#### **WORKSHOP HORMONE MONITORING IN ZOO ANIMALS**

## Tiergarten Nürnberg, Germany

15. - 16. October 2015

#### **Preliminary Program**

## Thursday, 15. October 2015

13:00 - 14:30

Hormone Monitoring: Tools for Population Management

Dr. Gabriela Mastromonaco - Canada

#### 14:30 - 15:00 Coffee Break

15:00 - 16:30

Non-invasive endocrine monitoring in urine and feces of mammals: the importance of validation.

Dr. Marina Ponzio - Argentina

#### 16:30 - 17:30 Discussion

### Friday, 16. October 2015

10:00 - 11:00

Measuring glucocorticoids resp. their metabolites in hair and faeces of polar bears (*Ursus maritimus*)

Ana Hein, TIHO Hannover & Zoo Karlsruhe, Germany

11:00 - 12:00

Reproductive Hormone Manipulation: Applications in Zoo Species

Dr. Gabriela Mastromonaco, Canada

12:00 - 13.00

FINAL DISCUSSION

## **Hormone Monitoring: Tools for Population Management**

Dr. Gabriela Mastromonaco Curator of Reproductive Programs & Research, Toronto Zoo Canada

On-going declines in wildlife populations present a challenge to biologists and animal managers working with free-ranging and captive populations. The

sustainability of genetically and demographically stable populations is problematic due to constant environmental pressures, reduced or fragmented populations, disease concerns, and other anthropogenic factors. Reproductive and stress hormone levels are important indicators of population health and growth, and thus, non-invasive hormone analysis can be instrumental in helping wildlife biologists understand the dynamics of natural populations. Hormone profiles have been established for numerous species in diverse taxa using a variety of sample types, including blood, saliva, urine, and feces, as well as using novel substrates currently being developed (hair/fur, feathers, skin sheds and claws). Work in our laboratory has focused on developing endocrinological tools to monitor, understand and implement solutions for the long-term sustainability of threatened species.

## Non-invasive endocrine monitoring in urine and feces of mammals: the importance of validation.

Dr. Marina F Ponzio Investigadora Adjunta CONICET Profesora Adjunta FCM-UNC Instituto de Fisiología INICSA-CONICET Universidad Nacional de Córdoba Córdoba-Argentina

Traditionally, studies on reproductive physiology and stress response in mammals have been based on the determination of endocrine activity of hormones associated with these processes in plasma. However, extraction of blood samples is, in itself, a procedure that can modify plasma cortisol levels and in turn negatively impact the expression of the hypothalamic-pituitary-gonadal axis. Furthermore, plasma levels of certain hormones fluctuate widely as a result of pulsatile secretion and / or circadian rhythms and therefore each blood sample provides a static data of a parameter that is variable, so that multiple samples would be required for a reliable notion of the hormone secretory activity over time.

For this reason, an efficient alternative developed in recent years, is the non-invasive monitoring of steroid hormone metabolites excreted in various matrices as feces, urine, saliva, hairs etc. Its usefulness has been extensively tested in many species. With the implementation of animal welfare policies and research methodologies based on the 3 Rs this technique is also being used in laboratory animals. Its main benefit is that with the use of animal handling, stress and risks associated with repetitive puncture is avoided. The sample collection may be performed over prolonged periods of time and finally, the assays used are relatively simple, efficient and easy to adapt from one species to another. Using the non-invasive technique we can study topics as diverse as reproductive cycles, seasonal variations in hormone levels, sexual and behavioral differences associated with hormones, association between hierarchical positions, thyroid function, effects of environmental toxins on endocrine function, stress and hormone levels with effects on reproduction and the effects of human activities on animal welfare. However, implementation of the laboratory techniques associated

with this discipline emphasizes its previous validation, aspects that will be considered in this dissertation.

# Measuring glucocorticoids resp. their metabolites in hair and faeces of polar bears (Ursus maritimus)

Vet. Med. Anna Hein Tierärztliche Hochschule Hannover & Zoo Karlsruhe Germany

In the field of zoo and wildlife medicine especially non-invasive methods for measuring glucocorticoids (GCs) are being used in order to evaluate an individual's physical condition referring to stress. Particularly the analysis of faecal glucocorticoid metabolites (FGM) or the recently investigated measurement of hair cortisol concentrations (HCC) have proved to be useful.

As demonstrated in previous studies (Macbeth et al. 2012; Bechshøft et al. 2011, 2012; Shepherson et al. 2013) the analysis of GCs is also possible in polar bear hair and faeces.

However, GC values in these studies differ widely what among others could be attributed to differences of the particular applied laboratory method (sample preparation, extraction, assay etc.).

Furthermore the interpretation of the GC levels obtained from polar bear hair or faeces remains very difficult because no species-specific base lines have been established for polar bears, also relations between stressful events and faecal/hair GCs of polar bears are only insufficiently known.

That for, during this study methods of measuring HCCs and FGMs in polar bears will be analytically and biologically validated.

Additionally an investigation of long-term cortisol profiles of zoo polar bears will help to understand seasonal variations in the release of GCs and enable to approach reference values for this species.

Analytical validation of the assays will be conducted through reapeated measurements of opportunistically obtained hair and faecal samples of polar bears in European Zoos according to laboratory standards (specificity, linearity, accuracy, limit of determination/detection).

To biologically validate the measurement of FGMs, faeceal samples will be collected in the context of transports (resp. other stressful events), when an increase of GCs can be expected.

For setting up long-term cortisol profiles, hair samples of polar bears from European institutions will be collected for at least 12 months and analysed for cortisol. Hair will be sampled through repeated shavings of the same body region. For the interpretation and biological validation of the measured HCCs possible stressful events (e.g. fights, mating, changes of enclosure) will be recorded by the keepers parallel to the hair sampling.

The establishment of repeatable analysing methods as well as the investigation of cortisol base lines and circannual fluctuations in captive polar bears is essential for the assessment and understanding of cortisol levels from wild bears, since a validation and establishment of reference values is hardly possible under field conditions.

In the course of the project different methods of analysing glucocorticoids in polar bear hair and faeces will be investigated, validated and applied.

#### Reproductive Hormone Manipulation: Applications in Zoo Species

Gabriela Mastromonaco, PhD Curator of Reproductive Programs & Research, Toronto Zoo

Zoos and aquariums care for a wide range of species with diverse social dynamics and reproductive strategies. Maintaining healthy and sustainable populations in captivity can be challenging due to the constraints of captive environments, including space limitations, availability of genetically compatible mates, and less understood factors, such as stress. Reproductive hormones play a key role not only in the breeding success of the animals, but also in their health and welfare. With the onset of circulating reproductive hormones come sexual maturity and associated changes in ovarian/testicular activity, anatomical features and behaviour. As a result, management of reproductively active animals can be difficult for a variety of reasons, including lack of or too much breeding success, sexual aggression, and potential for reproductive pathologies. Manipulation of natural reproductive hormones levels using exogenous hormones in the form of injectables or implants provides unique methods for modifying reproductive function with the goal of improving animal breeding, health and welfare.