Non-invasive testosterone measurement reveal seasonality in males of free living white rhinoceros, *Ceratotherium simum simum*

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The breeding program of the two subspecies of the white rhinoceros suffers from low breeding success. Only 8% of the world-wide F_1 population of the southern (*Ceratotherium simum cottoni*) and only 15 % of the founder population of the northern subspecies (*Ceratotherium simum cottoni*) have reproduced in captivity. While female reproductive behaviour has already been studied intensively in the zoo, mating and reproductive behaviour in the wild, and particularly of the males, has only poorly looked into. However, an understanding of the males contribution to reproduction is crucial for optimising captive propagation of wildlife species because reproduction failure has been related to male infertility in many zoo maintained animals. Therefore we studied the reliability of a non-invasive method for monitoring the testosterone level in the blood of the white rhinoceros in order to link mating and reproduction behaviour to testosterone profiles of the animals.

The study was conducted on a South African game farm, in Northern Transvaal. The farm was 30.000 ha in size and inhabited a population of 58 white rhinoceros. Five territorial males were monitored continuously over a period of two years by following their tracks together with an experienced game tracker. Single fresh tracks were followed until the animal was sighted. During the tracking dung heaps could be reliably assigned to individuals, and the time of defecation was estimated. The faecal samples were collected approx. two times per week along the tracks of an animal, and were stored at -12° C in Methanol until the final analyses took place. Additionally, faecal samples of females and subadult and juvenile males were collected during the study period as the animals were sighted defecating. Blood samples and simultaneous faecal samples were collected from adult, subadult and juvenile males as the animals were tranquillised in order to analyse the connection between serum testosterone and faecal metabolites concentration. Additionally, an GnRH analogous, Buserelin 100 µg, was administered to one adult male and the faecal samples of the following four days were collected completely. All faecal samples were analysed with enzyme-immuno essays by using the ELISA technique.

After application of Burserilin the testosterone values increased from a baseline of 30 – 70 ng/g faeces to 165 ng/g within a 24 h period. This high level dropped slowly within the next 4 days. This shows that an increase in testosterone level as expected after Burserilin application could be reliably detected by our method. Furthermore, testosterone levels obtained from blood and faecal samples showed a significant correlation, which enables us to predict the testosterone concentration by the faecal metabolites. Faecal testosterone concentrations are also significantly higher in males than in subadult males and females, what allows to distiguish between different age and sex classes. The male testosterone levels showed a clear seasonality with increased levels between August and November, and a maximum in October. These elevated testosterone levels were observed in both years and may indicate the onset of the mating season in the white rhinoceros.

We conclude that faecal testosterone analyses are a suitable tool for the study of male reproduction and might help to discover potential reproduction failure of male white rhinoceroses. The discovery of sesonality in male reproductive physiology may be an important step in order to understand factors that limit reproduction in this species. Future analyses of mating and territorial behaviour will show how this hormonal seasonality correlates to behavioural patterns and reproduction.