

Non-invasive gonadal sex determination in white rhinoceros (*Ceratotherium simum*)

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White rhinoceros are under constant threat due to illegal poaching. Moreover, the low reproduction rate found in this species in captivity is a subject of interest and concern to many research groups. An appropriate management of every rhino population is essential to ensure a future for the species. Although sex determination is mainly visual in rhinoceros, sometimes it might be useful to have a tool to determine sex non-visually, for example when it is difficult to identify the sex, as might be the case in free-ranging rhinos. Androgens and oestrogens proportion seem to determine gonadal sex in all the species. Since a correct identification of the individuals of a rhino population is essential for a proper management, our main objective was to develop a statistical model to determine the sex of the rhinos based on faecal oestrogen and androgen concentrations. A total of 572 faecal samples from 15 white rhinoceroses were collected in three different habitats: captivity (Zoo of Madrid; n = 3); semi-captivity (Bioparc of Valencia; n = 4) and wild population (South African Reserve; n = 8). We have used a non-invasive enzyme immunoassay to analyse androgens (A: 4-androsten-17 β -ol-3-one) and oestrogens (E: oestrone sulphate). Assay sensitivity was 4.9 pg/well and 2.9 pg/well for androgens and oestrogens respectively. The variation coefficient for androgens was 4.32 % (intra-assay) and 7.87 % (inter-assay), and 8.89 % and 10.22 % for oestrogens. Assay precision was determined by 86.70 % androgens recovery and 89.50 % oestrogens recovery. The results obtained did not show a statistical difference between males and females in regard to mean hormone concentrations (A (ng/g): males = 128.20 \pm 22.15; females = 85.39 \pm 7.18; E (ng/g): males = 200.02 \pm 14.90; females = 285.06 \pm 29.71). We modelled individual gonadal sex using mean androgen and oestrogen concentrations of each animal by a logistic model. We compared sensibility and sensitivity in three models: model I: included oestrogens/androgens ratio; model II included oestrogen and androgen values and model III included oestrogen, androgen, and the interaction between both variables. Our results showed that model II had the best balance identifying the sex in rhinos from hormonal faecal concentrations ("when A > 1.46E - 222 ng/g it is a male"), with a 74.6 % of success. We conclude that androgen and oestrogen faecal concentrations are powerful tools to predict the sex in white rhinoceroses.