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75 **Reproductive Biology**

Non-invasive measurement of faecal testosterone metabolites revealed seasonality in free living male white rhinoceros, *Ceratotherium simum simum*

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The capture breeding program of the southern white rhinoceros (*Ceratotherium simum simum)* suffers from low reproduction rates. Only 8% of the world-wide F_1 has reproduced in captivity. Mating and reproductive behaviour of males, particularly in the wild has been poorly investigated. Understanding principles of male reproduction is crucial for optimising captive propagation of wildlife species. To link reproduction behaviour to testosterone profiles of the animals, we studied the reliability of a non-invasive method for monitoring of the testosterone metabolite levels in faeces.
The study was conducted on a South African game farm in Northern Transvaal. To stimulate testosterone secretion a GnRH analogoue (Burserelin 100 µg) was administered to one adult male and the faecal samples were collected over a period of four days. Furthermore, five territorial males were monitored continuously over a period of two years by following their tracks together with an experienced game tracker. During tracking, dung heaps could be reliably assigned to individuals, and the time of defecation was estimated. The faecal samples were collected approximately twice per week along the tracks of an animal. In addition, faecal samples of females, subadult and juvenile males were collected. All faecal samples were stored at -22° C in methanol until analysis. When individual animals were immobilised to mark them, blood samples were collected. All samples were analysed for testosterone metabolites with an enzyme-immunoassay (EIA) using an antibody directed against testosterone-17ß-HS-BSA.
After application of Burserelin testosterone metabolite levels increased from a baseline of 30 – 70 ng/g faeces to 165 ng/g within 24 h and dropped to basal levels within the next 4 days. This demonstrates that an increase in peripheral testosterone levels, as expected after Burserilin application, could be reliably detected when faecal metabolites were measured. Furthermore, the comparison between testosterone levels in blood and faecal metabolites revealed a significant correlation. Faecal metabolite concentrations of adult males were significantly higher than in subadult males and females and demonstrated a clear seasonality in two consecutive years with increased levels between August and November (maximum in October) which may indicate the onset of the mating season.
We conclude that faecal testosterone analyses are a suitable tool to characterise the gonadal status of males and might help to discover potential reproduction failure of male white rhinoceroses. The indication of seasonality in male reproductive physiology may be an important step towards understanding of factors that limit reproduction in this species.