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MONITORING FECAL PROGESTOGEN METABOLITES IN THE SUMATRAN RHINOCEROS (*Dicerorhinus sumatrensis*) BY ENZYME IMMUNOASSAY

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The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is listed as critically endangered by the IUCN and is also listed in Appendix I of CITES. This species has proven to be difficult to breed in captivity, with no offspring produced in over 100 years. Measuring fecal progestogen metabolites by radioimmunoassay (RIA) using a monoclonal progesterone antibody (provided by J. Roser) that cross-reacts with several fecal progestogen metabolites, has proven useful in monitoring the reproductive cycle of numerous rhino species, including the Sumatran rhinoceros. This information may enable animal managers to improve reproductive performance of this endangered species. However, few laboratories associated with facilities that hold Sumatran rhinos are equipped to conduct assays involving radioactivity (i.e. RIA). Therefore, the purpose of this study was to develop an enzyme immunoassy (EIA) protocol comparable to the existing RIA for use in monitoring fecal progestogen metabolites of the female Sumatran rhinoceros.

Fecal samples (n=170) were collected over a period of 182 days from an 8 year old female Sumatran rhinoceros housed at the Cincinnati Zoo and Botanical Garden, Ohio. This female had previously been shown to exhibit regular ovarian cyclicity, as evidenced by ultrasound examinations and RIA analysis of fecal samples. The samples were frozen immediately after collection and sent to the Conservation and Research Center in Front Royal, Virginia for analysis.

The samples were dried, crushed and extracted with ethanol (92% extraction efficiency). As a preliminary validation, parallelism was determined between serial dilutions of pooled fecal extracts and the standard curves of both the RIA and a new EIA method which used a biotinylated label (provided by F. Schwarzenberger) and the same progesterone monoclonal antibody described above. Each sample was then analyzed for progestogen metabolites using both assays. Over the sampling interval, the progestogen profile initially reflected a partial luteal phase, followed by a prolonged elevation in progestogens which was diagnosed as a pregnancy by ultrasound. The pregnancy was lost after 37 days of elevated progestogens and was followed by 3 cycles (identified as prolonged increases followed by gradual decreases in progestogens) with an average length of 20 days (based on the interval between nadir values). The progestogen patterns generated from both assays were similar, with a correlation coefficient of 0.705.

These results demonstrate that EIA with an appropriate broad-spectrum antibody can be used to monitor the reproductive cycle of the female Sumatran rhinoceros by measuring the excretion of fecal progestogen metabolites. This assay not only will simplify the processing of samples collected from captive animals but will also allow field researchers to monitor the reproductive cycles of animals in conditions where it is impossible to gain access to laboratories equipped to handle radioactivity.