USING URINARY HORMONE ANALYSIS TO PREDICT GENDER AND ASSESS FETAL VIABILITY IN THE INDIAN RHINOCEROS (Rhinoceros unicornis)

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Abstract

The high incidence of stillbirth (16% of all births) within the global captive propagation program for Indian rhinoceros (Rhinoceros unicornis) has been a major impediment to achieving a sustainable population.[2] Many factors have been linked to stillbirth incidence in domestic livestock including: parity, sex of offspring and gestation length. Recent analysis of studbook data indicates primiparous Indian rhinoceros dams experience less perinatal death and stillbirths compared to pluriparous dams.[2,3] While sex ratio at birth remains fairly equal in this species (54% male), the majority of stillbirths (67%) have been associated with male calves.[2]

As fetal gender may be a contributing factor in stillbirth in the Indian rhinoceros, knowledge of the sex of calves in utero could help identify those dams at a potentially higher risk. Knowing the gender of an expected calf would provide institutions and the rhinoceros taxon advisory group (TAG) more lead time to plan for housing requirements and subsequent breeding recommendations if viable births occur. Developing a non-invasive means to determine sex of an Indian rhinoceros fetus could potentially benefit this species and the zoo community.

In this study, we examined if urinary hormone analysis of maternal testosterone (T), cortisol (C) and corticosterone (C_1) could be used to establish physiologic markers to predict gender, parturition date and potentially assess fetal viability during pregnancy in the Indian rhinocersos. While urinary C has been validated for Indian rhinoceros,[1] it has not yet been examined during gestation, and the pattern of excretion as it relates to timing of parturition and birth outcome is unknown. To validate urinary C_1, samples collected during a previously conducted adrenocorticotropic hormone (ACTH) stimulation test were utilized.[1]

Longitudinal urine samples were collected starting on day of breeding (3 dams) or AI (3 dams), continued throughout gestation and for 1 wk post parturition. Of the six pregnancies monitored, three resulted in live births of female calves at 477.67 ± 6.96 days (range 465-489 days) post-breeding (n = 2) or AI (n = 1); two resulted in live births of male calves at 481 ± 1 days post-breeding (n = 1) or AI (n = 1); and one resulted in birth of a male calf at 490 d post-AI that exhibited signs of respiratory distress and died ~12 hr post-parturition. Although sample size was low, we found no difference (P = 0.452) in gestation length between dams carrying female versus male calves.

Significantly higher concentrations of urinary T were excreted from dams carrying male versus female calves during all months of gestation, with the largest difference observed during month 11 of gestation. Similarly, glucocorticoids C and C_1 were excreted in higher concentrations during all months of gestation in dams pregnant with male calves when compared to those pregnant with female calves ($P < 0.05$). Peak urinary C was measured during the 30 days prior to parturition in dams carrying a female calf, whereas dams pregnant with male calves excreted peak C during the 60 days prior to birth.

As urinary C_1 has the potential to signal independently of C, and because in some species the fetus preferentially secretes C_1 versus C, we also sought to validate urinary C_1 in this species and determine its utility as a prognostic tool for fetal viability. Pharmacologic validation of C_1 was shown via a 12-fold increase in urinary C_1 concentrations (baseline 3.45 ± 0.50 ng/mg vs. peak 44.17 ng/mg corticosterone) measured 16 hr after ACTH injection in a 32-yr-old Indian rhinoceros bull.

The data from this study provided useful information showing that urinary hormone excretion can be used to determine the sex of the fetus in Indian rhinoceros. However, as none of the pregnancies evaluated in this study resulted in stillbirth and only one birth was associated with perinatal death, sex of the calf could not be shown to be associated with an increased risk of stillbirth in this study. Evaluation of hormonal data, fetal sex and calf viability from additional pregnancies is warranted to determine key factors associated with reproductive success in Indian rhinoceros.

Key words: Fetal gender, glucocorticoids, pregnancy, Rhinoceros unicornis, testosterone, urinary hormones

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LITERATURE CITED

1. Capiro JM, Stoops MA, Freeman AW, Clawson D, Schook M. Effects of management strategies on glucocorticoids and behavior in Indian rhinoceros (Rhinoceros unicornis): Translocation and operant conditioning. Zoo Biol. 2014;33:131-143.

2. von Houwald F, Pagan O, Rieches R. International studbook for greater one-horned or Indian rhinoceros. Zoo Basel Switzerland. 2014. p. 66.

3. Wyss F, Wenker C, Robert N, Clauss M, von Houwald F. Why do greater one-horned rhinoceroses (Rhinoceros unicornis) die? An evaluation of necropsy reports. Proc Int Conf Dis Zoo Wild Anim; 2012. p. 54-61.