# NON-INVASIVE ENDOCRINE MONITORING OF REPRODUCTION AND STRESS IN THE WILD ANIMALS: ANALYZING HORMONE METABOLITES IN URINE AND FAECES USING ENZYMEIMMUNOASSAY

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## Introduction

Hormone analysis is the most precise of the indirect methods for monitoring the functional status of the reproductive and stress event (Hodges *et al.,* in print). Since many wildlife species are easily stressed, intractable and also potentially dangerous, the repeated capture and restraint necessary for regular blood sampling is both undesirable and Impractical. Therefore, non-invasive methods, based on the measurement of hormone metabolites in urine and faeces are preferable, particularly as they have been shown to be as accurate and reliable as blood hormone analysis for monitoring the reproductive cycle and stress in many animal species (Heistermann *etal.* 1995b).

However, since correct interpretation of hormonal data requires at least some knowledge of the physiology of the species in question, monitoring methods based on non-invasive hormonal analysis first need to provide the basic physiological information (hormone metabolism, pattern of secretion and excretion). On which their subsequent application depends. Therefore, Hodges (1996) states that the choice of methods depends largely on the type of information required, the species in observation and circumstances under which the study will be carried out.

Since, species can largely vary in this respect (Palme ef *al.* 1993; Brown 2006), a careful validation of hormone measurements for each species is required if the results are to be accurate and reliable with respect to reproductive and stress assessment (Heistermann ef *al.* 1995a). This paper will inform the procedures of application non-invasive hormone metabolites analysis in urine and feaces.

## Characterization of hormone metabolites

Species can differ markedly in the metabolism of hormones, and thus may excrete largely different metabolites of the same parent compound. Moreover, species can also vary in terms of route of hormone excretion. There are also differences in the time course of hormone excretion, i.e., the time lag between the secretion of the parent compound into the bloodstream and the excretion of the corresponding metabolites. This time lag depends mainly on the route of excretion, the hormone in question and the species. In this regard, radiometabolism studies can clarify the metabolic fate and route of hormone excretion and thus provide valuable information for the development of appropriate assays.

In order to characterize the hormone metabolites excreted into urine and faeces, urinary and faecal samples at the peak of hormone radiolabelled from radiometabolism study could be performed on:

1. High Presure Liquid Chromatography (HPLC), and then further with
2. Gas Chromatography/Mass spectro-photometry (GC/MS)

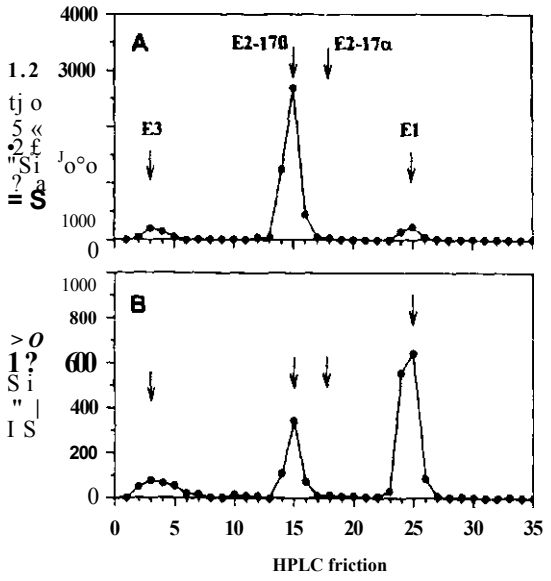


Figure 1. HPLC profiles of metabolised oestrogens in (A) urine and (B) faeces after i.v. injection of 3H-oestradiol-17p in a female Sumatran rhinoceros (cited from Heistermann *et al.*, 1998).

### Sample preparation

Sample preparation included several processes for preparing the samples before hormone analysis for both faecal and urine samples. These processes included a) drying and pulverization of feces, b) hydrolysis of urine and c) sample extraction (both faeces and urine).

Sample preparation for analysis is also depended on the protocol of sample collection. Samples can be collected and preserved by freezing immediately or in ethanol 90%.

Figure 2. Faecal drying process in freeze dryer

Figure 3. Pulverization of dry faeces

### Hormone assay

Ali measurements were carried out by competitive, double-antibody, enzymeimmuno-assay procedures (EIA) as already described in detail by Hodges *et al.* (1997) and Heistermann *et al.* (1993). The enzymeimmunoassays were performed on microtitreplates coated with a goat anti rabbit IgG (1pg/well; Quartett Immunodiagnostika und Biotechnologie GmbH, Berlin) and using hormone-specific antibody as second antibody and enzyme- or biotin-labelled hormones as competitive tracers. Sample extracts were diluted in assay buffer and duplicate 50 pi aliquots were taken to assay.

Figure 4. EIA hormone analysis procedure

Validation of the assay is very important to be conducted for each assay being used. Sensitivity of the assay was determined at the concentration of 90% binding. Parallelism test, serial dilutions of sample extracts from the expected follicular and luteal phase of the ovarian cycle and from the expected stress and non stress condition should be determined to give displacement curves parallel to that of the hormone standard before using the assay in order to find a proper assay as required. Intraassay and interassay coefficient of variation for QC high and QC low have to be noted and calculated as the values of assay accuracy.

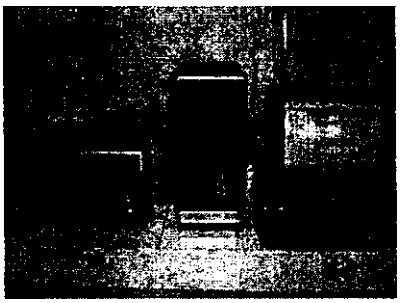

Figure 5. Reading the plate using microplate reader connected to computer with software Gen5

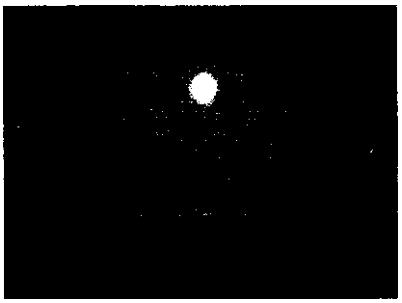

Figure 6. Result will be displayed including standard curve and actual hormone concentration of each sample.

## Conclusion

Measurement of hormone metabolites in faeces and in urine enables non-invasive monitoring of the reproduction and stress in the wild animals.

## Acknowledgement

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