Meeting report

International Society for Wildlife Endocrinology: the future of endocrine measures for reproductive science, animal welfare and conservation biology

André Ganswindt1,2,*, Janine L. Brown3, Elizabeth W. Freeman4, Andrew J. Koub5, Linda M. Penfold6, Rachel M. Santymire7, Mandi M. Vick8, Nadja Wielebnowski9, Erin L. Willis10 and Matthew R. Milnes11

1Department of Zoology and Entomology, Mammal Research Institute, University of Pretoria, Pretoria, Republic of South Africa
2Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, Republic of South Africa
3Center for Species Survival, Smithsonian Conservation Biology Institute, Front Royal, VA, USA
4New Century College, George Mason University, Fairfax, VA, USA
5Department of Conservation and Research, Memphis Zoological Society, Memphis, TN, USA
6SEZARC/White Oak Conservation Center, Yulee, FL, USA
7Department of Conservation and Science, Lincoln Park Zoo, Chicago, IL, USA
8Cleveland Metroparks Zoo, Cleveland, OH, USA
9Chicago Zoological Society, Chicago, IL, USA
10Department of Natural Sciences, Mars Hill College, Mars Hill, NC, USA
*Author for correspondence (aganswindt@zoology.up.ac.za).

Hormone analysis is a precise and widely accepted tool for monitoring reproductive function and responses to stressors. Although hormones are present and can be measured in various biological matrices, non-invasive methods have gained popularity over the past 30 years as a more practical approach for assessing ovarian, testicular and, more recently, adrenocortical activity in intractable wildlife species. Non-invasive hormone monitoring also has been key to understanding basic mechanisms related to observed behaviours of captive and free-ranging animals. Despite the increasing popularity of this research field, wildlife endocrinologists have not had a specific forum for sharing and discussing their latest findings, technical developments and common challenges. To provide such a communication platform, the International Society for Wildlife Endocrinology (ISWE) was established in 2010, followed by an international meeting held on 3–4 November 2011 at the Toronto Zoo, Canada. Over several sessions, keynote speakers and participants discussed recent developments of new and innovative methods for hormone monitoring, as well as the latest advances in basic endocrinology as applied to adrenal function, reproductive physiology, animal health, ecology and evolution. Here, we introduce ISWE to the scientific community and discuss how this new society will serve as a resource for wildlife endocrinologists worldwide.

Keywords: non-invasive hormone monitoring; behavioural endocrinology; environmental endocrinology; conservation endocrinology; reproductive hormones; stress hormones

1. INTRODUCTION

Collectively, we know very little about the endocrinology of the majority of mammals on the planet, with only 2 per cent having been described in any detail; even less is known about birds, reptiles, amphibians and invertebrates [1,2]. Yet, hormone analyses are key to understanding basic physiological functioning, such as reproduction, metabolic activity, health and well-being. Hormones are present and can be measured in several biological matrices, including blood, saliva, urine, hair and faeces. The choice of which to use depends on a range of factors, including the type of information required, the assay techniques available, species differences in hormone metabolism and excretion, and the practicality of sample collection. For intractable or free-ranging wildlife species, non-invasive approaches based on urine and faecal analyses offer tremendous advantages and are the preferred option in most situations.

As reviewed by Monfort [1], urinary steroid monitoring began in the 1970s to assess ovarian cycles and pregnancy of captive non-human primates, followed by their adaptation in the early 1980s to zoo species. In the late 1980s, methods to measure faecal steroids were developed to track reproductive activity in a diverse array of species. By the early 1990s, measurements of faecal adrenal glucocorticoid metabolites began to be used as indicators of stress in managed and free-ranging populations. Owing to the rapid loss of our planet’s biodiversity, there is a growing international interest within the scientific community in understanding the physiology (including regulative endocrine mechanisms), behaviours and stressors that impact the survival of threatened species in captivity and in the wild, which can be accomplished using various endocrine methods.

Historically, researchers actively engaged in wildlife endocrinology have not had a specific forum for sharing protocols and resources or discussing their latest developments, research findings and common challenges. Furthermore, there has been a lack of informational resources for investigators interested in adopting non-invasive approaches in newly established research programmes. With this underlying motivation, the International Society for Wildlife Endocrinology (ISWE) was created in 2010 after two planning workshops hosted by the Smithsonian’s Conservation Biology Institute in 2008 and 2009. An inaugural international gathering was held at the Cincinnati Zoo and Botanical Garden in 2010, followed by a meeting in November 2011 at the Toronto Zoo, which brought together attendees from nearly a dozen countries across six continents. The purpose of this report is to introduce ISWE to the scientific community at large, and identify our group as a global resource for conservation and wildlife endocrinology research.
2. MAJOR THEMES OF THE 2011 INTERNATIONAL SOCIETY FOR WILDLIFE ENDOCRINOLOGY MEETING

(a) Advances in basic endocrinology as applied to reproduction

Non-invasive monitoring of gonadal steroid hormones (oestrogens, progestagens and androgens) has become a widely appreciated technique that can provide insight into the reproductive characteristics of species in which invasive sampling is not feasible. Its diverse applicability has led to an increased utilization in the field of environmental endocrinology, where scientists strive to understand the underlying mechanisms that explain observations of altered fitness (e.g. intersex individuals, delayed or suppressed reproduction and development) in some species or populations.

In his keynote address to open the meeting, Prof. Glen van der Kraak (University of Guelph, Canada) used a series of case studies to illustrate how endocrinology has increased our understanding of the physiology of wildlife species and also how it has been used to define the actions of natural and anthropogenic stressors in the environment. The presentation surveyed the impacts of the biocide, tributyltin, on marine gastropods as well as the impacts of human-use pharmaceuticals and industrial processes on reproductive fitness in fish. A further example highlighted the association between exposure to a potential endocrine disruptor used as an agricultural herbicide, atrazine, and the development of intersex in the African clawed-frog (*Xenopus laevis*) in its native habitat.

Multiple presentations then discussed recent advances in basic endocrinology as applied to reproduction. For example, Andre Ganswindt (University of Pretoria, South Africa) described longitudinal profiles of excreted oestrogen and progestagen metabolites throughout gestation in aardvarks (*Orycteropus afer*), revealing new evidence for the existence of delayed implantation. Tamara Keeley (Taronga Western Plains Zoo, Australia) presented hormonal evidence of an autonomous ovary in the highly endangered Tasmanian devil (*Sarcophilus harrisii*), and Marina Poncio (Universidad Nacional de Córdoba, Argentina) showed results for cycle manipulation in female chillas as a first step towards establishing an effective assisted reproduction protocol. To better understand factors that promote reproduction in captive kori bustards (*Ardeotis kori*), Linda Penfold (SEZARC/White Oak Conservation Center, USA) showed how non-invasive faecal testosterone analysis can be used to predict the fertility potential of male and female birds.

Janine Brown (Smithsonian Conservation Biology Institute, USA) illustrated the variability in oestrus cycle characteristics among a large herd of female Asian elephants (*Elephas maximus*) highlighting the need for more institution-specific management strategies to maximize elephant health and reproduction. Ratna Ghosal (Indian Institute of Science, India) described behavioural and endocrine correlates associated with musth in wild male Asian elephants and identified subtle yet significant differences in musth-related hormone patterns between two phenotypes (tuskers and tuskless males).

(b) Advances in basic endocrinology as applied to adrenal function

Methods for determining adrenocortical activity as a measure of stress have been gaining traction in animal welfare, ethology and conservation [3]. In his keynote address, Prof. Rudy Boonstra (University of Toronto at Scarborough, Canada) highlighted the important role of the stress axis in allowing individuals to cope with environmental change and uncertainty, as well as its central role in evolutionary adaptations to ecological pressures on a species level. Through studies on northern and temperate mammals, he showed how fluctuations in predator pressure or food supply affect both individual fitness and population demography and outlined how aspects of the stress axis have evolved to produce particular life-history strategies.

Andre Ganswindt then described the validation of enzyme-immunoassays for non-invasive monitoring of adrenocortical function in African buffalo (*Syncerus caffer*), to examine the potential effect of stress on the transmission path of foot and mouth disease from wild ungulate to livestock species in Africa. Sergey Naidenko (Severtsov Institute of Ecology and Evolution, Russia) identified interesting species differences in seasonal variation of glucocorticoid levels among several felid species living under extreme temperature fluctuations (winter: $-40^\circ$C, summer: $+40^\circ$C) in the Russian far east, and Kathleen Hunt (New England Aquarium, USA) showed how analysis of corticosterone and thyroxine may serve as a potential tool for triage, monitoring of recovery and readiness for release of stranded Kemp’s Ridley sea turtles (*Lepidochelys kempii*) after recovery from cold-stunning.

(c) Innovative approaches and method development

The unique challenges of working in a field environment, inter-specific variation and animal welfare issues create a constant need for developing novel methods for studying the endocrinology of free-ranging and captive wildlife. One ubiquitous challenge is the anthropogenic influence on habitat. This was highlighted by a talk from Krista Milich (University of Illinois, IL, USA) and colleagues, who showed differences in the relationship between habitat quality and female reproduction in the endangered red colobus monkey (*Procolobus rufomitratus*) of Kibale National Park, Uganda. Highlighting the use of unconventional taxa, Michela Sugni (University of Milan, Italy) exposed sea urchins (*Paracentrotus lividus*) to varying concentrations of oestradiol to study hormonal mechanisms regulating ovarian development and reproduction, and found it did not promote gonadal maturation in this species as hypothesized.

In the wild, a major challenge is determining the identity of individual faecal samples. Using a unique approach based on headspace solid-phase microextraction and gas chromatography mass spectrometry, Martin Dehnhard (Leibniz Institute for Zoo and Wildlife Research, Germany) investigated whether odour profiles differed between individuals of two species: Iberian lynx (*Lynx pardinus*) and brown bear (*Ursus arctos*), but found that, unless diets differed, odour profiles were indistinguishable. Another presentation by
Michael Lepschy (University of Veterinary Medicine, Austria) reported on the excretion and deposition of cortisol into guinea pig hair, which indicated possible local production of glucocorticoids in the hair follicle itself, thus raising questions about its use for evaluating chronic stress in various species. Finally, Grace Fuller (Cleveland Metroparks Zoo and Case Western Reserve University, USA) presented a method for quantifying melatonin in saliva from nocturnal prosimians to understand how they are physiologically affected by zoo exhibit lighting design. The findings indicate that melatonin production may be suppressed owing to the brightness of dark phase lights often used in nocturnal houses, suggesting that improved lighting designs may be necessary for some exhibits.

(d) Standardization and information sharing

The advent of new endocrine techniques brings about questions of standardization. By using different biological matrices or by modifying the analysis methods, the absolute hormone values revealed may not be directly comparable [4]. For example, the choice of antibody introduces significant variation when measuring hormone metabolites [5]. To address this concern, an inter-laboratory comparison of progesterone-enzyme-immunoassays versus radio-immunoassays in Bovidae and Hylotragidae species was conducted by Diana Armstrong (Lincoln Park Zoo, USA). Findings confirmed that different assays often produce similar temporal results but measured concentrations and signal quality varies substantially, underlining the importance to test different assays to determine the most suitable for each species. Therefore, a major goal of ISWE is to develop an endocrinology database as a means of sharing methodologies and results of wildlife studies. Edward Wilkerson, an information specialist from Lincoln Park Zoo, is currently working with ISWE to create a robust, user-friendly data repository for current endocrine projects. We envision this repository will serve as the foundation for information sharing across a diverse assemblage of scientists with interests in wildlife endocrinology.

(e) Assay reagent resources

One of the technical hurdles that must be overcome in using immunoassays is identification of an antibody that will recognize the hormone of interest, especially when measuring faecal and urinary steroids that are often metabolized before excretion. As previously mentioned, challenges arise when endocrine laboratories use different assay systems thus limiting direct comparisons of quantitative data across studies. Following this, a major goal of ISWE is to improve access and/or distribution of existing resources (i.e. create a repository and distribution network for existing antibodies and labelled antigens) and develop new resources (i.e. new antibodies through partnerships with commercial and not-for-profit laboratories) so that researchers can maintain consistency in assay methods. We therefore created regional consortia (e.g. European, US and Australasian) among ISWE members to share in the costs of producing new materials for use by the global wildlife endocrine community. Other groups will validate these new assay systems for a diverse array of species and share this knowledge through our web portal. In this way, ISWE will facilitate stronger collaborations among endocrinologists and assist in standardization of wildlife endocrinology methods.

3. FUTURE DIRECTIONS

The international attendance and overall success of the first two ISWE conferences (2010 and 2011) further underscored the need for a specific forum to share both existing knowledge and new ideas in the field of wildlife endocrinology. The continued growth and expansion of this meeting has brought opportunities for new collaborations, support of student development, conserving and pooling of resources, and exploration of new approaches for monitoring hormones in wildlife. Future conferences hope to attract an even larger and broader group of colleagues to share knowledge and to introduce the possible applications of non-invasive endocrinology research to newcomers. The field of wildlife endocrinology has been expanding at a rapid pace, yet the need for new tools and techniques is greater than ever in our quest to understand more about how to manage and conserve the diversity of species on our planet. The ISWE provides a much-needed forum to bring together those in zoos and aquaria, academia and private institutions working towards a better understanding of the biology of wildlife.

We thank all conference participants and ISWE members for their commitment, and Toronto Zoo for hosting the meeting.