

Influences of age, time of year, and sociosexual status on serum testosterone concentration in captive black rhinoceros (*Diceros bicornis*).

Bruce W. Christensen,¹ DVM, Linda Penfold,² PhD, Marcie Oliva,² RVT, Margo Macpherson,¹ DVM, PhD, DACT, Malgorzata Pozor,¹ Med. Vet., PhD, DACT, Mats Troedsson,¹ DVM, PhD, DACT, DECAR

1. University of Florida, College of Veterinary Medicine, Gainesville, Florida

2. White Oak Conservation Center, Yulee, Florida

Little is known regarding male reproductive physiology in rhinoceros species. Captive breeding of rhinoceros has met with limited success. Male reproductive hormones are essential to spermatogenesis, development of primary and secondary sexual characteristics, and libido, all of which play key roles in an individual's ultimate fertility. The purpose of this study was to measure serum testosterone concentrations in captive black rhinoceros and to establish baseline values for this species based on age, time of year and sociosexual status. We hypothesized that serum testosterone in male rhinoceros will show variation with age, time of year, and sociosexual status.

A retrospective study was conducted using samples collected from male, black rhinoceros housed at 10 facilities in the United States. A total of 382 serum samples were collected from 23 rhinoceros. Testosterone concentrations were measured in serum samples using an enzyme immunoassay [heterologous EIA with a polyclonal anti-testosterone-6-carboxymethyl oxime antiserum (R156/7) and testosterone conjugate (testosterone-3-carboxymethyl oxime: horseradish peroxidase)] that was previously validated for use with black rhinoceros serum in our laboratory (interassay variation < 15%; intraassay variation < 10%). Samples were grouped into juvenile (< 6 years) and adult (6-26 years) categories to evaluate the effect of age. Monthly values from all individuals were evaluated together to give an average monthly value for all rhinoceros in the study. To evaluate the effect of sociosexual status, we grouped samples into the following categories: isolated, housed with other males, housed directly with females, visual/tactile contact with females (separated by barrier), or contact with both males and females. Statistical analyses were performed using Kruskal-Wallis all-pairwise comparisons test. Significance was set at $P < 0.05$.

Significant rises ($P < 0.05$) in testosterone concentrations were noted between juveniles (35.84 ± 9.60 pg/ml), and adults (87.53 ± 10.12 pg/ml). Juvenile samples were therefore excluded from the remainder of the correlative evaluations. No correlation was found between time of year and testosterone concentrations. Grouped samples showed no significant difference between sociosexual groupings. Individual males, however, showed higher numerical concentrations when housed with females than when housed separated by a barrier or isolated completely. In addition, one male housed with a single female showed a significant rise in testosterone concentrations when a second female was introduced (88.16 ± 3.80 pg/ml and 117.35 ± 7.60 pg/ml, respectively; $P < 0.05$).

These data suggest that captive, male, black rhinoceros in North America do not show a fluctuation in their serum testosterone concentration correlated with time of year. They appear to undergo hormonal puberty around the age of 6 years. This correlates well with anecdotal evidence of when young male rhinoceros start breeding in captivity. These data also suggest that sociosexual environment may play a role in serum testosterone concentration in captivity. Further studies are indicated to confirm this.

Key words: *rhinoceros; testosterone; sociosexual; seasonal; puberty*