



Editorial

Special issue: Biomarkers of reproductive health in wildlife

Founded in 2010, the International Society for Wildlife Endocrinology (ISWE) is a non-profit organization whose mission is to advance the science of wildlife endocrinology, expand our understanding of animal physiology, and apply that information to improving management and conservation of the world's diverse species. ISWE has a rich history of developing innovative endocrine monitoring techniques to answer fundamental questions about why some animals thrive while others do not. Hormones play a key role in shaping animal health and fitness; therefore, endocrine monitoring can provide valuable insights into the biological functioning of an individual and what factors affect it.

A key to species survival is reproduction, which involves the interplay of multiple hormone systems. Monitoring reproductive function through hormonal analyses can provide insights into the health of both wild and captive populations. It can inform our understanding of life history strategies, behavior, and selection pressures acting on a population. This knowledge is of utmost importance in the face of increasing anthropogenic threats, such as climate change, pollution, poaching, and habitat loss. Knowledge about basic reproductive biology is also essential for conservation breeding programs to manage pairing animals for mating to developing more advanced assisted reproductive techniques.

This special issue showcases recent advances by ISWE members in developing and using endocrine biomarkers to assess reproduction in a wide range of wildlife species (references in **bold**). Developing reliable, biologically relevant, and, whenever possible, noninvasive biomarker tests is challenging, particularly when working with wildlife species in captive and wild environments. The articles in this issue reveal the diverse strategies used to overcome these challenges and develop a better understanding of reproductive biology, both *in situ* and *ex situ*. A key paper in this special issue is the review by **Ghosal et al. [1]**, which provides a comprehensive overview of reproductive biomarkers used in diverse species and outline exciting future directions in the field.

When studying the reproductive physiology of wildlife, the first challenge is to develop, validate, and optimize appropriate monitoring tools for a particular species. Traditional approaches to studying endocrinology, such as blood sampling, are difficult to use with many wildlife species. Founding and current members of ISWE have played key roles in pioneering novel, non-invasive techniques for studying reproductive endocrinology in a wide range of species. They have developed, adapted, and optimized techniques such as urinary and fecal hormone monitoring [2] to investigate numerous hormones and biomarkers [3]. The papers in this special issue illustrate validation and optimization steps for different species, hormones, and substrates. For example, **Roth et al. [4]** validated an assay for monitoring prolactin in white, black, and Sumatran rhinoceroses, and **Atkinson et al. [5]** validated an assay for monitoring progesterone in blubber samples from humpback whales.

Keeley et al. [6] demonstrate the range of validation steps necessary when working with unusual species in unusual locations; in this case, sensitive ghost bats roosting in caves. It is important to note that every species is different; therefore, methodological validation and optimization must be conducted for each species, hormone, and substrate [7].

The second step is to establish foundational knowledge about the reproductive biology of a species. We lack basic information about the reproductive physiology of the vast majority of species, with only a small fraction described in any detail [8]. Many studies in this issue focused on unraveling the complexity of female reproductive endocrinology. For example, **Cantarelli et al. [9]** characterize ovarian activity in chinchillas, and **O'Hanlon et al. [10]** map endocrine changes during pregnancy in okapi. **Van den Berghe [11]** found that some biomarkers are more reliable than others for identifying the fertile period in African wild dogs. One benefit of non-invasive endocrine monitoring is that it enables researchers to collect repeated samples and construct longitudinal hormone profiles over prolonged periods of time, which can provide insights about unusual reproductive traits. Both **Bateman-Jackson et al. [12]** and **Wauters et al. [13]** capitalized on this to improve our understanding of embryonic diapause in wolverines and giant pandas, respectively.

Armed with appropriate tools and a good understanding of basic reproductive biology, biologists can start predicting reproductive events or outcomes. Several studies in this special issue examined longitudinal measures of hormone biomarkers to predict reproductive outcomes. For example, **Brown et al. [15]** found significant differences in hormone levels between laying and non-laying whooping cranes during key reproductive stages, and **Wauters et al. [13]** were able to identify endocrine differences between pregnancy and pseudopregnancy in giant pandas. However, individual variation poses a major challenge to developing robust, reliable biomarker tests [14]. A common constraint in wildlife studies is sample size – both the number of collected samples and the number of animals evaluated. These must be considered carefully when optimizing and refining reproductive techniques to ensure a proper understanding of healthy hormone profiles. Without understanding individual variation, there is a risk of misinterpreting normal hormone fluctuations.

Although most studies focused on classic reproductive steroid hormones (progesterone, estradiol, testosterone), others investigated less-studied hormones. For example, **Hart et al. [16]** characterized changes in DHEA during pregnancy in primate species, identifying key differences in DHEA associated with fetal health and pregnancy outcomes. Both **Prado et al. [17]** (African elephants) and **Roth et al. [4]** (rhino species) explored the use of prolactin as a biomarker of reproductive status and overall health. Interestingly, **Dedato et al. [18]** found

that fecal progesterone metabolite concentrations, which are widely used to diagnose pregnancy in other species, could not distinguish between parturient and non-parturient red pandas. This highlights the importance of reporting unexpected or negative results, especially for biomarkers that typically work in other species.

A major benefit of hormone analyses is that they can provide a cost-effective, non-invasive tool to monitor wildlife reproduction, health, and welfare across an array of species and situations. In animals under human care, those analyses offer valuable insights into understanding how management factors impact reproductive success, as demonstrated by **Schwarzenberger and Pannrucker [19]** for white rhinoceros. In wild, free-ranging animals, **Keeley et al. [6]** (ghost bat) and **Atkinson et al. [5]** (humpback whale) showed how progesterone analyses can be used to detect pregnancy, which is key to understanding threats to fitness and survival. This information can then be used to predict population growth or identify times or locations where breeding individuals need extra protection.

ISWE remains steadfast in its mission to create robust endocrine monitoring techniques and comprehensive endocrine databases that can illuminate a path toward understanding and safeguarding the rich diversity of wildlife species. These tools are even more relevant today as we face increasing threats to habitats and the species therein. Animals often do not outwardly advertise negative biological states; thus, hormone monitoring can effectively shine a light on what is happening inside an animal so that mitigating steps might be taken to prevent adverse outcomes.

CRediT authorship contribution statement

Tamara Keeley: Conceptualization, Writing – review & editing. **Janine L Brown:** Conceptualization, Writing – review & editing. **Kerry V Fanson:** Conceptualization, Writing – original draft, Writing – review & editing. **Franz Schwarzenberger:** Conceptualization, Writing – review & editing. **Beth M Roberts:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] R. Ghosal, K.L. Edwards, T.L. Chiarelli, K.V. Fanson, A. Ganswindt, T. Keeley, et al., Biomarkers of reproductive health in wildlife and techniques for their assessment, *Theriogenol. Wild* 3 (2023) 100052.
- [2] K. Hodges, J. Brown, M. Heistermann, D. Kleiman, K. Thompson, Endocrine monitoring of reproduction and stress. *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*, 2010, pp. 447–468.
- [3] D.C. Kersey, M. Dehnhard, The use of noninvasive and minimally invasive methods in endocrinology for threatened mammalian species conservation, *Gen. Comp. Endocrinol.* (2014).
- [4] T.L. Roth, E.M. Donelan, L.A. Rispoli, T. Reilly, Prolactin enzyme-linked immunosorbent assay for rhinoceroses – another tool for assessing reproductive function and dysfunction in this taxon, *Theriogenol. Wild* 2 (2023) 100035.

- [5] S. Atkinson, V. Melica, S. Teerlink, K. Mashburn, J. Moran, H. Pearson, Use of hormones in assessing reproductive physiology of humpback whales (*Megaptera novaeangliae*) from Juneau, Alaska, *Theriogenol. Wild* 3 (2023) 100050.
- [6] T. Keeley, M. O'Connell, L. Kelman, B. Laming, C. Knuckey, Validation of non-invasive hormone analysis techniques to assist in the future identification of maternal roosts of ghost bats (*Macroderma gigas*), *Theriogenol. Wild* 3 (2023) 100060.
- [7] C. Touma, R. Palme, Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation, *Ann. N. Y. Acad. Sci.* 1046 (2005) 54–74.
- [8] A. Ganswindt, J.L. Brown, E.W. Freeman, A.J. Kouba, L.M. Penfold, R. M. Santymire, et al., International Society for Wildlife Endocrinology: the future of endocrine measures for reproductive science, animal welfare and conservation biology, *Biol. Lett.* 8 (2012) 695–697.
- [9] V.I. Cantarelli, G. Mastromonaco, G. Galeano, M. Fiore de Cuneo, M.F. Ponzio, Use of urinary biomarkers of ovarian function in domestic Chinchilla lanigera: assessing protocols for exogenous regulation of the hypothalamic-pituitary-gonadal axis, *Theriogenol. Wild* 4 (2024) 100074.
- [10] J. O'Hanlon, R. Moge, S.L. Walker, S. Roffe, H. Clarke, K.L. Edwards, Utilising routine non-invasive faecal samples for the detection of oestrus and early gestation in okapi (*Okapi johnstoni*), *Theriogenol. Wild* 3 (2023) 100047.
- [11] F. Van den Berghe, M.C.J. Paris, Z. Sarnyai, A. Ganswindt, D.B.B.P. Paris, Peri-ovulatory changes in behavior and fecal hormone metabolite concentrations but not vaginal cytology or vaginocopy are indicative for the fertile period in female African wild dogs (*Lycaon pictus*), *Theriogenol. Wild* 2 (2023) 100032.
- [12] H.L. Bateman, L.K. McCaw, J.L. Seguin, W.F. Swanson, A.E. Rivas, R. Boonstra, et al., Assessing reproduction in wolverines (*Gulo gulo*): fecal progesterone levels in zoo and wild females, *Theriogenol. Wild* 3 (2023) 100054.
- [13] J. Wauters, K.S. Wilson, T. Cools, C. Vancsok, T. Bouts, B. Mulot, et al., Pregnancy length and health in giant pandas: what can metabolic and urinary endocrine markers unveil? *Theriogenol. Wild* 3 (2023) 100063.
- [14] T.D. Williams, Individual variation in endocrine systems: moving beyond the 'tyranny of the Golden Mean', *Philos. Trans. R. Soc. B: Biol. Sci.* 363 (2008) 1687–1698.
- [15] M.E. Brown, G.H. Olsen, C.L. Keefer, N. Songsasen, Egg production and endocrine profiles of female whooping cranes (*Grus americana*) maintained ex situ are improved in naturalized enclosures, *Theriogenol. Wild* 2 (2023) 100034.
- [16] E.N. Hart, G.P. da Silva, R.S.C. Takeshita, Dehydroepiandrosterone and dehydroepiandrosterone-sulfate: biomarkers of pregnancy and of fetal health, *Theriogenol. Wild* 3 (2023) 100056.
- [17] N.A. Prado, M. Krcmarik, S. Paris, L.L. Rockwood, J.L. Brown, Abnormal prolactin secretion is associated with changes in metabolic health biomarkers in acyclic female African elephants (*Loxodonta africana*), *Theriogenol. Wild* 3 (2023) 100062.
- [18] M.N. Dedato, J. Magerman, O. Berke, E. Curry, G.F. Mastromonaco, Understanding pregnancy-related hormones in female red pandas, *Theriogenol. Wild* 3 (2023) 100053.
- [19] F. Schwarzenberger, C. Pannrucker, The effects of animal transfers on the reproductive success of female white rhinoceroses (*Ceratotherium simum simum*) kept in European zoos, *Theriogenol. Wild* 3 (2023) 100049.

Kerry V. Fanson^{a,*}, Janine L. Brown^b, Tamara Keeley^c, Beth M. Roberts^d, Franz Schwarzenberger^e

^a School of Agriculture, Biomedicine and Environment, La Trobe University, Melbourne, Victoria, Australia

^b Smithsonian's National Zoo and Conservation Biology Institute, Front Royal, VA, USA

^c School of Agriculture and Food Sustainability, University of Queensland, Brisbane, Queensland, Australia

^d Department of Biological Sciences and Pathobiology - Endocrinology, University of Veterinary Medicine, Vienna, Austria

^e Conservation and Research Department, Memphis Zoo, Memphis, TN, USA

* Correspondence to: La Trobe University, Kingsbury Drive, Bundoora, Victoria 3086, Australia.

E-mail address: k.fanson@latrobe.edu.au (K.V. Fanson).