APPLICATION NOTE #201410

Reproductive Hormone Extraction from Black Rhinoceros Faeces using the Omni Micro Homogenizer (µHb) and Omni Tip™ Plastic Generator Probes

This Application Note was developed based on the following citation: Elizabeth W. Freeman, Jordana Meyer, Jed Bird, John Adendorff, Bruce Schulte, Rachel Santymire. Impacts of environmental pressures on the reproductive physiology of subpopulations of black rhinoceros (Diceros bicornis bicornis) in Addo Elephant National Park, South Africa. Conservation Physiology. 2014. Vol 2, Issue 1.doi: 10.1093/conphys/cot034

The black rhinoceros is an endangered species with population numbers dramatically decreasing in the 20th century by more than 97%. Successful conservation efforts increased the black rhinoceros population from 2,410 in 1995 to 4,880 individuals in 2010. A component of the ongoing conservation efforts are studies aimed at understanding black rhino reproductive physiology. There is still a poor understanding of the factors that impact black rhino reproductive success, due in some part to their long gestation periods and low population densities. With this in mind, non-invasive techniques have been developed to monitor reproduction in free-ranging black rhinoceros populations.

The study performed by Freeman *et al* involved the extraction and quantification of the reproductive hormones, progestagen and androgen, from black rhino faeces collected at the Addo Elephant National Park in South Africa. Hormone levels were correlated with season, location, climate, age and reproductive status of the animal. While the publication encompasses a comprehensive review of black rhinoceros hormone levels as a function of environment, for the purpose of this notes we simply summarize the Omni micro homogenizer based hormone extraction procedure and correlation of hormone levels with temperature as described in the study.

Materials & Methods

Equipment

- **Omni Micro Homogenizer (uHb)** (Cat #H115)
- **Omni Tip™ Disposable Hard Tissue Generator Probes** (Cat #30750H)

Sample Preparation

A total of 231 faecal samples were collected from black rhinos from July 2007 to November 2010 and positively identified to a specific animal. Faecal samples were correlated to specific animals by anatomical features and ear notching through the use of a motion activated camera. Samples were collected from 144 male and 87 female animals. Average monthly temperature data were provided by the South African Weather Service. Faecal samples were mixed and 0.5 +/- 0.02 g of wet faeces was weighed, logged and then placed in a 16 mm x 100 mm polypropylene tube. 5 mL of 70% propanol was added to each sample and homogenized for 1 minute using the Omni micro homogenizer (µHb) equipped with a disposable plastic Omni Tip hard tissue generator probe. The resulting homogenate was filtered and a 1 mL aliquot was transferred to a clean 16 mm x 100 mm polypropylene tube, allowed to air dry then heated to 72°C for 30 minutes.

Hormone quantification

Faecal extracts were reconsitituted in 1 mL of 0.2 M NaH_2PO_4, 0.2 M $NaHPO_4$ and 0.14 M NaCl. Glass beads were added and the sample was vortexed then sonicated for 20 min. The sample was then diluted prior to enzyme immunoassays.

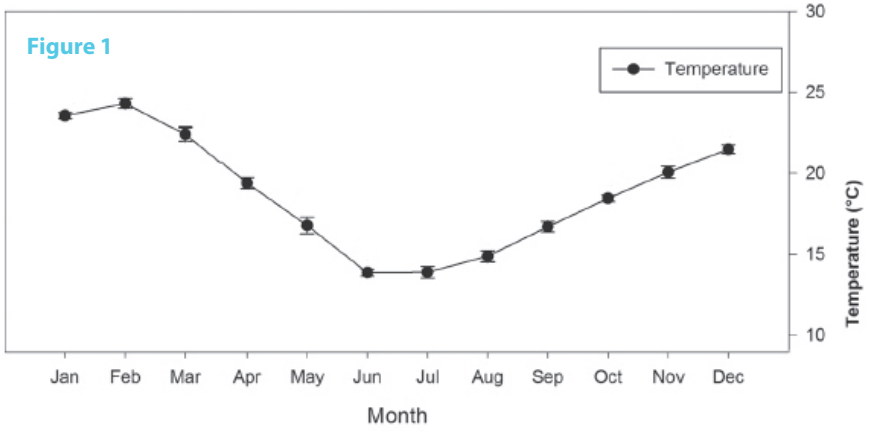
Faecal progestagen metabolite (FPM) was analyzed by enzyme immunoassay of progesterone polyclonal antiserum at a 1: 10,000 dilution and horseradish peroxidase at a 1: 40,000 dilution. Faecal androgen metabolite was analyzed by testosterone enzyme immunoassay at a 1: 10,000 dilution and horseradish peroxidase at a 1:30,000 dilution.

Example Results

In this study the authors investigate an array of variables including animal location, temperature, precipitation, sex and age. Figure 1 shows the average temperature variation as a function of month from 2005-2011.

continued

Figure 1



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Example Results (cont.)

These average monthly temperatures were then compared with the Faecal progestagen metabolite and Faecal androgen metabolite levels to determine if there was a correlation between temperature and reproductive physiology. A linear mixed-effects model was used to determine if temperature predicted the concentration of faecal progestagen metabolite. Since multiple faecal samples were collected from the individual animals the animal identity was used as the random effect. Data were further analyzed using a t-test or ANOVA. As shown in figure 2, the measured faecal progestagen metabolite concentrations had a linear relationship with average monthly temperature for non-pregnant females (FPM = -18.19 + 5.53 x temperature, r2 = 0.25, P = 0.03).

In contrast to FPM in female black rhino, faecal androgen metabolite concentrations were shown to not vary with respect to month or temperature in male black rhinos.

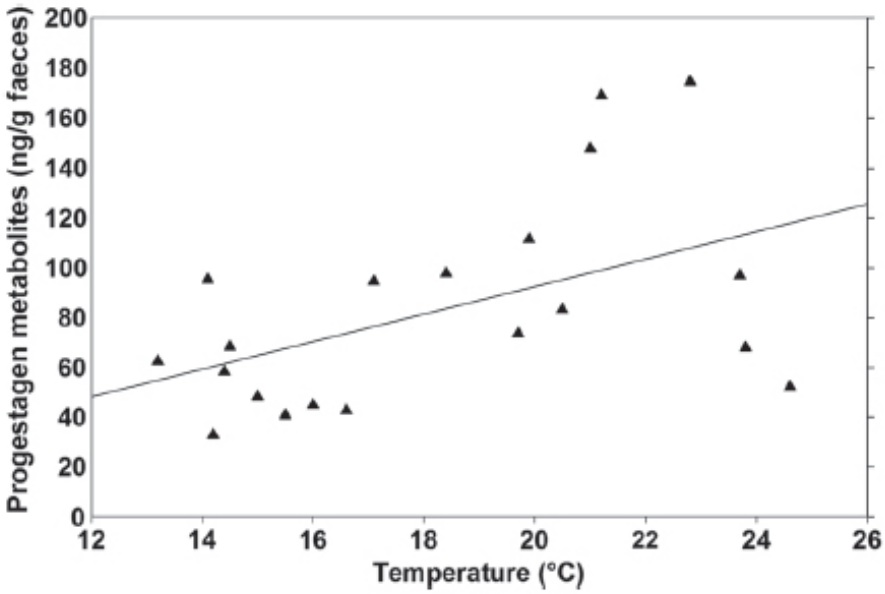

Figure 2 Faecal progestagen metabolite concentrations as a function of temperature.

Part Numbers Referenced

Omni Micro Homogenizer (uHb): H115

Omni Tip Disposable Hard Tissue Generator Probes: 30750H

Omni uHb Homogenizer

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