NON-INVASIVE MONITORING REPRODUCTIVE STATUS IN THE SUMATRAN RHINOCEROS: EIA PREGNANOLONE (5-P-30H) FOR FAECAL HORMONE ANALYSIS IN THE FEMALE SUMATRAN RHINO

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Introduction

Information on the reproductive biology of the Sumatran rhinoceros is very limited. and for many aspects, it does not even exist. In contrast, there is much information on the reproductive biology of the other rhino species (African black rhino: Garnier *et al*. 2002; African white rhino: Patton *et al*. 1999; Indian rhino: Schwanenberger et *al*. 2000).

In this respect, the establishment of reliable and practical methods for reproductive monitoring is important. As shown for other species of rhinoceros, the use of non-invasive techniques based on analysis of urinary and faecal reproductive hormone metabolites can provide reliable information on female reproductive status and, in particular, valuable basic data on the characteristics of the ovarian cycle and pregnancy. An assessment of female reproductive status is important in order to support breeding programs for many species in captivity. In this respect, the information gained is particularly important for maximizing natural breeding by providing the basis for an improved breeding management.

As a basis to assist the breeding management of the Sumatran rhinoceros in captivity, the overall aim of this part of the study was to provide information on the fertility status of individual female Sumatran rhinoceros. To this end, the specific objectives was to establish and validate reliable methods of faecal hormone analysis for monitoring female endocrine function.

Materials and Methods

Animals

Two adult female Sumatran rhinoceros (ages: 9 and 18 years) served as subjects in this study. The females were maintained at different places in Sabah, Malaysia (Gelogob#40), and Taman Safari Indonesia (TSI), Indonesia (Bina#32).

From each of the females, regular faecal was collected during the different study periods. Sample collection was performed according to the protocol reported by Hindle *et* el. (1992) and Heistermann et *al*. (1993). The complete sample collection period was from 1996-2005.

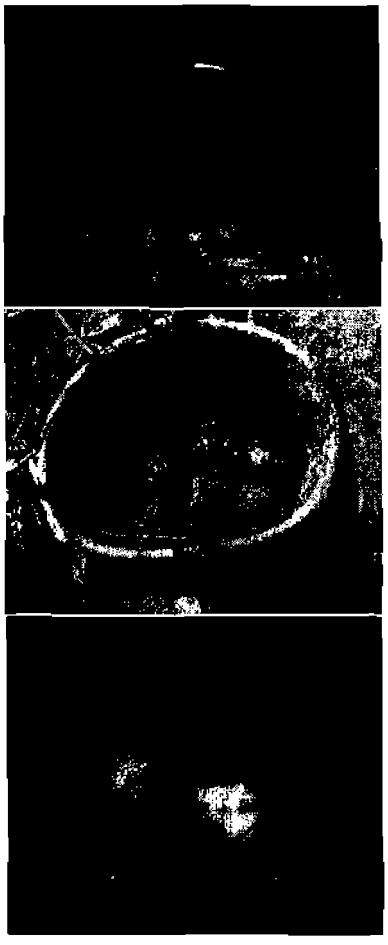

Figure 1. Feacal sample collection in the Sumatran rhino

Faecal Extraction

An aliquot of the faecal powder of each sample, representing approximately 50 mg (exact weight noted), was extracted with 3 ml of 80% MeOH by vortexing for 15 minute in a 15 ml plastic tube using a multi-tube vortexer (Multi-Tube Vortexer, SMI®, USA). The efficiency of the whole extraction procedure was determined randomly.

Hormone assay

Pregnanolone (5-P-30H) was determined using the streptavidin-biotin technique. The antiserum used was raised in rabbit against 5α-pregnane-3β-ol-20-one-3HS-BSA (supplied by E. Mostl, Vienna), 5α-pregnane-3β-ol-20-one-3HS coupled to biotin was used as label (supplied by E. Mostl, Vienna), and 5α-pregnane3α-ol-20-one as standard. Extracts were diluted 1:20 to 1:90 (during oestrous cycle) in assay buffer and duplicate 50 µl aliquots were taken to assay. Sensitivity of the assay was 9.8 pg/well at 90% binding. Serial dilutions of faecal extracts from the follicular and luteal phase of the ovarian cycle gave displacement curves parallel to that of the 5-P-30H standard, Intraassay coefficient of variation for QC high and QC low were 7.0% (n=16) and 9.1% (n=17), while, interassay coefficient of variation for QC high and QC low were 7.8% (n= 42) and 9.39% (n= 42), respectively.

Pregnanediol in faeces was determined as described by Heistermann *et al.* (1993) as follow: The assay used an antiserum against pregnanediol-3-glucuronide-BSA. Biotinylated pregnanediol-3-glucuronide (prepared by E. Möstl, Vienna) in conjunction with peroxidase (POD) labelled streptavidin (No. S-5512, Sigma chemie) was used as conjugate, and pregnanediol was used as standard, although the assay was originally designed to measure the glucuronide. Exctracts were diluted 1:10 in assay buffer and duplicate 50 µl aliquots were taken to assay. Assay sensitivity at 90% binding was 6.3 pg per well. Serial dilutions of samples from the follicular and luteal phase of the ovarian cycle were parallel to the pregnanediol standard curve, Intraassay coefficient of variation was 7.5% (QC high, n=16) and 8.8% (QC low, n=17). Interassay coefficient of variation for QC high and QC low were 7.6% (n=14) and 11.7% (n=14)

Results and Discussion

Hormone profile of the reproductive cycles Bina #32 (TSI)

Concentrations of Pd in faeces were generally low (10-20 µg/g dry faeces) before the E2 elevations and increased to 30-60 µg/g during the presumed luteal phase. However, the Pd profile was less clear in the second cycle as compared to the first. HPLC analyses of Pd immunoreactivity in the luteal phase samples showed a similar profile as found in the radioactive sample, indicating that the measurement of Pd was non-specific (see insert, Figure 2).

The more specific measurement of faecal **5-P-30H** immunoreactivity (see insert, Figure 2) revealed a clear cyclic pattern for both cycles with consistently low levels during the presumed follicular phase (3-8 µg/g dry faeces) and three- till fivefold elevated concentrations (15-25 µg/g dry faeces) during the presumed luteal phase. Based on the results, it appeared that measurement of 5α-P-30H immunoreactivity gave clearer profiles compared to Pd and is thus superior for monitoring reproductive function in the female Sumatran rhinoceros. Therefore, for monitoring reproductive status in the other female rhinos, the 5-P-30H only assay was applied. Oestrous cycle length was 23 days according to the interval between **5-P-30H** rises.

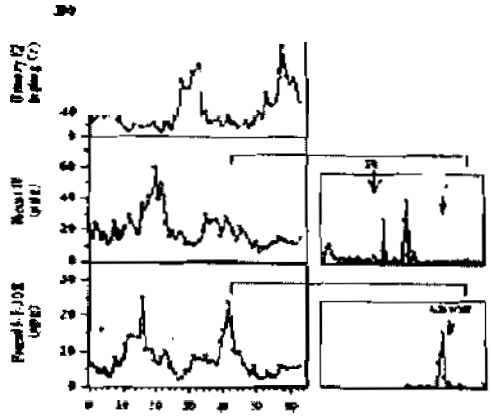

Figure 2. Profile of oestradiol-17β (E2) immunoreactivity in urine and pregnanediol (Pd) and 5α-pregnane-3α-ol-20-one (5-P-30H) immunoreactivity in faeces, Insert on the right show respective HPLC profiles of Pd and 5-P-30H immunoreactivity obtained from a

mid-luteal phase sample. Arrow indicate elution positions of authentic 3H-Pd (Pd) and 5α-pregnane-3α-ol-20-one (5-P-30H) tracers

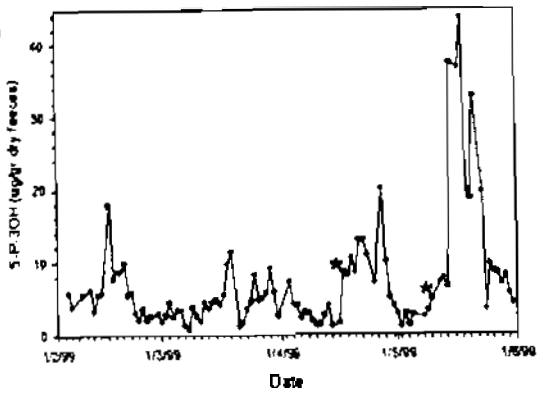

Figure 3. Faecal 5-P-30H profile in female Gologob in Sepilok rhino center, Sabah (February-May 1999)

Figure 3 shows the faecal 5-P-30H profile of Gologob during four months of monitoring. During the first two months, Gologob showed an erratic pattern of 5-P-30H excretion, values being almost constantly low and there was no clear evidence for cyclic ovarian activity during this period. By mid of April 1999, the profile indicated two clear periods of defined 5-P-30H elevations, separated by a period of low levels. This suggests the presence of two ovarian cycles, although there was no report on oestrous behaviour. According to the rises of 5-P-30H, cycle length was defined as being 23 days.

Conclusion

Measurement of 5-P-30H immunoreactivity in faeces enables non-invasive monitoring of the oestrous cycle in the Sumatran rhinoceros.

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