

**Proceedings of the Fourth Annual Conference of  
the International Society of Wildlife Endocrinology  
14-16 October 2013  
Lincoln Park Zoo, Chicago, IL, USA**



Lincoln Park  
Zoo



2013 Annual Conference, 14-16 October, Chicago IL, USA

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## Comparing exhibit and education armadillos using behavioral and physiological measures of welfare

### Authors:

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### Topic:

Animal health and welfare

### Abstract:

The AZA Animal Welfare Committee has deemed research on education program animals a priority. However, there are only limited data on potential welfare impacts, positive or negative, of using animals for education programs. Armadillos are commonly maintained in zoos for both education (Ed) and exhibit (Ex), providing a model species for examining possible differences. Fecal samples were collected for 60 d from education (n=28), exhibit (n=16) and off-exhibit (OE; n=13) armadillos. Fecal glucocorticoid metabolites (FGM) were quantified using a commercial corticosterone radioimmunoassay kit (MP Biomedicals), validated with an ACTH challenge. In addition, behavior (1 min scan sampling; 20 min-observations; n=20/animal) data were collected from a subset (n=23) of armadillos. Possible effects of management type, species, sex, age, enclosure size, substrate type and depth, light cycle, and frequency or duration of handling on FGM and behavior were analyzed using a General -and Generalized- Linear Mixed Model, respectively. After controlling for management type, increased handling events per week [ $F_{(1, 1059)}=10.20$ ,  $p=0.001$ ] were associated with increased FGM. Deeper substrates of any variety tended to be associated with lower FGM [ $F_{(1, 46.9)}=2.37$ ,  $p=0.13$ ] and those with no substrate had higher FGM compared to those with cardboard, dirt, or straw ( $p < 0.05$ ). Shallow substrate depth and high handling frequency were also associated with increased undesirable behavior, especially in EX animals ( $p < 0.05$ ). Education animals displayed more locomotion [ $F_{(2, 633)}=2.72$ ,  $p=0.067$ ] than OE animals and more object investigation behavior [ $F_{(2, 12.5)}=12.82$ ,  $p=0.004$ ] than EX animals [ $F_{(2, 12.5)}=12.82$ ,  $p=0.004$ ]. The results of this study suggest that the frequency of handling and substrate provided in the enclosure have the most significant effect on FGM and behavior, and should be considered carefully in husbandry decisions, regardless of management type.



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## **Effects of environmental stressors on the health of female black rhinoceros (*Diceros bicornis bicornis*) in Addo Elephant National Park, South Africa**

### **Authors:**

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### **Topic:**

Animal health and welfare

### **Abstract:**

Black rhinoceros are an icon for international conservation, yet relatively little is known about their ecology due to their secretive nature. To overcome these challenges, non-invasive techniques were used to monitor the Southwestern arid subspecies (*Diceros bicornis bicornis*) in two sections of Addo Elephant National Park, South Africa, Main Camp and Nyathi. These sections were separated by a highway and the numbers of elephants, predators and tourists were higher in Main Camp. Because biotic factors could impact rhino health, our goal was to investigate how population demographics and hormonal activity varied between the sections and with respect to season, climate and rhino age. Fecal samples (n=231) were analyzed using EIA for progesterone (FPM; #CL425, C. Munro, UC Davis) and glucocorticoid (FGM; #CJM006, C. Munro, UC Davis) metabolite concentrations and were screened for parasites. Mean FPM concentrations from pregnant females were seven times higher ( $P < 0.05$ ) than samples from non-pregnant rhinos. Positive relationships were found between monthly temperatures and FPM from non-pregnant females ( $P < 0.05$ ) and percentage of calves born ( $P < 0.05$ ). The majority of calves (40%) were conceived in the spring, but no seasonal patterns were found with respect to conception and parturition months. FGM concentrations were lowest ( $P < 0.05$ ) in the winter. Females in Main Camp and Nyathi had similar ( $P > 0.05$ ) FPM concentrations; however, Main Camp rhinos had higher FGM concentrations and lower prevalence of ciliates compared to rhinos in Nyathi ( $P < 0.05$ ). Females in Main Camp also had a longer inter-calving interval and were less likely to be pregnant ( $P < 0.05$ ). These data suggest that Main Camp females may be experiencing lower population health than Nyathi rhinos, possibly due to biotic stressors such as predators, competitors and/or tourists. This knowledge can help guide SANParks conservation strategies for the successful management of black rhinos.





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## **The effect of atypical life histories on hair cortisol concentrations in captive chimpanzees**

### **Authors:**

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**Presenter and contact person:** Hani D. Freeman, hani.freeman@gmail.com

### **Topic:**

Animal health and welfare

### **Abstract:**

Chimpanzees exist in a wide range of settings in the United States including their use as pets and performers. As such, they may experience very atypical life histories, including high exposure to humans rather than conspecifics during important developmental stages of their lives. We sought to assess the impact on of these atypical histories through a variety of behavioral and physiological measures. We collected between 2-4 hair samples from 68 chimpanzees (30 former pet/performers) currently living at accredited zoos or sanctuaries to obtain a long-term measure of cortisol. We used a cortisol enzyme immunoassay (#R4866, C. Munro, University of California, Davis, CA). Using a unique index (Chimpanzee-Human Index, CHI) that we developed to characterize the degree of human and chimpanzee exposure these chimpanzees experienced throughout their lives, we compared how atypical histories relate to hair cortisol concentrations. Overall, males had higher cortisol concentrations compared with females ( $F = 20.27$ ,  $df = 66$ ,  $p < .00001$ ). There was a significant effect of the adult CHI value as well as the lifetime CHI value on average hair cortisol concentrations ( $F = 2.97$ ,  $df = 7$ ,  $p = .012$ ;  $F = 2.0$ ,  $df = 35$ ,  $p = .03$ ). These results indicate that chimpanzees who spent more time with conspecifics as adults and throughout their lifetime, express lower hair cortisol concentrations later in life, suggesting they are less susceptible to generalized social stressors compared with chimpanzees that have less typical early histories. These findings help characterize some of the long-term effects borne by chimpanzees maintained as pets and performers. Future research should refine methods for assessing the long-term consequences of atypical early histories experienced by chimpanzees and determine means to address the challenges they may face as they adapt to more standardized care and housing later in life.



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## **Population assessment of wild Belizean felids through non-invasive genetic, glucocorticoid metabolite, parasite and diet analyses**

### **Authors:**

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### **Topic:**

Animal health and welfare

### **Abstract:**

Some Belizean felids, such as jaguars, are threatened with extinction because of habitat destruction and/or human persecution. Factors impacting the well-being of these cats remain unknown. Human activities around protected areas in Belize are increasing and so are levels of human-felid conflict (H-FC). Potential consequences of this conflict are an increase in stress that negatively impacts health, or heightening animal aggression resulting in more conflict. Our goals were to examine the effects of human disturbance and H-FC on Belizean felids, and identify sources of deleterious stress to formulate holistic management plans. Using a detector dog, we collected scat for DNA, fecal glucocorticoid metabolite (FGM), parasite and diet analyses of Belizean felids from areas of varying human disturbance. Scat surveys were conducted every 4 days, which was shown in a prior hormonal degradation study to be an adequate interval. DNA was analyzed to assign individual identity to the scat. Parasites and diet remains were identified using differential separation techniques. Feces for FGM assessment were freeze-dried and homogenized, and the steroids extracted by boiling the sample in ethanol (90%v/v). FGM were measured using two immunoassays: EIA cortisol R-4866 (C. Munro) or RIA <sup>125</sup>I corticosterone (MP Biomedicals), as previously described for each species. Over 300 scat samples were collected, but only 30% amplified for DNA. Five felid species were detected: jaguar, puma, ocelot, jaguarundi and domestic cat. Techniques proved valid for assessing FGM in these species, and concentrations in general were lower than in zoo-housed individuals, yet causes of these differences remain inconclusive. We found no evidence of felids preying on livestock. Geographical distribution of samples and physiological measures suggest that the degrees of disturbance in study areas did not compromise overall animal well-being. This study shows the feasibility of assessing FGM in free-ranging felids and how it could be applied to conservation.





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## **Role of life history traits in hyperprolactinemic female African elephants (*Loxodonta africana*)**

### **Authors:**

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### **Topic:**

Animal health and welfare

### **Abstract:**

In 2004, a third of captive African elephants with abnormal cycles were found to produce excessive amounts of prolactin. Since then the hyperprolactinemic rate in reproductively aged females has increased significantly to over three quarters. This is concerning not only because hyperprolactinemia is thought to be contributing to the high rates of infertility in the population, but because of the potential long-term health impacts that could be diminishing quality of life for affected individuals. We have examined several physiological factors known to be associated with hyperprolactinemia-induced infertility in women, but found no such relationships in African elephants. Human studies have suggested a role of life events in uncovering a person's vulnerability to an endocrine disorder. We categorized life events as change situations involving entrances and exits (i.e. transfers, and exposures to births and deaths of conspecifics). The symptoms of hyperprolactinemia often have an acute onset with close temporal associations to important life events experienced by the patient. Therefore, the specific aim of this study is to examine the life history of hyperprolactinemic female African elephants to determine if such an association can be found. We hypothesize that hyperprolactinemic elephants may be susceptible to social and environmental stressors as are hyperprolactinemic human patients, and therefore will have experienced an increased number of life events when compared to normal cycling elephants. We have compiled life history information for female African elephants in North American zoos, regarding exposures to births, deaths, and the number of transfers each elephant experienced. Preliminary results indicate that on average African elephant females have experienced 2.47 transfers (range: 0-11), 2.42 births (range: 0-11), and 2.50 deaths (range: 0-15). We will compare this information to prolactin status (normal, low, moderate, hyper) in order to better understand the etiology of this condition in the captive population.



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## **Monitoring adrenocortical activity, fertility, and behavior in two newly introduced gorillas (*Gorilla gorilla gorilla*)**

### **Authors:**

<sup>1</sup>Raechel Jacobs; <sup>2</sup>Steven Ross; <sup>2</sup>Kathy Wagner; <sup>2</sup>Maureen Leahy; <sup>3</sup>Susan Meiers; <sup>2</sup>Rachel M Santymire

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### **Topic:**

Animal health and welfare

### **Abstract:**

In April 2010, a sexually mature female western lowland gorilla (*Gorilla gorilla gorilla*) was moved to Lincoln Park Zoo (LPZ; Chicago, IL) for breeding following the Gorilla Species Survival Plan<sup>®</sup> recommendations. The objectives of this study were to: 1) monitor changes in behavior and hormones during the introduction of the female to the silverback and his troop; 2) monitor stress hormones (via fecal glucocorticoid metabolite analysis; FGM) of the male and female during the different stages of introduction; and 3) monitor reproduction events using behavior and fecal progesterone metabolite (FPM; female) and fecal androgen metabolite (FAM; male) analyses. Hormones were monitored prior to, during and immediately following the social introduction and quantified using an enzyme immunoassay (EIA) using cortisol polyclonal antiserum (R4866), progesterone monoclonal antiserum (CL425), and testosterone polyclonal antiserum (R156/7). Behavioral data were also collected using instantaneous scan sampling every 30 seconds for ten minutes. Results demonstrated that mean FGM ( $20.61 \pm 0.83$  ng/g) for the male was elevated ( $P=0.002$ ) during the introduction compared to the pre- and post-introduction phases ( $11.31 \pm 0.48$  ng/g and  $12.42 \pm 0.65$  ng/g, respectively). For the female, mean FGM ( $17.91 \pm 1.07$  ng/g) was lower ( $P<0.001$ ) post-introduction than during the pre- and introduction phases ( $30.50 \pm 3.42$  ng/g and  $27.38 \pm 1.51$  ng/g, respectively). The stress response during the introduction phase was not sufficiently elevated to suppress reproductive hormones as evidenced by normal FPM cyclicity ( $32.5 \pm 8.1$  days long, range 15-71 days,  $n=5$  cycles) and a confirmed pregnancy (mean FPM concentration:  $414.5 \pm 26.3$  ng/g) in the post-introduction phase. In conclusion, this study demonstrates the importance of both behavioral and physiological monitoring of zoo animals and suggests that the potential stress of social introductions may not be disruptive to concurrent reproductive success in this species.



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## **Assessment of animal welfare in semen collection in tigrina (*Leopardus tigrinus*) through serum cortisol**

### **Authors:**

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**Presenter and contact person:** Rodrigo Neca Ribeiro, rodrigonribeiro@hotmail.com

### **Topic:**

Animal health and welfare

### **Abstract:**

Semen collection in felids is needed to assess male fertility and for use in assisted reproduction programs. Wild animals must be anesthetized to be submitted to electroejaculation. The objective of this work was to evaluate animal welfare using serum cortisol as a biomarker, after capture and sedation and after electroejaculation. Adult male tigrinas (*Leopardus tigrinus*, n= 12) were submitted to semen collection on different occasions (n=32) at the Itaipu Captive Breeding Wild Animal Center (CASIB) in Foz do Iguaçu-PR, Brazil. The animals were captured using a hand net (time 0 min), anesthetized with tiletamine-zolazepam (6,7mg/kg) and xylazine (1mg/kg), both IM, and clinically evaluated and electroejaculated. In all procedures, semen samples were obtained. Blood was collected from the jugular vein before (time 20 min) and after electroejaculation (time 60 min). Serum cortisol concentrations were determined by chemiluminescence. Some samples (15%, n=12) were also analyzed using a radioimmunoassay, resulting in a high correlation (r=0,97; p=0,0002) between methods. The assay used was Immulite cortisol, a solid phase chemiluminescent enzyme immunoassay, with polyclonal rabbit anti-cortisol antibody. Serum cortisol (mean+SEM) decreased (p=0.0009) after electroejaculation (5.01+0.53 mg/dl[time 60 min] versus 3,02+0,52mg/dl [time 20min]), suggesting that the anesthesia protocol was effective and the acute stress generated by capture was greater than the stress generated by semen collection.



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## **Aerial temperature as crucial factor for the Siberian tiger (*Panthera tigris altaica*) well-being in Russian Far-East**

### **Authors:**

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### **Topic:**

Animal health and welfare

### **Abstract:**

Winter conditions in Russian Far-East are extremely severe with low air temperatures (less than  $-35^{\circ}\text{C}$ ) and deep snow (more than 70-100 cm) making the movements of the carnivores and their hunting very difficult. The aim of this study was to evaluate if aerial temperatures were related to adrenal activity as a measure of animal welfare in Siberian tigers in Russian Far-East. This study was conducted in Volokolamsk Breeding Center of Moscow Zoo, Novosibirsk Zoo and on six experimental areas in the wild at the Russian Far-East. In captivity tigers' feces ( $n=10$  animals, 360 samples) were collected weekly few hours after defecation to describe the seasonal patterns of cortisol metabolites. In the wild, feces samples ( $n=187$  samples) and aerial temperatures were collected during the winter months from known Siberian tiger routes in the forest. Fecal extraction was conducted with 90% methanol (0,1 g of feces : 0,9 ml). Cortisol metabolites concentration was determined by previously validated commercial ELISA kits (Immunotech, Moscow, Russia) that were validated previously. Significant negative correlation was described for aerial temperature and feces cortisol metabolites level in each study site (wild, Volokolamsk and Novosibirsk;  $p \leq 0,06$ ). This correlation was more obvious when the temperature differences were higher and the lowest temperatures were below  $-10^{\circ}\text{C}$ . The increase of cortisol metabolites level may be related with the increase of metabolic rate in this tropical species under the low temperatures. There was a tendency that experiencing the same low temperatures animals from southern populations had higher cortisol metabolites level than northern ones ( $p < 0,1$ ). In captivity there were no seasonal differences in fecal cortisol metabolites, because environmental conditions are relatively more constant than wild conditions. An effect of temperature should be considered in wildlife studies trying to estimate the influence of crucial factors on animal welfare. This study was supported by RFBR №13-04-01465.

## **Blow-samples as substratum to extract and quantify reproductive and adrenal hormones of beluga whales (*Delphinapterus leucas*)**

### **Authors:**

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### **Topic:**

Methodologies

### **Abstract:**

The primary objective of this study was to determine if it is possible to measure cortisol, progesterone and testosterone using blow-samples collected from beluga whales (*Delphinapterus leucas*), as has already been done for other cetacean species. Subsequent goal was to evaluate the effects of blood sampling on blow-cortisol levels. Samples were collected from a dyad of beluga whales (*Delphinapterus leucas*) housed at the Oceanographic Park, Valencia, Spain. Sampling was carried out in the early morning using routine husbandry training and on a voluntary basis. Blow-samples (female=40, male=34) were collected with polypropylene jars three times a week from June to August 2012. Samples were processed via radioimmunoassay (RIA), supported by RFO-Università di Bologna. The assay procedure included petroleum ether extraction for progesterone and ethyl ether extraction for testosterone as well as cortisol. Self-made polyclonal rabbit anti-progesterone (1:20000 final dilution), anti-testosterone (1:70000) and anti-cortisol (1:40000) antibodies were used. The average cortisol concentrations measured  $27.43 \pm 3.48$  pg/ml ( $\pm$ SEM) for female and  $12.08 \pm 1.79$  pg/ml ( $\pm$  SEM) for male. Male's average testosterone levels measured  $9.48 \pm 2.45$  pg/ml ( $\pm$  SEM). Female's progesterone average was  $79.39 \pm 17.52$  pg/ml ( $\pm$  SEM). An estrous cycle was monitored, blow progesterone concentrations increased after GnRH administration to induce ovulation. Weekly progesterone concentrations suggest the cyclical nature of progesterone blow-values that were comparable with the follicular development monitored by trans-abdominal ultrasonography. Non-invasive samples were also collected immediately before and after the blood sampling procedures. Higher ( $p < 0.011$ ) cortisol concentrations have been detected from blow samples collected after blood sampling (i.e. 9.94 vs. 18.97 pg/ml, 11.07 vs. 19.77 pg/ml ( $\pm$ SEM)). Overall, these data confirm that beluga's blow-samples are a viable alternative substratum to extract and quantify reproductive and adrenal hormones.



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## **Detection of pregnancy and fertility status in big cats and ungulates using an enzyme immunoassay based on 5 $\alpha$ -pregnane-3 $\alpha$ -ol-20-one**

### **Authors:**

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### **Topic:**

Methodologies

### **Abstract:**

Development of non-invasive steroid hormone assays using fecal samples is crucial for detection of pregnancy and monitoring of fertility status in big cats and ungulates, and thus facilitates conservation and management of wild animals. Due to changes in metabolism and excretory pattern, animals excrete different steroid metabolites in feces and urine. The present study is an attempt to develop a common enzyme immunoassay for 5 $\alpha$ -pregnan-3 $\alpha$ -ol-20-one one of the predominant progesterone metabolites in the feces samples of big cats (Asiatic lion, Indian tiger, leopard and jaguar) and ungulates (black buck and cattle). The present ELISA showed a high sensitivity and low cross reactivity to other hormones compared to commercially available RIA kits based on progesterone antibody. Fecal progesterone measured by developed ELISA and commercial RIA kits showed identical pattern and high correlation ( $r = 0.96$ ,  $n = 38$ ). However, the progesterone values were significantly higher in the ELISA than in the RIA ( $P < 0.0001$ ). Fecal progesterone metabolites concentrations could be monitored for big cats and ungulates using the developed ELISA. Concentrations of 5 $\alpha$ -pregnan-3 $\alpha$ -ol-20-one in feces of lions ( $P < 0.001$ ), tigers ( $P < 0.001$ ), jaguar ( $P < 0.001$ ), black buck ( $P < 0.001$ ) and cattle ( $P < 0.001$ ) were significantly higher compared to the concentrations in non-pregnant animals all across the pregnancy period. Further, pseudo-pregnancy could also be detected in leopards using this ELISA. The present ELISA may prove to be a universal and versatile assay for assessment of fecal progesterone metabolite profiles in a wide range of animals and including other species of big cats and ungulates.





2013 Annual Conference, 14-16 October, Chicago IL, USA

## **An improved method for enzyme immunoassay analysis of androgens in polar bear (*Ursus maritimus*) urine using enzymatic hydrolysis**

### **Authors:**

<sup>1</sup>Karen J Steinman; <sup>1</sup>Justine K O'Brien; <sup>2</sup>Terri L Roth; <sup>1</sup>A J Ho; <sup>2</sup>Erin Curry; <sup>3</sup>G Alan Fetter; <sup>1</sup>Todd R Robeck

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**Presenter and contact person:** K J Steinman, karen.steinman@seaworld.com

### **Topic:**

Methodologies

### **Abstract:**

Validation of androgen enzyme immunoassays (EIAs) in the polar bear has been performed for fecal extracts and neat urine using the polyclonal antibody R156/7 (C. Munro, UC Davis, CA, USA). Because the former antibody cross-reacts with testosterone (100%) and 5 $\alpha$ -dihydrotestosterone (57%) but exhibits low (<0.5%) cross-reactivity with testosterone metabolites commonly found in mammalian urine, it was hypothesized that hydrolysis treatment of polar bear urine (PBU) prior to EIA may provide a better index of androgen secretion during different reproductive states. Enzymatic hydrolysis (EH) of urinary conjugated steroids using  $\beta$ -glucuronidase/arylsulfatase ( $\beta$ G) followed by steroid extraction has been reported in other species. The objective of this study was to validate the analysis of androgens in PBU following EH without subsequent extraction. Acidic buffers are recommended for EH; to determine if buffers and/or pH negatively influenced EIA parameters,  $\beta$ G (0.02ml) was added to known concentrations of testosterone (T, range 600–1.95 pg/well; n=4) reconstituted in three different buffers: (i) 0.2M phosphate buffered saline (PBS), pH 7.0 (PBS-7.0); (ii) 0.2M PBS, pH 5.0 (PBS-5.0); (iii) 1M acetate buffer, pH 5.0 (AB-5.0). Samples were incubated (16 h, 37°C) then assayed. Correlation of standards +  $\beta$ G (r, P) and recoveries of T (% $\pm$ sd, r<sup>2</sup>) reconstituted in each buffer were: PBS-7.0, r=0.992, P<0.05, 128.1 $\pm$ 20.9%, r<sup>2</sup>=0.998; PBS-5.0, r=0.998, P<0.05, 113.5 $\pm$ 13.5%, r<sup>2</sup>=0.998; and AB-5.0, r=0.997, P<0.05, 174.1 $\pm$ 15.8%, r<sup>2</sup>=0.998. Percent binding of pure buffer (% $\pm$ sd) +  $\beta$ G was 87.0 $\pm$ 5.4%, 89.1 $\pm$ 1.5%, and 60.6 $\pm$ 8.7% (n=8) for PBS-7.0, PBS-5.0 and AB-5.0, respectively. Due to lower non-specific binding, PBS-5.0 was selected as the EH buffer. Androgen concentrations of neat PBU and those of hydrolyzed PBU assayed directly and after extraction in diethyl-ether were highly correlated (r $\geq$ 0.954), but were ~ seven-fold lower for the former. These results indicate that accurate analysis of PBU androgen concentrations can be achieved following EH without an extraction step.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Pregnant, or not pregnant, that is the question**

### **Authors:**

<sup>1</sup>Lara C Metrione

<sup>1</sup>South-East Zoo Alliance for Reproduction and Conservation

**Presenter and contact person:** Lara C Metrione, Lara.Metrione@sezarc.com

### **Topic:**

Methodologies

### **Abstract:**

More than 12 years of serum progesterone data from female white rhinos (*Ceratotherium simum*) at White Oak previously provided benchmark values for pregnancy diagnosis and even approximations of gestation progress when breeding dates were uncertain. Recently however, as pregnancy diagnoses for white rhinos at other institutions were attempted, remarkable disparities in non-pregnant serum progesterone concentrations were noted. This study used a polyclonal progesterone enzyme immunoassay to assess serum progesterone among 19 non-pregnant females housed at six institutions in order to better characterize the observed variability. The assay was validated by evaluations of parallelism and recovery. Average non-pregnant serum progesterone concentrations ranged from 1.5 to >100 ng/mL, with 17 of the females distributed throughout concentrations <33 ng/mL and two females with average concentrations >100 ng/mL. Within institutions, average non-pregnant serum progesterone concentrations differed by as little as 3.6 ng/mL to more than 97 ng/mL. Average non-pregnant serum progesterone concentrations even increased from 3.4, 3.3, and 14 ng/mL to 10.3, 20, and >100 ng/mL in three females, respectively, after they were moved from one institution to another. Among eight cycling females, variations in both average baseline (1.1-15.9 ng/mL) and average luteal (4.6-25.1 ng/mL) progesterone concentrations were noted, and the amplitude of increase from average baseline to average luteal values in those individuals ranged from 1.8 to 10.1 ng/mL. The magnitude of erraticism in non-pregnant serum progesterone concentrations among this subset of zoo-managed white rhinos poses a significant challenge for pregnancy diagnoses. Future studies will consider other progesterone antibodies and assay systems and the possible effects of diet on progesterone concentrations. In the meantime, these data highlight the necessity of analyzing pre-breeding samples in addition to post-breeding samples for each animal in order for labs to accurately diagnose pregnancy in white rhinos.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## What about running an HPLC immunogram?

### Authors:

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### Topic:

Methodologies

### Abstract:

Measurement of steroids in fecal samples has become a widely used, non-invasive method for evaluating reproductive status and stressful situations in animals. To characterize the fecal metabolites of steroid hormones reacting with given immunoassays, HPLC (High Performance Liquid Chromatography) separations are performed (mainly using reverse phase systems). The eluent is fractionized and an aliquot of each fraction measured in respective immunoassays. Samples derived from radiometabolism studies (animals injected with radiolabeled steroid hormones) are most helpful, as they enable an easy detection of the naturally occurring metabolites by additional measurement of the radioactivity in the fractions. A coelution of radioactive and immunoreactive peaks is a strong indication that formed metabolites are recognized by the antibody of the assay. The present contribution is meant as a synopsis of respective results of a multitude of performed radiometabolism experiments (more than 20 different mammalian and bird species). It will focus on metabolites of glucocorticoids and progesterone. HPLC immunograms proved that steroid hormones are heavily metabolized and thus only negligible amounts (if at all) of the parent compounds are excreted in the feces. They also yielded interesting information about pronounced species and sex differences in formed metabolites. In some species (e.g., red squirrels or cats) polar metabolites prevailed (mostly resisting an enzymatic hydrolysis), whereas in others (ruminants) almost all metabolites are unconjugated. In addition, such methods helped to elucidate a co-measurement of androgen with cortisol metabolites (for example in dogs and elephants). However, HPLC separations need expensive equipment, special expertise and they are time consuming to perform. Thus, they can only be recommended in special occasions, ideally in combination with radiometabolism studies. In any case, most important for a sound application of non-invasive methods in a given species is the proof that meaningful changes of plasma steroid hormones are reflected in the measured fecal metabolites.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Quantification of cortisol, progesterone and testosterone in saliva-samples collected from walrus (*Odobenus rosmarus divergens*)**

### **Authors:**

<sup>1</sup>Pier Attilio Accorsi; <sup>2</sup>Raffaella Tizzi; <sup>3</sup>José Antonio Esteban; <sup>1</sup>Gabriella Postiglione  
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**Presenter and contact person:** Pier Attilio Accorsi, pierattilio.accorsi@unibo.it

### **Topic:**

Methodologies

### **Abstract:**

Evaluation and improvement of well-being of captive marine mammals can be enhanced by monitoring their endocrine conditions. Our specific objectives were to: a) validate a radioimmunoassay for salivary cortisol, progesterone and testosterone of walrus (*Odobenus rosmarus divergens*), b) evaluate the usefulness of non-invasive sampling to obtain basal cortisol levels, and c) evaluate if salivary cortisol concentrations increase following blood collection. Five walrus (*Odobenus rosmarus divergens*) one male and four females, housed at the Oceanographic park, Valencia, Spain, were used as the subjects of the study. Saliva samples (n=167) were collected with sterile cotton wool two times a week during the early morning from unrestrained animals using routine husbandry training methods. The sampling period lasted three months from June through August 2012. Steroid hormones were extracted from saliva samples and measured via radioimmunoassay (RIA) supported by RFO-Università di Bologna, using petroleum ether extraction for progesterone and ethyl ether extraction for testosterone and cortisol. Self-made polyclonal rabbit anti-progesterone (1:20000 final dilution), anti-testosterone (1:70000) and anti-cortisol (1:40000) antibodies were used. Salivary cortisol mean concentration detected for females was  $167.89 \pm 43.41$  pg/ml (SEM), and  $42 \pm 13.68$  pg/ml (SEM) in male. Mean salivary progesterone concentration for females was  $918.72 \pm 128.67$  pg/ml (SEM). Male's testosterone mean concentrations measured  $64.07 \pm 13.46$  pg/ml. A high degree of parallelism ( $p < 0.01$ ) was observed between the standard and the diluted samples hormones' curves, the recovery of the hormones varied from 81.27% to 82.88%. Cortisol concentrations detected from saliva samples collected immediately after the blood sampling were considerably higher than those detected in saliva samples collected before it (e.i. 5654.10 vs. 127.74 pg/ml, 743.45 vs. 59.07 pg/ml, 1119.83 vs. 7.68 pg/ml). Results indicate that walrus saliva sampling can be used as non-invasive steroids monitoring method. Future works may be able to include comparisons of saliva and blood steroids levels.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Effects of sample collection, processing, and storage treatment for validation of an enzyme immunoassay to monitor testosterone in circulation in male sea turtles (*Chelonia mydas* and *Caretta caretta*)

### Authors:

<sup>1</sup>Katie Graham; <sup>1</sup>Catharine J Wheaton; <sup>2</sup>Natalie Mylniczenko; <sup>2</sup>Charlene Burns; <sup>1</sup>Tamara Bettinger

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**Presenter and contact person:** Katie Graham, Katie.M.Graham@Disney.com

### Topic:

Methodologies

### Abstract:

Accurate measurement of circulating testosterone concentration is necessary when assessing contraceptive implant efficacy to reduce testosterone for management of male sea turtles in an aquarium environment. This study validated an enzyme immunoassay (EIA; antisera R156/7, Munro, UC Davis, CA) for testosterone measurement in two male sea turtles by comparing the effects of 1) blood sample collection of serum (untreated, thrombin) or plasma (lithium heparin, sodium citrate, EDTA); 2) sample storage condition (fresh, frozen, 4°C for one week); 3) extraction (unextracted, ethyl ether) and long-term storage of extracts (reconstituted in phosphate buffer or 80% methanol); 4) sample processing (untreated, homogenized); and 5) contraception treatment (dose: four-4.7 mg deslorelin (Suprelorin®) implants in *Chelonia mydas* vs non-contracepted control *Caretta caretta*) in monthly samples collected November 2011-April 2013. Serial dilutions from seasonal baseline and peak periods were parallel with the standard curve only in fresh unextracted, and previously frozen, ether-extracted samples. Following processing, but independent of storage condition, samples were observed to partially gel producing liquid and non-pipettable gel fractions. Sample homogenization did not alter testosterone concentrations producing values comparable to the liquid fraction in gelled samples (student *t*, *P*>0.05). Testosterone concentrations in frozen ether extracts reconstituted in buffer or 80% methanol did not change following three months storage. Seasonal increases in testosterone were observed in the non-contracepted male (*Caretta caretta*) beginning in late fall and peaked in late winter to approximately eight-times concentrations observed during seasonal lows. Deslorelin was not effective when administered after the start of the seasonal increase in testosterone (February 2011, December 2011). However, the seasonal increase was not observed following a third treatment (December 2012). In summary, this testosterone EIA was validated for use with male sea turtles. Results showed serum (untreated or with thrombin) demonstrated parallelism and the most consistent measurements across storage and processing treatments tested.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **The validity of commercial ELISA assays for quantification of fecal corticosterone metabolites in mice**

### **Authors:**

<sup>1</sup>Klas SP Abelson; <sup>1</sup>Otto Kalliokoski; <sup>1</sup>Anne Charlotte Teilmann; <sup>1</sup>Jann Hau

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**Presenter and contact person:** Klas SP Abelson, klasab@sund.ku.dk

### **Topic:**

Methodologies

### **Abstract:**

Commercially available ELISA assays are popular among investigators that quantify fecal corticosterone or cortisol metabolites (FCM) for stress assessment in animals. However, such assays are generally designed for measuring serum glucocorticoids in their native form. These are found in very low levels in feces. Thus, in feces, the assays mainly detect immunoreactive glucocorticoid metabolites. Since different assays contain antibodies of different origin, the detection level and cross-reactivity towards different metabolites and other steroids differ considerably between assays. Thus, the validity of one assay for FCM quantification in stress assessment is not necessarily the same for another assay. The present study was designed to investigate corticosterone in serum and FCM levels in feces of laboratory mice, as quantified in four different ELISA assays (DRG EIA-4164, Demeditec DEV9922, Enzo ADI-900-097 and Cayman EIA kit 500655). Assays were chosen based on origin of antibody (donkey or rabbit anti-sheep IgG), detection level (25-500 pg/ml) and variation in cross-reactivity (mainly regarding differences against progesterone, deoxycorticosterone or 11-dehydrocorticosterone). As expected, all four assays could detect higher serum corticosterone levels in mice treated with ACTH, compared to untreated mice. Unexpectedly though, the measured concentration of serum corticosterone differed significantly between assays, in both groups of mice. In fecal samples, there was an expected tendency towards higher FCM levels in ACTH mice than in non-treated mice, but only in three out of four assays. Similar to serum samples, the measured concentration of FCM also differed between assays. In conclusion, commercially available corticosterone ELISA assays are useful for FCM quantification in most cases, but validation of the assay is necessary before being applied in stress assessment. Furthermore, the ELISA assays can accurately determine relative differences in serum corticosterone levels and FCM levels between samples, but the precision in determining the actual value of the concentration is low.





2013 Annual Conference, 14-16 October, Chicago IL, USA

## **The effect of high caloric diets on fecal glucocorticoid measurements as an indicator of stress in laboratory mice**

### **Authors:**

<sup>1</sup>Otto Kallioikoski; <sup>1</sup>Anne Charlotte Teilmann; <sup>1</sup>Kirsten R. Jacobsen; <sup>1</sup>Jann Hau; <sup>1</sup>Klas S.P. Abelson

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**Presenter and contact person:** Otto Kallioikoski, otto.kallioikoski@gmail.com

### **Topic:**

Methodologies

### **Abstract:**

A new golden standard for gauging stress in living beings is through measuring glucocorticoids in excreta. Non-invasively, an integrative measure is gained which is representative of a preceding time period of a certain length. Minimally affected by the sampling process and momentary fluctuations in hypothalamic-pituitary-adrenal (HPA) axis functioning, this methodology has quickly gained ground and is today considered superior to blood biomarkers by many. Now that the method is established there is a need, however, to identify the limits of the technique. An issue raised by many is the unknown effect of differing diets on fecal glucocorticoid measurements. With an energy dense diet, leading to the production of less excreta, will low concentrations of glucocorticoids be artificially inflated, and vice versa? In a controlled laboratory setting we have been able to explore this effect in mice. When standard mouse chow – high in dietary fiber – is replaced with energy-denser, pelleted diets, intestinal passage time is increased and fecal mass is significantly reduced. The latter, in turn, leads to an overestimation of HPA axis activity, which appears unaltered by the change in diet. We have previously shown the effect on these measurements when using a pelleted diet high in dietary fat. Now we are able to extend these conclusions by examining the effects of a carbohydrate-rich diet. Whereas the process can still be considered a “black box” where shifts in the produced metabolites due to the altered diets cannot fully be accounted for, we can increase the transparency to a degree. We can point to problematic settings where these measurements will be misleading, we can infer underlying processes, and we can confirm the hypothesis that a change in diet will bring about a change in fecal glucocorticoid levels that is wholly independent of HPA axis functioning.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Validation of salivary cortisol: using saliva to measure stress in dromedary camels (*Camelus dromedarius*)**

### **Authors:**

<sup>1</sup>Yasmine N. Majchrzak; <sup>1</sup>Gabriela Mastromonaco; <sup>2</sup>Wendy Korver; <sup>1</sup>Gary Burness  
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**Presenter and contact person:** Yasmine N. Majchrzak, yasminemajchr@trentu.ca

### **Topic:**

Methodologies

### **Abstract:**

Salivary cortisol is a non-invasive method for measuring stress that is gaining popularity in many fields. Given its recent emergence, it has yet to be tested in various taxa and validation by an ACTH challenge is therefore required before proper studies can be conducted. We investigated the use of blood, feces and particularly saliva for measuring stress in dromedary camels. Dromedary camels (*Camelus dromedarius*) are a pseudo-ruminant due to their 3 chambered stomachs but maintain the digestive processes characteristic of a true ruminant. Ruminating animals are a group of species in which measuring salivary cortisol can be difficult due to their unique digestive mechanisms. We propose that saliva is an effective method for monitoring and measuring stress in species that ruminate. An ACTH stimulation test was performed on 3 male camels by taking saliva samples every two hours for 24 hours and fecal samples when available for 72 hours post injection. The samples were analyzed using enzyme immunoassay (EIA) method, involving antiserum and horseradish peroxidase conjugate (Cortisol R4866; C. Munro, University of California, Davis, CA, USA). In saliva, the average baseline cortisol level for the three camels was  $1.12 \pm 0.32$  ng/ml and increased to  $17.69 \pm 7.09$  ng/ml two hours after the ACTH injection. Cortisol levels continuously decreased throughout the day and returned close to baseline within 6 hours ( $1.94 \pm 0.76$  ng/ml). Fecal samples showed no spikes in cortisol, potentially caused by the long gut passage time in camels. The results indicate that saliva is more accurate than feces at measuring short term stress and less invasive and difficult than blood. We present evidence that saliva can be used when monitoring species in captivity, and can help identify biotic and abiotic factors that may trigger short term stress in camels and other species.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Advances in non-invasive hormone monitoring of birds and mammals from southern South America: Development of a technical platform for reproductive and stress research in Argentina**

### **Authors:**

<sup>1</sup>Juan M. Busso; <sup>2</sup>Rubén D. Ruiz; <sup>3</sup>Janine L. Brown; <sup>1</sup>Raúl H. Marin; <sup>4</sup>Alvina Lèche; <sup>2</sup>Marina F. Ponzio; <sup>1</sup>María F. Dominchin; <sup>5</sup>Gabina V. Eguizábal; <sup>1</sup>Stefanía Pellegrini; <sup>5</sup>Daniel Villarreal; <sup>6</sup>Rupert Palme

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**Presenter and contact person:** Marina F. Ponzio, mponzio@mater.fcm.unc.edu.ar

### **Topic:**

Miscellaneous

### **Abstract:**

In continental terms, southern South America is a peninsula. The dominant feature of this portion is the Andes Mountains, which is associated with the Monte, Gran Chaco and Patagonian areas. To understand basic animal reproductive and stress physiology, expertise in tracking gonadal and adrenocortical activities is essential. This presentation describes the approach used by our team to develop a technical platform for reproductive and stress hormone research by applying non-invasive monitoring techniques to explore the biology of several endemic avian and mammalian species. One of our goals was to revise the protocols and the advances of studies conducted in Argentina. From 1998 to date, our staff has received international research training and six Ph.D. studies are being developed by local students. This research endeavor expects to improve our professional skills, to detect steroidal metabolites by specific immunoassays, and establish a collaborative net-work. We are also conducting collaborative experiments investigating reproductive and stress functions of chinchilla (*Chinchilla lanigera*) from the Andes, collared anteater (*Tamandua tetradactyla*) from the Monte, and mara (*Dolichotis patagonum*) from Patagonia. Results of some of these studies suggest a preferential route of steroidal metabolite excretion that varies depending on the rodentia suborder. We are also conducting experiments in Japanese quail (*Coturnix coturnix japonica*), as a laboratory animal model for wild birds, to understand reproductive and adrenocortical activity under different environmental conditions, including measuring the effects of confounding factors (e.g., photoperiod, diet) on fecal steroid metabolites. Results demonstrated that not only diet but also photoperiod affected non-invasive monitoring of avian adrenocortical activity. At present, highly-trained local researchers are able to accurately assess endocrine state of these captive- or zoo-housed animals in conservation programs, based on their normative endocrine data.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Monitoring of male and female reproductive function in southern African spiny mice based on fecal hormone analysis

### Authors:

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**Presenter and contact person:** André Ganswindt, [aganswindt@zoology.up.ac.za](mailto:aganswindt@zoology.up.ac.za)

### Topic:

Reproduction

### Abstract:

No detailed long-term data on the endocrine correlates of reproductive function exists for the southern African spiny mouse (*Acomys spinosissimus*), or any other *Acomys* species, as limited blood volumes make frequent collections of individual plasma samples for hormone monitoring impractical. To provide an alternative non-invasive approach, the aim of this study was to examine the suitability of two group-specific enzyme immunoassays for monitoring male and female reproductive function in the southern African spiny mouse using feces as a hormone matrix. Fourteen non-pregnant and one pregnant female and 24 male spiny mice were wild-caught at the Goro Game Reserve, Limpopo Province, South Africa, and subsequently monitored under permit and controlled conditions at the University of Pretoria. Fecal samples were collected every second day for up to six weeks and extracted with 1ml of 80% ethanol in water. Resulting extracts were measured for immunoreactive 20-oxopregnanes (antibody against 5 $\beta$ -pregnane-3 $\alpha$ -ol-20-one-3HS, label: 5 $\alpha$ -pregnane-3 $\beta$ -ol-20-one-3,17-dione-thioether-DADOO-Biotin) or 17-oxo-androgens (antibody against 5 $\alpha$ -androstane-3 $\alpha$ -ol-17-one-HS, label: 5 $\alpha$ -androstane-3,17-dione-thioether-DADOO-Biotin), respectively. Minimum sample mass for hormone monitoring required, as well as the rate of respective hormone metabolism post-defecation was additionally investigated using separately collected fecal material. Thirteen out of the 14 non-pregnant females exhibited elevated fecal progesterone metabolite (FPM) concentrations with eight individuals showing indications of a luteal phase. Two females showed two post-ovulatory luteal phases with estimated cycle lengths of 16 and 18 days, respectively. The pregnant female showed an elevation of 231% in mean FPM concentrations compared to the overall mean baseline hormone concentration determined for the 14 non-pregnant females. Males exposed to a long photoperiod, simulating summer-related breeding activity, exhibited a 47.8% increase in fecal androgen metabolite (FAM) levels compared to males exposed to a short photoperiod. Collectively, the data clearly demonstrate that reproductive endocrine function can be reliably monitored in male and female spiny mice by measuring respective fecal hormone metabolites.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Characterizing urinary PGF<sub>2α</sub> metabolites (PGFM) during the estrous cycle and pregnancy in the Giant Panda**

### **Authors:**

<sup>1</sup>Beth M Roberts; <sup>2</sup>Janine L Brown; <sup>3</sup>Rebecca J Snyder; <sup>4</sup>Barbara S Durrant; <sup>5</sup>David C Kersey; <sup>1</sup>Andrew J Kouba

<sup>1</sup>Conservation and Research Department, Memphis Zoo, Memphis, TN, USA; <sup>2</sup>Center for Species Survival Smithsonian Conservation Biology Institute, Front Royal, Virginia, USA; <sup>3</sup>Zoo Atlanta, Atlanta, GA, USA; <sup>4</sup>San Diego Zoo Institute for Conservation Research, San Diego, CA, USA; <sup>5</sup>College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA, USA

**Presenter and contact person:** Beth M Roberts, broberts@memphiszoo.org

### **Topic:**

Reproduction

### **Abstract:**

Prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) is a hormone involved in reproductive processes including corpora luteal regression, maternal recognition of pregnancy, embryo implantation, and parturition. Recently, the measurement of urinary PGF<sub>2α</sub> metabolites (PGFM) has become a non-invasive marker of pregnancy in wild felids. The objectives of this study were to: 1) determine the characteristics of the post-ovulatory urinary PGFM profile of giant panda females; and 2) evaluate the differences between non-bred (NB), bred but non-parturient (BN), and parturient (BP). PGFM was measured using The DetectX<sup>®</sup> 13, 14, dihydro-15-keto-prostaglandin F<sub>2α</sub> enzyme Immunoassay kit (PGFM: K022-H1, Arbor Assays). Urine samples were serially collected over multiple years from four adult captive females held in four U. S. Zoological institutions. Nineteen estrous cycles were assessed: NB (n=4), BN (n=7), and BP (n=7). Several characteristics of the PGFM profile were found to be similar for all groups including: 1) basal levels ranged from 3-15 ng/mg creatinine; 2) 18-35 days after the start of the secondary rise in urinary progestogens PGFM activity increased 5 to 10 fold for approximately 10 days; and 3) PGFM peaked a second time as progestogens declined to baseline. Results indicate that the interval between the start of the first PGFM peak and the second is shorter for BP than for NB and BN (24 ± 2 versus 34 ± 4 days) and the concentration between peaks is higher for the BP than for the NB and BN profiles (28 ± 7.71 versus 13.54 ± 5.54 ng/mg creatinine). For the BP profiles, the second peak corresponds to parturition with levels increasing markedly 10-24 hours in advance of birth. Results suggest that the synchronous monitoring of urinary progestogens and PGFM may allow for a more accurate, non-invasive, prediction of reproductive outcome and parturition timing than previously available in Giant pandas.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Fecal progesterone and estradiol monitoring of ovarian activity in African lions (*Panthera leo*) following treatment with varying number and duration of efficacy of deslorelin (Suprelorin®) implants**

### **Authors:**

<sup>1</sup>Catharine J Wheaton; <sup>1</sup>Katie Graham; <sup>2</sup>Natalie Mylniczenko; <sup>2</sup>Donald Neiffer; <sup>1</sup>Tamara Bettinger

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**Presenter and contact person:** Catharine J Wheaton, Catharine.J.Wheaton@Disney.com

### **Topic:**

Reproduction

### **Abstract:**

Effective, reversible contraception that does not produce unwanted health effects is critical for successful management of female African lions (*Panthera leo*) in zoos. In this study we evaluated the efficacy of 4.7 mg (6-month) and 9.4 mg (12-month) deslorelin (Suprelorin®) implants on the suppression of ovarian activity via fecal progesterone (P4) and estrogen (E2) enzyme immunoassay (EIA) (4-7 samples/week, antisera; P4 #CL425, E2 R0008, Munro, UC Davis, CA). Two socially housed adult female lions each received two-4.7 mg deslorelin implants after 13 days pretreatment with megestrol acetate (Ovaban, 3.6 mg/kg SID for 21 days). Ovaban pretreatment reduced fecal P4 to 7% of baseline concentrations for 10 weeks before returning to 60% of average pretreatment concentrations. Fecal E2 concentrations and patterns remained unaffected. A gonadotropin-releasing hormone (GnRH) challenge at two months post-treatment revealed inhibition of LH release compared to an untreated control (LH EIA; antisera #518-B7, Roser, UC Davis, CA; Witness® LH, Synbiotics, Kansas City, MO). However, follicle-stimulating hormone (FSH) tests did not validate (Feline FSH ELISA #ERKF4007, Endocrine Technologies; FSH RIA, BET Labs, Lexington, KY). A third-4.7 mg implant inserted immediately following the GnRH challenge failed to reduce fecal P4 or E2 to nadir levels, and reproductive behaviors continued to be observed in both females. Insertion of three-9.4 mg implants at month six also failed to suppress E2 or further alter P4 concentrations. In contrast to a previous report, our results show that deslorelin treatment did not alter fecal E2 patterns or suppress E2 concentrations (Kruskall-Wallis,  $P > 0.05$ ), but may work by inhibiting LH production or release, preventing ovulation and reducing measurable fecal P4 by 40% (Kruskall-Wallis,  $P < 0.05$ ). Continued, long-term E2 activity in the ovary may produce unwanted health effects. Further study to determine an effective contraception treatment protocol that eliminates E2 production by the ovary is warranted in this species.





2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Comparing body condition and metabolic biomarkers to determine relationships with ovarian acyclicity in female African elephants (*Loxodonta africana*) in zoos**

### **Authors:**

<sup>1</sup>Janine L Brown; <sup>1</sup>Kari A Morfeld

<sup>1</sup>Smithsonian Conservation Biology Institute, National Zoological Park

**Presenter and contact person:** Kari A Morfeld, karimorfeld@yahoo.com

### **Topic:**

Reproduction

### **Abstract:**

A previous study found a high body mass index (BMI) was positively correlated with ovarian inactivity in African elephants, suggesting reproductive problems may be caused in part by metabolic derangements associated with excessive body weight. To determine whether obesity and related metabolic disruptions are a problem in zoo African elephants, body condition score (BCS), insulin, glucose, and leptin levels were compared between breeding-aged cycling (n=23) and non-cycling (n=23) elephants. Cycling status was determined by serum progesterone analysis. Serum concentration of insulin was measured by enzyme immunoassay (EIA) using a bovine insulin EIA kit (Merckodia Inc.) and serum leptin measured by radioimmunoassay (RIA) using a multi-species leptin RIA kit (Linco Research Inc.). Serum glucose was assessed using an automated glucose analyzer. To assess body condition, a new BCS index (5-point scale with 1 = thinnest and 5 = fattest), validated with ultrasound measures of fat depth, was used. The mean BCS of non-cycling elephants was higher than that of cycling elephants ( $4.39 \pm 0.58$  vs.  $3.73 \pm 0.93$ ,  $P = 0.009$ ). There were differences in the serum concentrations of leptin ( $4.03 \text{ ng/mL} \pm 1.63$  vs.  $3.16 \text{ ng/mL} \pm 0.88$ ,  $P = 0.041$ ) and insulin ( $0.65 \text{ mg/mL} \pm 0.31$  vs.  $0.48 \text{ mg/mL} \pm 0.18$ ,  $P = 0.032$ ) for non-cycling females in the BCS=5 category. Serum glucose did not differ between cycling and non-cycling elephants ( $P = 0.892$ ); however, the G:I was lower in the non-cycling group ( $69 \pm 53$  vs.  $227 \pm 96$ ,  $P = 0.019$ ). The odds ratio for the BCS coefficient was 3.15 [95% CI: 1.36, 7.26], suggesting that with each 1 point increase in BCS, an elephant is approximately 3 times more likely to be non-cycling. These results demonstrate ovarian acyclicity is associated with a high BCS and perturbations in metabolic markers.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Characterizing the estrous cycle and delayed implantation profile of gestation in giant anteaters through non-invasive monitoring of fecal hormone metabolites**

### **Authors:**

<sup>1</sup>Katrina K Knott; <sup>1</sup>Beth M Roberts; <sup>1</sup>Morgan A Maly; <sup>2</sup>Carrie K Vance; <sup>1</sup>Jennifer DeBeauchamp; <sup>1</sup>Jackie Majors; <sup>3</sup>Peter Riger; <sup>4</sup>Heather DeCaluwe; <sup>1</sup>Andrew J Kouba  
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**Presenter and contact person:** Katrina K Knott, kknott@memphiszoo.org

### **Topic:**

Reproduction

### **Abstract:**

Declining population numbers and loss of genetic diversity threaten the ex situ and in situ conservation of giant anteaters. For captive populations, reproductive success is hindered by inappropriate husbandry due to the absence of external physical indicators of estrus and pregnancy in females, and the aggressive behaviors of males. Furthermore, it is unknown whether giant anteaters exhibit a delayed implantation strategy during gestation. Our objective was to characterize the estrous cycle and reproductive endocrinology of gestation to improve reproductive management of giant anteaters in captivity. Fecal samples were collected from 7 females 3-5 times weekly for 2 years, during which time 3 of the females became pregnant. Concentrations of estrogens (R4971, R522-2) progestogens (CL425), and glucocorticoids (CJM006) were examined in fecal extracts by enzyme immunoassay using antibodies and conjugates provided by C. Munro (U. California-Davis). Ovarian hormone profiles showed no evidence of estrous cycling before 1.8 years of age. Estrous cycle length of nulliparous females (age, <4 years) were shorter ( $47.3 \pm 4.3$  days) with lower luteal phase progestogen concentrations ( $49.4 \pm 2.9$  ng/g) compared to a multiparous female (age, 6-7 years, cycle length,  $62.5 \pm 2.6$  days; luteal phase progestogens,  $136.8 \pm 1.8$  ng/g). Pregnancy ranged from 175-196 days. A biphasic increase in progestogens was observed during pregnancies with an initial 16-fold increase above baseline lasting 76-112 days after breeding, and followed by a 26-fold rise in progestogens 78-118 days prior to parturition. Concentrations of estrogens and glucocorticoids were also highest during late pregnancy. Endocrine profiles suggest delayed implantation during gestation consistent with many other xenarthrans. Fecal hormone monitoring is a useful tool to prepare for male: female introductions and determine pregnancy in giant anteaters. Differences in estrous cycle characteristics among age classes and protracted gestation, however, must be considered to best improve breeding success and neonatal survival.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## An Endocrine Indicator of Maternal Success in Phocid Seals

### Authors:

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**Presenter and contact person:** Kelly Robinson, kjr33@st-andrews.ac.uk

### Topic:

Reproduction

### Abstract:

Concentration of phocid seal reproductive effort into brief, energy-costly annual episodes in colonies should select for mothers recognizing and expending resources only on filial pups. Nevertheless maternal care in phocid seals is highly variable. Grey seals (*Halichoerus grypus*) can exhibit mother/pup separation, abandonment, inconsistent ability to recognize offspring, adoption of unrelated pups, pup theft and infanticide, with direct impacts on pup survival and maternal reproductive success. The hormone oxytocin is responsible for the correct expression of maternal behavior, and maintaining social bonds. Plasma concentrations of oxytocin were measured in mother/pup pairs on two breeding colonies with different levels of maternal attendance using an ELISA. Mothers exhibiting “poor” maternal behavior were predicted to have lower basal oxytocin levels during the breeding season than those that raised their pups to weaning. Successfully pupping mothers had higher oxytocin concentrations than “poor” mothers who lost their pups through abandonment or death during lactation ( $p < 0.01$ ). In addition, “poor” mothers had levels of oxytocin that were not significantly different to those found in non-breeding individuals ( $p = 1$ ), while “successful” mothers had higher plasma concentrations than non-breeding animals ( $p < 0.001$ ). These results provide the first physiological explanation for why some grey seal mothers fail to express effective maternal behavior. Unexpectedly, there was consistent evidence that dependant pups had plasma oxytocin concentrations three times greater than those of their mothers ( $p < 0.001$ ). After weaning, pup oxytocin levels fell by 50%. We suggest that this is not caused by milk ingestion. Pups play an active role in maintaining the mother/pup bond, stimulated by elevated oxytocin levels from sustained contact with the mother and maintained via a positive feedback loop. This hypothesis is supported further by prior behavioral studies on the two colonies, with pups from the colony with greater amounts of mother/pup contact having higher oxytocin concentrations than the other ( $p < 0.001$ ).



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Urinary metabolite monitoring of ovarian activity to characterize the natural estrus cycle in the critically endangered chinchilla (*Chinchilla lanigera*)**

### **Authors:**

<sup>1</sup>María G Galeano; <sup>2</sup>Christine Gilman; <sup>2</sup>Gabriela F Mastro Monaco; <sup>1</sup>Marta Fiol de Cuneo; <sup>1</sup>Marina F Ponzio

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### **Topic:**

Reproduction

### **Abstract:**

Excessive hunting and habitat fragmentation greatly reduced chinchilla populations in the wild and today it's almost extinct in the wild. Understanding basic reproductive physiological function is essential for a successful conservation program, yet these characteristics are unknown or poorly studied in this species. Our objective was to characterize the natural fluctuations in ovarian endocrine activity in female chinchillas throughout the sexual cycle, using urinary biomarkers of ovarian function. Urine samples were collected from 9 females, 3 times per week for a period of 4 months during the active reproductive season. Vaginal opening was also recorded as an external sign of estrus. Each sample was analyzed for creatinine (crt, Sigma #C3613), pregnanediol glucuronides and estrone conjugates (PdG R13904, EC R522-2, C. Munro) using EIA. A longitudinal profile was obtained for each hormone and female, using an iterative process to calculate baseline and peak hormonal levels. Baseline and peak levels for EC and PdG were (ng/mg crt)  $7.9 \pm 0.7$ ,  $28.1 \pm 4.4$  and  $890.5 \pm 189.3$ ,  $2272.0 \pm 246.6$ , respectively. The length of the natural sexual cycle in the female chinchilla was determined as  $34.8 \pm 4.4$  days (range 16.8-49) for EC and  $32.1 \pm 3.6$  days (range 18.7-56) for PdG. Maximal EC concentrations used as an indicator of estrus length lasted  $6.7 \pm 0.9$  days (range 4.7-11). Follicular and luteal phases were  $3.9 \pm 0.4$  and  $7.1 \pm 0.6$  days, respectively. A total of  $3.9 \pm 0.3$  and  $3.7 \pm 0.3$  sexual cycles were detected throughout the study period using PdG and EC assessments. Vaginal opening was not synchronous with elevations in hormones above baseline levels and therefore, cannot be used as a reliable indicator of estrus. It is clear that urinary endocrine analysis is a valuable tool in the efforts to elucidate the reproductive physiology of the endangered chinchilla.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Reproductive hormones and behaviors are indicative of reproductive performance in female whooping cranes (*Grus americana*)**

### **Authors:**

<sup>1</sup>Megan E Brown; <sup>2</sup>Sarah J Converse; <sup>1</sup>Carol L Keefer; <sup>3</sup>Janine L Brown; <sup>2</sup>Jane N Chandler; <sup>3</sup>Nucharin Songsasen

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**Presenter and contact person:** Megan E Brown, brownme@si.edu

### **Topic:**

Reproduction

### **Abstract:**

Over the past decade, Patuxent Wildlife Research Center in Laurel, MD has released on average 18 whooping crane chicks into wild populations each year. However, reproductive performance of adult birds at this facility is lower than desired, with a 60% fertility rate of eggs laid from 2000 to 2010 as compared to the 95% fertility rate observed in wild populations. During two successive breeding seasons, six adult whooping crane pairs were monitored through behavioral observations and fecal hormone analyses to determine potential causes of this poor reproductive performance. All pairs were observed for 75 minutes each morning at natural sunrise from March 30<sup>th</sup> until June 15<sup>th</sup>. Fecal samples were collected three times per week February 4<sup>th</sup> until July 1<sup>st</sup>, and evaluated for gonadal (estrogen and progesterone) and adrenal (corticosterone) hormone metabolites. Antibodies were obtained from MP Biomedicals (estradiol and corticosterone) and Coralie Munro (progesterone, CL425). At  $\alpha=0.10$ , females that laid eggs exhibited higher estradiol concentrations during the breeding season ( $p=0.0630$ ). There were no differences in corticosterone concentrations between the two groups, ( $p=0.4645$ ) indicating poor reproductive performance may not be associated with elevated adrenal activity. Each pair was evaluated for proportion of time spent performing reproductive behaviors compared to total active time. Overall, successful pairs showed higher proportions of interactive reproductive behaviors than unsuccessful pairs ( $p=0.0089$ ). Evaluated separately, successful females exhibited a higher average rate of reproductive behaviors. Individual behaviors, specifically unison calls, precopulatory behavior (marching), and number of copulation attempts were higher in laying females compared to nonlaying females ( $p=0.0251$ ,  $0.0428$ ,  $0.0045$  respectively). The findings from this study showed that a female's reproductive capacity may be indicated through reproductive behavior, as well as overall gonadal hormone activity during the breeding season.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Predicting mating outcomes in old females: an assessment of anti-Müllerian hormone

### Authors:

<sup>1</sup>Ned J. Place; <sup>1</sup>Kristen A. Roosa; <sup>1</sup>Devin A. Zysling

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### Topic:

Reproduction

### Abstract:

Anti-Müllerian hormone (AMH) is routinely measured in women as part of an evaluation for infertility, especially when assisted reproductive technologies (e.g., IVF) are being considered. A low serum AMH concentration has some predictive value in identifying patients that may not respond favorably to gonadotropin stimulation for the purposes of follicle development and egg retrieval. For women, age-specific AMH tables have also been generated to estimate the age of menopause years and decades before onset. To determine if AMH concentration can predict mating outcomes in older, non-human females, we measured AMH in 9-month-old Siberian hamsters (*Phodopus sungorus*). Our prior investigations of reproductive aging in hamsters have determined that a substantial proportion of females experience reproductive failures when first mated at 9 months of age. Therefore, 2 weeks prior to their first pairing with males, we collected small blood samples from 28 females to measure serum AMH concentrations using an ELISA previously validated for *P. sungorus*. Females were paired with males for 10 days and subsequently divided into four groups based on outcomes: mated with live pups ( $n=10$ ), mated with implantation sites but no live pups ( $n=11$ ), mated but no implantation sites or pups ( $n=5$ ), and no evidence of mating ( $n=2$ ). Mean AMH concentration was highest in the group of females that produced live pups ( $2.5 \pm 0.5$  ng/mL), but the majority of females in this group had an AMH concentration that overlapped with females that failed to produce live litters ( $1.5 \pm 0.2$  ng/mL). In general, a high AMH concentration ( $>3.5$  ng/mL) was associated with a favorable reproductive outcome, whereas mated females with a low AMH concentration ( $<1.0$  ng/mL) almost always failed to produce live young. Overall, AMH concentrations at the extremes of the age-specific range were predictive of mating outcomes in older animals.





2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Assessment of reproductive cycling and stress in captive fennec foxes (*Vulpes zerda*) using non-invasive hormone monitoring**

### **Authors:**

<sup>1</sup>Corinne P Kozlowski; <sup>1</sup>Karen L Bauman; <sup>1</sup>Colleen Knobbe; <sup>1</sup>Shannon Santangelo; <sup>1</sup>Alice Seyfried; <sup>1</sup>Cheryl S Asa

<sup>1</sup>Saint Louis Zoo

**Presenter and contact person:** Corinne P Kozlowski, kozlowski@stlzoo.org

### **Topic:**

Reproduction

### **Abstract:**

The population of fennec foxes (*Vulpes zerda*) has declined significantly in AZA institutions due to low reproductive success and attrition. Since fennecs serve multiple institutional roles, they are often housed under a variety of conditions. This has led managers to suspect a correlation between husbandry, stress and reproductive success. However, little information is available regarding the reproductive and stress physiology of fennecs, and no systematic study of the relationship between husbandry and reproductive success has been conducted. As a first step towards understanding this connection, we characterized reproductive cycling and quantified stress hormone levels in five female fennecs at the Saint Louis Zoo. Fecal samples were collected weekly for at least 24 months, and progesterone and glucocorticoid levels were analyzed through radioimmunoassay (Coat-A-Count® Progesterone <sup>125</sup>I Kit, Diagnostic Products Corporation; DA Corticosterone <sup>125</sup>I Kit, ICN MP Biomedicals). During this time, none of the females became pregnant, but 4 of the 5 females underwent at least one non-pregnant cycle. One female each cycled four times, three times, twice, and once. The remaining female failed to cycle. Baseline ( $134.7 \pm 7.5$  ng/g) and pseudopregnancy ( $2286.1 \pm 31.48$  ng/g) progesterone levels did not differ among females. Pseudopregnancy duration varied from 31 to 76 days ( $53.3 \pm 6.9$  days), and the inter-cycle interval ranged from 43 to 238 days ( $140.0 \pm 32.4$  days). Although most females ovulated between February and April, pseudopregnancies also occurred between May and July, as well as November. Fecal glucocorticoid concentrations ranged from 9.1 to 605.3 ng/g ( $121.8 \pm 4.7$  ng/g) and varied significantly among females. In particular, the only non-cycling female had significantly higher levels of glucocorticoids than several cycling females. These data provide additional information regarding the reproductive physiology of female fennec foxes, as well as suggest a possible link between elevated glucocorticoid secretion and suppression of reproductive cycles.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Could fecal progestins or glucocorticoid levels provide clarity in determining cheetah pregnancy vs. pseudopregnancy?

### Authors:

<sup>1</sup>Corinne B Pisacane; <sup>1</sup>Lance J Miller

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### Topic:

Reproduction

### Abstract:

The cheetah (*Acinonyx jubatus*) population in North American zoos is currently not sustainable due to less than 20% successfully reproducing annually. However, in the wild, cheetahs reproduce at rates of approximately 90%. One of many challenges surrounding breeding cheetahs involves determining whether the animal is actually pregnant or is demonstrating "pseudopregnancy". Early detection of pregnancy would be valuable for both animal management and scientific purposes. However, it is currently difficult to differentiate between the two reproductive states by progestin values shortly after breeding. In the current study we explore both progestin and glucocorticoid levels in cheetah fecal samples across 6 true pregnancies and 6 pseudopregnancies. Daily fecal samples were collected from both pregnant and "pseudopregnant" cheetahs at the San Diego Zoo Safari Park between 2005-2012. Fecal samples were frozen until analysis; then dried, pulverized, weighed and extracted. Progestin and glucocorticoid values were determined by radioimmunoassay (RIA) using Quidel CL425 progesterone antibody and <sup>3</sup>H-progesterone (Perkin Elmer) to compete with endogenous progesterone. Glucocorticoid values were determined by RIA using 3R3-PB corticosterone antibody (MP Biomedicals) and <sup>3</sup>H-corticosterone (Perkin Elmer) to compete with endogenous glucocorticoid metabolites. Averages and medians were calculated based on five day time periods throughout each pregnancy and "pseudopregnancy". Mann-Whitney U test of significance was utilized to determine significant differences between the types of pregnancies examining the different time periods. Results suggest that detection of true pregnancy can be established by examining the values between days 41 and 46. Earlier detection of pregnancy in cheetahs, and potential reasons for "pseudopregnancy" could help facilitate future breeding efforts for cheetahs across North American zoos increasing the sustainability of the population.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Characterization of reproductive status of Mexican gray wolves (*Canis lupus baileyi*) using non-invasive hormone monitoring**

### **Authors:**

<sup>1</sup>Amanda M Murti; <sup>1</sup>Corinne P Kozlowski; <sup>1</sup>Cheryl S Asa

<sup>1</sup>Saint Louis Zoo

**Presenter and contact person:** Amanda M Murti, murti@stlzoo.org

### **Topic:**

Reproduction

### **Abstract:**

Some genetically valuable Mexican gray wolves (*Canis lupus baileyi*), an endangered subspecies of the gray wolf, fail to reproduce in captivity for unknown reasons. Hormone monitoring can be used to help identify reproductive problems in these individuals. The goal of this study was to identify differences in the profiles for pregnancy and pseudopregnancy, both natural and Ovuplant-stimulated, in Mexican wolves. Ovuplant, a short-acting GnRH agonist, was used to stimulate ovulation in some individuals in conjunction with artificial insemination. Non-invasive fecal hormone monitoring was used to characterize the physiology of 7 females at the Endangered Wolf Center in Eureka, MO. On average, weekly samples were obtained for most individuals for at least one full breeding season from 2005 to 2011. Fecal progesterone levels were analyzed through radioimmunoassay. The average progesterone level for natural pseudopregnancy was  $159.3 \pm 6.7$  ng/g ( $\pm$  SE, n=5 profiles), Ovuplant pseudopregnancy  $330 \pm 21.6$  ng/g ( $\pm$  SE, n=4 profiles), Ovuplant pregnancy  $228.1 \pm 21.4$  ng/g ( $\pm$  SE, n=4 profiles), natural pregnancy  $217.6 \pm 16.8$  ng/g ( $\pm$  SE, n=2 profiles) and the average baseline value was  $53.6 \pm 1.3$  ng/g ( $\pm$  SE, n=13 profiles). The maximum progesterone level for Ovuplant pseudopregnancy was ( $2515 \pm 21.7$  ng/g), Ovuplant pregnancy ( $1811.5 \pm 21.4$  ng/g), natural pseudopregnancy ( $528.5 \pm 6.7$  ng/g) and natural pregnancy ( $715.9 \pm 16.8$  ng/g). Area under the curve (AUC) calculations was performed for each female, and General Linear Models were used to assess the relationship between AUC and cycle type (pregnancy or pseudopregnancy), presence or absence of Ovuplant, year, and individual. AUC values were log-transformed to establish normality. Although progesterone values in Ovuplant-stimulated cycles tended to be higher, there were no statistically significant differences found between progesterone profiles from pregnant or pseudopregnant females, or cycles induced by Ovuplant. These data provide a valuable foundation for future research on Mexican wolf reproduction.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **The efficacy of etonogestrel implants for ovarian suppression and the use of commercial kits for detection of fecal gonadal steroids in female ocelots (*Leopardus pardalis*)**

### **Authors:**

<sup>1</sup>Marcisnei Luiz Zimmermann; <sup>1</sup>Marcel Henrique Blank; <sup>1</sup>Helber Daniel Parchen; <sup>1</sup>Renato Herdina Erdmann; <sup>1</sup>Nei Moreira; <sup>2</sup>Rodrigo Neca Ribeiro; <sup>3</sup>Zalmir Silvino Cubas; <sup>3</sup>Wanderlei Moraes; <sup>3</sup>Marcos José Oliveira

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**Presenter and contact person:** Rodrigo Neca Ribeiro, rodrigonribeiro@hotmail.com

### **Topic:**

Reproduction

### **Abstract:**

Implants can be used in ART techniques to induce ovarian activity suppression before gonadotropin stimulation. Evaluating the effect of using them in small felids will possibly aid with future AI attempts. Additionally, identifying low cost and simple methods could further promote research in the area of reproduction and conservation of felines. This study aimed to evaluate the action of a progestin (etonogestrel, Implanon<sup>®</sup>, Organon Brazil's Industry and commerce, São Paulo - SP), normally used for human use, over ovarian activity of females of ocelots. Fecal hormonal concentrations were determined using commercial enzyme immunoassay techniques (EIA) kits for estradiol (DRG<sup>®</sup> Estradiol ELISA - EIA-2693) and progesterone (DRG<sup>®</sup> Progesterone ELISA - EIA- 1561). Five females at the Bela Vista Biological Refuge from ITAIPU Binational (Foz do Iguaçu - PR) were used in the study which compared ovarian activity before and after the insertion of the implant. Considering that the estrous cycle of these wild cats lasts 7 to 51 days, fecal metabolites of estrogens and progestins were measured over a 60 day period. The results demonstrate there was a significant difference, in accordance with the non-parametric test of Mann-Whitney, in ovarian activity during the period of the implant with estrogens concentrations decreasing from  $1950.69 \pm 709,88$  ng/g to  $692.33 \pm 275.14$  ng/g ( $p = 0.0051$ ) and progesterone concentrations increasing from  $5.57 \pm 1.5$  µg/g to  $10.72 \pm 5.88$  µg/g ( $p = 0.0407$ ). This work points out the efficacy of the use of etonogestrel in female ocelots to reduce ovarian activity and the efficiency of these commercial kits for fecal hormone analysis.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **The impact of secondary sex characteristics (SSC) and social behavior to cortisol and sexual hormones in female primates**

### **Authors:**

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### **Topic:**

Stress

### **Abstract:**

Primates living in multi-male–female associations develop SSC during sexual active periods. During such periods socio-sexual environments of females (e.g., male contact, female competition) affect ovarian function and the activity of the hypothalamic-pituitary-adrenal axis. The aim of this study was to investigate the impact of the social environment on female chimpanzee (**C**; n=6) cycles and the socio-endocrine impact of SSC in two macaque species, Barbary macaques (**B**; n=24) and Japanese macaques (**J**; n=19) females. In **C** morphological changes of anogenital swellings were scored using three categories, pre-tumescence, maximum-tumescence, post-tumescence. In **B** quantitative measurements of swelling parameters (width, height, depth, labial width) were determined using a video imaging technique. In **J** coloration scores of sex-skins were classified using color tables. **C** were single or paired housed (no-mating-competition), **B** were treated with contraceptives (no-mating-competition), **J** were intact (mating-competition). The latter two lived under semi-free conditions. Concentrations of fecal estradiol, progesterone, and cortisol metabolites were analyzed using an enzyme-immunoassay (Veterinary-Medicine-University Vienna). **C** females housed with males showed decreased cortisol concentrations during late and decreasing tumescence of SSC. Paired housed females had shorter cycle lengths compared to single housed. In **B** multiple regressions showed a negative relationship between SSC size and cortisol respectively a positive between swelling size and intersexual socio-positive contact. Females with enlarged SSC had lower progesterone levels and increased estradiol-progesterone ratios. In **J**, a light-red (lactating) and a dark-red (non-lactating) group was discerned. Intensity of redness increased during the breeding season and correlated with the amount of socio-sexual behavior and reproductive success in non-lactating females. They had significantly elevated cortisol and sex steroid titers compared to the lactating group. In conclusion, reduced cortisol excretion rates in females are associated with exaggerated SSC and intensified male contact when male mating competition for females is diminished.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Challenges in assessing total corticosteroid concentrations in serum of bachelor African elephants (*Loxodonta africana*)

### Authors:

<sup>1</sup>Cayman Lynn Adams; <sup>2</sup>Russ Hart; <sup>1</sup>Linda M Penfold

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### Topic:

Stress

### Abstract:

Assessing corticosteroids in African elephant (*Loxodonta africana*) can be a key part of properly understanding bachelor group interactions. Corticosteroid analysis can be used to better establish social groups and improve captive management. However, elephants, like many vertebrates, have corticosteroid binding globulin (CBG) in their blood, which can affect the ability to measure total circulating corticosteroids. Initial attempts to measure corticosteroids by enzyme-linked immunoassay yielded poor recoveries of 41% ( $r=0.979$ ) corticosterone (UC Davis Ab) and 2% ( $r=0.9765$ ) cortisol (UC Davis Ab), even though parallelism criteria were met. Ether extraction of the serum only marginally improved the recovery for corticosterone (55%), though gave better results for cortisol (45%). These recoveries, however, were still unacceptable. To test whether CBGs were interfering with the accurate measurement of corticosteroids, duplicate pooled serum samples were analyzed using a corticosterone assay (Arbor Assays) with and without the use of the dissociation reagent supplied with the kit resulting in recoveries of 92% ( $r=0.9988$ ) with dissociation reagent and 19% ( $r=0.9907$ ) without dissociation reagent. Using these results, a dissociation reagent (Arbor Assays) was added to the assay system for cortisol using the UC Davis Ab. Equal parts dissociation reagent was added to serum, which was immediately vortexed for 10 sec and then diluted with EIA buffer (x20) before assaying. This time the recovery of corticosteroid was 106% ( $r=0.9979$ ). Results show that a standard EIA protocol using UC Davis cortisol Ab can be adapted for African elephant serum to circumvent CBG interference. This approach may have implications for other vertebrate species where CBG prevents accurate measurement of corticosteroids. It is recognized that this method measures total vs. free corticosteroids, but to date physiologically meaningful results related to bachelor elephant interactions have been generated using this modified EIA.





2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Influence of adrenocorticotrophin hormone challenge and external factors (age, sex, and body region) on hair cortisol concentration in Canada lynx (*Lynx canadensis*)**

### **Authors:**

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<sup>1</sup>Trent University; <sup>2</sup>Toronto Zoo, Canadá

**Presenter and contact person:** Christine Terwissen, [christineterwissen@trentu.ca](mailto:christineterwissen@trentu.ca)

### **Topic:**

Stress

### **Abstract:**

Land use changes are a significant factor influencing the decline of felid populations; however, more research is needed to understand how these factors influence lynx in the wild. To our knowledge no one has validated hair analyses in a felid or prior to evaluations in the wild. The objective of this study was to evaluate whether increased adrenocorticotrophin hormone (ACTH) during the period of hair growth results in elevations in cortisol that are measurable in hair, validate the enzyme immunoassay used, and determine if there are variations in hair cortisol between age, sex and body regions of Canada lynx. An EIA was validated for the analysis of hair cortisol (Cortisol R4866; C. Munro, University of California, Davis, CA, USA). Extraction of exogenous cortisol in lynx hair samples resulted in procedure efficiency of  $81.9 \pm 9.4\%$  ( $x \pm SE$ ). The recovery of known concentrations of cortisol from lynx hair extracts was  $76.39 \pm 2.2\%$ . The measured hormone concentrations in the spiked samples correlated with the expected concentrations ( $r = 0.99$ ,  $p < 0.01$ ). Serial dilutions of pooled hair extract showed parallel displacement with the cortisol standard curve ( $r = 0.99$ ,  $< 0.01$ ). We quantified hair cortisol concentration in captive animals for the ACTH challenge and collected samples from fur auction houses to compare body region variations. Lynx ( $n=3$ ) hair cortisol concentration increased by an average of 44.67 % following an ACTH challenge (20IU/kg weekly for 5 weeks), validating the use of an EIA to quantify hair cortisol values in Canada lynx fur. Based on our analysis of sampled lynx pelts, we found that hair cortisol did not vary between age and sex but varied within the foot/leg region to a greater extent than between individuals. It is therefore suggested that a standardized hair cortisol sampling location be adopted in future studies.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Impact of novel scent stimuli on fecal glucocorticoid and androgen metabolites in mated coyote pairs (*Canis latrans*)

### Authors:

<sup>1</sup>Christopher J Schell; <sup>2</sup>Julie K Young; <sup>3</sup>Elizabeth V Lonsdorf; <sup>1</sup>Jill M Mateo; <sup>4</sup>Rachel M Santymire

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**Presenter and contact person:** Christopher J Schell, cschell@uchicago.edu

### Topic:

Stress

### Abstract:

Coyotes are highly aggressive to neighboring conspecifics during territorial incursions and challenges to dominance. For mated pairs, repeated instances of aggression during the breeding season might increase glucocorticoid and androgen concentrations that can be transmitted to the offspring *in vitro*. Consequently, alterations of parental hormones may modify traits of offspring. Our objective was to determine whether repeated presentation of novel conspecific scents during the breeding season increased vigilance and aggression, glucocorticoids (via fecal glucocorticoid metabolite, FGM, analysis), and androgens (via fecal androgen metabolite, FAM, analysis) in male and female coyotes. We conducted a study on captive, mated coyotes ( $n=8$  pairs) from February to March 2011 at the National Wildlife Research Center in Millville, UT. Four treatment pairs were provided with novel scents during pregnancy, while control pairs were presented with distilled water. Scents were applied to the interior of treatment pens once every 5 days over a 20-day period. Fecal samples were collected 2-3 days per week for 2 months ( $n=588$  total). We extracted FGMs and FAMs from dry feces and analyzed samples via cortisol and testosterone enzyme immunoassays (EIAs). We used polyclonal cortisol antiserum (R4866), testosterone antiserum (R156), and horseradish peroxidase (HRP) provided by C. Munro (University of California, Davis, CA). Our results did not demonstrate an effect of our scent stimuli on FGMs or FAMs for females (FGMs:  $F_{2,8}=0.952$ ,  $P=0.413$ ; FAMs:  $F_{2,8}=1.062$ ,  $P=0.376$ ) or males (FGMs:  $F_{2,8}=2.376$ ,  $P=0.135$ ; FAMs:  $F_{2,8}=3.649$ ,  $P=0.058$ ). However, treatment pairs investigated and marked the scent-affected areas more frequently ( $P<0.05$ ) than controls. FGM and FAM concentrations decreased in both sexes as each pair approached parturition regardless of treatment condition. Future work will examine the potential long-term effects of coyote parental physiology and behavior on temperament trait development and hormones in offspring.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Physiological, social and ecological mechanisms of survival in Belding's ground squirrels (*Urocitellus beldingi*)**

### **Authors:**

<sup>1</sup>Jill M. Mateo; <sup>1</sup>Andy Dosmann; <sup>1</sup>Katherine Brooks

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**Presenter and contact person:** Jill M. Mateo, [jmateo@uchicago.edu](mailto:jmateo@uchicago.edu)

### **Topic:**

Stress

### **Abstract:**

For most species little is known about the proximate mechanisms underlying trade-offs among growth, maintenance and reproduction, which in turn affect fitness and survival, especially in free-living animals experiencing species-typical stressors. Belding's ground squirrels (*Urocitellus beldingi*) are group-living rodents that live in high elevation meadows and are active only 3-4 months each summer. Males disperse as juveniles whereas females are philopatric, thus living near kin and favoring the evolution of cooperative behaviors. Both sexes experience stressors such as extreme weather, predators, food shortages and social instability. In humans and some primates, social support and kin networks can lead to improved health, reduced stress responses and enhanced survival, but this phenomenon has not been studied in shorter-lived species. Using a 19-year demographic dataset, with extensive pedigrees and reproductive histories, as well as repeated non-invasive cortisol monitoring ( $\leq 20$  fecal samples collected from marked individuals over six summers) and behavioral observations, we test how these factors affect survival to the following year. Metabolites were assayed with cortisol solid phase component system <sup>125</sup>I-cortisol Corticote<sup>®</sup> radioimmunoassay kits (MP Biomedicals, Irvine, CA) and assayed in duplicate and re-analyzed if the coefficient of variation exceeded 20%. For both sexes, individuals are more likely to survive if they reproduce, have higher body weights, and lower corticoids. For females, but not males, the presence of kin alive in the meadow is associated with higher rates of reproduction and survival and lower vigilance and corticoids. Both body weight and corticoids are heritable, and there is significant directional selection on each trait (positive and negative, respectively). Lower corticoids, which promote energy storage, are an important fitness trait for this obligate hibernator. In this philopatric species, living near kin may not only facilitate nepotism but also affects physiology and survival, perhaps with social networks buffering against stressors.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **The impact of social and ecological conditions on physiological markers in red colobus monkeys (*Procolobus rufomitratus*) in Kibale National Park, Uganda**

### **Authors:**

<sup>1</sup>Krista M Milich; <sup>2</sup>Rachel M Petersen; <sup>3</sup>Colin A Chapman; <sup>2</sup>Janice M Bahr  
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**Presenter and contact person:** Krista M Milich, kmilich@uchicago.edu

### **Topic:**

Stress

### **Abstract:**

We examined the effects of habitat quality, group size, and social rank on reproductive, stress, and energetic indicators in red colobus monkeys (*Procolobus rufomitratus*) living in Kibale National Park, Uganda, and in adjacent unprotected forest fragments. Fecal (n=175) and urine (n=60) samples were collected opportunistically from 3 red colobus groups inside the park and 3 groups in fragments, including 2 park groups with identified individuals. Reproductive hormone (testosterone, estradiol, and progesterone; non-commercial kit following Bahr et al. 1983) and stress hormone (glucocorticoid; MP Biomedicals Corticosterone kit) concentrations in fecal samples and energetic indicators (C-peptide; EMD Millipore Human C-peptide kit) in urine samples were determined by radioimmunoassays. We tested three main hypotheses: 1) individuals in the park have higher reproductive hormone and c-peptide concentrations and lower glucocorticoid concentrations than individuals in the fragments, 2) individuals in a large social group (120 individuals) have higher reproductive hormone, C-peptide, and glucocorticoid concentrations than in a small group (60 individuals), and 3) the alpha male has higher testosterone, c-peptide, and glucocorticoid concentrations than subordinate males. The alpha male had higher testosterone (P=0.04) and glucocorticoid (P=0.03) concentrations than the other males and females in the fragments had lower progesterone concentrations (P=0.04) than females in the park. These results indicate that there are physiological correlates to primate social systems and ecological conditions, such as higher testosterone and glucocorticoids in high ranking males and lower reproductive hormone concentrations for females living in poor environments. Furthermore, this study provides evidence of sex differences in the stress response, which correlates with variation in male and female sexual strategies.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **The immediate and long-term relationship between social play behavior and fecal glucocorticoid metabolites in wild chimpanzees (*Pan troglodytes*)**

### **Authors:**

<sup>1</sup>Matthew R Heintz; <sup>2</sup>Elizabeth V Lonsdorf; <sup>3</sup>Carson M Murray; <sup>4</sup>Rachel M Santymire

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**Presenter and contact person:** Matthew R Heintz, mheintz@lpzoo.org

### **Topic:**

Stress

### **Abstract:**

Social play behavior is ubiquitous among mammals yet the behavioral and physiological benefits of play remain relatively unknown. Play occurs most often in infants when individuals are in a relaxed setting and include repeated behavioral movements that can sometimes result in the loss of control. This practice can improve their physiological response to unexpected events; potentially yielding both immediate and long-term physiological benefits to play behavior. In this study, we compared social play behavior in infant chimpanzees (*Pan troglodytes*) with fecal glucocorticoid metabolites (FGM) to assess both immediate and long-term benefits of play. Behavioral observations have been conducted in Gombe National Park, Tanzania since the late 1970's. Fecal samples were collected opportunistically and analyzed using a cortisol enzyme immunoassay. We compared immature individual social play percentage with FGM values (daily, monthly mean, variance) to assess immediate benefits of social play. To examine potential long-term benefits of social play, we compared FGM mean and variance of adolescents and adults with their social play percentage during infancy. We found that FGM monthly mean was positively associated (GLMM:  $F_{1,64}=11.16$ ,  $p=0.0014$ ) with social play percentage when individuals were in small groups. However, we did not find any support for social play to be related to FGM variance during infancy or later in life. Overall, these results demonstrate that while social play has the immediate benefit of serving as a eustress, it does not appear to function as adapting an individual's stress response in the long-term.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Monitoring fecal glucocorticoid metabolites in native Australian marsupials

### Authors:

<sup>1</sup>Meredith J Bashaw; <sup>2</sup>Kerry P Fanson

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### Topic:

Stress

### Abstract:

Australia hosts a unique mammalian fauna, including the majority of the world's marsupial species. Many of these marsupials are threatened with extinction. Monitoring stress physiology provides insight into how animals perceive their environment and can be used to assist conservation efforts, improve animal welfare, understand behavior, and increase general knowledge about a species. The goal of this study was to validate assays for monitoring adrenal activity via feces in 12 native Australian marsupial species: southern bettong, woylie, mountain pygmy possum, northern and southern hairy-nosed wombats, potoroo, eastern and western grey kangaroos, bilby, koala, numbat, and Tasmanian devil. We compared the performance of five glucocorticoid enzyme immunoassays (GC EIAs) to identify which most effectively monitored stress responses in each species. We used either adrenocorticotrophic hormone administration or stressful events (e.g., transfer) for biological validation; an assay was considered valid if a peak ( $>1.5$  SD above mean) was detected in the 3 days following the event. For all species, at least one EIA detected a GC peak in 50% or more individuals, but validation success varied. For two species, all five EIAs identified GC peaks in 75%-100% of individuals. For three species, no EIA measured a GC response in more than 50% of the individuals. The  $5\alpha$ -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA was most effective in the marsupial species tested, identifying peaks in 100% of individuals for 6 species and at least one individual of every species. The cortisol-HRP EIA was least effective for these marsupials, identifying peaks in 100% of individuals for 2 species and less than half the individuals in 7 species. The results highlight variability in GC metabolite profiles and illustrate the methodological challenges of GC validation experiments.





2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Non-invasive monitoring of stress in Iberian lynx (*Lynx pardinus*) and Eurasian lynx (*Lynx lynx*) using fecal glucocorticoid measurement**

### **Authors:**

<sup>1</sup>Susanne Pribbenow; <sup>1</sup>Katarina Jewgenow; <sup>2</sup>Rodrigo Serra; <sup>2</sup>Alexandre Azevedo; <sup>3</sup>Sergey Naidenko; <sup>4</sup>Martin Dehnhard

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**Presenter and contact person:** Susanne Pribbenow, pribbenow@izw-berlin.de

### **Topic:**

Stress

### **Abstract:**

The level of stress is an important indicator of animal's well-being, as stress has harmful effects on reproduction, growth and immune function. The development of species specific enzyme immunoassays (EIA) to monitor fecal glucocorticoid metabolites (fGM) contributes a powerful tool to assess an animal's endocrine state non-invasively. The first aim was to identify a suitable EIA for fGM monitoring in Iberian lynx using Eurasian lynx as a model by assessing the suitability of six different EIAs for detecting quantitative changes in fGM concentrations in response to an ACTH challenge test conducted in two captive Eurasian lynx (one female and one male) and characterizing fecal cortisol metabolites in a male Eurasian lynx, that received an injection of <sup>3</sup>H-cortisol. Using HPLC analyses radiolabeled metabolites were compared with immunoreactive metabolites also including fecal extracts of Iberian lynx for species comparison. The second aim was to biologically validate the established EIA for monitoring adrenocortical activity of six captive Iberian lynx (3 females and 3 males) after a translocation and during acclimatization to new enclosures in relation to behavior rated by the keepers. The ACTH challenge revealed that our 11-hydroxyetiocholanolone EIA is the most sensitive to reflect acute elevations in both sexes of the Eurasian lynx. HPLC immunograms demonstrated that the 11-hydroxyetiocholanolone EIA measured amounts of immunoreactivities corresponding to the radiolabeled peaks with strong similarities across both lynx species. Additionally, HPLC and GC-MS analyses identified the 11-hydroxyetiocholanolone metabolite in feces of Eurasian and Iberian lynx. Longitudinal fGM profiles of Iberian lynx revealed increases in concentrations associated with management events. In contrast, elevated fGM concentrations after translocation were only measured in a single male. Our results show that the 11-hydroxyetiocholanolone EIA is a reliable tool to assess fGM in both the Eurasian and the Iberian lynx and could facilitate to improve husbandry and management.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Social and developmental factors affect fecal glucocorticoid levels across the first year of life in infant rhesus macaques (*Macaca mulatta*)**

### **Authors:**

<sup>1</sup>Tara M Mandalaywala; <sup>2</sup>Michael Heistermann; <sup>1</sup>Dario Maestriperi

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**Presenter and contact person:** Tara M Mandalaywala, tmandalaywala@uchicago.edu

### **Topic:**

Stress

### **Abstract:**

Although not subject to the same pressures as reproductively capable adults, primate infants face their own challenges and stressors, from learning how to navigate the social milieu to extracting resources from caregivers. How these early life challenges affect underlying changes in stress physiology (i.e. adrenocortical activity) is poorly understood. We studied how hormonal indicators of stress fluctuated in infant rhesus macaques. Fecal samples (n = 226) were collected from known, individually identifiable free-ranging infants (n = 47) on Cayo Santiago, Puerto Rico, when animals were between 6 and 12 months of age, and fecal glucocorticoid metabolites (fGCM) were measured using a group-specific 11-hydroxyetiocholanolone enzyme immunoassay validated for rhesus macaques. Investigation of the effects of social and developmental factors on fGCM levels revealed that: 1.) infants of middle-ranking mothers had higher fGCM levels than infants of high or low-ranking mothers ( $p = 0.050$ ), and 2.) fGCM levels decreased from 6 months to 12 months of age ( $p < 0.00$ ). In adult primates, dominance rank can affect cortisol in a variety of manners; some evidence suggests that individuals with unstable ranks (e.g. middle-ranking individuals) are more heavily stressed. Our data suggest that exposure to increased maternal stress leads to higher physiological stress in middle-ranking infants. As weaning is hypothesized to be stressful for the offspring, the high fGCM levels at 6 months followed by a decrease might be physiological evidence for the stress of weaning conflict, which peaks around 6 months in rhesus macaques. Further analysis of behavioral data collected in conjunction with the fecal samples is necessary to explore these questions further. It is clear, however, that investigation of fGCM levels in infant subjects can shed new light on the development of adult physiology and behavior, and help us explore infant specific questions, such as parent-offspring conflict, from a new perspective.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Using non-invasive techniques to study the stress physiology of the Virunga mountain gorillas (*Gorilla beringei beringei*)

### Authors:

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**Presenter and contact person:** Winnie Eckardt, weckard@emory.edu

### Topic:

Stress

### Abstract:

Only 880 mountain gorillas remain in two isolated populations (Virunga and Bwindi populations). Although both populations are increasing due to intensive conservation efforts, they face significant stressors which pose threats to the long-term viability of the subspecies. Only one study of basic stress physiology and sources of stress ( $N_{\text{animals}}=9$ ) has been conducted. Our project thus aimed to provide the first comprehensive understanding of stress physiology in mountain gorillas and to understand the effect of significant sources of stress—both anthropogenic and natural—on stress physiology and health. We focused on the Virunga population, which: 1) ranges at elevations up to ~12,000 feet and thus experiences extreme environmental conditions; 2) is surrounded by one of the highest human population densities in Africa, and 3) has experienced a significant increase in density in recent years. Over an 18-month period representing seven seasons, we collected ~10,000 fecal samples from ~100 gorillas living in nine groups. We aimed to collect weekly baseline samples and *ad libitum* samples for 3-5 days after potential stress events. Samples were frozen each day at  $-20^{\circ}\text{C}$  and hormones were extracted at an on-site laboratory within 2-3 months of collection. Fecal glucocorticoid metabolites (FGM) were measured using a cortisol enzyme-immunoassay (R4866; C. Munro, University of California, Davis). Preliminary analysis provides biological validation of the assay, as extreme stressors such as getting caught in a snare, were associated with elevated FGM ( $\mu_{\text{BSL}}=26.2\text{ng/g}$ ;  $\mu_{\text{Stress}}=45.1\text{ng/g}$ ). Seasonal fluctuations in FGMs suggest an effect of environmental influences such as rain, temperature and elevation, on stress physiology. Future research will include examining the relationship between FGMs and gastrointestinal parasite to examine how stress may impact health. Such findings will provide important information to the larger conservation community to develop policies and make informed decisions on wild ape management and protection.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Peak glucocorticoid metabolite levels within 45 minutes of capture during biological validation of EIA assays in White-tailed Ptarmigan

### Authors:

<sup>1</sup>David Paul Benson; <sup>1</sup>Jessica L Williams; <sup>1</sup>Samuel Jordan; <sup>2</sup>Rupert Palme

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**Presenter and contact person:** David Paul Benson, [dbenson@marian.edu](mailto:dbenson@marian.edu)

### Topic:

Stress

### Abstract:

In many wild species, it is not possible to use invasive validation techniques such as adrenocorticotrophic hormone (ACTH) challenge, dexamethasone suppression test, or a radiometabolism study to relate glucocorticoid metabolites from fecal samples (fGCM) to stress. In this case, a “capture challenge” may provide sufficient biological validation. We obtained an initial fecal sample from wild White-tailed Ptarmigan in Glacier National Park, MT, USA, then captured the birds and took a blood sample. Birds were released and followed for three hours while fecal samples were collected. Two enzyme immunoassays for cortisone, one previously published in Rettenbacher *et al.* 2004 (RETT), and the other a new cortisone assay (NC) developed using the same label and immunogen as RETT, were used to measure fGCM. Although both assays appear to be valid measures of stress in White-tailed Ptarmigan, RETT may be preferred because while the mean percent increase above baseline was not significantly different statistically between the two assays, using RETT all 16 individuals had “after capture” values that were over 70% above baseline. Further, RETT appears to measure GCMs in the urinary component more effectively than NC. Although most individuals’ GCMs rose to a peak within 90 minutes then returned to near or even below initial level by 180 minutes, in at least some cases, peak stress levels were measured in 45 minutes following the stressor. Capture was attempted unsuccessfully for one male, who showed the slowest and mildest increase in GCMs of any of the males sampled during the breeding season. Although the number of individuals in this study was small and the difference was not statistically significant, females without chicks had a smaller mean increase above baseline (132.5%; n=4) than females with chicks (3547.1%; n=4).



2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Assessment of capture stress, entanglement stress and stranding stress in leatherback sea turtles (*Dermochelys coriacea*)**

### **Authors:**

<sup>1</sup>Hunt K; <sup>1</sup>Innis C; <sup>1</sup>Merigo C; <sup>1</sup>Rolland R

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**Presenter and contact person:** Kathleen Hunt, huntk@neaq.org

### **Topic:**

Stress

### **Abstract:**

As part of a multi-species study on stress physiology of sea turtles, we compared corticosterone and thyroxine of 16 healthy leatherback sea turtles (*Dermochelys coriacea*), vs. 11 distressed leatherbacks found in a variety of life-threatening situations, with the goal of assessing relative impact of different stressors. The healthy animals were captured at sea for satellite tag attachment during 2007-2012; blood samples were obtained an average of 30 min after initiating capture and thus probably reflect some degree of capture stress. The distressed animals included eight turtles entangled in fishing gear, one turtle trapped in a weir net (e.g., confined but not injured), and two turtles stranded on shore; these turtles had been subjected to these stressors for unknown periods of time before sampling. Plasma samples were analyzed for corticosterone and free thyroxine using MP Biomedicals <sup>125</sup>I radioimmunoassay kits #07-120102 and #06B-257214, respectively (Solon, OH, USA); both assays have been validated for this species. Plasma mean corticosterone levels were significantly higher in distressed leatherbacks (11.22 ng/ml  $\pm$  2.23) than in healthy leatherbacks (3.99 ng/ml  $\pm$  0.62; t-test, P = 0.002). Generally, corticosterone was highest in stranded and entangled turtles, intermediate in the turtle in the weir net, and lowest in the healthy turtles. Thyroxine levels were undetectably low in all healthy captured turtles, but were detectable in the majority of entangled turtles, indicating that swimming while entangled may represent a metabolic challenge for leatherbacks. Overall, live capture of leatherbacks at sea seems to represent only a mild stressor for healthy animals. In contrast, entanglement and stranding appear to be more severe stressors, and entanglement in particular may carry a significant metabolic cost.



2013 Annual Conference, 14-16 October, Chicago IL, USA

## Correlations among plasma corticosterone and multiple physiological metrics in a songbird community

### Authors:

<sup>1</sup>Travis E Wilcoxon; <sup>1</sup>David J Horn; <sup>2</sup>Sarah J Huber; <sup>1</sup>Brianna M Hogan; <sup>1</sup>Cody N Hubble; <sup>1</sup>Madeline H Knott; <sup>1</sup>Sarah Plants; <sup>3</sup>Samantha J Wassenhove  
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**Presenter and contact person:** Travis E Wilcoxon, twilcoxen@millikin.edu

### Topic:

Stress

### Abstract:

Physiological ecologists are regularly searching for effective ways to determine the impacts of variable environmental conditions within habitats on the physiology of animals living in those habitats. A long-running physiological product of choice for assessing stress in vertebrates is measurement of glucocorticoids in blood samples. We conducted a 2-year, community-level study of the effects of supplemental feeding of a traditional commercial bird seed blend on the health of over 2,000 free-living birds from 10 species of passerines and near-passerines and utilized many physiological metrics to assess the health of the birds. Total captures ranged from 86 to 224 birds from each species with a 34% recapture rate over the course of the study. Naturally, species varied in body size; therefore, we ensured that blood samples from each capture comprised less than 1% of the bird's body weight. Included in this assessment were corticosterone measurement via enzyme immunoassay (Cayman Chemical, Ann Arbor, MI), blood smears for leukocyte counts, feather quality assessment, total antioxidant capacity, and three measurements of innate immune function. Birds at sites with supplemental food had significantly lower baseline corticosterone levels than birds at control sites without supplemented food following three months of supplementation ( $p = 0.023$ ). Baseline corticosterone levels were inversely correlated to total antioxidant capacity ( $p = 0.004$ ), inversely correlated to the bactericidal capability of the blood ( $p < 0.032$  in all three measures of innate immunity), and positively correlated with heterophil to lymphocyte ratios ( $p = 0.027$ ). These correlations among multiple physiological products and baseline corticosterone levels further our understanding of the influence of glucocorticoids on the physiology of free-living birds. Further, the differences between birds with supplemental food and those without supplemental food suggests that commercial bird seed availability significantly influences the health of wild birds.





2013 Annual Conference, 14-16 October, Chicago IL, USA

## **Application of non-invasive method for monitoring stress hormones for the study of interspecies chemical communication in the house mouse (*Mus musculus musculus*)**

### **Authors:**

<sup>1</sup>Tatiana K Laktionova; <sup>1</sup>Anna E Voznesenskaya; <sup>1</sup>Maria A Klyuchnikova; <sup>1</sup>Vera V Voznessenskaya

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### **Topic:**

Stress

### **Abstract:**

Predator-prey relationships provide an excellent model for the study of interspecies chemical communication. Our earlier research showed that chemical cues from the natural predator *Felis catus* (intact urine, L-felinine) significantly suppresses reproduction in *M. m. musculus*. To study possible mechanisms underlying suppressed sexual behavior and reduced reproductive effort we monitored plasma corticosterone and compared our findings with the results of non-invasive monitoring of fecal glucocorticoid (GC) metabolites. Blood samples (0,075 ml) were taken from orbital sinus on 2-d, 5-th and 8-th day of pregnancy. To monitor fecal GC metabolites we used the technique established by Touma et al (2004). On completion of exposures to L-felinine (0.05%) fecal material was collected from each animal over 24 h period. We observed clear elevation of plasma corticosterone in mice in response to cat urine (681,25±135,16 ng/ml; p<0.001; n=8) and to guinea pig urine (278,87 ng/ml±96,91 ng/ml), but not to water treatment (92,75±43,51 ng/ml). As a positive control we used the "open arena" test. Mice responded to this kind of treatment with elevated corticosterone (371,25±175,05 ng/ml) but we observed habituation during the course of consecutive placements(183,75±86,34 ng/ml and 96, 25±34,61ng/ml). At the same time mice did not habituate to consecutive exposures to cat odor (706,25±123,63 ng/ml and 716,25±105,55 ng/ml). Long lasting exposure of mice to predator chemical cues showed significant elevation of corticosterone. In water treatment group of mice concentration of GC metabolites was 203, 85 ± 47, 74 ng/0.2g feces; in felinine treatment group – 702, 15 ± 122, 24 ng/0.2g feces (n= 13, p<0.001). The response of laboratory naive animals to predator scents and failure to habituate to the stimulus indicate the innate nature of the response. Chronically elevated GCs in response to cat odor exposure, especially at early stages of pregnancy may be a reason for the induction of pregnancy block. Supported by RFBR 12-04-32079.