%)	42±2	5±1

* 1 jill did not display oestrus until July 2004 after which she could not have been followed up

Table 5: Summary of efficacy results in the treated groups (*Exp. 3*)

Treatment			Animals				Efficacy of treatment	
Drug	Dose	Date	Number	Body weight (kg; mean±SD)	Age (years; mean±SD)	Number of jills showing oestrus	Days (mean±SD) elapsed between treatment and first oestrus	Date of the first oestrus after treatment
PROL	40 mg	February 20, 2002	5	740±55	2.1±1.0	5/5	99±40	Apr-July 2002
MPA	15 mg	February 20, 2002	4	725±96	0.8±0.0	4/4	94±18	May-June 2002
hCG	100 IU	at first oestrus in March - April 2002	4	825±171	1±0.4	4/4	53±9	May-June 2002
srGnRH	4.7 mg	February 20, 2002	4	875±50	1.6±1	4/4	698±122	July 2003-May 2004

Table 6: Reversibility results in the treated groups after mating at the first post-treatment oestrus. (*Exp. 3*)

Treatment group	Number of jills showing oestrus post-treatment and mated	Number of jills showing anovulation	Post-mating events: Number of jills with pseudo-pregnancy	Post-mating events: Number of jills with pregnancy (FERTILITY %)	Post-mating events: Length of pregnancy (days; mean±SD)	Post-mating events: Number of kittens born (mean±SD)
PROL	5/5	1/5 (20%)	1/5 (20%)	3/5 (60%)	41±1	5±2
MPA	4/4	0	1/4 (25%)	3/4 (75%)	39±3	4±1
hCG	4/4	0	1/4 (25%)	3/4 (75%)	42±1	6±3
srGnRH	4/4	0	4/4 (100%)	0	na	na
na: not applicable				p=0.216	p=0.069	p=0.328

In the hCG group, four jills showed spring oestrus from mid-March to mid-April. hCG treatment triggered ovulation followed by luteinisation and pseudopregnancy. Fecal P_4-met content of such jills was low (< 500 ng/g) before treatment, started to increase after hCG administration, remained elevated (≥ 500-800 ng/g) for 28 to 36 days, and then dropped to the baseline value (< 500 ng/g). All four jills returned to oestrus again in May or June, 53±9 days after hCG administration: three of them conceived and delivered 2 to 8 kittens after a normal pregnancy of 40 - 43 days and one of them became pseudopregnant. After mating, the fecal P_4-met profiles of pregnant animals (n=3) and of the one with pseudopregnancy were similar to those found in controls.

Compared to untreated controls and to the hCG group, MPA, PROL and $_{sr}$GnRH administrations clearly postponed the expression of first post-treatment oestrus. In all females of the MPA, PROL and $_{sr}$GnRH groups, low P_4-met concentrations (< 500 ng/g) confirmed ovarian quiescence.

Gestagen-treated jills returned to oestrus in April to July 2002, e.g. after an ovarian quiescence of 94±18 and 99±40 days in the MPA and PROL groups, respectively. They were mated at their first oestrus inducing 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, the others delivered 3 to 6 kittens after a normal pregnancy). Their fecal P_4-met profiles confirmed the clinical observations, and were similar to those found in (pseudo)pregnant animals of the untreated control group. In the fifth PROL-treated jill, vulval swelling disappeared after mating (in July); however, continuously low (< 500 ng/g) fecal P_4-met content proved that this female failed to ovulate.

All $_{sr}$GnRH-treated jills (n=5) displayed intensive oestrus signs with vulval swelling within 4 days after implant insertion. Two weeks after treatment, oestrus stopped spontaneously. However, the P_4-met concentrations remained low (< 500 ng/g) thought-out the 10-months sampling period (which ended in late September 2002), thus proving lack of ovulation. Four jills showed oestrus 17 to 27 months post-treatment (between July 2003 and May 2004). The duration of the ovarian quiescence therefore was 698±122 days. 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 (in 2004) and all of them became pregnant and delivered 5 to 6 kittens after a normal pregnancy of 42±2 days.

Fertility at the first oestrus after treatment (i.e. the percentage of jill delivering kittens versus the total number of females mated), demonstrated reversibility of treatments, and was 75, 60, and 75 % in groups treated with MPA, PROL and hCG, respectively while being 100 and 60% in the control group during the spring and summer periods, respectively. When fertility was compared between groups (MPA, PROL, hCG and control), no significant differences could be detected (p=0.216). Lengths of pregnancies and numbers of the kittens born were also not different between groups (p=0.069 and p=0.328, respectively). $_{sr}$GnRH treated females failed to conceive after mating at their first post-treatment oestrus.

Both gestagen treatments caused progressive hormonal alopecia in one case of each group. In the MPA treated group, alopecia around the vulval area and on the back occurred 33 days after treatment, and was associated with poor body condition (body weight was 380 g). In the PROL treated jill, alopecia around the vulval area occurred 45 days after treatment (*Figure 19*) and stopped after a few weeks (68 days post-treatment).

Figure 19: Female ferret with alopecia around the vulval area. These signs occurred 45 days after PROL treatment (*Exp. 3*) (photo by *Gy. Proháczik*)

Four out of the 5 $_{sr}$GnRH treated females showed light alopecia on the tail from 166 days post-treatment for about 6 weeks. Moreover, one jill, treated with MPA, showed purulent vaginal discharge, high fever (41 ^{0}C), and bad general condition when prematurely delivering. After appropriate medical therapy, she recovered. No side effect was recorded in the hCG treatment group; furthermore, $_{sr}$GnRH implants were well tolerated by the animals.

Clinical conditions (pathologies) interfering with treatment evaluation observed in 3 of the 25 jills are detailed below.

- From the 4th week after treatment, one of the MPA-treated jills showed a serious body condition loss of undefined clinical origin: its body weight decreased to 380 g, which is lower than the critical minimum (420 g; *Donovan, 1986*) for oestrus and regular ovarian activity in the ferret.
- A jill, in the hCG group, showed the first oestrus of the breeding season only in June, and after its hCG-induced pseudopregnancy no oestrus was detected again until the end of the observation period in 2002.

These individuals failed to return to oestrus and regular ovarian activity in this breeding season presumably due to poor body condition (MPA-treated jill), or due to the season-dependent decrease of photo-stimulation in late August (hCG-treated jill), rather than due to the treatment received. Hence these females were not included in the efficacy and reversibility evaluation.

- One further jill died of enterotoxaemia 630 days (1.5 years) after the $_{sr}$GnRH treatment before showing heat after treatment. This disease was unlikely to be related to the treatment. Hence in this female, neither the exact lengths of the ovarian silence (efficacy of the treatment) nor the reversibility of the treatment could be determined.

Therefore, these three cases were excluded from the final efficacy and reversebility assessment, and at the end, data generated by 4, 5, 4 and 4 jills in the MPA-, PROL-, $_{sr}$GnRH- and hCG groups, respectively, were evaluated for these parametes and were compared to those of the 5 jill in the untreated control group.

4.3.3. Discussion and conclusions

Efficacy, reversibility and safety of 4 different hormonal treatments for suppression of ovarian activity in female ferrets were compared in this study. All treatments proved useful, but with various durations of efficacy, different degrees of reversibility, and different safety levels.

The two long-acting forms of gestagens (15 mg of MPA and 40 mg of PROL per jill, s.c.) proved efficient to prevent ovarian activity for the same duration, 3 to 5 months

(94±18 and 99±40 days, respectively). These treatments, when given before the breeding season (in mid-February) postpone the first wave of follicular development for several months until ovarian activity recurred due to the stimulating photoperiod in summer. As ferrets are seasonal breeders, the timing of progestagen treatments is important to suppress follicular development for the complete breeding season (from March until August, depending on the climate and weather condition of the country) using a single treatment. Based on results reported by *Oxenham* (*1990*), PROL treatments given in late March (about 6-7 weeks later than in our study) increase the probability that jills will not show oestrus again due to the fact that the end of the period of efficacy coincides with the end of the breeding season. Ferrets kept in an artificial environment (under stimulating photoperiod during the whole year, e.g. in a flat) may however behave as the jills did in our study and may return into heat 3 to 5 months after treatment, implying that an additional treatment would be needed each year for long-term breeding control.

The duration of efficacy of hCG treatment was shorter than that of the two gestagens. Jills resumed ovarian activity 53±9 days post-treatment. hCG is used for induction of ovulation in jills to prevent hyperoestrogenism caused by prolonged oestrus. 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 (698±122 days, about 23±4 months) was detected in the group treated with the srGnRH (4.7 mg/jill). The suppression of ovarian function in this group was significantly longer than that in the other three groups ($p<0.05$). 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. This long-term efficacy is in agreement with a study done with deslorelin treated male ferrets. The authors concluded that this treatment was a suitable alternative method for surgical castration (*Schoemaker et al., 2008a*).

To date, reversibly of hormonal treatments for breeding control in ferrets has not been documented. In our study, fertility of the hCG, MPA and PROL treated females at the first oestrus after treatment was not different from the fertility of control jills. In the control group, a clear difference in breeding success (100% and 60%) was seen between spring and summer breeding, respectively. This difference was to be expected as after the first delivery and nursing, jills have less ability to conceive and nurse kittens in the

second part of the breeding season. Reversibility of fertility after $_{sr}$GnRH 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 of safety, both gestagens treatments caused progressive hormonal alopecia in one case of each group. In the PROL group, this case was not serious and the jill recovered after a few weeks. In contrast, the jill of the MPA group suffered from a concomitant poor body condition and weight loss for several weeks post-treatment. Such alopecia has not been previously mentioned in the literature in this species. Moreover, one MPA treated jill delivered prematurely and showed purulent vaginal discharge and fever after breeding her at the next oestrus post-treatment. Based on these data, PROL proved safer than MPA in this species. Almost all jills (4/5) of the $_{sr}$GnRH group showed light alopecia on the tail 6 moths after treatment (in August). The cause of such alopecia is unknown, but it was harmless and regressed spontaneously without medical treatment.

We can conclude that the four hormonal treatments were efficient medication for oestrus control in ferrets. Based on our results, clear differences were seen in the length of the follicle-suppressive property between the Deslorelin implant (long lasting suppression of about 23 months) and the two synthetic gestagens used (shorter suppression of about 3 to 5 months). With the exception of the $_{sr}$GnRH, all treatments were well reversible and fertility at the first oestrus was not different from that of the control group. Fertility after $_{sr}$GnRH treatment also proved to return to normal, but only at the second oestrus post-treatment. As regards safety of treatment, the Deslorelin implant and hCG were the best and MPA the worst.

4.4. Experience with a non-traditional treatment of hyperoestrogenism in ferrets (*Exp. 4*)

After gonadectomy, ferrets may show elevated E_2 concentrations principally as a result of reactivated ovarian remnants (females) and nodular hyperplasia of the adrenocortex in both genders (i.e. hyperadrenocorticism). Clinical sings of hyperadrenocorticism include a range of cutaneous, reproductive, or behavioral symptoms, all related to the elevated concentrations of sexual steroids. Cutaneous signs are characterized by bilaterally symmetric alopecia beginning over the tail and progressing forward along the body. Sometimes pruritus may occur as well. Reproductive abnormalities (depending on which sex steroid is elevated) may include swelling of the vulva (oestrus) in females, and dysuria in males (*Rosenthal and Peterson, 1996b; Coleman et al., 1998*). Behavioral abnormalities may include increased mounting behavior or aggression in both genders, and marking behavior in males. Longstanding cases may show mild anemia and petechiation (as a result of the suppressive effect of E_2 on the bone marrow), muscle wasting, and other non-specific signs such as lethargy and posterior paresis.

The objective of the study was to determine whether a $_{sr}$GnRH (i.e. Deslorelin implant), has any value in the therapy of hyperoestrogenism of adrenocortical origin. For realistic monitoring we needed reference values, therefore blood samples for E_2 determination were also taken from intact, healthy (untreated control) females.

4.4.1. Material and methods

4.4.1.1. Animals and design

The study was conducted on domestic ferrets (*Mustela putorius furo*) ($n_{control}$=14 and $n_{alopecic\ neutered}$=3; age: 9 months to 4 years; body weight: 800 to 1000g) of mild to moderate body condition, from private veterinary practices in Budapest and Veszprém. Owners gave their permission for the veterinary manipulations (blood sampling and/or treatment) in each case.

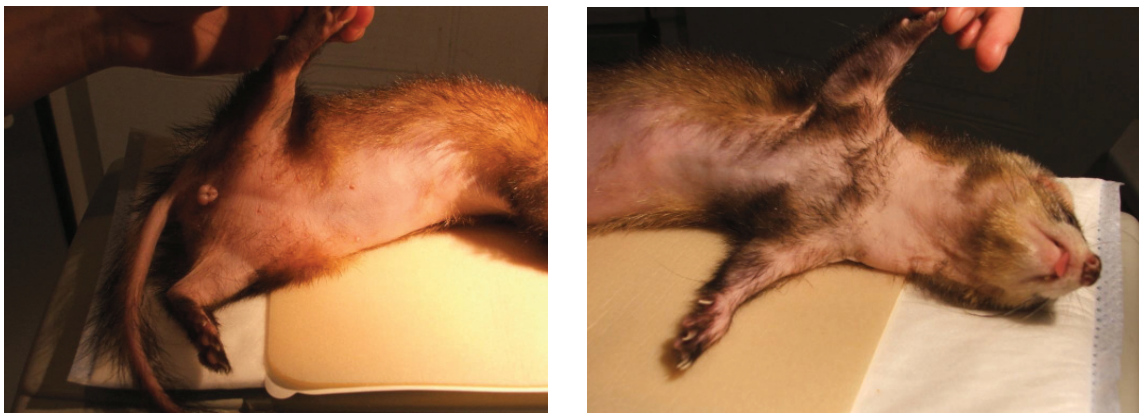
Healthy ferrets (n=14) were used as control animals. These females were hospitalized for ovariectomy, either 3 to 10 days after the beginning of heat (n=5), or 9 to 21 days after hCG induced ovulation (n=6), or out of breeding season (in winter when

ferrets were in anoestrus (n=3). The stage of ovarian function was confirmed by morphological examination of ovaries removed. Blood samples were taken at the beginning of the operative manipulation.

The case history of each neutered ferret (n=3), showing pathognostic signs of endocrine alopecia, is detailed below. Each ferret had been showing clinical sings for 3 (Emmy) to 8 weeks (Pipi and Peti) when blood samples were taken to determine E_2 concentrations. At that time, they were subcutaneously treated with 4.7 mg srGnRH (Deslorelin implant®, Peptech Animal Health, North Ryde, Australia). Blood samples for E_2 determination were again collected approximately one month after the insertion of the implant.

The first ferret (Emmy) was a 4 year old female. She was neutered at the age of one year. After the ovariohysterectomy, she lost the hair on her tail periodically every year and had a swollen vulva every spring. Oestrus signs and hair loss resolved spontaneously every year, but at four years old, the hair loss remained persistent. First, her hair thinned at the base of the tail, inside the legs and around the genitals, than gradually it was lost over most of the body (*Figure 20*). Body condition was very bad despite her good appetite. Later, she lost her appetite and became apathetic. As a first step, she was treated with 150 IU hCG three times, seven days apart to exclude ORS. Despite this hormonal treatment, oestrus signs did not disappear and the clinical signs did not change.

Figure 20: One female ferret (Emmy) with clinical signs of hypero-estrogenism (her hair thinned at the base of the tail, inside the legs and around the genitals, than gradually it was lost over most of the body) before Deslorelin implant insertion treatment (*Exp. 4*) (photo by *A. Proháczik*)



The second ferret (Pipi) was a three year old female. She was neutered during her pregnancy when she was two years old. Next spring after the ovariohysterectomy, she

had developed a swollen vulva and began losing the hair on her tail and then on the dorsal side of her neck (*Figure 21*). This hair loss was dramatic. She had pruritus, her body condition was very bad, and she had no appetite. At first, it was suggested that she had ORS. Exploratory laparoscopy was done, but no hormone producing ovarian remnant could be seen in the abdominal cavity. A suitable volume of blood for E_2 determination could not be collected due to her bad body condition.

The third ferret (Peti) was a four year old male. Three years after his castration, typical endocrine alopecia was observed on the dorsal side of the neck, on the tail and on the fingers (*Figure 22*). His appetite and body condition were quite good. No weight loss was detected. Neither ectoparasite nor fungal infections were found. According to the case history and clinical signs, the suggested diagnosis was hyperadrenocorticism in each ferret with hormonal alopecia.

Figure 21: One female ferret (Pipi) with clinical signs of hyperoestrogenism (she had swollen vulva and began losing the hair on her tail) before Deslorelin implant insertion (*Exp. 4*) (photo by *A. Proháczik*)

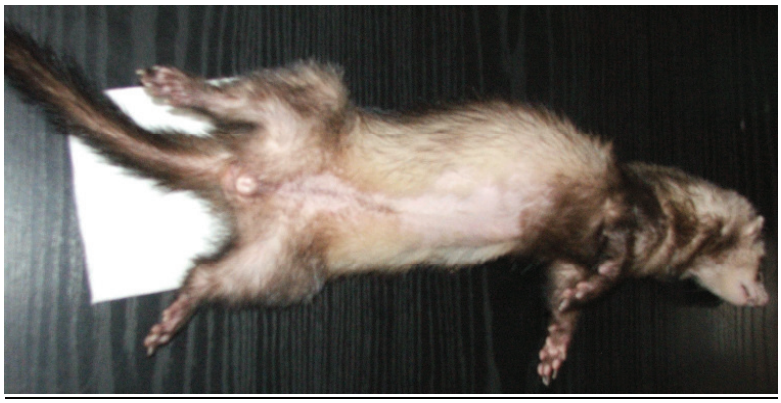
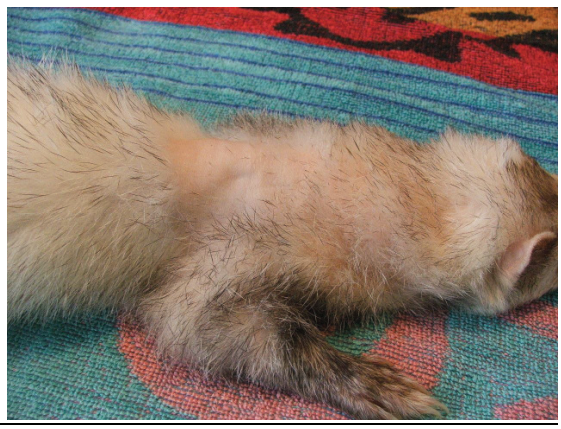


Figure 22: One male ferret (Peti) with typical endocrine alopecia on the dorsal side of the neck, on the tail and on the fingers before Deslorelin implant insertion (*Exp. 4*) (photo by *A. Proháczik*)



4.4.1.2. Handling of blood samples. Statistical procedures

Blood samples (2 mL) from each ferret were taken from the jugular vein into heparinized tubes for E_2 determination.

For the presentation of results the group means and their standard errors (SEM) were expressed. The results were subjected to Student's t-test (for comparison of group means between healthy control females in oestrus and alopecic ferrets before Deslorelin implant insertion, and between healthy control females in anoestrus / luteal phase and previously alopecic ferrets after Deslorelin implant insertion and between ferrets before and after Deslorelin implant insertion), or a single trait analysis of variance (ANOVA; for comparison of 3 group means in healthy control females). If ANOVA revealed significant differences, the least significant differences were planned to be calculated at 5% ($LSD_{P<0.05}$) for further comparison. Bonferroni's t-test was used in all pairwise multiple comparison procedures.

4.4.2. Results

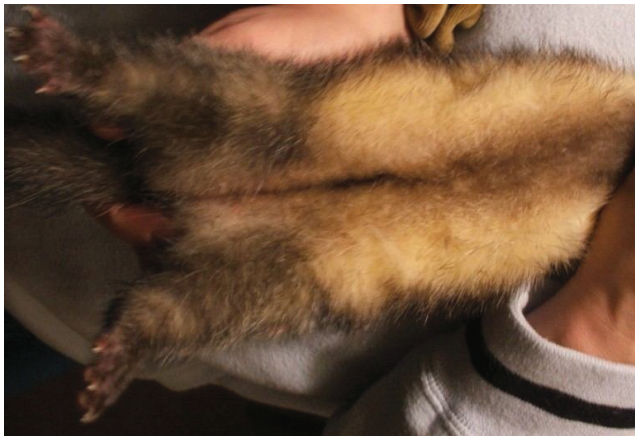
The morphological examination of ovaries from healthy ferrets showed the expected physiological characteristics. Ovaries of females in heat (n=5) contained 3 to 9 tertiary follicles, whilst those of ferrets treated with hCG had 2 to 10 well developed corpora lutea. Animals, spayed in anoestrus, did not have any follicle- or CL-like structures on their ovaries. Control females in oestrus had significantly higher E_2 concentrations than control females out of the breeding season or in the luteal phase ($p=< 0.001$).

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 (*Figure 23*). 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.

Figure 23: One female ferret (Pipi) 2 months after Deslorelin implant insertion (hair growth had resumed, good appetite had returned, and body condition had improved) (*Exp. 4*) (photo by *A. Proháczik*)



4.4.3. Discussion and conclusions

In this practice-oriented study, 14 healthy, untreated control females at different stages of the ovarian cycle and 3 neutered ferrets (n=2 females and n=1 male) with suggested hyperadrenocorticism were included. All three neutered ferrets showed clinical signs of hormonal disorder (typical alopecia and swollen vulva in females). One female (Emmy) had periodically occurring alopecia for four years before her hair loss became permanent. The possible explanation of this phenomenon is that alopecia and other clinical signs of adrenocortical diseases may remit during autumn and winter in response to the decline in LH associated with the shortening photoperiod (*Fox and Marini, 1998*). However, at an advanced stage, the seasonal disappearance of the clinical signs stops and they become permanent (*Rosenthal et al., 1993*).

In our study, based on the clinical signs (oestrus in females and hormonal alopecia in both genders), plasma E_2 concentrations were measured. ORS in the females was excluded based on the unsuccessful hCG treatments for induction of ovulation and exploratory laparoscopy for detecting retained ovarian tissue. The diagnosis therefore was hyperadrenocorticism in both females. In the castrated male, after excluding ectoparasite or fungal infections, the diagnosis was also hyperadrenocorticism.

In our study, elevated plasma E_2 concentrations similar to the healthy control females in oestrus were measured in each sampled ferrets with hormonal alopecia. The

assay for E_2 determination was validated for ferrets in the author's laboratory and the normal range of E_2 in 14 healthy (untreated control) ferrets was established.

Recently deslorelin was used as a long-lasting treatment of hyperadrenocorticism in ferrets (*Wagner et al., 2005*). Deslorelin is used more and more commonly in the reproductive management and veterinary praxis. It was showed that deslorelin reversibly suppressed reproductive function in several species (e.g. male and female dogs, female eastern grey kangaroos, female cats and ferrets) for extended periods (*Munson et al., 2001; Gobello, 2006; Herbert et al, 2006; Schoemaker et al., 2008a*). Moreover, seeing that such treatment caused long-term reduction of circulating FSH and LH concentrations to very low or undetectable levels, it was successfully used in the treatment of urinary incontinence in neutered female dogs (*Reichler et al., 2003*). References of studies using deslorelin treatment are also available in farm animals for ovulation induction, ovarian function suppression etc. (*Kraeling et al., 2000; Silvestre et al., 2009*). *Delbecchi and Lacasse* (*2006*) showed that such treatment could temporarily suppress the return of ovarian cycles in cows. This study described that deslorelin significantly reduced serum concentrations of E_2 and P_4 as compared with untreated cows.

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 ≥ 1.5 years, the three ferrets with clinical signs of hyperadrenocorticism included in this study were subcutaneously treated with a Deslorelin implant (4.7 mg per animal, irrespective of body weight). Our results indicate that such treatment was able to alleviate clinical signs and elevated E_2 concentrations in the affected animals for a long period (>19 months).

In summary, decreasing E_2 levels and improving clinical signs after Deslorelin implant insertion prove that E_2 was the cause of hair loss in neutered ferrets with hormonal alopecia. E_2 was probably produced by the adrenal gland. The secretion of sex steroids in ferrets (particularly E_2 production) was efficiently suppressed by 4.7 mg deslorelin for >19 months.

4.5. Metabolic and endocrine characteristics of pregnancy toxemia in the ferret (*Exp. 5*)

Pregnancy toxemia caused by negative energy balance in late gestation is commonly observed in ewes and nanny goat (*Henze et al., 1998; Rook, 2000; Van Saun, 2000; Kulcsár et al, 2006b*), occasionally in beef cows (*Rook, 2000*), and also in monogastric species (rabbits, guinea pigs, dogs and in ferrets; *Bell, 2004; Pare and Murphy, 1997; O'Rourke, 1997; Batchelder et al., 1999; Dalrymple, 2004; Lewington, 2007a*). The background of the disease is the result of fetal carbohydrate- or energy-demand exceeding maternal supply during the last trimester of pregnancy. In the ferret, pregnancy toxemia usually occurs between days 32 and 42 of gestation, especially just before the whelping date. It is more common in primiparous female ferrets carrying >10 kittens (*Batchelder et al., 1999; Lewington, 2007a*) or among females carrying average litters and fed adequate diets, but where an accidental fast occurs during this period (*Bell, 2004*).

Negative energy balance increases lipid mobilization, which results in hepatic lipidosis with subsequent impairment of hepatocellular function, glucose deficiency with intermittent hypoglycemia and accumulation of ketone bodies. The shift of energy metabolism in a catabolic direction is characterized by wide range of endocrine changes, such as insufficient pancreatic β-cell function with a coinciding increase in insulin resistance, impairments in growth hormone - insulin-like growth factor-1 (IGF-1) axis, and increased peripheral inactivation of thyroid hormones. As a result, low levels of insulin, IGF-1, leptin, T_4, and T_3 are measure in the blood. These endocrine consequences are clearly demonstrated in peri-parturient and postpartum dairy cows (*Pethes et al., 1985; Sartin et al., 1988; McGuire et al., 1991; Harmon, 1992; Bauman, 2000; Kadokawa et al., 2000; Kahl et al, 2000; Meikle et al., 2004; Balogh et al., 2008; Bossaert et al., 2008; Lucy, 2008*), and during the late gestation also in twin-pregnant, hyperketonemic ewes (*Henze et al., 1998; Van Saun, 2000; Kulcsár et al., 2006b*).

Whilst the pathology and clinical biochemistry of pregnancy toxemia are known in ferrets, our understanding of the endocrine background is still incomplete. We were interested in whether endocrine changes described in the pregnancy toxemia of ewes are present in the ferret. The objective of the current study was to measure the levels of glucose, ketone bodies (BHB) and three hormones (insulin, T_4 and T_3) regulating the

energy homeostasis in blood from sick females, and to compare these parameters to those of healthy, non-pregnant female ferrets (control).

4.5.1. Material and methods

4.5.1.1. Animals, study design and sampling

The study was conducted on female ferrets (*Mustela putorius furo*) (n=18; age: 9 to 35 months; body weight: 800 to 1000g) of mild to moderate body condition, from private veterinary practices in Budapest. Owners gave their permission for blood sampling and necropsy in all cases.

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 estimated (Medi-Test Combi-9 strips, Macherey-Nagel, Duren, Germany).

Healthy female ferrets (n=14) were used as control animals. These ferrets were hospitalized for ovariectomy, either 3 to 10 days after the beginning of heat (n=5), or 9 to 21 days after, with hCG inducted ovulation (n=6), or out of breeding season (in winter when females were in anoestrus; n=3). The stage of ovarian function was confirmed by morphological examination of ovaries removed. Blood samples were taken at the beginning of the operative manipulation.

Blood samples (2 mL) from each female were taken from the jugular vein into heparinized and fluoride-containing tubes. Samples were cooled and centrifuged within 3 hours. Plasma was harvested immediately, and stored at either +4 ^{o}C (≤48h) until BHB and glucose determinations or at -20 ^{o}C until insulin, T_4 and T_3 measurements.

4.5.1.2. Statistics

For presentation of results the group means and their SEMs were calculated. The results were subjected to Student's t test (pair-wise comparison of group means), or a single trait analysis of variance (ANOVA; for comparison of 3 group means in healthy

controls). If ANOVA revealed significant differences, the least significant differences were planned to be calculated at 5% ($LSD_{P< 0.05}$) for further comparison.

4.5.2. Results

Necropsy confirmed the suspected diagnosis of pregnancy toxemia: 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. The morphological examination of ovaries from healthy ferrets showed the expected physiological characteristics. Ovaries of females in heat (n=5) contained 3 to 9 tertiary follicles, whilst those of ferrets treated with hCG had 2 to 10 well developed CLs. Animals, spayed in anoestrus, did not have any follicle- or CL-like tissue on their ovaries.

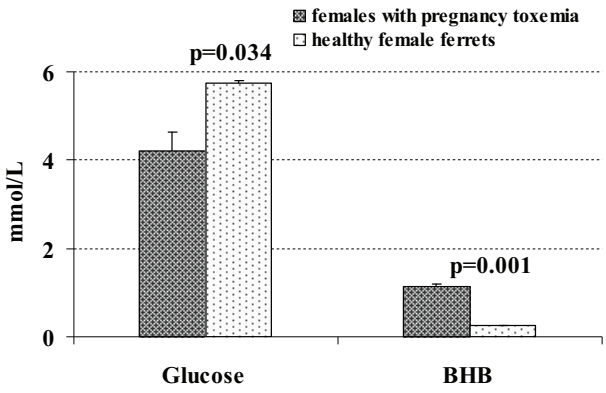


Figure 24 The plasma glucose and ketone (βOH-butyrate, BHB) levels in late pregnant jills suffering from pregnancy toxemia (n=4) and from healthy non-pregnant (control) females (n=14) (mean ± SEM)

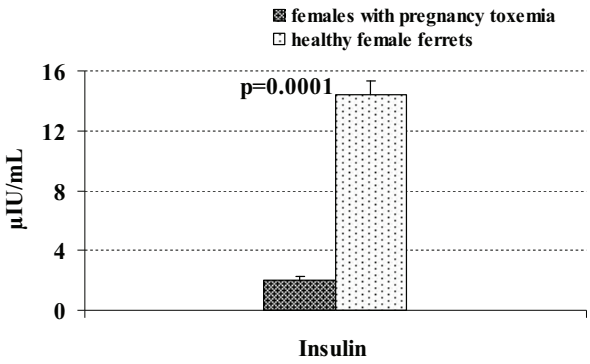
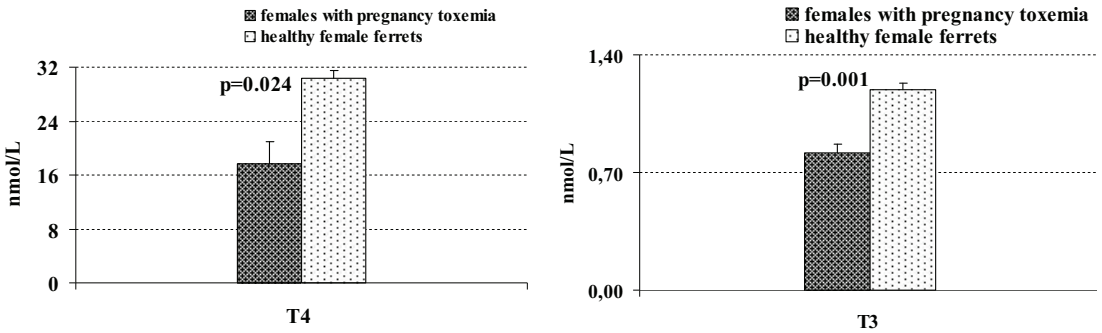


Figure 25 The plasma insulin, T_4 and T_3 levels in late pregnant ferrets suffering from pregnancy toxemia (n=4) and from healthy non-pregnant (control) female ferrets (n=14) (mean ± SEM)



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; *Figure 24*). Significant differences were found also in the endocrine parameters (*Figure 25*): 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). There were no differences among the three sub-groups of controls (data not shown).

4.5.3. Discussion and conclusions

In this study, the endocrine characteristics of ketonemia in ferrets with pregnancy toxemia were studied. Plasma glucose, BHB, insulin, T_4 and T_3 levels in sick animals 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. These tendencies were comparable to those of ruminant ketonemia in late pregnancy. Although, our results generally correlated with reference ranges found in the literature (*Fox, 1998a; Garibaldi et al., 1988*), variability was seen, probably due to the several different technical procedures among laboratories.

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.

During pregnancy, fetuses have a large glucose demand that is satisfied by the mother. If the fetal demand and the mother supply become imbalanced due to fasting of the mother or increased nutritional demands of the rapidly developing fetal placental

unit, females suffer from negative energy balance and succumb to severe hypoglycemia (*Batchelder et al., 1999; Dalrymple, 2004*). In our sick animals, the blood glucose concentration was lower than the normal fasting blood glucose level although the differences between the sick and healthy animals only just reached the significance level. This was most likely due to the terminal stage of the sick animals. In this disease, initially, glucose concentration is low, but in terminal stage, it may rise even above normal levels (*Fox et al., 1998*).

During the decompensation of the negative energy balance (fat mobilization), liver function may be overwhelmed and hepatic lipidosis may develop. The impaired function of the liver together with enhanced mobilization of free fat acids from triglycerides causes ketogenesis. Consequently, the level of ketones increases in the blood. This phenomenon was observed previously (*Lewington, 2007a*), and was also confirmed in our study. In female ferrets with pregnancy toxemia, the plasma level of this parameter (BHB) related to the energy metabolism was elevated (1.13 ± 0.07 mmol/L) compared to the control group (0.25 ± 0.02 mmol/L). Moreover, in the sick female ferrets, hepatic lipidosis was observed post-mortem.

The response to fasting (negative energy balance) incorporates hormonal signals which initiate energy preservation. Insulin, T_4 and T_3 are important hormones in the regulation of energy homeostasis. Our data in ferrets were pro-rata similar to the ewes with pregnancy toxemia (*Kulcsár et al., 2006b*) in that hypoinsulinemia and decrease in the T_4 and T_3 concentrations were detected. In ferrets, hypoinsulinemia may be linked to the lower than normal secretory capacity of pancreatic β-cell function in the same way as in ruminants (*Harmon, 1992; Henze et al., 1998; Van Saun, 2000*). In pregnant ewes, the therapeutic effect of insulin treatment demonstrated that insulin plays a causative role in the pathogenesis of ovine ketosis (*Henze et al., 1998*). Based on this observation, the survival rate of the affected ferrets could probably be increased with the inclusion of insulin treatment in therapeutic protocol.

The peri-parturient changes of thyroid hormones and further, their involvement in the metabolic adaptation to negative energy balance and in the pathogenesis of ketosis have been intensely studied in ruminants (*Pethes et al., 1985; Meikle et al., 2004; Huszenicza et al., 2006*). In these studies, decreases in thyroid hormone levels have been described and explained by the increased degree of their inactivation in peripheral tissues, and/or alternatively by the decreased capacity of T_4 activation, diminishing the

transformation of T_4 to T_3. Decrease in the T_4 and T_3 concentrations was also detected in our ferrets with pregnancy toxemia.

To summarize the results, 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.

5. 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.

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

- Female ferrets displaying **oestrus** during 9±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 lutal 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_4-met concentrations can be detected. The source of the elevated fecal P_4-met excretion is the ovary; the length of this fecal P_4-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 detrimental 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*).

5.2. 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).*

5.3. Endocrine diagnostic importance

An ELISA system was validated for quantitative determination of fecal P_4-met content providing an available, non-invasive diagnostic tool for the ferret health- and reproduction management (*Exp.1).*

5.4. Therapeutic importance

- Subcutaneous Deslorelin implant (4.7 mg deslorelin acetate per animal) suppresses oestrus and cyclic ovarian function for a longer 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_2 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).

References

Agu G.O., Rajkumar K., Murphy B.D. (1986): Evidence for the dopaminergic regulation and a luteotropic complex in the ferret. Biol. Reprod. 35, 508-515.

Amstislavsky S., Terovskaya Y. (2000): Reproduction in mustelids. Anim. Reprod. Sci. 60-61, 571-581.

Amstislavsky S., Aalto J., Järvinen M., Lindeberg H., Valtonen M., Zudova G., Ternovskaya Y. (2004): Transfer of European mink (*Mustela lutreola*) embryos into hybrid recipients. Theriogenology. 62, 458-467.

Amstislavsky S, Lindeberg H, Ternovskaya Y, Zavjalov E, Zudova G, Klochkov D, Gerlinskaya L. (2009): Reproduction in the European mink (*Mustela lutreola*): oestrous cyclicity and early pregnancy. Reprod Domest Anim. 44, 489-498.

Bakker J., Baum MJ. (2000a): Neuroendocrine regulation of GnRH release in induced ovulators. Front. Neuroendocrinol. 21, 220-262.

Bakker J., Baum M.J. (2000b): Effect of gonadal steroids on pituitary LH secretion and mediobasal hypothalamic GnRH mRNA in ferrets. J. Reprod. Fertil. 119, 315-321.

Bakker J., Kelliher K.R., Baum M.J. (2001): Mating induces GnRH neuronal activity in anosmic female ferrets. Biol. Reprod. 64, 1100-1105.

Ball R. S. (2006): Issues to Consider for Preparing Ferrets as Research Subjects in the Laboratory. Intitute for Laboratory Animal Research Journal. 47, 348-357.

Balogh O., Szepes O., Kovacs K., Kulcsar M., Reiczigel J., Alcazar J. A., Keresztes M., Febel H., Bartyik J., Fekete S., Fesus L., Huszenicza Gy. (2008): Interrelationships of growth hormone *Alu*-I polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows. Vet. Med. Cz. 53, 604-616.

Batchelder M.A., Bell J.A., Erdman S.E., Marini R.P., Murphy J.C., Fox J.G. (1999): Pregnancy toxemia in the European ferret (*Mustela putorius furo*). Lab. Anim. Sci. 49, 372–379.

Bateman H.L., Bond J.B., Campbell M., Barrie M., Riggs G., Snyder B., Swanson W.F. (2009): Characterization of basal seminal traits and reproductive endocrine profiles in North American river otters and Asian small-clawed otters. Zoo Biology 28, 107-126.

Baum M.J., Lynch H.J., Gallagher C.A., Deng M.H. (1986): Plasma and pineal melatonin levels in female ferrets housed under long or short photoperiods. Biol Reprod. 34, 96-100.

Bauman D.E. (2000): Regulation of nutrient partitioning during lactation: homeostasis and homeorhesis revised. In: Cronje P.J. (ed.): Ruminant physiology: digestion, metabolism and growth and reproduction. CAB Publishing, New York. pp.311-327.

Behrend E.N., Weigand C.M., Whitley E.M., Refsal K.R., Young D.W., Kemppainen R.J. (2005): Corticosterone and aldosterone-secreting adrenocortical tumor in a dog. J. Am. Vet. Med. Assoc. 226, 1659, 1662–1666.

Bell J.A. (1999): Ferret nutrition. Vet. Clin. North Am. Exot. Anim. Pract. 2, 169–192.

Bell J.A. (2004): Periparturient and neonatal diseases. In: Quesenberry K.E. and Carpenter J.W. (eds.): Ferrets, Rabbits, and Rodents. Clinical medicine and surgery. 2nd revised ed. USA, W. B. Saunders Company, pp 51-67.

Bennett R.A., Pye G.W. (2007): General surgery. In: Lewington, J.H. (ed.): Ferret husbandry, medicine and surgery. Saunders Elsevier, Edinburgh, 2nd ed. pp. 430-439.

Bernard S.L., Leathers C.W., Brobst D.F., Gorham J.R. (1983): Estrogen induced bone marrow depression in ferrets. Am. J. Vet. Res. 44, 657-661.

Bertschinger H. J., Asa C.S., Calle P.P., Long J.A., Bauman K., DeMatteo K., Jöchle W., Trigg T.E., Human A. (2001): Control of reproduction and sex related behavior in exotic wild carnivores with the GnRH analogue deslorelin: preliminary observations. J. Reprod. Fert. Suppl. 57, 275-283.

Bertschinger H. J., Trigg T.E., Jöchle W., Human A. (2002): Induction of contraception in some African wild carnivores by downregutation of LH and FSH secretion using GnRH analogue deslorelin. Reprod. Suppl. 60, 41-52.

Bielinska M., Kiiveri S., Parviainen H., Mannisto S., Heikinheimo M., Wilson D.B. (2006): Gonadectomy-induced adrenocortical neoplasia in the domestic ferret (*Mustela putorius furo*) and laboratory mouse. Vet Pathol, 43, 97-117.

Bissonnette T. H. (1932): Modification of mammalian sexual cycles; reactions of ferrets (*Putorius vulgaris*) of both sexes to electric light added after dark in November and December. Proc. Roy. Soc. Lond. 110, 322-336.

Blatchley F.R, Donovan B.T. (1976): Progesterone secretion during pregnancy and pseudopregnancy in the ferret. J. Reprod. Fertil. 46, 455-456.

Bossaert P., Leroy J.L.M.R., de Vliegher S., Opsomer G. (2008): Interrelation between glucose-induced insulin response, metabolic indicators, and the time of first ovulation in high-yielding dairy cows. J. Dairy Sci. 91, 3363-3371.

Bravo P.W., Stabenfeldt G.H., Lasley B.L., Fowler M.E. (1991): The effect of ovarian follicle size on pituitary and ovarian responses to copulation in domesticated South American camelids. Biol Reprod. 45, 553-559.

Brown J. L. (1997): Fecal steroid profiles in black-footed ferrets exposed to natural photoperiod. J. Wildl. Manage. 61, 1428-1436.

Carroll R.S., Erskine M.S., Doherty P.C., Lundell L.A., Baum M.J. (1985): Coital stimuli controlling luteinizing hormone secretion and ovulation in the female ferret. Biol. Reprod. 32, 925-933.

Carroll R.S., Erskine M.S., Baum M.J. (1987): Sex difference in the effect of mating on the pulsatile secretion of luteinizing hormone in a reflex ovulator, the ferret. Endocrinology, 121, 1349-1359.

Carroll R.S., Baum M.J. (1989): Evidence that oestrogen exerts an equivalent negative feedback action on LH secretion in male and female ferrets. J. Reprod. Fertil. 86, 235–245.

Carter D.S., Herbert J., Stacey P.M. (1982): Modulation of gonadal activity by timed injections of melatonin in pinealectomized or intact ferrets kept under two photoperiods. J. Endocrinol. 93, 211-222.

Cavestany, D., Kulcsár, M., Crespi, D., Chilliard, Y., La Manna, A., Balogh, O., Keresztes, M., Delavaud, C., Huszenicza, Gy., Meikle, A. (2009): Effect of prepartum energetic supplementation on production and reproductive characteristics, and metabolic and hormonal profiles in dairy cows under grazing conditions. Reprod. Domest. Anim., 44. 663-671.

Chemineau P., Guillaume D., Migaud M., Thiéry J.C., Pellicer-Rubio M.T., Malpaux B. (2008): Seasonality of reproduction in Mammals: intimate regulatory mechanisms and practical implications. Reprod. Domest. Anim., 43 (Suppl. 2), 40-47.

Church B. (2007): Ferret-polecat domestication: genetic, taxonomic and phylogenetic relationship. In: Lewington, J.H. (ed.): Ferret husbandry, medicine and surgery. Saunders Elsevier, Edinburgh, 2nd edition. pp. 122-150.

Coleman G.D., Chavez M.A., Williams B.H. (1998): Cystic prostatic disease associated with adrenocortical lesions in the ferret (*Mustela putorius furo*). Vet. Pathol. 35, 547-549.

Crawford R.L., Jensen D., Allen T. (2002): Information resources for ferrets. September 1991-July 2002. Animal Welfare Information Center Resource Series No. 15. Beltsville MD: USDA, NAL.

Dalrymple E.F. (2004): Pregnancy toxemia in a ferret. Can. Vet. J. 45, 150-152.

Daniel J.C.Jr. (1970): Coincidence of embryonic growth and uterine protein in the ferret. J. Embryol. exp. Morphol. 24, 305-312.

Daniel J.C.Jr. (1976): Plasma progesterone levels before and at the time of implantation in the ferret. J. Reprod. Fertil. 48, 437-438.

Dawson A. B., Friedgood H. B. (1940): The time and sequence of preovulatory changes in the cat ovary after mating or mechanical stimulation of the cervix uteri. Anat. Rec. 76, 411–429.

DeClue A.E., Breshears L.A., Pardo I.D., Kerl M.E., Perlis J., Cohn L.A. (2005): Hyperaldosteronism and hyperprogesteronism in a cat with an adrenal cortical carcinoma. J. Vet. Intern. Med. 19, 355–358.

Dehnhard M., Naidenko S., Frank A., Braun B., Göritz F., Jewgenow K. (2008): Non-invasive monitoring of hormones: a tool to improve reproduction in captive breeding of the eurasian lynx. Reprod. Domest. Anim. 43 (Suppl. 2), 74-82.

Delbecchi L., Lacasse P. (2006): Suppression of estrous cycles in lactating cows has no effect on milk production. J. Dairy Sci. 89, 636-639.

DeNardo G.A., Becker K., Brown N.O., Dobbins S. (2001): Ovarian remnant syndrome: revascularization of free-floating ovarian tissue in the feline abdominal cavity. J. Am. Anim. Hosp. Assoc. 37, 290-296.

Desmarchelier M., Lair S., Dunn M., Langlois I. (2008): Primary hyperaldosteronism in a domestic ferret with an adrenocortical adenoma. J. Am. Vet. Med. Assoc. 233, 1297-1301.

De Wit M., Schoemaker N.J., van der Hage M.H., Afonso P.M., Kirpensteijn J. (2001): Signs of estrus in an ovariectomized ferret. Tijdschr. Diergeneeskd. 126, 526-528.

D'Hooghe T.M., Bambra C.S., Raeymaekers B.M., Koninckx P.R. (1996): Increased incidence and recurrence of recent corpus luteum without ovulation stigma

(luteinized unruptured follicle syndrome?) in baboons with endometriosis. J. Soc. Gynecol. Investig. 3, 140–144.

D'Occhio M.J., Fordyce G., Whyte T.R., Jubb T.F., Fitzpatrick L.A., Cooper N.J., Aspden W.J., Bolam M.J., Trigg T.E. (2002): Use of GnRH agonist implants for long-term suppression of fertility in extensively managed heifers and cows. Anim. Reprod. Sci. 74, 151-162

Donovan B.T. (1986): Is there a critical weight for oestrus in the ferret? J. Reprod. Fertil. 76, 491-497.

Douglas D.A., Pierson R.A., Murphy B.G. (1994): Ovarian follicular development in mink (*Mustela vison*). J. Reprod. Fertil. 100, 583-590.

Duffy D.M., Stouffer R.L. (2002): Follicular administration of a cyclooxygenase inhibitor can prevent oocyte release without alteration of normal luteal function in rhesus monkeys. Hum. Reprod. 17, 2825-2831.

Elofson L., Lagerkvist G., Gustafsson H., Einarsson S. (1989): Mating system and reproduction in mink. Acta Agric. Scand. 39, 23-41.

Enders A.C., Schlafke S. (1972): Implantation in the ferret: epithelial penetration. Am. J. Anat. 133, 291-315.

Faigl V., Keresztes M., Márton A., Schneider Z., Korvin L., Nagy S., Novotniné Dankó G., Árnyasi M., Kulcsár M., Jávor A., Cseh S., Huszenicza Gy. (2007): Melatonin- illetve fénykiegészítés-alapú ciklusindukciós technikák kiskérődzőkben: élettani vonatkozások és gyakorlati alkalmazás. Irodalmi áttekintés. Magyar Állatorvosok Lapja 129, 219-230.

Felberbaum R., Diedrich K. (1999): Ovarian stimulation for in vitro fertilization/intracytoplasmic sperm injection with gonadotropins and gonadotropin-releasing hormone analogues: agonist and antagonists. Hum. Reprod., 14 (Suppl. 1), 207-211.

Forsberg M. (1992): Seasonal breeding and the significance of carnivorous fur bearing animals. Nordiska Jordbruksforskares Forening, Rapport Nr. 75, Jordbrugsforlaget, Nordisk Kulturfond, Copenhagen, pp. 17-38.

Fox J.G. (1998a): Normal clinical and biological parameters. In: Fox J.G. (ed.) Biology and Diseases of the Ferret. 2nd ed. Williams and Wilkins, Philadelphia, pp. 183–210.

Fox J.G. (1998b): Taxonomy, history, and use. In: Fox J.G. (ed.): Biology and Diseases of the Ferret. 2nd ed. Williams and Wilkins, Philadelphia, pp. 3-18.

Fox J.G., Bell J.A. (1998): Growth, reproduction and breeding. In: Fox JG (ed.): Biology and diseases of the ferret, 2nd 2nd ed. Williams and Wilkins, Philadelphia, pp. 211-227.

Fox J.G., Marini R.P. (1998): Disease of the endocrine system. In: Fox J.G. (ed.): Biology and diseases of the ferret. 2nd ed. Williams and Wilkins, Philadelphia, p. 294.

Fox J.G., McLain D.E. (1998): Nutrition. In: Fox J.G. (ed.): Biology and diseases of the ferret. 2nd ed. Williams and Wilkins, Philadelphia, pp. 151–172.

Fox J.G., Pearson R.C., Bell J.A. (1998): Disease of the genito-urinary system. In: Fox J.G. (ed.): Biology and diseases of the ferret. 2nd ed. Williams and Wilkins, Philadelphia, pp. 247-272.

Gad S.C. (2000): Pigs and ferrets as models in toxicology and biological safety assessment. Int. J. Toxicol. 19, 149-168.

Garibaldi B.A., Pecquet Goad M.E., Fox J.G., Murray R. (1988): Serum thyroxine and triiodothyronine radioimmunoassay values in the normal ferret. Lab. Anim. Sci. 38, 455-458.

Gobello C. (2006): Dopamine agonists, anti-progestins, anti-androgens, long-term-release GnRH agonists and anti-estrogens in canine reproduction: a review. Theriogenology 66, 1560-1567.

Göritz F., Dehnhard M., Hildebrandt T.B., Naidenko S.V., Vargas A., Martinez F., López-Bao J.V., Palomares F., Jewgonow K. (2009): Non cat-like ovarian cycle in the Eurasian and the Iberian lynx – ultrasonographical and endocrinological analyses. Reprod. Domest. Anim., 44 (Suppl. 2), 87-91.

Gudermuth D.F., Newton L., Daels P., Concannon P. (1997): Incidence of spontaneous ovulation in young, group-housed cats based on serum and fecal concentrations of progesterone. J. Reprod. Fertil. Suppl. 51, 177–184.

Hall J.A., Meisterling E.M., Benoit A.M., Cooper D.A., Coleman D.A., Lerner S.P., Lewis P.E., Dailey R.A. (1993): Factors contributing to the formation of experimentally-induced ovarian cysts in prepubertal gilts. Domest. Anim. Endocrinol. 10, 141–155.

Hammond J., Marshall F.H.A. (1930): Oestrus and pseudopregnancy in the ferret. Proceedings of the Royal Society of London. Series B, 105, 607-630.

Harcourt-Brown F. (2002): Textbook of rabbit medicine. Oxford: Butterworth-Heinemann, p 57.

Harmon D.L. (1992): Impact of nutrition on pancreatic exocrine and endocrine secretion in ruminants: a review. J. Anim. Sci. 70, 1290-1301.

Hart J.E. (1990): Endocrine pathology of estrogens: species differences. Pharmacol. Ther. 47, 203-18.

Heap R.B., Hammond J.Jr. (1974): Plasma progesterone levels in pregnant and pseudopregnant ferrets. J. Reprod. Fertil. 39; 149-152.

Heffelfinger D.J. (2006): Ovarian remnant in a 2-year-old queen. Can. Vet. J. 47, 165-167.

Henze P., Bickhardt K., Fuhrmann H., Sallmann H.P. (1998): Spontaneous pregnancy toxaemia (ketosis) in sheep and the role of insulin. Zentralbl. Veterinarmed. A. 45, 255-266.

Herbert J. (1969): The pineal gland and light induced oestrus in ferrets. J. Endocrinol. 43, 625–636.

Herbert J. (1972): Initial observations on pinealectomized ferrets kept for long periods in either daylight or artificial illumination. J. Endocrinol. 55, 591–597.

Herbert J. (1989): Light as a multiple control system on reproduction in mustelids. In: Seal, U.S., Thorne, E.T., Bogan, M.A., Anderson, S.H. (eds), Conservation

biology and the black-footed ferret. Yale University Press, New Haven, pp. 138-159.

Herbert J., Stacey P.M., Thorpe D.H. (1978): Recurrent breeding seasons in pinealectomized or optic-nerve-sectioned ferrets. J. Endocrinol. 78, 389-397.

Herbert C.A., Trigg T.E. (2005): Applications of GnRH in the control and management of fertility in female animals. Anim. Reprod. Sci. 88, 141-153.

Herbert C.A., Trigg T.E., Cooper D.W. (2006): Fertility control in female eastern grey kangaroos using the GnRH agonist deslorelin. 1. Effects on reproduction. Wildlife Research 33, 41-46.

Hillyer E.V. (2004): Urogenital Disease. In: Hillery E.V., Quesenberry K.E.: Ferrets, Rabbits and Rodents Clinical medicine and surgery. W.B. Saunders Company, United States of America, pp. 50-51.

Howard J.W. (1979): Control of estrus in ferrets. Vet. Rec. 104, 291.

Huszenicza Gy., Kulcsar M., Korodi P., Bartyik J., Rudas P, Ribiczei-Szabo P., Nikolie J.A., Samanc H., Ivanov I., Gvozdie D. (2006): Adrenocortical and thyroid function, hormone and metabolite profiles and the onset of ovarian cyclicity in dairy cows suffering from various forms of ketosis. Acta Veterinaria (Beograde). 56, 25-36.

Jallageas M., Boissin J., Mas M. (1994a): Differential photoperiodic control of seasonal variations in pulsatile luteinizing hormone release in long-day (ferret) and short-day (mink) mammals. J. Biol. Rhythms. 9, 217-231.

Jallageas M., Mas N., Boissin J., Maurel D., Ixart G. (1994b): Seasonal variations of pulsatile luteinizing hormone release in the mink (*Mustela vison*). Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 109, 9-20.

Jekl V., Hauptman K., Jeklová E., Dorrestein G.M., Knotek Z. (2006): Hydrometra in a ferret – A case report. Vet. Clin. North Am. Exot Anim. Pract. 9, 695-700.

Jewgenow K., Göricz F., Vargas A., Dehnhard M. (2009): Seasonal profiles of ovarian activity in Iberian lynx (*Lynx pardinus*) based on urinary hormone metabolite analysis. Reprod. Domest. Anim., accepted for publication

Joseph M.M., Mead R.A. (1988): Size distribution of ferret luteal cells during pegnancy. Biol. Reprod. 39, 1159-1169.

Juvancz I., Paksy A. (1982): Medical biometry (In Hungarian). Medicina Kiadó, Budapest

Kadokawa H., Blache D., Yamada Y., Martin G.B. (2000): Relationships between changes in plasma concentrations of leptin before and after parturition and the timing of first post-partum ovulation in high-producing Holstein dairy cows. Reprod. Fertil. Dev. 12, 405-411.

Kahl S., Elsasser T.H., Blum J.W. (2000): Effect of endotoxin challenge on hepatic 5'-deiodinase activity in cattle. Domest. Anim. Endocrinol. 18, 133-143.

Kintner P.J., Mead R.A. (1983): Steroid metabolism in the corpus luteum of the ferret. Biol. Reprod. 29, 1121-1127.

Kluth G.A. (1993): Oestrus control in ferrets. Vet. Rec. 133, 252.

Kociba G.J., Caputo C.A. (1981): Aplastic anemia associated with estrus in pet ferrets. J. Am. Vet. Med. Assoc. 178, 1293-1294.

Koepfli K.P., Deere K.A., Slater G.J., Begg C., Begg K., Grassman L., Lucherini M., Veron G., Wayne R.K. (2008): Multigene phylogeny of the Mustelidae: resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. BMC Biol. 14, 6-10.

Kraeling R.R., Barb C.R., Rampacek G.B., Thompson D.L.Jr., Gibson J.W., Sullivan S.A., Simon B., Burns P.J. (2000): Luteinizing hormone response to controlled-release deslorelin in estradiol benzoate primed ovariectomized gilts. Theriogenology, 53, 1681-1689.

Kulcsár M., Danko G., Delavaud C., Mircu C., Nikolic A.J., Gáspárdy A., Cernescu H., Chilliard Y., Cseh S., Rudas P., Huszenicza Gy. (2006a): Endocrine characteristics of late pregnant hyperketonaemic ewes and their reproductive performance following the induction of ovarian cyclicity out of the breeding season. Acta Vet. Hung. 54, 235-249.

Kulcsár M.., Dankó G., Magdy H.G.I., Reiczigel J., Forgach T., Proháczik A., Delavaud A., Magyar K., Chilliard Y., Solti L., Huszenicza Gy. (2006b) Pregnancy stage and number of fetuses may influence maternal plasma leptin in ewes. Acta Vet. Hung. 54, 221-234.

Kurose N., Abramov A.V., Masuda R. (2000): Intrageneric diversity of the cytochrome B gene and phylogeny of eurasian species of the genus *Mustela* (Mustelidae, Carnivora). Zoolog. Sci. 17, 673-9.

Lagerkvist G., Einarsson E.J., Forsberg M., Gustafsson H. (1992): Profiles of oestradiol-17 beta and progesterone and follicular development during the reproductive season in mink (*Mustela vison*). J. Reprod. Fertil. 94, 11-21.

Larter N.C., Rajamahendran R., Sivakumaran K. (1994): Immunoreactive faecal progestins as indicators of reproductive status. Vet Rec. 134, 474-475.

Lawler D.F., Johnston S.D., Hegstad R.L., Keltner D.G., Owens S.F. (1993): Ovulation without cervical stimulation in domestic cats. J. Reprod. Fertil. Suppl. 47, 57–61.

Lawrence H.J., Gould W.J., Flanders J.A., Rowland P.H., Yeager A.E. (1993): Unilateral adrenalectomy as a treatment for adrenocortical tumors in ferrets: five cases (1990-1992). J. Am. Vet. Med. Assoc. 203, 267-270.

Lewington, J.H. (2007a): Pregnancy toxaemia. In: Lewington, J.H. (ed.): Ferret husbandry, medicine and surgery. Saunders Elsevier, Edinburgh, 2nd ed. pp. 94-95.

Lewington, J.H. (2007b): Reproduction and genetics. In: Lewington, J.H. (ed.): Ferret husbandry, medicine and surgery. Saunders Elsevier, Edinburgh, 2nd ed. pp. 86-121.

Lewington, J.H. (2007c): Disease of special concern. In: Lewington, J.H. (ed.): Ferret husbandry, medicine and surgery. Saunders Elsevier, Edinburgh, 2nd ed. pp. 258-288.

Lewington, J.H. (2007d): Classification, history and current status of ferrets. In: Lewington, J.H. (ed.): Ferret husbandry, medicine and surgery. Saunders Elsevier, Edinburgh, 2nd ed. pp. 3-14.

Li X., Fox J.G., (1998): Neoplastic diesaes. In: Fox J.G. (ed.): Biology and Diseases of the Ferret. 2nd ed. Williams and Wilkins Press, London, pp 405-448.

Li Z., Sun X., Chen J., Leno G.H., Engelhardt J.F. (2006a): Factors affecting the efficiency of embryo transfer in the domestic ferret (*Mustela putorius furo*). Theriogenology. 66, 183-90.

Li Z. Sun X., Chen J., Liu W., Wisely S. M., Zhou Q., Renard J-P., Leno G.H., Engelhardta J.F. (2006b): Cloned ferrets produced by somatic cell nuclear transfer. Dev. Biol. 293, 439–448.

Lindeberg H. (2008): Reproduction of the female ferret (*Mustela putorius furo*). Reprod Domest Anim. 43 (Suppl 2), 150-156.

Lipman N.S., Marini R.P., Murphy J.C., Zhibo Z., Fox J.G. (1993): Estradiol-17 beta-secreting adrenocortical tumor in a ferret. J. Am. Vet. Med. Assoc. 203, 1552-1555.

Lodé T., Guiral G., Peltier D. (2005): European Mink–Polecat Hybridization Events: Hazards From Natural Process? J. Hered. 96, 89-96.

Lucy M. C. (2008): Functional differences in the growth hormone and insulin-like growth factor axis in cattle and pigs: Implications for postpartum nutrition and reproduction. Reprod. Domest. Anim. 43 (Suppl. 2), 31-39.

Ludwig L., Aiken S. (2004): Soft Tissue Surgery. In: Quesenberry K.E. and J. W. Carpenter (eds.): Ferrets, Rabbits, and Rodents. WB Saunders, Philadelphia, pp. 121-134

Marmi J., López-Giráldez J. F., Domingo-Roura X. (2004): Phylogeny, evolutionary history and taxonomy of the Mustelidae based on sequences of the cytochrome b gene and a complex repetitive flanking region. Zool. Scr. 33, 481-499.

Mattheij J.A., Swarts H.J. (1995): Induction of luteinized unruptured follicles in the rat after injection of luteinizing hormone early in pro-oestrus. Eur. J. Endocrinol. 132, 91–96.

McGuire M.A., Beede D.K., Collier R.J., Buonomo F.C., DeLorenzo M.A., Wilcox C.J., Huntington GB, Reynolds CK. (1991): Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin insulin-like growth factor (IGF)-I and IGF-II, and thyroid hormones in plasma of lactating Holstein cows. J. Anim. Sci. 69, 2050-2056.

McKibbin P.E., Rajkumar K., Murphy B.D. (1984): Role of lipoproteins and prolactin in luteal function in the ferret. Biol. Reprod. 30, 1160-1166.

McRae A.C. (1992): Effect of ovariectomy on blastocyst expansion and survival in ferrets (*Mustela putorius furo*). Reprod. Fertil. Dev. 4, 239-47.

Mead R.A., Joseph M.M., Neirinckx S. (1988): Optimal dose of human chorionic gonadotropin for inducing ovulation in the ferret. Zoo. Biol. 7, 263-267.

Mead R.A., Neirinckx S. (1990): Photomanipulation of sexual maturation and breeding cycle of the steppe polecat (*Mustela eversmanni*) and other techniques for more rapid propagation of the species. J. Exp. Zool. 255, 232-238.

Meikle A., Kulcsár M., Chilliard Y., Fébel H., Delavaud C., Cavestany D., Chilibroste P. (2004): Effects of parity and body condition score at calving on endocrine and

reproductive parameters of the dairy cow under grazing conditions. Reproduction 127, 727-737.

Miwa Y., Nakata M., Kurosawa A., Sasai H., Sasaki N. (2008): Adrenal diseases in ferrets in Japan. J. Vet. Med. Sci. 70, 1323-6.

Moller O.M. (1973): The fine structure of the lutein cells in the mink (*Mustela vison*) with special reference to the secretory activity during pregnancy. Z. Zellforsch. Mikrosk. Anat. 138, 523-544.

Möstl E., Lehmann H., Wenzel U. (1993): Gestagens in the faeces of mink and cats for monitoring corpus luteum activity. J. Reprod. Fertil. Suppl. 47, 540-541.

Munson L., Bauman J.E., Asa C.S., Jöchle W., Trigg T.E. (2001): Efficacy of the GnRH analogue deslorelin for suppression of oestrous cycles in cats. J. Reprod. Fertil. Suppl. 57, 269-73.

Murphy B.D. (1979): The role of prolactin in implantation and luteal maintenance in the ferret. Biol. Reprod. 21, 517-521.

Murphy B.D. (1989): Reproductive physiology of female mustelids. In: Seal U.S., Thorne E.T., Bogan M.A., Anderson S.H. (eds): Conservation biology and the black-footed ferret. Yale University press, New Haven, CT, pp 107-123.

Nagy P, Solti L, Kulcsár M, Reiczigel J, Huszenicza Gy, Abaváry-Mihály K, Wöfling A (1998): Progesterone determination in equine plasma using different immunoassays. Acta Vet. Hung. 46, 501-513.

Olson P.N., Thrall M.A., Wykes P.M., Husted P.W., Nett T.M., Sawyer H.R. (1984): Vaginal cytology. Part 1. A useful tool for staging the canine estrous cycle. Compendium for the Continuing Education for Practicing Veterinarians, 6, 288-298.

Orcutt C.J. (2003): Ferret urogenital diseases. Vet. Clin. North Am. Exot. Anim. Pract. 6, 113-38.

O'Rourke D.P. (1997): Disease problems of guinea pigs. In: Hillyer E.V. and Quesenberry K.E. (eds.): Ferrets, Rabbits, and Rodents. WB Saunders, Philadelphia, pp. 245-254.

Oxenham M. (1990): Oestrus control in the ferret. Vet. Rec. 126, 148.

Palme R., Fischer P., Schildorfer H., Ismail M.N. (1996): Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Anim. Reprod. Sci. 43, 43–63.

Palme R., Robia C., Messmann S., Hofer J., Möstl E. (1999): Measurement of fecal cortisol metabolites in ruminants: a non-invasive parameter of adrenocortical function. Wien. Tierärztl. Mschr. 86, 237–241.

Pare J.A., Murphy J.P. (1997): Disorders of the reproductive and urinary systems. In: Hillyer E.V. and Quesenberry K.E. (eds.): Ferrets, Rabbits, and Rodents. WB Saunders, Philadelphia, pp. 183-193.

Patterson M.M., Rogers A.B., Schrenzel M.D., Marini R.P., Fox J.G. (2003): Alopecia attributed to neoplastic ovarian tissue in two ferrets. Comp. Med. 53, 213-7.

Pelican K.M., Wildt D.E., Pukazhenthi B., Howard J. (2006): Ovarian control for assisted reproduction in the domestic cat and wild felids. Theriogenology. 66, 37-48.

Pethes Gy., Bokori J., Rudas P., Frenyó V.L., Fekete S. (1985): Thyroxin, triiodothyronine, reverse-triiodothyronine, and other physiological characteristics of periparturient cows fed restricted energy. J. Dairy Sci. 68, 1148-1154.

Petrie A., Watson P. (1999): Statistics for veterinary and animal science. Balckwell Science Ltd, Oxford, UK.

Pollock C.G. (2004): Urogenital disease. In Quesenberry K.E., Carpenter J.W. (eds): Ferrets, Rabbits, and Rodents. Clinical medicine and surgery. Saunders Elsevier, Edinburgh, 2nd ed. pp. 41-49.

Porter K.B., Tsibris J.C., Nicosia S.V., Murphy J.M., O'Brien W.F., Rao P.S., Spellacy W.N. (1995): Estrogen-induced guinea pig model for uterine leiomyomas: do the ovaries protect? Biol. Reprod. 52, 824-832.

Purcell K., Brown S.A. (1999): Essentials of ferrets: a guide for practitioners. Philadelphia, American Animal Hospital Association; 2nd ed., pp 41-43; 136-148; 186-187.

Quesenberry K.E., Rosenthal K.L. (2004): Endocrine disease. In: Quesenberry K.E., Carpenter J.W. (eds.): Ferrets, Rabbits and Rodents Clinical medicine and surgery. W.B. Saunders Press, United States of America, pp. 86-87.

Ramer J.C., Benson K.G., Morrisey J.K., O'Brien R.T., Paul-Murphy J. (2006): Effects of melatonin administration on the clinical course of adrenocortical disease in domestic ferrets. J. Am. Vet. Med. Assoc. 229, 1743-1748.

Reichler I.M., Hubler M., Jöchle W., Trigg T.E., Piché C.A., Arnold S. (2003): The effect of GnRH analogs on urinary incontinence after ablation of the ovaries in dogs. Theriogenology. 60, 1207-1216.

Robinson A. (1918): XIII. The formation, rupture, and closure of ovarian follicles in ferrets and ferret-polecat hybrids, and some related phenomena. Transactions of the Royal Society. Edinburgh. 52, 303-362.

Romagnoli S., Concannon P.W. (2003): Clinical use of progestins in bitches and queens: a review. In: Concannon P.W., England G., Verstegen J., Linde-Forsberg C. (eds.): Recent advances in small animal reproduction. International Veterinary Information Service, Ithaca NY (www.ivis.org).

Rook J.S. (2000): Pregnancy toxemia of ewes, does, and beef cows. Vet. Clin. North Am. Food Anim. Pract. 16, 293-317.

Roots C. (2008): Domestication. Greenwood guides to the animal world. Greenwood Press. United States of America, p. 128.

Rose J., Huang J.L., Mead R.A. (1993): A role of ovarian steroids in development of uterine binding sites for prolactin in the ferret. Biol. Reprod. 48, 1266-1273.

Rosenthal K.L. (1997): Adrenal gland disease in ferrets. Vet. Clin. North Am. Small Anim. Pract. 27, 401-18.

Rosenthal K.L., Peterson M.E., Quesenberry K.E., Hillyer E.V., Beeber N.L., Moroff S.D., Lothrop C.D.Jr. (1993): Hyperadrenocorticism associated with

adrenocortical tumor or nodular hyperplasia of the adrenal gland in ferrets: 50 cases (1987-1991). J. Am. Vet. Med. Assoc. 203, 271-275.

Rosenthal K.L., Peterson M.E. (1996a): Evaluation of plasma androgen and estrogen concentrations in ferrets with hyperadrenocorticism. J. Am. Vet. Med. Assoc. 209, 1097-1102.

Rosenthal K.L., Peterson M.E. (1996b): Stranguria in a castrated male ferret. J. Am. Vet. Med. Assoc. 209, 62-63.

Rossmeisl J.H.Jr., Scott-Moncrieff J.C., Siems J., Snyder P.W., Wells A., Anothayanontha L., Oliver J.W. (2000): Hyperadrenocorticism and hyperprogesteronemia in a cat with an adrenocortical adenocarcinoma. J. Am. Anim. Hosp. Assoc. 36, 512–517.

Ryan K.D. (1984): Hormonal correlates of photoperiod-induced puberty in a reflex ovulator, the female ferret (*Mustela furo*). Biol. Reprod. 31, 925-935.

Ryan K.D., Siegel S.F., Robinson S.L. (1985): Influence of day length and endocrine status on luteinizing hormone secretion in intact and ovariectomized adult ferrets. Biol. Reprod. 33, 690–697.

Ryland L.M., Lipinski E. (1994): A technique for vasectomizing male ferrets. Canine Practice. 19, 25-27.

Sartin J.L., Kemppainnen R.J., Cummins K.A., Williams J.C. (1988): Plasma concentrations of metabolic hormones in high and low producing dairy cows. J. Dairy Sci. 71, 650-657.

Sato J.J., Hosoda T., Wolsan M., Tsuchiya K., Yamamoto M., Suzuki H. (2003): Phylogenetic relationships and divergence times among mustelids (Mammalia: Carnivora) based on nucleotide sequences of the nuclear interphotoreceptor retinoid binding protein and mitochondrial cytochrome-b genes. Zoolog. Sci. 20, 243-64.

Schoemaker N.J., Schuurmans M., Moorman H., Lumeij J.T. (2000): Correlation between age at neutering and age at onset of hyperadrenocorticism in ferrets. J. Am. Vet. Med. Assoc. 216, 195-197.

Schoemaker N.J., Teerds K.J., Mol J.A., Lumeij J.T., Thijssen J.H., Rijnberk A. (2002): The role of luteinizing hormone in the pathogenesis of hyperadrenocorticism in neutered ferrets. Mol. Cell. Endocrinol. 197, 117-125.

Schoemaker N.J. (2003): Hyperadrenocorticism in ferrets. PhD dissertation. Faculty of Veterinary Medicine. Utrecht.

Schoemaker N.J., van der Hage MH.., Flik G., Lumeij J.T., Rijnberk A. (2004): Morphology of the pituitary gland in ferrets (*Mustela putorius furo*) with hyperadrenocorticism. J. Comp. Pathol. 130, 255-265.

Schoemaker N.J., van Deijk R., Muijlaert B., Kik M.J., Kuijten A.M., de Jong F.H., Trigg T.E., Kruitwagen C.L., Mol J.A. (2008a): Use of a gonadotropin releasing hormone agonist implant as an alternative for surgical castration in male ferrets (*Mustela putorius furo*). Theriogenology. 70, 161-167.

Schoemaker, N.J., Kuijten, A.M., Galac, S. (2008b) Luteinizing hormone-dependent Cushing's syndrome in a pet ferret (*Mustela putorius furo*). Domest. Anim. Endocrinol. 34, 278-283.

Schwarzenberger F., Möstl E., Palme R., Bamberg E. (1996a): Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim. Reprod. Sci. (Netherlands) 42, 515-526.

Schwarzenberger F., Son C.H., Pretting R., Arbeiter K. (1996b): Use of group-specific antibodies to detect fecal progesterone metabolites during the estrous cycle of cows. Theriogenology 46, 23-32.

Selman P.J., Mol J.A., Rutteman G.R., Van Garderen E., Van den Ingh T.S., Rijnberk A. (1997): Effects of progestin administration on the hypothalamic-pituitary-adrenal axis and glucose homeostasis in dog. J. Reprod. Fertil. Suppl. 51, 345-354.

Senger P.L. (2003): Pathways to pregnancy and parturition. 2nd ed. Current Conceptions Inc., Washington State University, Pullman, WA.

Sherrill A., Gorham J. (1985): Bone marrow hypoplasia associated with estrus in ferrets. Lab. Anim. Sci. 35, 280-286.

Siklódi B., Barna-Vetró I., Solti L. (1995): Latent immunization to produce high affinity monoclonal antibodies to progesterone. Hybridoma. 14. 79-84.

Silvestre F.T., Bartolome J.A., Kamimura S., Arteche A.C., Pancarci S.M., Trigg T., Thatcher W.W. (2009): Postpartum suppression of ovarian activity with a Deslorelin implant enhanced uterine involution in lactating dairy cows. Anim. Reprod. Sci. 110, 79-95.

Sirivaidyapong, S., Swangchanuthai, T. (2003): The estrous cycle and estrogen toxicity during estrus in female ferret. Proceedings of 41st Kasetsart University Annual Conference, Subject: Animals and Veterinary Medicine, February 3, 2003-February 7, 2003, Kasetsart University: Bangkok, Thailand, pp. 554-562.

Snedecor GW, Cochran WG (1982): Statistical methods. 7th ed. The Iowa State University Press, Ames.

Talwar G.P. (1997): Fertility regulating and immunotherapeutic vaccines reaching human trials stage. Hum. Reprod. Update. 3, 301-310.

Tauson A.H. (1997): Prolactin profiles of pregnant, lactating and non-mated female mink (*Mustela vison*). J. Reprod. Fert. Suppl. 51. 195-201.

Thomson D.L.Jr. (2000): Immunisation against GnRH in male species (comperative aspects) Anim. Reprod. Sci. 60-61, 459-469.

Thorpe D.H. (1967): Basic parameters in the reaction of ferrets to light. In: Wolstenholme GEW, O'Connor M (eds.): Effects of External Stimuli on Reproduction. CIBA Found Study Group 1967, Vol. 26; Boston: Little. Brown & Co. pp 53-70.

Tsutsui T., Stabenfeldt G.H. (1993): Biology of ovarian cycles, pregnancy and pseudopregnancy in the domestic cat. J. Reprod. Fertil. Suppl. 47, 29-35.

Van Goethem B, Schaefers-Okkens A, Kirpensteijn J. (2006): Making a rational choice between ovariectomy and ovariohysterectomy in the dog: a discussion of the benefits of either technique. Vet. Surg. 35, 136-143.

Van Saun R.J. (2000): Pregnancy toxaemia in a flock of sheep. J. Am. Vet. Med. Assoc. 217, 1536-1539.

Villars T.A., Erskine M.S., Lambert G.M, Jacobson D., Weaver C.E., Baum M.J., (1990): Endocrine correlates of mating-induced reductions in estrus behaviour in an induced ovulator, the ferret. Horm. Behav. 24, 198-214.

Vinke C.M., Deijk R., Houx B.B., Schoemaker N. J. (2008): The effects of surgical and chemical castration on intermale aggression, sexual behaviour and play behaviour in the male ferret (*Mustela putorius furo*) Applied Animal Behaviour Science. 115, 104-121.

Wagner R.A., Dorn D.P. (1994): Evaluation of serum estradiol concentrations in alopecic ferrets with adrenal gland tumors. J. Am. Vet. Med. Assoc. 205, 703-707.

Wagner R.A., Bailey E.M., Schneider J.F., Oliver J.W. (2001): Leuprolide acetate treatment of adrenocortical disease in ferrets. J. Am. Vet. Med. Assoc. 218, 1272-1274.

Wagner R.A., Piché C.A., Jöchle W., Oliver J.W. (2005): Clinical and endocrine responses to treatment with deslorelin acetate implants in ferrets with adrenocortical disease. Am. J. Vet. Res. 66, 910-914.

Wallach I.D., Boever W.J. (1983): Diseases of exotic animals: medical and surgical management. WB Saunders, Philadelphia, p. 93

Waston A.D., Church D.B., Emslie D.R., Middleton D.J. (1989): Comparative effects of proligestone and megestrol acetate on basal plasma glucose concentrations and cortisol responses to exogenous adrenocorticotrophic hormone in cats. Res. Vet. Sci. 47, 374-376.

Weiss C.A., Scott M.V. (1997): Clinical aspects and surgical treatment of hyperadrenocorticism in the domestic ferret: 94 cases (1994-1996). J. Am. Anim. Hosp. Assoc. 33, 487-93.

Weiss C.A., Williams B.H., Scott J.B., Scott M.V. (1999): Surgical treatment and long-term outcome of ferrets with bilateral adrenal tumors or adrenal hyperplasia: 56 cases (1994-1997). J. Am. Anim. Hosp. Assoc. 215, 820-823.

Westfahl P.K. (1993): Comparison of luteinized unruptured follicles and corpora lutea: steroid hormone production and response to luteolytic and luteotropic agents. Biol. Reprod. 48, 807–814.

Wheler C.L., Kamieniecki C.L. (1998): Ferret adrenal-associated endocrinopathy. Can. Vet. J. 39, 175-176.

Wildt D.E., Seager S.W., Chakraborty P.K. (1980): Effect of copulatory stimuli on incidence of ovulation and on serum luteinizing hormone in the cat. Endocrinology. 107, 1212-1217.

Williams E. S., Thorne E. T., Kwiatkowski D. R., Lutz K., Anderson S. L. (1992): Comparative vaginal cytology of the estrus cycle of black-footed ferrets (*Mustela nigripes*), Siberian polecats (*M. eversmanni*), and domestic ferrets (*M. putorius furo*). J. Vet. Diagn. Invest. 4, 38-44.

Young K.M. (1998): Reproductive biology of the black-footed ferret (*Mustela nigripes*). PhD dissertation. The Faculty of Graduate Studies of the University of Guelph, Guelph.

Young K.M., Brown J.L., Goodrowe K.L. (2001): Characterization of reproductive cycles and adrenal activity in the black-footed ferret (*Mustela nigripes*) by fecal hormone analysis. Zoo Biol. 20, 517-536.

Zaidi J., Jurkovic D., Campbell S., Collins W., McGregor A., Tan S.L. (1995): Luteinized unruptured follicle: morphology, endocrine function and blood flow changes during the menstrual cycle. Hum. Reprod. 10, 44–49.

6. The candidate's publications related to the present dissertation

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

***Proházik A.**, Kulcsár M., Huszenicza G.* (2009): Metabolic and endocrine characteristics of pregnancy toxemia in the ferret. Vet. Med. Cz. 54, pp. 75-80. (last known **IF,** 2008: 0.659)

***Proházik A.**, Kulcsár M., Huszenicza G.* (2009): Deslorelin treatment of hyperoestrogenism in neutered ferrets (*Mustela putorius furo*). A case report. Vet. Med. Cz. 54, pp. 89-95. (last known **IF,** 2008: 0.659)

***Proházik 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 (last known **IF,** 2008: 1.240)

***Proházik A.**, Kulcsár M., Huszenicza G., Glávits R.* (2009)*:* Reproductive features and fecal progesterone metabolite profile in female ferrets. Reprod. Domest. Anim. ongoing publication (last known **IF,** 2008: 1.526)

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

***Proházik 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. (last known **IF,** 2008: 0.088)

***Proházik 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. (last known **IF,** 2008: 0.088)

***Proházik 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. (last known **IF,** 2008: 0.088)

***Proházik 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. (last known **IF,** 2008: 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.

Further publications not related to the current thesis

Fodor K., Fekete S., **Proháczik A.,** Andrásofszky E. (2003): Comparison of preference of cat and ferret towards raw protein sources. 7th ESVCN Conference, Hannover, Germany, Proceeding. p. 68.

Fodor K., **Proháczik A.,** Andrásofszky E., Fekete S. (2004): A vadászgörény, mint kísérleti állat. Biomodellek 2004. Ferret as model animal in biomedical research. Laboratory Animal Conference, Budapest.

Fekete S., Fodor K., **Proháczik A.,** Andrásofszky E. (2005): Comparison of feed preference and digestion of three different commercial diets for cat and ferret. J. Anim. Physiol. Anim. Nutr. (Berl). 89, 199-202.

Kulcsár M., Dankó G., Magdy, H.G.I., Reiczigel J., Forgach T, **Proháczik A.**, Delavaud, A., Magyar K., Chilliard, Y., Solti L., Huszenicza Gy. (2006): Pregnancy stage and number of fetuses may influence maternal plasma leptin in ewes. Acta Vet. Hung. 54, 221-234.

Fodor K., **Proháczik A**., Andrásofszky E., Hábor K. and Fekete S. (2006): Determination of transit time in ferret and domestic cat (in Hungarian with English summary). Magyar Állatorvosok Lapja. 128, 674-679.

……

Proháczik A. (2002): Egzotikus lakótársak: a vadászgörény. Veszprémi Állatvédők Lapja. Január.

Proháczik A. (2003): A vadászgörényről…. Váróterem. 1, 40-41.

Proháczik A. (2003): Egzotikus nagymacskák és házicicák. Váróterem. 1, 44-46.

Proháczik A. (2003): Egzotikus lakótársak: ormányos és a mosómedve. Veszprémi Állatvédők Lapja. November.

Proháczik A. (2004): A vadászgörény. In: A család állatorvosa; kedvenceink egészségkönyve, Kossuth Kiadó Rt. Budapest. pp. 133-148.

Proháczik A. (2004): A ormányos-, és mosómedve. In: A család állatorvosa; kedvenceink egészségkönyve, Kossuth Kiadó Rt. Budapest. pp. 149-156.

Proháczik A. (2004): A majmok. In: A család állatorvosa; kedvenceink egészségkönyve, Kossuth Kiadó Rt. Budapest. pp. 157-161.

Proháczik A. (2004): Egzotikus lakótársak: A vörösfülű ékszerteknős tartásáról. Veszprémi Állatvédők Lapja. Február.

Proháczik A. (2004): Egzotikus lakótársak: otthonunkban a zöld leguán. Veszprémi Állatvédők Lapja. Április.

Acknowledgements

First, I would like to express my great appreciation to *Prof. Dr. Gyula Huszenicza* for his continuous help and encouragement throughout the years and without whom I would have ever succeeded to reach my professional goals.

A special thank has to be granted to *Dr. Margit Kulcsár* for her contribution in the experiments and above all, for her kind and loving personality.

It is my honor to acknowledge *Dr. Róbert Glávits* for the histological examinations and his generous help and advices, and the great comments to *M.A. Driancourt* at INTERVET Pharma R&D (Beaucouzé, France) to improve my work.

This thesis would have never been accomplished without the academic staff, co-workers, fellow students and technicians in my alma mater whose support needs to be recognized: *Prof. László Solti* as the head of the department, *Drs. Jenő Reiczigel* and *András Gáspárdy* for their help in the statistical evaluations, *Drs. Vera Faigl, Mónika Keresztes* and *Alíz Márton* for their cooperation in the laboratory and for sharing the winding road of PhD students with me, *Alice Vonáné Nagy* for her great analytical work and friendship, *Erzsébet Kiss* and *Ibolya Czigányné Simon* for their outstanding laboratory assistance.

My reviewers, *Prof. László Zöldág, Prof. Zoltán Zomborszky* and *Dr. János Gál* deserve all my appreciation for their time and effort to revise my work and for their precious advices so that the final format of this thesis could hopefully be also the best one.

The financial support was granted by the *National Foundation for Basic Research (Hungary, project number: OTKA-T/046826).*

Last, but not least I would like to acknowledge my mother, my father and my sister for their endless sacrifices, love and support so that I could stand where I am now. They supported me and my ferrets days and nights for several years. They loved me even if I had more that 100 ferrets in their garden (and in their garage and house). My father endless prepared and repaired the cages for my ferrets. My mother and my sister nonstop cleaned and feed my ferrets when I did not have time. All my family collected more than thousand fecal samples from my ferrets and did notes about their reproductive behaviors. And it was not enough…my sister and my mother sacrificed them to help me in the lab to prepare the fecal samples for the analysis. Once it happened that we were closed in night, because it was so late that nobody thought my sister and me work there.

Finally, I would like to acknowledge all my friends and colleagues who believed in me and in my ferret project from the beginning until the end.

Budapest, 7th September, 2009

Angella Proháczik