



**International Society for Wildlife Endocrinology (ISWE)**  
**2<sup>nd</sup> Annual Meeting**  
**Toronto Zoo**  
**November 3-4, 2011**



**PRESENTATION ABSTRACTS:**

**Thursday, November 3:**

**Session Moderator:** Janice Brown, Smithsonian Conservation Biology Institute

**Keynote Address I:**

**Environmental Endocrinology: Applications to Understanding the Fitness of Wildlife**

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Over the last decade there has been the emergence of environmental endocrinology as a discipline within umbrella of the environmental sciences. This area has developed as scientists have tried to understand the underlying mechanisms that explain the observations of altered fitness (e.g. intersex individuals, delayed reproduction and development) reported for wildlife species. In part the field has grown as more scientists have begun to address questions of wildlife species. The discipline has benefited enormously from the increased accessibility of immunoassay methods that enable scientists to measure hormones but also from the portability of the molecular biology toolbox that have allowed the measurement of gene expression in diverse species. By far the biggest growth in the discipline has come from the expansion of experimental techniques in the field as scientists have moved from measurements of individual hormones in blood or fecal samples to more elaborate experiments involving the tracing of individuals over time and detailed assessment of endocrine systems in wildlife species tested under field conditions. In this presentation, I will use a series of case studies to illustrate how environmental endocrinology has increased our understanding of the physiology of wildlife species but also how this has been used to define the actions of natural and anthropogenic stressors in the environment. The recognition that man-made chemicals can impact the endocrine system of wildlife species has had the broadest impact on environmental endocrinology and has led to the recognition that these compounds represent a global threat to wildlife, particularly those in the aquatic environment. The talk will quickly survey the impacts of tributyltin on marine gastropods to the impacts of human-use pharmaceuticals and industrial processes on fish. A second broad example will explore factors contributing to the prespawning mortality and the reduced reproductive success in sockeye salmon in Western Canada. This example will serve to illustrate how advances in the tracking of individuals over vast differences can be used to better understand the etiology of the disease and the stressors impacting reproduction. The final example will explore the association between exposure to the herbicide atrazine and the development of intersex in the African clawed-frog *Xenopus laevis* in its native habitat. Collectively these studies will show how studies of endocrine physiology have applications in better understanding the fitness of wildlife species in their native habitats.

## Session I: Method Development

### Data Integrity for the Wildlife Endocrinology Database

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Data integrity refers to how closely data stored in a database correspond to the reality represented by those data. A database facilitates analysis, management and sharing of data. It is important to make certain that stored data are clear, concise and accurately represented. A database can cease to function when a database field is mistyped. Another category of problems arises pertaining to field relationships. Two fields may individually store appropriate values which when compared to each other fall outside of acceptable limits. What fields are required to fulfill the requirements for a completed entry, how to manage free form text entry and the ramifications of empty fields all contribute to the degree with which data integrity is maintained. For three years, we have been developing the Wildlife Endocrinology Information Network system which uses SQL Server as its database engine along with ASP.Net and DotNetNuke to manage the web front end. Our goal is to build a robust, user-friendly database that will share wildlife endocrinology data across the world. Our objectives are to: 1) demonstrate some basic techniques which are employed in the Wildlife Endocrinology Database to minimize data entry errors through field validation, screen validation and database field validation; 2) show how fields may be compared to stored values to ensure that data fall within acceptable limits; 3) examine the consequences of blank fields, N/A fields, fields with a value of zero and how it affects numerator/denominator relationships during analysis; 4) discuss techniques to allow free form text entry which will allow data comparison; and 5) consider various formats to store references such as literary citations, web pages and emails so that they are kept up to date thereby minimizing 'dead' links. Each step of data manipulation from data entry to data storage and retrieval requires that data fulfill required validation. By using built-in and customized tools the system can achieve a high degree of data integrity.

### Validation of an Assay for Measurement of Salivary Melatonin in Nocturnal Primates

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Melatonin coordinates the body's internal timekeeping system and is an emerging biomarker for circadian disruption. We aimed to validate a method for quantifying melatonin in saliva from nocturnal prosimians to understand how they are physiologically affected by zoo exhibit lighting design. Two pygmy lorises (*Nycticebus pygmaeus*) and one potto (*Perodicticus potto*) were conditioned for voluntary collection of saliva using honey-flavored swabs. To eliminate matrix interference, saliva samples were first extracted using an organic solvent and then quantified using a commercial RIA kit (Labor Diagnostika Nord). Serially diluted extracts displayed parallelism with the standard curve ( $t=2.103$ ,  $p=0.054$  for pygmy loris;  $t=2.046$ ,  $p=0.063$  for potto). Recovery of hormone from saliva spiked with 6 pg/ml or 20 pg/ml of standard averaged 94.5% for potto and 102% for pygmy loris. Light phase melatonin levels (mean pg/ml  $\pm$  SE) were  $2.64 \pm 0.16$  ( $n=3$  samples) for pygmy loris compared to  $3.55 \pm 0.54$  ( $n=5$ ) during the dark phase; values for potto were  $2.92 \pm 0.2$  (light,  $n=2$ ) and  $3.20 \pm 1.12$  (dark,  $n=2$ ). Although melatonin levels were slightly higher in darkness, we suspect nocturnal melatonin production may be suppressed due to the brightness of the dark phase lights used to exhibit these subjects. To address this concern and biologically validate the assay, we are

currently analyzing data from experiments examining acute suppression of melatonin under different lighting conditions. These methods may be applied to a wide range of species in future studies and will inform strategies for lighting design in the captive environment.

### **An Inter-lab Comparison of Progesterone EIA versus RIA in Bovidae and Hylobatidae Species**

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As the need for intensive management of an increasing number of species increases, so too does the role of non-invasive endocrinology in wildlife conservation. However, the increasing demand for hormonal analysis to identify reproductive, health and welfare issues is overwhelming, motivating endocrinology labs to pool resources. Our objective was to compare a progesterone EIA and RIA on takin pregnancy and estrous cycle length (ECL) analysis and on gibbon [white-cheeked (WCG) and buff-cheeked (BCG)] and siamang (SIA) ovarian cyclicity analysis. All samples were extracted using Lincoln Park Zoo protocols and analyzed using Coralie Munro's CL425 progesterone EIA. Dried extracts were sent to St. Louis Zoo for analysis on progesterone (Coat-A-Count) RIA. For the takin, both assays produced similar ( $R^2=0.885$ ) results and mean progesterone values ( $P > 0.05$ ; RIA,  $360.3 \pm 36.9$  ng/g; EIA,  $591.5 \pm 71.8$  ng/g). Interestingly, the ECL ( $n=7$ ; RIA,  $25.2 \pm 1.1$  days; EIA,  $26.0 \pm 1.2$  days) was similar ( $P > 0.05$ ). However, although the follicular phase was similar ( $P > 0.05$ ; RIA,  $6.5 \pm 1.3$  days; EIA,  $4.6 \pm 1.0$  days), the luteal phase was longer ( $P=0.051$ ) in EIA ( $18.5 \pm 1.4$  days) compared to RIA ( $14.1 \pm 1.5$  days) with one ECL not being detected by the RIA. Both assays detected the pregnancy easily with a 20-fold difference for the RIA and a 40-fold difference for the EIA in mean progesterone values. For the WCG and BCG, the similarity between the assays was reduced ( $R^2=0.14$  and  $R^2=0.12$ , respectively); however, results proved similar ( $R^2=0.66$ ) in SIA. Despite the hormonal pattern, mean progesterone values did vary ( $P < 0.001$ ) in all three species (WCG: RIA,  $61.5 \pm 2.8$  ng/g; EIA  $278.8 \pm 29.0$  ng/g; SIA: RIA,  $75.2 \pm 3.3$  ng/g; EIA,  $1210.1 \pm 166.5$  ng/g; BCG: RIA,  $49.2 \pm 1.8$  ng/g; EIA,  $146.2 \pm 14.1$  ng/g). Although the ovarian cycle length did not vary ( $P > 0.05$ ) between assays for WCG ( $n=2$ ; RIA,  $23.5 \pm 5.5$  days; EIA,  $24.5 \pm 2.5$  days) or SIA ( $n=3$ ; RIA,  $21.3 \pm 1.2$  days; EIA,  $23.7 \pm 0.9$  days), none could be detected by the RIA for BCG ( $n=2$ ; EIA,  $22.5 \pm 2.5$  days). Differences determined here suggest that the crossreactivities to progestin metabolites vary between assays; therefore, it is important to test different assays to determine the most suitable for each species. Here, we illustrate the importance of developing a collaborative, multi-institutional approach to wildlife endocrinology.

### **What about Hair Cortisol: a Radiometabolism Study in Guinea Pigs**

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Measurement of hair cortisol has become popular to evaluate chronic stress in various species. However, a sound validation is missing up to now. Therefore in a study in guinea pigs the excretion and the deposition of radioactivity in the hair after repeated injection of <sup>3</sup>H-cortisol was studied. In total 8

adult animals (4/4) were intraperitoneally administered 243.6 kBq <sup>3</sup>H-cortisol on 3 successive days (24h apart). Beginning after the first injection all voided excreta were frequently collected for four days. Peak radioactivity in urine was detected in the first samples after each injection (after 3.5 ± 1.5 hours). In feces maximum radioactivity was reached 7.8 ± 1.8 hours post each injection. Excretion into the feces or urine did not show a marked accumulation of radioactive metabolites in the course of the multiple-dose administration. Performed HPLCs of fecal samples showed only minor variation between individuals and sexes. Major radioactive peaks and several congruent immunoreactive peaks in both a cortisol and an 11-oxoetiocholanolone enzyme immunoassay (EIA) were present. After the 2nd injection, hair was shaved off the animals' back. Four days after the last injection newly grown hair from the same areas was obtained. Following methanol extraction of pooled hair samples radioactive and unlabelled glucocorticoid metabolites (GCM) were characterized by HPLC. Only small amounts of radioactivity were present in the hair samples and injected <sup>3</sup>H-cortisol was totally metabolized. However, a cortisol and cortisone EIA detected large amounts of unlabelled GCM, including high levels at the position of the cortisol standard measured by the cortisol EIA (radioactivity was absent in this fraction). Furthermore both EIAs revealed large amounts of immunoreactivity present together with a radioactive peak at the elution position of cortisone. Our findings show for the first time that only very small amounts of systemically administered radioactive glucocorticoids are deposited into the hair of guinea pigs, while measurement of large amounts of unlabelled GCM suggests local production of GC in the hair follicle. Further studies have to clarify whether GC synthesis in the hair follicle is susceptible to systemic suppression or stimulation.

**Session Moderator:** Nadja Wielebnowski, Chicago Zoological Society

**Session II: Innovative Endocrinology**

**Estradiol in the Reproductive Biology of Edible Sea Urchins: Which is the Function?**

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Sea urchins are key-elements of the marine ecosystems and their gonads are commercially overexploited as food. They are also historical and extensively used models in embryology. Despite this, the specific hormonal mechanisms regulating sea urchin gonad development and reproduction are scarcely known. To overcome this gap of knowledge, in this research we focused on the sex steroid estradiol (E2), a well-known regulator of many reproductive aspects in vertebrates and invertebrates and whose presence as well as seasonal and sex-specific variations were previously reported in different echinoderm tissues. In order to understand E2 role we set up an experiment of direct hormone administration, using the edible sea urchin *Paracentrotus lividus* as model. We injected three different concentrations (2 ng/mL, 20 ng/mL and 200 ng/mL) of 17β-estradiol for 2 and 12 weeks (2 injections/week) and we checked the effects on different reproductive parameters. The lowest concentration was close to the "physiological" one. Results showed a lack of dose-dependent increase of endogenous hormone levels in both body fluids and gonads, thus suggesting the presence of potent homeostatic mechanisms: these latter are particularly activated over a critical threshold and work much more efficiently in short-term period and in specific tissues. These homeostatic mechanisms do not involve enzymes such as aromatase, sulfotransferase and acyltransferase, whose activities were not influenced by the hormone treatment. Despite the actual increase of endogenous E2, both short- and long-term hormonal treatment did not induce marked variations in most the reproductive parameters, such as sex-ratio, Gonad Index and maturative stage. Only lipid content resulted negatively correlated

with E2 level in the gonad. Overall our present results contribute to a better knowledge of sea urchin endocrinology and suggest that E2 does not markedly influence their reproduction and, particularly, it does not promote gonad maturation, as commonly reported for many vertebrates and also suggested for other echinoderms.

### **Habitat Quality and Hormone Profiles in Female Red Colobus Monkeys (*Procolobus rufomitratus*) in Kibale National Park, Uganda**

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Understanding the factors influencing female reproductive function is essential for the conservation of wild species. Using the endangered red colobus monkey (*Procolobus rufomitratus*) of Kibale National Park, Uganda as a model, we investigate the relationship between habitat quality and female reproduction. Specifically, we investigate estradiol, progesterone, and cortisol concentrations in disturbed vs undisturbed areas. The study areas included unprotected forest fragments, previously logged areas that are now protected in the park, and undisturbed protected areas. Global Positioning System, Geographic Information System, and Remote Sensing technologies were used to create maps of the ground cover in each group's home range. Approximately 1500 fecal and urine samples were collected noninvasively from habituated and identified focal females and were analyzed using radioimmunoassay for hormone concentrations. Results indicated that females in unprotected forest fragments had lower reproductive hormone concentrations than females in protected forest ( $p < 0.05$ ), but females in previously logged areas of the protected forest did not have significantly different hormone profiles from those in unlogged areas of the park. Furthermore, behavioral observations of feeding ecology and behavioral profiles suggest that females in previously logged areas may be adjusting their diet and activity profiles to cope with living in degraded habitats. Understanding the reproductive physiologies and behaviors of red colobus monkeys and examining how these relate to habitat quality is crucial for the proper management of this endangered species.

### **Does the Odour (or/scent) of Faeces allow Individual Identification in Brown Bears and Iberian Lynx?**

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Due to the difficulty of directly observing wildlife species it is often impossible to allocate faecal samples to particular individuals. We aimed to investigate whether odour profiles can be used to identify faecal samples from a certain individual and to distinguish it from samples of other individuals. In the Scandinavian brown bear project adult bears were GPS radio tracked and scat samples of individual bears were collected during the mating season at their resting sites ~24 hours after deposition. Often females are accompanied by their cubs or an adult male. Therefore sample assignment to the female could prove to be erroneous. The same problem arises when Iberian lynxes are housed in pairs and pregnancy diagnosis based prostaglandin F<sub>2</sub> metabolite (PGFM) analyses is

required; the sample from the male generates basal (non-pregnant) PGFM concentrations and causes confusion. We used headspace solid-phase micro-extraction and gas chromatography mass spectrometry to investigate the presence/absence (qualitative) of compounds as well as the relative abundance (quantitative) of faecal volatile substances (odour profiles). In an individual bear the qualitative pattern of faecal volatiles remained constant over periods of several weeks. Only when food preferences of bears changed e.g. towards cranberries in late summer odour profiles changed completely. A blind test was performed using faecal samples from a known individual and a sample from another unknown bear; from the odour profile it was easily possible to identify which animal the sample originated. Opposite results were obtained from Iberian lynx fed on a standardized diet (rabbits). Qualitative and quantitative composition of odour profiles of individuals remained constant over weeks and did not differ between individuals. Thus, based on their odour profiles no reliable differentiation between individual lynx was feasible making it impossible to identify false samples (e.g. from cubs) that conflict with interpretations of reproductive hormone profiles from adult females. We assume that individualisation of faecal samples in bears is possible mainly based on different individual food preferences generating unique odour profiles. In lynx a standardized diet prevents the expression of distinct individual odour profiles, therefore feeding of inert markers in would be necessary to differentiate between them.

### Session III: Recent Advances in Basic Endocrinology as Applied to Adrenal Function

#### **Corticosterone and Thyroxine Profiles of Kemp's Ridley Sea Turtles (*Lepidochelys kempii*)**

##### **Recovering from Cold-Stunning**

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Kemp's ridley sea turtles (*Lepidochelys kempii*), a critically endangered species, frequently strand on the shores of Cape Cod (MA) in late autumn in a state of "cold-stunning," exhibiting low body temperature and related clinical issues. Stranded turtles are transported to the New England Aquarium (Boston, MA) for treatment and rehabilitation. We hypothesized that cold-stunned sea turtles might exhibit high corticosterone ("stress hormone") and/or low thyroxine (which affects metabolic rate and behavior), and that monitoring of both hormones may be useful for treatment. In a retrospective analysis, we assayed 87 archived plasma samples from 56 cold-stunned juvenile Kemp's ridley sea turtles, for corticosterone and free thyroxine (fT4). Upon admission, mean corticosterone was the highest yet reported for sea turtles ( $39.3 \pm 2.5$  ng/ml; mean  $\pm$  SEM), likely reflecting a combination of capture stress and cold-stunning stress. Corticosterone at admission was negatively correlated with white blood cell count, but uncorrelated with blood glucose. fT4 on admission was usually undetectable. There were no differences in either hormone between survivors and nonsurvivors on admission. After 18+ days in recovery, surviving turtles' corticosterone dropped significantly to levels typical of baseline in other species ( $0.9 \pm 1.0$  ng/ml), while fT4 increased significantly ( $1.3 \pm 1.5$  pg/ml). During recovery, corticosterone was positively correlated with blood glucose and was uncorrelated with white blood cell count. Turtles that showed persistent deficits in feeding and/or activity during recovery had significantly lower fT4 than turtles with no such deficits. The "high corticosterone, low fT4" endocrine profile seen on admission may be a useful marker of cold-stunning in this and in other species. Further experiments are necessary to determine whether low thyroid hormones play a causal role in deficits in feeding and activity during recovery. Monitoring of both hormones may be useful for triage, monitoring of recovery, and assessing readiness for release.

## **Identification of a Suitable Enzyme Immunoassay for Assessment of Adrenocortical Endocrine Function in African Buffalo (*Syncerus caffer*) based of Faecal Analysis**

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The African buffalo is a large, gregarious bovine generally common in most sub-Saharan ecosystems, and one of the most successful grazers in Africa. Susceptible to many diseases shared with cattle, it is often regarded as a constraint to the expansion of livestock activities. Although intensively studied with regards to its distribution, feeding habits, and infectious diseases, little is known regarding the levels of stress experienced by animals affected by injury, disease, or other socio-ecological and anthropogenic factors. In order to fill this gap, a reliable method for determining stress-related physiological responses in African buffalo is required. Our aim was to examine the suitability of two group-specific enzyme-immunoassays (EIAs) for monitoring adrenocortical function in captive African buffalo based on faecal glucocorticoid metabolite (GCM) analysis. The two EIAs detect 11,17-dioxoandrostanes (11,17-DOA) and GCM's with a 5 $\beta$ -3 $\alpha$ -ol-11-one structure (3 $\alpha$ ,11oxo-CM), respectively, and have successfully been used for monitoring adrenocorticoid activity in several other species, including cattle. We performed an ACTH challenge on one male (~180 kg, 2 years of age) and one female (~300 kg, 5 years of age) buffalo at the Mokopane Biodiversity Conservation Centre, South Africa. Prior to intramuscular injection of synthetic ACTH (150 IU per individual), each study animal was kept in a separate enclosure for 5 days with visual and olfactory contact with the remaining herd. Blood samples were taken during the separation process as well as prior to and post ACTH treatment. Blood samples were analysed with a Coat-A-Count® Cortisol radio-immunoassay (Siemens) and both animals showed a 3-fold elevation in serum glucocorticoid levels 40 min post-injection. Faeces were collected 5 days prior and 4 days following ACTH injection and both EIAs detected a peak in faecal GCM levels 7-22 h after treatment. Faecal 11,17-DOA levels increased 10-fold (female) and 15-fold (male) above baseline, and faecal 3 $\alpha$ ,11oxo-CM concentrations increased 9-fold (female) and 12-fold (male) above pre-injection levels, indicating that both EIAs are suitable for measuring GCM's in African buffalo faeces. The ability to reliably assess adrenocortical endocrine function in African buffalo now provides a solid basis to further examine endocrine responses to putative stressful circumstances in this iconic African species.

## **Session IV: Recent Advances in Basic Endocrinology as Applied to Reproduction**

### **Monitoring of Reproductive Steroid Hormones in Female Aardvarks (*Orycteropus afer*) – Evidence for Embryonic Diapause**

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*Delayed implantation or embryonic diapause*, a condition of temporary suspension of mammalian embryo development, is an evolutionary strategy to enhance the survival rate of neonates. It occurs in about 100 different mammals, and can be divided into two functionally distinct types: facultative and



obligate diapause. The occurrence of facultative diapause seems to be associated with metabolic stress for the dam and her ability to nourish developing embryos, whereas obligate diapause is believed to be a mechanism for synchrony of parturition with environmental conditions favorable to neonatal survival. Although the hormonal control of diapause differs remarkably between species, high levels of progesterone often characterize termination of delayed implantation. A promising candidate to enlarge the group of animals showing embryonic diapause is the Aardvark; a vaguely pig-like appearing, nocturnal, obligate myrmecophagous mammal native to Africa. Due to the marginal mass gain during the first two months of gestation, it is conceivable that embryonic diapause may also occur in Aardvarks, but so far, no endocrinological data exists to support this hypothesis. In this study, we therefore used fecal steroid analysis for monitoring hormone levels in captive Aardvarks to a) describe longitudinal profiles of excreted estrogen and progestagen metabolites throughout gestation and b) reveal endocrinological evidence for the possible existence of delayed implantation.

Between Oct 2002 and Nov 2010, 1232 fresh fecal samples were collected (approximately three times weekly) from 2 female Aardvarks, housed at Brookfield Zoo, USA. Following extraction (0.5g wet feces with 5ml of 80% ethanol in water), resulting extracts were analyzed for immunoreactive estrogen and progestagen concentrations using enzyme-immunoassays for estradiol (antibody R4972, UC Davis, USA) and progesterone (monoclonal antibody CL425, UC Davis, USA).

Collectively, two successful pregnancies and one stillbirth with gestation length of 239, 238, and 234 days, respectively, were monitored in a total of two animals. During preconception periods, fecal estrogens showed a clear cyclic pattern (n = 4-7 cycles per animal) with an average cycle length of 10-11 days, indicating a seasonally polyestrous pattern of reproduction. In all three cases, fecal progestagen levels were only marginally elevated during the first 80-93 days of pregnancy, suggesting the possible existence of obligate diapause in Aardvarks.

### **An Effective and Reversible Contraceptive Option for the Management of Captive Cotton-Top Tamarins (*Saguinus oedipus*): Method Validation and Results from a Study of Long Acting Subcutaneous Levonorgestrel (LNG) Gel Depot**

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Cotton-top tamarins (*Saguinus oedipus*) are a critically endangered species that have been bred successfully in captivity for many years. With captive females producing twins every 29 weeks (Ziegler et al., 1987), combined with a history of contraceptive failures and non-recommended births, the Cotton-top Tamarin SSP<sup>®</sup> has been faced with some unique challenges in reproductive management for more than two decades. To address these issues and to provide an alternative to the currently accepted contraception combination treatment (Depo Provera<sup>®</sup> (medroxyprogesterone acetate) injection followed by MGA (melengestrol acetate) implant), we have developed and tested the use of the synthetic progestin levonorgestrel (LNG) delivered in a biodegradable, injectable, gel matrix. This gel matrix (polylactic-co-glycolic acid, triethyl citrate and N-methylpyrrolidine) forms a biodegradable depot at the injection site and provides slow release of the LNG. The matrix components and LNG concentration were adjusted in 4 gel formulations with a goal to minimize the immediate post-injection burst release (increase in fecal LNG concentrations) while maximizing duration of contraceptive efficacy. LNG gel depot treatment (68.44 ± 8.61 mg/kg) successfully eliminated ovarian cycles, indicated by the



reduced fecal pregnanediol-3-glucuronide (PdG) and estrone conjugate (E<sub>1</sub>C) concentrations for 198.8 ± 70.3 days (range 19 to 50 weeks). This contraceptive treatment method is demonstrated to be an effective and reversible contraceptive option for management of this critically endangered species in captivity.

#### Friday, November 4:

Session Moderator: Karen Goodrowe, Point Defiance Zoo & Aquarium

Keynote Address II:

### **The Ecology of Stress: Insights from Natural Populations**

RUDY BOONSTRA

*Centre for the Neurobiology of Stress, University of Toronto at Scarborough, Toronto, ON, Canada*

The neuroendocrine system is a major pathway in vertebrates that integrates environmental change and through which life history decisions to reproduce, to grow, or to put energy into storage are implemented. The goal of individuals is to maximize lifetime reproductive fitness and the functioning of the stress axis plays a central role in the neuroendocrine system in making this happen. At the individual level, the stress axis plays a key role in allowing animals to cope with change and challenge in the face of both environmental certainty and uncertainty. When the environment becomes unpredictable, increased stress can cause organizational effects that program offspring through maternal effects and can result in both individual and population effects. At the species level, the stress axis plays a central role in evolutionary adaptations to particular ecological pressures and an understanding of differences among species is essential to life-history adaptations. In this talk, I will discuss examples from the research of our lab showing how mammals cope with certainty (seasonal changes) and uncertainty (fluctuating predator pressure or food supply). For the latter, I will then present evidence showing that strong maternal effects result that then affect both the individual fitness and the population demography. Finally, I will discuss some examples showing how aspects of the stress axis have evolved to produce particular life history strategies. I will argue that our ability to predict what how the stress axis should function, given that we know a species' habitat and life history, is limited.

#### Session IV: Recent Advances in Basic Endocrinology as Applied to Reproduction (cont'd)

### **Investigation of Individual and Group Variability in Estrous Cycle Characteristics in Female Asian Elephants (*Elephas maximus*) at the Oregon Zoo**

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Evaluating ovarian cycle activity through longitudinal progestagen monitoring is important for improving the breeding management of captive elephants and understanding the impact of life events (births, deaths, and transfers) on reproductive function. This study summarized longitudinal serum progestagen data from eight Asian mainland elephants (*Elephas maximus indicus*) and one Bornean elephant (*E. maximus borneensis*) at the Oregon Zoo over a 20-year period. Our data show that estrous cycle characteristics were more varied than previously reported for this species, with an overall duration of 12 to 19 weeks, luteal phase duration of 4 to 15 weeks, and follicular phase duration of 2 to 12 weeks. In general, there was more cycle variability across than within individuals. In particular, the Borneo female

exhibited consistently longer cycle lengths and higher progestagen concentrations compared to herdmates. Variability in duration of the follicular phase was greater than that of the luteal phase. In addition, there was a negative and significant relationship between luteal and follicular phase duration, suggesting a possible regulatory role of the follicular phase in maintaining a relatively consistent cycle duration within individuals. Cycle durations did not appear to change over time, and the first pubertal cycle was similar to subsequent cycles. Major life events (births, deaths, and changes in herd structure) had minimal effect on cycle dynamics in this group of elephants. Our next goal is to extend this database analysis to other facilities holding Asian elephants and evaluate hormonal relationships on an individual, herd, and population basis. Understanding the complexities of herd reproductive characteristics could facilitate the development of more institution-specific management strategies to maximize the health and reproduction of captive elephants.

### **Endocrine and Behavioural Correlates of Musth in the two Phenotypes of Male Asian Elephants (*Elephas maximus*)**

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Male Asian elephants exhibit phenotypic variation in secondary sexual characters, with respect to the presence of tusks - males possessing tusks (tuskers) and others without tusks (tuskless males, locally known as makhnas). Tusks appear to be an ornamental trait, probably conferring a sexual advantage to the males in attracting mates, but nothing is currently known about alternative strategies followed by makhnas to compensate for the lack of tusks. Both tuskers and tuskless males show periods of heightened sexual activity, thereby expressing physical signs of musth typically characterized by copious secretions from swollen temporal glands and continuous discharge of urine over lengthy periods of time. The state of musth is associated with distinct changes in the endocrine milieu, but till date it has not been investigated whether the levels of reproductive or adrenocortical steroids of musth males differ between the two phenotypes. Therefore, our aim is to describe the behavioral characteristics and endocrine correlates of musth in both phenotypes of Asian elephants, through non-invasive monitoring of androgen and glucocorticoid metabolites in elephant feces.

The study is carried out on wild and semi-captive animals in Mudumalai Tiger Reserve, Tamil Nadu and Kaziranga National Park, Assam, India. Since April 2011, fresh fecal samples have been collected by following adult males (>20yrs), during musth (M) and non-musth (NM), and collection continues until June 2011. So far, 73 samples (tusker: 15/29 M/NM, tuskless: 14/15 M/NM) have been collected from 69 different bulls. Feces will be lyophilized and approximately 0.05g dry material extracted with 3ml (80% ethanol in water). The extracts will be analyzed using two enzyme immunoassays, measuring epiandrosterone (EA) and metabolites with a 5 $\beta$ -3 $\alpha$ -ol-11-one structure (3 $\alpha$ ,11oxo-CM), which have been shown to reliably reflect testicular and adrenocortical endocrine function respectively in elephant bulls. Preliminary results for tuskers (n = 3/12 M/NM) revealed no musth-related changes in both EA levels (1.08/1.2  $\mu$ g/g DW M/NM), and 3 $\alpha$ ,11oxo-CM concentrations (0.13/0.20  $\mu$ g/g DW M/NM). Since only 15 samples of tuskers are currently analyzed, it is difficult to substantiate the initial findings. Moreover, the results for tuskless bulls are pending. Therefore conclusions will be drawn after hormone analysis is completed.

## **Seasonal Changes in Steroid Hormone Profiles, Body Weight, Semen Quality, and Accessory Sex Organs In Captive African Wild Dogs (*Lycaon pictus*) in South Africa**

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Many endangered African wild dog (*Lycaon pictus*) populations are fragmented which facilitates inbreeding. In South Africa, to conserve and prevent inbreeding in this species a reintroduced meta-population has been established. Characterization of reproductive seasonality in wild dogs could assist the on-going reintroduction program. The objectives of this study were to: 1) assess year-round male reproduction through fecal (FT) and serum testosterone (ST) concentrations, body weight, total testis (TTV) and prostate (PV) volume, preputial gland measurements, and ejaculate analysis; 2) characterize seasonal estrous cyclicity and gestation through fecal progestagens (P) and behavioral observations. FT concentrations (n=14) were significantly higher late winter to early summer ( $246.4 \pm 14.5$  ng/g) than mid-summer to early winter ( $218.6 \pm 13.4$  ng/g). ST was significantly higher in mid-summer ( $1.4 \pm 0.3$  ng/ml; n=4) than late winter ( $0.7 \pm 0.1$  ng/ml; n=18). Body weight did not differ by season. Number of spermatozoa per ejaculate was greatest in late summer ( $301.4 \pm 39.3 \times 10^6$ ; n=5). Other semen parameters reached peak levels in mid-summer (pH: 7.4; progressive motility:  $85.0 \pm 0.1$  %; live spermatozoa:  $81.0 \pm 16$  %; normal morphology:  $71.5 \pm 8.2$  %; n=4). TTV and PV were significantly greater during mid and late summer (TTV:  $36.7 \pm 4.2$  cm<sup>3</sup>; PV:  $12.0 \pm 1.9$  cm<sup>3</sup>; n=9) than late winter (TTV:  $25.2 \pm 1.9$  cm<sup>3</sup>; PV:  $5.8 \pm 0.8$  cm<sup>3</sup>; n=21). Preputial pendulance was significantly greater in mid and late summer ( $7.1 \pm 0.5$  cm; n=9) than late winter ( $5.9 \pm 0.2$  cm; n=21). Baseline P was  $6.2 \pm 2.5$  µg/g and peak P was  $14.7 \pm 2.8$  µg/g (n=4). Female wild dogs (n=4) were seasonally monoestrous with primary cyclicity in mid to late summer and secondary cyclicity in mid-winter. The luteal period was 21-42 days (n=3) and gestation was  $71.0 \pm 0.1$  days (n=2). Copulations resulting in viable pregnancies (n=2) occurred in late summer. African wild dogs are reproductively seasonal. Improvement in male reproductive parameters occurs during summer. Female cyclicity occurs primarily during summer with a secondary adaptive period in mid-winter. Information on reproductive seasonality generated in this study may assist on-going wild dog reintroductions in South Africa.

## **Seasonal Changes in Hormonal Status in Tropical Cat Species at the Northern Edge**

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Four cat species lives at the Russian Far East near the border with North Korea and China: Siberian tiger (*Panthera tigris altaica*), Amur leopard (*Panthera pardus orientalis*), bengal cat (*Prionailurus bengalensis euptilura*) and Eurasian lynx (*Lynx lynx stroganovi*). All of them but not the last are tropical species. They live at the northern edge of the species range under extreme conditions of seasonal temperature changes (from -40 to +40 C). These changes need to be corresponded with the changes in metabolic rate and behavioral traits. We tried to analyze the seasonal changes in glucocorticoids (GK) level and to correspond them with weather conditions. We collected feces samples in the wild (Russian Far East) and in captivity (Moscow and Novosibirsk zoo, biological station Tchernogolovka). All samples were collected shortly after excretion excluding the winter period when the old frozen samples were also used for the

analyses. Extraction of steroids was conducted with 90% methanol during 30 minutes and supernatant was used for the analyses. Commercial kits (Immunotech, Russia) for cortisol assay were validated for measurements of GK in feces of these cat species. Tigers and leopards did not show any sex and seasonal differences in feces GK level in captivity, but wild tiger had higher GK levels than captive animals. It may be related to the increase of animals' movements following the main prey (wild boars). Wild leopards showed the same level of feces GK as the captive ones. The movements of leopard main prey (Sika deer) are very restricted in winter and leopard winter movements are much smaller than in tiger. The Bengal cat is poorly adapted to the snow conditions and decreases their activity dramatically to the winter period. Feces GK level increases significantly in winter especially in females which are much smaller than males. To sum up the severe winter conditions (snow cover and low temperatures) are critical only for the small cat but for the big cats the hunting strategy plays an important role in GK level regulation.

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**Session Moderator: Mandi Vick, Cleveland Metroparks Zoo**

**Session IV: Recent Advances in Basic Endocrinology as Applied to Reproduction (cont'd)**

**Homonal Evidence of the Autonomous Ovary in the Tasmanian Devil (*Sarcophilus harrisi*)**

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The Tasmanian devil is the largest extant carnivorous marsupial. Marsupials are unique in that young are born in an embryonic form and continue their development within the pouch. Devils are seasonally restricted, with peak breeding occurring during the first oestrous cycle in Austral Autumn (March-May). Young are born after a short two week gestation and only 2 days after implantation. The objective of this study was to use non-invasive hormone monitoring to develop a method of pregnancy detection for the Tasmanian devil to assist captive management. Faecal progesterone (PM) and prostaglandin F<sub>2α</sub> metabolite (PGFM) concentrations were quantified by enzyme-immunoassay for 10 confirmed pregnancies and 6 non-mated oestrous cycles. The duration and faecal PM concentrations of the luteal phase were not different between groups (P < 0.5). An acute increase in faecal PGFM concentrations was detected in both pregnant and non-conceptive females during the last third of the luteal phase, which declined in parallel with PM concentrations. The similarities in the patterns of progesterone secretion and the production of PGFM regardless of reproductive state provide evidence of an autonomous ovary. Hormonal changes associated with the maintenance of pregnancy appear to occur regardless of conception in this species. This may be related to the short gestation and minimal physical contact with the growing embryo and the uterus. This also suggests that it is unlikely that pregnancy can be detected by non-invasive measures.

**Use of Urinary Biomarkers of Ovarian Function in the Chinchilla: a Comparative Study of Two Ovulation Induction Protocols**

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Intensive hunting for fur placed chinchilla populations at the brink of extinction (IUCN critically endangered and Appendix I of CITES). A hybrid of the two chinchilla taxa has been bred for fur production, providing a unique model to develop procedures that could be applied to their endangered counterparts. However, a key aspect for the implementation of captive breeding programs is the study of the species' reproductive physiology. Attempts to obtain repeated blood samples from chinchillas were unsuccessful because of small vein size and their stress-susceptible nature. Therefore, non-invasive techniques provided a unique opportunity, allowing long-term endocrine monitoring. The objective of this study was to use urinary biomarkers of ovarian function to assess two protocols for exogenous regulation of the hypothalamus-hypophyseal-gonadal axis. Females were induced using eCG alone (Novormon, Syntex, 30 I.U., n=6) or GnRH (Gonasyn, Syntex, lecorelin acetate 0.2 mL, n=4). Hormones assessed in 24 h urine longitudinal samples before and after injection included creatinine (colorimetric assay from creatinine standard set, Sigma #C3613), pregnanediol glucuronide (PdG, C. Munro R13904), estrone conjugate (EC, C. Munro R522-2), LH and FSH (NIDDK-anti rat LH-RIA-S118rabbit AFPC697071P; NIDDK-anti rat FSH-RIA-11 AFP-C0972881, National Hormone & Peptide Program, CA, USA). Comparative profiles of progesterone (P) and estradiol (E) metabolites (I125 RIA kits, Coat-A-Count, Siemens) and the occurrence of vaginal opening (as external sign of oestrus) were also determined. After eCG injection, vaginal opening was observed after  $4 \pm 0.6$  days in 93.1 % of the females. Elevation of urinary PdG and EC metabolites above baseline occurred after 8 and 10 days respectively, reaching values of  $2720 \pm 1109.8$  and  $22.5 \pm 9.7$  ng/mg creatinine. Ovarian stimulation using GnRH resulted in less pronounced peaks of hormonal activity, indicating a lower response to the treatment (PdG:  $1463 \pm 620.8$  EC:  $19.5 \pm 6.7$  ng/mg creatinine). Vaginal opening occurred after  $3.1 \pm 0.9$  days in 76.9 % of the females. Similar profiles were obtained using E or P determinations, yet metabolite concentrations were significantly lower. Occurrence of LH and FSH activity peaks were variable among individuals. These data provide promising results for manipulating the cycle in female chinchillas, a first step towards establishment of a protocol for assisted reproduction.

### **Spontaneous Ovulation in Jaguars (*Panthera onca*)**

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In preparation for artificial insemination (AI) procedures, Jacksonville Zoo keepers trained two jaguar females to stand cooperatively for vaginal stimulation when estrous behaviors were observed. To follow the females' ovarian activity, fecal samples were collected three times per week and analyzed by enzyme immunoassay for fecal progesterone and estradiol metabolites. Between May 2010 and February 2011, training and vaginal stimulation did not occur during every estrus. Nonetheless, ovulation occurred regularly in both cats (5 and 4 ovulations, respectively), suggesting spontaneous ovulation in at least 40-50% of the cycles. Spontaneous and induced ovulatory cycles did not differ ( $p > 0.05$ ) in luteal progestagen concentrations. Cycle length ( $72 \pm 5$  days) in these females was longer than previously reported for jaguars ( $47 \pm 5$  days; Wildt et al., 1979), but the length of known spontaneous cycles ( $76.7 \pm 5.2$  days;  $n = 3$ ) did not differ ( $p > 0.05$ ) from that of presumably induced cycles ( $71.0 \pm 11.9$  days;  $n = 3$ ). In general, elevations in estradiol metabolite concentrations were observed during estrus for one female, but they occurred in the middle of some luteal and follicular phases for the second. A spike in estradiol metabolites was observed immediately following declining progesterone metabolite concentrations in the first female, possibly corresponding to the removal of progesterone

inhibition on the hypothalamic-pituitary-gonadal axis and stimulation of follicular development. These findings are important as ovulation in jaguars was believed to be induced (Sadleir, 1966; Wildt et al., 1979).

### **Using Endocrinology to Understand Basic Reproductive Biology and Develop Assisted Reproductive Techniques in an Endangered Equid, the Persian Onager (*Equus hemionus onager*)**

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Assisted reproduction can aid in preserving the genetic diversity of small populations. However developing advanced techniques first requires a sound understanding of reproductive biology in each wildlife species. Our objective was to use non-invasive hormone monitoring to understand reproductive patterns in 11 female onagers. Urine samples (n=3,086) diluted in PBS were assayed for estrone conjugates (1:50 dilution; antiserum R522<sup>a</sup>) and progesterone metabolites (1:125 dilution; antiserum CL425<sup>a</sup>). For both assays, intra- and inter-assay coefficients of variation averaged <15% and samples spiked with a standard demonstrated >90% recovery. Female onagers exhibited seasonal reproductive activity from June to November, with cycles averaging 25.1 ± 3.3 days (mean ± SD) and a luteal length of 15.1 ± 1.7 days. In addition, four lactating females exhibited varying degrees of estrous cycle suppression. These data were applied in conjunction with ultrasound examination of ovarian activity to follow natural reproductive cycles and time artificial insemination (AI) of three females. Using daily assay of urine samples, females were monitored until luteolysis occurred, after which ultrasound was conducted every 48 h until a 20 mm follicle was observed. An ovulation inducing agent (Deslorelin, 1 mg, IM) was administered and females inseminated 24 h later. Two of three females produced healthy foals after ~11 months gestation, resulting in the birth of the first wild equids produced by artificial insemination with fresh (n=1) and frozen-thawed (n=1) semen. Our future goal is to use this technique to manage genetic diversity in small populations of endangered equids.

<sup>a</sup>Antiserum obtained from Coralie Munro, University of California, Davis

### **Determining Biological Factors that Influence Mate Choice in the Endangered Black-footed Ferret**

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Black-footed ferrets (*Mustelanigripes*; BFF) were rescued from extinction when the last 18 individuals were brought into captivity to initiate a breeding program in 1985. Currently, >7,000 individuals have been produced and ~2,500 animals have been reintroduced into 19 sites in eight states, Mexico and Canada. BFF recovery is limited by habitat, disease and reduced fecundity (pregnancy rates dropping from 80% to 46%). Failed pregnancies are attributed either to female behavior (choosing to not mate with the male), failed ovulation and/or pregnancy. Because BFFs are induced ovulators, this species provides an opportunity to investigate cryptic female mate choice in mammals, in which females employ post-copulatory selection. Our goal was to examine whether female mate choice behavior and ovulation are influenced by specific male traits in the BFF population. Our objective was to examine the

role of male androgens, glucocorticoids and seminal quality on female ovulation and overall breeding success. Methods included fecal androgen and glucocorticoid metabolite analysis and semen collection via electroejaculation on six males with high ovulation success (> 50%) and three males with low ovulation success ( $\leq$  50%). Results demonstrated that mean FAM concentrations were similar ( $P > 0.05$ ) between males with high ( $18.1 \pm 2.4 \mu\text{g/g}$  dry feces) and low ovulation success ( $10.3 \pm 1.9 \mu\text{g/g}$  dry feces). However, preliminary analyses suggested a positive relationship ( $y = 0.004x - 4.384$ ,  $R^2 = 0.48$ ) between mean FAM concentrations and ovulation success. Mean FGM concentration did not vary ( $P > 0.05$ ) between the male groups (high,  $4.4 \pm 3.2 \mu\text{g/g}$  dry feces; low,  $4.5 \pm 2.8 \mu\text{g/g}$  dry feces). Seminal results did not suggest a relationship between mean sperm concentration (high,  $274.4 \pm 71.5 \times 10^6/\text{ml}$ ; low,  $276.2 \pm 71.3 \times 10^6/\text{ml}$ ), mean testicular volume (low,  $1.4 \pm 0.1 \text{ cm}^3$ ; high,  $1.2 \pm 0.1 \text{ cm}^3$ ), mean percent normal sperm (low =  $21.3 \pm 4.9\%$ ; high =  $21.9 \pm 5.5\%$ ), or mean sperm motility (low,  $48.3 \pm 9.3\%$ ; high,  $55.0 \pm 5.0\%$ ) with ovulation success. In conclusion, FAMs may be related to a male BFF's ability to induce ovulation in females and warrants further investigation. Funded by the Phoenix Zoo Science and Conservation Fund.

### **Differences in Fecal Testosterone Patterns of Breeding and Non-Breeding Male and Female Kori Bustards (*Ardeotis kori*)**

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To better understand how to manipulate breeding conditions to promote reproduction in captive kori bustards, baseline endocrine studies measuring fecal testosterone in male and female kori bustards were conducted. Feces collected weekly from males and females were analyzed for testosterone using validated enzyme linked immunoassay. Testosterone concentrations for wet and dry extracted fecal samples were significantly correlated ( $r=0.91$ ;  $P < 0.001$ ) so wet weight samples were used for simplicity. Results from adult males ( $n=5$ ), adult females ( $n=10$ ), immature males ( $n=10$ ) and immature females ( $n=10$ ) revealed seasonal patterns of elevated testosterone concentrations in fertile, but not non-fertile adult males and females ( $P > 0.05$ ). Two immature males showed no testosterone increase in their third year, but seasonal increases at four years of age, interpreted as onset of puberty. Adult females that were not maintained in a breeding group, or that did not produce eggs, did not demonstrate increases in testosterone compared to egg laying counterparts. In males, but not females, seasonal testosterone increases were accompanied by weight gain and vocalizations. Peaks in male fecal testosterone ranged from 10- to 22- fold higher than non-breeding season concentrations ( $181.5 \pm 19.1$  vs  $17.0 \pm 0.94 \text{ ng/g}$ ;  $P < 0.05$ ), with one older breeding male displaying peaks >1200 fold higher ( $P < 0.05$ ) than pre-season concentrations. Mean breeding season values for adult males not including data from this male were  $83.6 \pm 6.1 \text{ ng/g}$  vs non-breeding season values of  $12.3 \pm 0.73 \text{ ng/g}$  ( $P < 0.05$ ). In females, average breeding season testosterone concentrations were ~4-fold higher than non-breeding season ( $55.9 \pm 6.0$  vs  $14.5 \pm 1.8 \text{ ng/g}$ ), with peaks 10- to 30-fold higher. Results show that non-invasive fecal testosterone analysis can provide a means of predicting fertility potential of male and female kori bustards and might be utilized to assess effects of modifying captive environments to promote reproduction in this species.



**POSTER PRESENTATIONS:**

**Pregnancy diagnosis based on fecal PGFM as a supportive part of the Iberian lynx captive breeding program**

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PGFM (13,14-dihydro-15-keto-PGF<sub>2</sub>) is known as an inactive metabolite of prostaglandin F<sub>2</sub> $\alpha$  in blood serum. An EIA based on antibody against PGFM was shown to be an easy and reliable method for non-invasive pregnancy diagnosis in the Iberian lynx (and other felids) if applied approximately 3 weeks prior parturition. During the breeding seasons 2010 and 2011, the PGFM assay was used for pregnancy diagnosis within the Iberian lynx captive breeding centers in Spain and Portugal.

In 2010, eleven females mated, and six were correctly diagnosed as pregnant. In 2011, altogether 27 (of 29) female were mated in early spring including 10 very young potential mothers born in 2009 and 2010. Three females mated twice, one after a perinatal loss of cubs. Sixteen females were diagnosed as pregnant, nine as non-pregnant. Thus, pregnancy prognosis has been correct in most cases except one female who was kept together with a male. Here, some of the fecal sample came obviously from the male. Samples from males contained only basal PGFM concentrations that were misinterpreted as "non-pregnant". In three females an abortion was diagnosed according to a steep decrease of PGFM between d40 and d52. One female delivered her cubs prematurely at d62. This was also accompanied by a preceding PGFM drop.

Altogether, 26 of 40 cubs were born alive and nursed adequately by their mothers. Compared to 8 of 14 cubs in the breeding season 2010, this number represents a great progress for the captive breeding centers. But as in the past the younger expectant lynx mothers sadly turned out to be highly sensitive and problematic. Only one primiparous female raised a healthy cub, whereas the other young females did not conceived or aborted their offspring.

Altogether fecal PGFM based pregnancy diagnosis is a very important tool for captive breeding of the critically endangered felid species, Iberian lynx. To meet the requirements of birth management, PGFM diagnosis must be performed in at least ten consecutive samples to be collected till d50. For future applications, a short and easy PGFM assay shall be developed, to reduce the time between sampling and diagnosis. (funded by BMBF 033L046)