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Non-Invasive Monitoring of Reproduction in Zoo and Wildlife Species

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Abstract

Graham LH. Non-Invasive Monitoring of Reproduction in Zoo and Wildlife Species. ARBS Ann Ver Biomed Sci 2004;6:91-8. Hormones are involved in all aspects of reproduction and characterizing endocrine patterns associated with reproductive events is important for investigations of the reproductive biology of wildlife species. Non-invasive techniques to monitor gonadal function by quantifying reproductive hormone metabolites in the urine and feces have been developed for several wildlife species.
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Introduction

Accurate information about the reproductive biology of a species is necessary for the effective management of animals in captivity and in the wild. Accurately assessing the reproductive status of individuals is essential to successful captive breeding programs. Reproductive parameters such as estrous cycle length, the length of gestation, the length of lactational anovulation and age at the onset of puberty all profoundly affect the growth of wild populations. Knowing the effects of various social and/or environmental factors on these parameters can help wildlife managers predict the response of a population to different conditions and manage it accordingly. Hormones are involved in all aspects of reproduction and characterizing endocrine patterns associated with reproduction is an important first step in the study of the reproductive biology of any species.

Longitudinal collection of samples is usually necessary for the successful investigation of reproductive-endocrine relationships. Circulating hormone concentrations are the most accurate indicators of reproductive-endocrine relationships, however, most wildlife species are intractable which makes repeated blood collection very difficult. A less invasive alternative to monitoring hormone concentrations in the blood is measuring hormone metabolite concentrations in the urine and feces. Non-invasive monitoring of reproductive hormones has several advantages with the most obvious being that animal handling is unnecessary. Another advantage of non-invasive monitoring is that metabolite concentrations in the urine and feces are pooled values representing gonadal activity over the previous several hours. Thus, result hormone concentrations are usually two to four magnitudes higher than that of the parent hormone in the blood, which allows a wider range of assays and assay types to be employed. Also, short-term fluctuations in hormone concentrations tend to be dampened giving a clearer picture of hormonal events. However, because of species differences, techniques for non-invasive monitoring must be developed and validated for each species separately.

Route of Excretion

Studies using the injection of radiolabeled steroid hormones have indicated that the route of excretion of steroid hormones varies considerably among species, as well as between steroids within the same species. For example, the injection of radiolabeled steroids in the African elephant (*Loxodonta africana*) indicated that greater than 90% of the estradiol metabolites are excreted in the urine while progesterone metabolites are found in both the urine and feces (Wasser *et al.*, 1996). In contrast, greater than 95% of both estradiol and progesterone metabolites are excreted in the feces of the domestic cat (Felis catus) (Shille *et al.*, 1984; Brown *et al.*, 1994). In the baboon (*Papio cynocephalus cynocephalus*) 90% of estradiol is excreted in the urine with peak excretion 4.5 hours following injection (Wasser *et al.*, 1994) while only 55% of estradiol is excreted in the urine of the macaque (*Macaca fascicularis*) (Shideler *et al.*, 1993). Similar differences in steroid excretion are observed among species of rhinoceros. In the white rhinoceros (*Ceratotherium simum simum*) virtually all of the progesterone is excreted via the urine (Hindle & Hodges, 1990) while in the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) it is excreted almost exclusively in the feces (Heistermann *et al.*, 1998). The delay time between the circulation of steroids in plasma and their appearance in urine samples is usually only a few hours. However, fecal steroid metabolites usually have a lag-time of longer than 12 hours with the lag-time often correlating with the time necessary for the intestinal passage of bile to the rectum (Palme *et al.*, 1996).

For many species, it is difficult to obtain animals for infusion of radioactive hormones because of the dangers inherent in using radioactive substances. As a result, the primary route of steroid metabolite excretion is unknown for most species. In these species, the ease of sample collection usually determines if feces or urine is investigated for monitoring gonadal function. Measuring reproductive hormone metabolites in unprocessed urine to monitor ovarian function has been used for several decades in different species, especially primates. While it is relatively easy to aspirate urine from the non-porous floors in captive housing, collection from free-ranging animals is more problematic. However, several ingenious techniques for urine collection from free-ranging species have been reported. Monitoring pregnancy in feral mares (*Equus caballus*) was accomplished by measuring hormone metabolites in urine extracted from the soil (Kirkpatrick *et al.*, 1988). Placing recently soaked soil in gauze squares that were then placed in plastic bags and centrifuged separated the urine from the soil. The urine collected in the bottom of the plastic bag was poured off and frozen until analysis. Another inventive urine collection technique was used in feral horses (Kirkpatrick *et al.*, 1990) during the winter. Urine soaked snow was melted and urinary metabolites of reproductive hormones assayed. The urine-soaked snow was also indexed for creatinine to account for differences in urine concentration and dilution caused by the mixing of the urine in snow. In primates, filter paper has been

used as the vehicle for collection, shipment, storage and transfer of urine samples (Shideler *et al.*, 1995). Regardless of the advances in urine collection techniques, fecal collection is still perceived to be easier under a variety of conditions. Consequently, the last 2 decades has seen an increase in the number of investigations into the measurement of fecal steroid metabolites as a means of monitoring gonadal function in a variety of species.

Steroid Metabolism

Another challenge facing attempts to non-invasively monitor gonadal function is that steroid hormones are usually metabolized prior to elimination from the body. After circulating, steroids are metabolized by the liver and appreciable amounts excreted in the bile. During passage through the intestinal system steroid metabolites can then be further metabolized by intestinal bacteria and excreted in the feces or re-absorbed into enterohepatic circulation and transported via the blood into the kidney for excretion through the urine. Metabolism by intestinal bacterial plays a large role in determining the route of steroid excretion. For example, it has been shown in humans that estrogens are excreted primarily in the urine. However, a reduction in intestinal microflora by antibiotics impairs the bacterial steroid conjugate hydrolysis, which is necessary for the efficient re-absorption of estrogens from the gut. As a result, fecal estrogen excretion is increased while urinary estrogen excretion decreases (Adlercreutz *et al.*, 1979). Bacterial metabolism can also determine the steroid metabolite form excreted (Groh *et al.*, 1993). For example, the splitting of steroid conjugates is achieved mainly through various bacteria and sulfatases originate exclusively from bacteria. Furthermore, dehydroxylation reactions of steroids are found with intestinal microorganisms only, with dehydroxylating enzymes of mammalian origin unknown.

Different species can differ in the metabolism of hormones and thus may excrete different metabolites of the same parent compound. For example, the major urinary metabolite of estradiol is estradiol glucuronide in the white and black rhinoceros (*Diceros bicornis*) but estrone sulphate in the Indian rhinoceros (*Rhinoceros unicornis*). Similarly, the major urinary progesterone metabolite is 20a-dihydroprogesterone sulphates in the white rhinoceros, 20a-dihydroprogesterone glucuronide in the black rhinoceros and pregnanediol glucuronide in the Indian rhinoceros (Hodges, 1992). In most species feces contain a higher percentage of free than conjugated steroids. Estrogens are end products of steroid metabolism and are often found unchanged from the parent form in the feces. In contrast, progesterone is extensively metabolized prior to excretion in the feces and several 5 a and 5 b-pregnanes predominate (see review by Schwarzenberger *et al.*, 1996). Again, these progesterone metabolites can be species specific. For example, in mares and rhinos the fecal pregnanes are primarily of the 5 a-series while those of okapis (*Okapia johnstoni*) belong to the 5 b-series.

Most commercially available antibodies for use in immunoassays are developed to quantify steroid hormones in the blood and are very specific to the parent steroid form. Consequently, they are often unable to quantify the steroid metabolite forms found in the urine and feces. One alternative is to determine the nature of the major hormone metabolites for a species and develop an appropriately specific assay. However, the only way to accurately determine the metabolite forms of a parent steroid is to do a radiolabel infusion of the parent steroid and subsequent chromatographic analysis on the urine and/or feces. This approach is often impossible with rare and endangered species and might render the assays specific to only a few species because of species specificity in steroid metabolism. A more popular approach is the use of antibodies with significant cross-reactivities to a number of common steroid metabolites. Urinary steroid metabolites are usually conjugated and assays to quantify estrone conjugates (both sulphate and glucuronide) and pregnanediol-3-glucuronide in the urine have been used successfully to monitor ovarian function in a variety of mammalian species including primates and herbivores (see review by Lasley *et al.*, 1991) and Killer whales (*Orcinus orca*) (Walker *et al.*, 1988; Robeck *et al.*, 1993). Assays that have cross-reactivities with a broad range of pregnanediones and hydroxylated pregnanes have been used successfully to quantify

progesterone metabolites in the feces of a wide range of species including a variety of carnivore and artiodactyl species, black and white rhinoceros, hippopotamus (*Hippopotamus amphibius*) and elephants (see reviews by Schwarzenberger *et al.*, 1996, and Graham *et al.*, 2001).

Applications

Non-invasive monitoring techniques have been successful in delineating endocrine parameters associated with reproduction in a variety of species despite the challenges described above. The picture that has emerged indicates a wide range of endocrine patterns in wildlife species. For example, non-invasive monitoring has indicated that okapi have an estrous cycle length of only 2 weeks (Loskutoff *et al.*, 1982; Schwarzenberger *et al.*, 1999), killer whales have an estrous cycle length of 6 weeks (Walker, *et al.*, 1988; Robeck *et al.*, 1993) and elephants have an estrous cycle length of 13 to 16 weeks (Niemuller *et al.*, 1993; Heistermann *et al.*, 1997; Fiess *et al.*, 1999). Fecal steroid analysis has indicated that felids are primarily induced ovulators although incidences of spontaneous ovulations have been observed (see reviews by Graham & Brown, 1997, and Brown *et al.*, 2001). Fecal estrogen analysis has indicated New World primates are unusual in that maximal estrogen concentrations occur during the luteal phase and not during the follicular phase (Heistermann *et al.*, 1993; Pryce *et al.*, 1994; Ziegler *et al.*, 1996). Although germ cell production is less tightly coupled to hormonal variations in the male than in the female, non-invasive androgen monitoring in males has useful in characterizing seasonal patterns. Fecal and urinary steroid analysis has indicated seasonal influences on reproduction in females and/or males in several species, including the scimitar-horned oryx (*Oryx dammah*) (Morrow *et al.*, 1999), the black-footed ferret (Mustela nigripes) (Brown, 1997), Maned wolves (Chrysocyon brachyurus) (Velloso *et al.*, 1998), African wild dogs (Lycaon pictus) (Monfort *et al.*, 1997), Dall's sheep (Ovis dalli dalli) (Goodrowe *et al.*, 1996), Eld's deer (Cervus eldi thamin) (Monfort *et al.*, 1990) and Pere David's deer (Elaphurus davidianus) (Monfort *et al.*, 1991). Once the relationship between steroid metabolite concentrations and reproductive events has been characterized for a species, non-invasive endocrine monitoring can be used as a tool to enhance the understanding and management of the species both in captivity and in the wild. In zoos, the diagnosis of pregnancy before parturition can greatly improve the survival of neonates by giving the animal care providers an opportunity to prepare appropriate housing requirements for the expectant mother. Monitoring the onset of puberty can help prevent inbreeding in family-housed groups of animals. Assessing the presence or absence of ovarian cyclicity can help in determining the appropriate individual animals to be included in a captive breeding plan and what individuals are candidates for treatment of infertility.

Non-invasive endocrine monitoring has also been used successfully to assess various fertility control techniques. The effects of contraceptive treatments have been assessed by non-invasive endocrine analysis in Przewalski's horses (*Equus przewalski*) and banteng (*Bos javanicus*) (Kirkpatrick *et al.*, 1995), the Nile hippopotamus (Graham *et al.*, 2002) and various primates like the gorilla (*Gorilla gorilla*) (Goodrowe *et al.*, 1992). Estrous synchronization protocols in Sable antelope (*Hippotragus niger*) (Thompson & Monfort, 1999), Scimitar-horned oryx (Morrow & Monfort, 1998) and Mohor gazelle (*Gazella dama mhorr*) (Pickard *et al.*, 2001) have been developed with the assistance of non-invasive endocrine monitoring. Endocrine responses to ovulation induction protocols have been assessed in cheetah (*Acinonyx jubatus*) (Brown *et al.*, 1996), clouded leopards (*Neofelis nebulosa*) (Brown *et al.*, 1995), domestic cats (Graham *et al.*, 2000), tigers (*Panthera tigris*) (Graham *et al.*, 1996) and llamas (*Lama glama*) (Paul-Murphy *et al.*, 1991).

Finally, non-invasive endocrine monitoring has been applied to free-ranging animals as well as captive animals. Pregnancy diagnosis has been achieved non-invasively in free ranging elk (*Cervus elaphus nelsoni*) (Garrott *et al.*, 1998), feral horses (Kirkpatrick *et al.*, 1991[b]; Lucas *et al.*, 1991), dwarf mongooses (*Helogale parvula*) (Creel *et al.*, 1997), African wild dogs (Creel *et al.*, 1997) and African elephants (Foley *et al.*, 2001). Non-

invasive pregnancy diagnosis in moose (*Alces alces*) is now being used to assist in the understanding of the effects on reproduction of predator colonization and ecological carrying capacity (Berger *et al.*, 1999). Monitoring of ovarian cyclicity and pregnancy has also been achieved in free-ranging meerkats (*Suricata suricatta*) (Moss *et al.*, 2001), bison (*Bison bison*) (Kirkpatrick *et al.*, 1991[a]) and baboons (Wasser *et al.*, 1991).

Conclusions

Valuable information can be collected about the reproductive biology of a species non-invasively through the analysis of urinary or fecal steroid hormone metabolites. Care must be taken to validate non-invasive endocrine monitoring techniques for each species of interest because of the considerable differences among species in the route of excretion and the form of the excreted metabolite. Animals in captivity are ideal research subjects to characterize the relationships between fecal/urinary hormone metabolites and reproductive events because regular sample collection is possible and independent assessment of reproductive events is possible. Once the endocrine parameters associated with various reproductive events have been established for a species, the non-invasive endocrine monitoring can be used as a tool to assist in the husbandry of animals in captivity and to investigate social and ecological effects on reproduction in animals in the wild.

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