AZA Mid-Year Meeting, Long beach California

2022 Rhino Taxon Advisory Group Proceedings

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Ameliorating a proximate negative welfare condition in Woodland Park Zoo's greaterone-horned rhinos

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In December of 2020, staff noted abnormal gait and behavior in one of the Zoo's two younggreater one-horned rhinos (Taj). Further examination indicated that the Zoo's rhinos were presenting symptoms of chronic foot disease (CFD).

Staff engaged in researching chronic foot disease and its variable presentations. Research rapidlyfocused on Dr. Friederike von Houwald's nearly 30 years of work with this disease.

A comprehensive action plan was developed that incorporated changes in facilities, training, andspatial management and focused heavily on developing meaningful metrics and effective data collection techniques.

The Zoo moved forward with the first phase of planned facilities changes in the summer of 2021.The concrete/rubber floor of the "herd" room of the barn was removed and a new concrete floor was poured that would accommodate a two foot depth of hardwood chips. Simultaneously, a tilling program was initiated in the outdoor yards to mitigate compacted substrate. Chip installation for the first phase of the project was completed in early October of 2021.

Throughout the project, data was collected on a variety of foot conformation variables, sand compaction, substrate tilling, episodes of abnormal gait, body condition score, and medicationimpact.

Medication for Taj's discomfort stopped being necessary shortly after chips were installed in phase one. Since late October, there have been no instances of abnormal gait or signs of discomfort. Additionally, foot conformation has significantly improved along a suite of variablesin both rhinos.

The Zoo's decision to move forward with replacing sand with wood chips in all the outdoor yards was informed by the progress made in the remediation of CFD, our experiences with usinga non-abrasive wood chip substrate, and lessons learned about the interplay between sand and our Pacific Northwest climate.

“The Big Year” A Holistic Approach to Southern White Rhinoceros Enrichment
Ceratotherium simum simum

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Abstract

“The Big Year” is a holistic enrichment program that is aimed at creating an environmentthat closely reflects what a wild Southern White Rhinoceros would experience in their natural habitat through a whole calendar year. Traditional enrichment focuses on categories (i.e. sensory, manipulanda, foraging, social, structure) and adding objects or inputs to elicit these behaviors. These inputs often fall short of meeting the intricacies of a 24 hour experience. “TheBig Year” focuses on outcomes, or species specific behaviors, and how to elicit each behavior through the use of different inputs. Additionally, it focuses on the differences between the climate in Southern California and the climate in South Africa. Although there are many variances when comparing the two climates, the main one that “The Big Year” focuses on is rainfall. South Africa’s hottest months see the highest rainfall while Southern California’s hottestmonths see the lowest amount of rainfall. “The Big Year” is broken down into four “seasons”; Wet Summer (July, August, September), Transition to Dry Winter (October, November, December), Dry Winter (January, February, March), and Transition to Wet Summer (April, May, June). Each season has specific changes to the environment; i.e. wallows, drinking water, browse. Furthermore, there are changes to diets; i.e. types of hay, produce, how many times a day hay is fed out. To accomplish a more robust enrichment experience, there are multi-day “plays” that are particular to each season and designed to elicit select behaviors. “The Big Year”takes a multifaceted approach to enrichment that includes an examination of natural history and species specific behavior to create a holistic enrichment program.

Precision Medicine in the Black Rhinoceros: First-ever characterization of the rhinoceros immunome

Molly Corder[1,2], Weidong Zhou[2], Timothy P. Cleland[1,3], Parker Pennington[1], Jennifer Nagashima[1], Emanuel Petricoin[2], A. Alonso Aguirre [2], Budhan Pukazhenthi[1]

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The black rhinoceros (*Diceros bicornis*) is listed as critically endangered due to poaching pressures *in-situ* by the International Union for Conservation of Nature. The *ex-situ* populationserves as a genetic reservoir against impending extinction threats. However, many black rhinoceroses in human care (zoos) display unusual disease syndromes not typically observed among their wild counterparts. Affected animals often display clinical signs of

immuno-metabolic dysfunction and chronic inflammation. The etiology of these disease syndromes remains poorly understood. This study analyzed the proteome of peripheral blood mononuclear cells (PBMCs) to investigate the physiological underpinnings of these disease processes. Whole blood (n = 37 samples) collected longitudinally from 13 southern black rhinoceroses (*D. b. bicornis*) housed in the United States were processed for isolation of PBMCsusing a Ficoll® density gradient. Cell pellets were resuspended in lysis buffer to extract cellular proteins, digested with Trypsin, and processed for mass spectrometry. The resulting mass spectral profiles were mapped against the Uniprot black rhinoceros proteomic database with MetaMorpheus, a proteomic database search software. Spectral intensities were log transformed,fold change calculated ($\geq$1.5-fold and $\leq$0.5-fold) and analyzed for statistical significance ($P < 0.05$) using Student's *t*-test with unequal variance. Significant differences in PBMC protein expression were detected upon comparison of health status (healthy vs. diseased). THO complex4, fermitin family homolog 2, and calcium-transporting ATPase were up-regulated in healthy rhinos. Leukocyte elastase inhibitor, ribonuclease T2, eukaryotic translation initiation factor 4B, and serine/arginine-rich splicing factor 9 were down-regulated in healthy rhinos. Several of theseproteins are involved in immune function regulation in humans and animal models. Results also highlight the molecular mechanisms involved in the cell signaling architecture of the rhinoceros immune system. Findings demonstrate dysregulation of immune function in *ex-situ* managed southern black rhinos and could facilitate improvements in their medical management.

Toll-Like Receptors 7 and 8 and Their Potential for Sex-Sorting Rhinoceros Sperm.

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There may be a more user-friendly and cost-effective alternative to fluorescent activated sperm sorting for separating female (X-bearing) from male (Y-bearing) producing sperm. This alternatemethod relies on activating toll-like receptors 7 and 8 (TLR7/8) on the X-bearing sperm to inhibittheir ability to swim. In several species, incubation with TLR7/8 ligands results in the more motile Y-bearing sperm being in the upper half of the treatment solution and thus easily separated from the less motile X-bearing counterparts in the lower half. We first investigated whether rhinoceros sperm express the TLR7 and TLR8 proteins using cryopreserved sperm fromwhite (*Ceratotherium simum*) and greater one-horned (*Rhinoceros unicornis*) rhinoceros. We determined that TLR7 was mainly localized to the sperm heads, whereas TLR8 was observed primarily on the tails of sperm. Given the location of TLR8, we proceeded to incubate sperm with a TLR8 activator and then examined the concentration, motility, and the ratio of X- to Y-bearing sperm in the upper and lower halves of the sperm suspension. To ensure we observedmobility with as little bias as possible, a portable computer-assisted sperm analyzer (iSperm) with an app developed and validated for rhinoceros sperm was used. This instrument has allowed us to standardize assessment of concentration and motility, while reducing the time required to perform these evaluations (< 1 min per sample). Incubating sperm with the TLR8 activator reduced the velocity of the sperm ($P = 0.001$) without impacting the percentage of total or progressively motile sperm ($P \geq 0.2$) in both the upper and the lower layers. In spite of that, the ratio of female to male sperm within the upper and lower layers was 50:50 for control and treated samples alike. In conclusion, activation of TLR8 affected X- and Y-bearing rhino sperm indiscriminately under the conditions employed.

Validation of steroid hormone in nasal secretion for reproductive management in SouthernWhite Rhinoceros (*Ceratotherium simum simum)*

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Abstract: Monitoring of estrous cycles and reliable pregnancy diagnosis are important aspects ofreproductive management of threatened southern white rhinoceros (*Ceratotherium simum simum)* (SWR) in human care. Ultrasound and serum progesterone monitoring is the gold standard for reproductive assessment in SWR;[4-6,9] however, development of less invasive and technical methods of reproductive monitoring are warranted. While fecal progesterone analysis isnon-invasive,[1,2,7,8,11] rhinoceros use communal dung piles, making it challenging to identify and collect from specific individuals.

Nasal mucosa is under hormonal control and directly affected by hormonal changes.[10,12,13] This study collected paired nasal secretion and serum from SWR at The Wilds to determine if progesterone in nasal secretions could be used as a non-invasive biomarker to assist in reproductive management of ex-situ SWR. Extracted serum and nasal secretion progesterone concentrations were quantified by EIA using an anti-progesterone antiserum (CL425) and progesterone-horseradish peroxidase ligand. [3] The CL425 assay was validated for SWR nasal secretion extract by testing for parallelism. No significant difference in slopes was observed between CL425 progesterone standards and serially diluted SWR nasal secretion ($F(1,14) = 0.166$, $P = 0.69$). Efficiency of the nasal secretion extraction procedure was 88%, comparable tothe established efficiency for serum extraction using the same solvent. There was high correlation between progesterone values in same-day serum extract and nasal secretion extract extracts ($r = 0.775$, $P < 0.001$). This is the first study in SWR to validate measurement of progesterone in a novel biological matrix of nasal secretion for use in reproductive managementof SWR.

Socio-spatial Relationships in an Ex Situ White Rhino Bachelor Group

Lara C. Metrione,[1] Elizabeth W. Freeman[2†]

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In the wild, dominant, breeding white rhino bulls defend non-overlapping territories but allow non-breeding, subordinate bulls to occupy the same area. Zoo bulls that are not in a breeding situation are traditionally housed individually, but space limitations now necessitate creating bachelor groups.

This study documented the establishment of a zoo bachelor group by investigating the social behavior and spatial distribution of six bulls (two 3-, two 5-, and two 8-year-olds) housed in a 3.1-ha enclosure. Focal animal observations were conducted 30 min/day/bull, 2×/week for eight months, though the

3-year-olds were shipped to other zoos after five months. Rhino location, nearest neighbor distance (<2 body lengths, >2 body lengths), and frequencies of aggressive behavior (vocalizations, and physical

non-contact and contact displays) were recorded, along with the outcome of interactions to determine the dominance hierarchy. There was no evidence of spatial segregation. The 3- and 5-year-olds grouped together by age, while 8-year-olds were more independent. Aggressive vocalizations (0.4-1.3/h) were more frequent than physical aggression (0.2-0.4/h), and the number of physical contact displays did not greatly exceed non-contact displays. Eight-year-olds used aggressive vocalizations more, and 3-year-olds used physical displays more than other age groups. Aggressive vocalizations and physical displays increased ~3× after the youngest bulls were removed. When there were four bulls, a social hierarchy was established with a dominant 8-year-old, middle-ranking 5- and 8-year-olds, and a subordinate

5-year-old. Second and third-ranked bulls displayed more frequent aggressive behavior, and third- and fourth-ranked bulls were the recipients more often. Overall, this bachelor group was cohesive and aggression was infrequent. The use of aggressive vocalizations more than physical displays might be a strategy to minimize injury. The dominant bull was unlikely to engage in aggression, but the next bulls in the hierarchy might be important to monitor for conflict.

Premature Indications of Parturition in a Female Black Rhinoceros at Zoo Miami

Joseph T. Svoke (Zoo Miami), Michael Barnes (Zoo Miami), Linda Penfold (SEZARC) Presenter: Joseph T. Svoke (joseph.svoke@miamidade.gov)

The black rhinoceros at Zoo Miami had a successful copulation event on November 19, 2019 with pregnancy being confirmed via fecal hormone analysis on April 21, 2020. This birth would be "Circe's" fourth calf so the team was looking forward to a normal gestation and birth in 10+ months. However, we were proven wrong. On June 17th, 2020 the keepers reported udder development, though only 209 daysinto her pregnancy. Initially it didn't cause too much alarm, but on July 9th (231 days) the keepers reported vulva swelling and discharge which altered the feelings of the animal management and veterinarian teams; we were only about half way through her pregnancy. After several internal meetings and consulting others within the rhino community, there appeared no evidence to this scenario being seen previously in black rhinos (or at least no one had heard of it). While developing a plan of action to determine what may be causing these changes and the potential effects on the fetus, a former keeper mentioned that they recalled a similar situation happening with "Circe's" last pregnancy. After reading through her ZIMS record, it did appear that "Circe" was following a similar pattern to her 2014 calf. Withthis information and her fecal hormone chart looking like a normal pregnancy we decide to just monitor the situation, but kept a close eye on her progess. Luckily, after 461 days, Zoo Miami had a new healthy male black rhino calf to celebrate.

This presentation is intended to let the community know what we saw over time to help educate the greater rhino community on this unique situation.