Saving Species With Science®

CREW ReView

Lindner Center for Conservation and Research of Endangered Wildlife

Cincinnati Zoo & Botanical Garden • Fall 2018

Roth's Remarks

Dr. Terri L. Roth
VP of Conservation & Science and Director of CREW

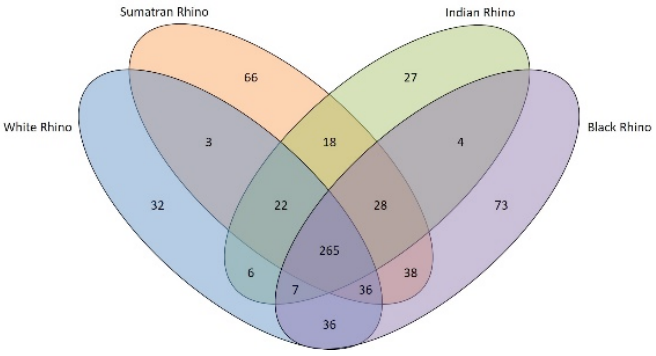
Voluntarily Making A Huge Impact

In 1996, when I interviewed for the CREW Director job, I spoke with a number of people in important positions associated with the Cincinnati Zoo & Botanical Garden including the Zoo's Director, the Zoo's Education Director (today's Zoo Director), the Institutional Animal Care and Use Committee Chair, prominent professors at the University of Cincinnati and Medical College associated with CREW, and the CREW Volunteer Chair (yes, really). Volunteers have always been an integral and vital part of CREW, so much so, that the opinion of the CREW Volunteer Chair was solicited in the hiring of a new CREW Director. That individual still volunteers at CREW today, and I often thank her for my job. The importance of the more than 130 volunteers at CREW has never wavered. These generous, talented individuals do everything from greeting guests at the front desk to socializing (and caring for) cats in the colony, from processing animal dung to making sterile media, and from assisting in surgery to designing scientific studies. In addition, there are others working as educators introducing students to CREW's work and talking to visitors at the CREW Public Exhibit and/or CREW cart each year. In fact, over the past five years, CREW volunteers have taught >7,000 students and introduced >280,000 visitors to CREW's research. During that same time frame, 69,305 volunteer hours were logged in, supporting CREW in too many ways to mention, with an economic value of $1,733,708. Perhaps just as important, these generous individuals are also dear friends and valuable colleagues, keeping CREW staff grounded while working side-by-side with us to advance our mission of *Saving Species With Science®*.

Love Hurts, Love Scars, Love Wounds

Habitat loss and poaching have driven the wild population of Eastern black rhino (*Diceros bicornis michaeli*) to less than 1,000 individuals. With such few individuals remaining, maintaining a sustainable captive population is crucial for black rhino conservation efforts. The North American Eastern black rhino captive population includes 21 breeding pairs, but several of these pairs have yet to produce calves. CREW scientists are investigating the role of reproductive physiology and courtship behaviors in breeding success. Fecal samples and breeding behavior recorded via GoPro cameras have been collected from six rhino pairs housed at six different U.S. institutions. To date, three of the pairs have successfully bred – a shout out to our black rhino pair Seyia and Faru for conceiving Kendi! Preliminary data indicate no difference in estrous cycle length or progesterone and testosterone fecal metabolite concentrations between successful and unsuccessful breeding pairs. Interestingly, horn clash, jousting, advancing, and following behaviors are more commonly found in successful breeding pairs than in unsuccessful pairs. Hence, successful courtship and breeding in black rhinos involves aggressive behaviors. These behaviors may be associated with the release of glucocorticoids, i.e., "stress" hormones, and an imbalance in the output of glucocorticoids may suppress the expression of these behaviors. To find out, CREW scientists are in the process of measuring and comparing fecal glucocorticoid metabolite concentrations in relation to courtship behavior in successful and unsuccessful breeding pairs. (*This project is supported by a grant from the Institute of Museum and Library Services.*)

MicroRNAs: Mini Messengers Revealing Megafauna Mysteries



Shared and species-specific miRNAs in serum of four rhino species.

MicroRNAs (miRNA) are small molecules made up of nucleotides (the same building blocks of DNA and RNA). MicroRNAs respond to different physiological states such as illness, reproductive status and food digestion, in turn influencing gene-expression controlling biological processes within an organism. Since miRNAs respond to physiological changes that occur within the body, they can reveal a great deal of information about what an individual is experiencing. For instance, in cows, changes in miRNAs can be used to diagnose pregnancy as early as eight days post-conception, and in humans, miRNAs can be used to predict the development of Alzheimer's disease. Rhinoceros species are impacted by several unique diseases that are difficult to diagnose, and CREW scientists are interested in determining if miRNA concentrations in rhino serum may provide insight into the health status of individuals. Circulating miRNAs within the serum of 27 rhinos of 4 species (African black, African white, Indian, and Sumatran) were sequenced, revealing the existence of 661 different miRNAs within the rhinoceros taxon. Of those, 168 have not previously been reported and may be unique to rhino species. The next step of this study is to investigate how miRNAs differ between healthy animals and those afflicted by iron overload disorder, a disease that is difficult to diagnose. CREW scientists are hopeful that miRNA biomarkers may bridge the gap in our knowledge of how to diagnose the disease and allow for earlier treatment. (*Supported by a grant from The Eppley Foundation for Research.*)

OptiXcell: The Answer to Optimizing Rhino Sperm Cryopreservation?

Semen cryopreservation is an important assisted reproductive technology for maintaining genetic variation in managed populations of endangered species. To date, the semen extender (media used for freezing semen) of choice for most species contains egg-yolk or other types of animal protein. Unfortunately, egg-yolk runs the risk of microbial contamination and could result in the spread of disease when the semen is thawed and used in procedures such as artificial insemination. CREW has been on a quest to find a vegan alternative to egg-yolk that will reduce or eliminate this risk but also protect rhino sperm during freezing. It has not been an easy adventure, as it turns out soy-derived products (loved by cat and cow sperm) limit rhino sperm motility, and coconut products have an out-right murderous relationship with rhino sperm. Thankfully, we believe the hero we've been hoping for has been found! OptiXcell is a commercially available animal-protein free extender that protects bull and buffalo sperm during freezing. CREW tested OptiXcell with semen collected from three rhino species: African black, African white, and Indian, and preliminary results are promising. Sperm frozen in OptiXcell displayed post-thaw quality equivalent to, and in some cases better than, sperm frozen with the traditional egg yolk extenders. OptiXcell offers an option that can help to prevent possible disease transmission, but also protects invaluable genetic material while it is being stored, relieving the worries associated with the traditional egg yolk options. (*Study supported by a gift from the Coombe Family Fund of the Greater Cincinnati Foundation.*)

Collaboration is Key to Conserving Rhinos

This Fall marks the completion of a four-year National Leadership Grant from the Institute of Museum and Library Services to apply and enhance assisted reproductive technologies (ART) for captive African and Asian rhinos to ensure their optimal genetic management and long-term sustainability. This collaborative effort between CREW and SeaWorld Busch Gardens Reproductive Research Center (SWBGRRC) involved partnering with 28 Association of Zoos and Aquariums (AZA)-accredited institutions and three privately owned facilities to achieve project goals. One primary objective was to build upon national rhino gamete rescue centers by banking substantial sperm numbers and increasing the individual males represented. While sperm sexing technology has been established in African white rhinos, the grant facilitated banking additional x-enriched samples from this species and enabled successful development of this technology in two new species, the African black and Indian rhino. In total, 77 multi-thermal gradient tubes of x-sorted and non-sorted sperm, and over 1400 straws of non-sorted sperm were banked from 27 male rhinos representing three species. Additionally, the first use of a mobile laboratory (ST Genetics) to sort and freeze rhino sperm occurred over this past year. Previously, semen had to be hand carried on an airline flight from each zoo where it was collected to the SWBGRRC lab in San Diego for sorting, whereas the mobile lab could be reached within a 1.5 hour drive. In addition to minimizing the time and hence stress that sperm samples had to endure from collection to sorting, the mobile lab employed three next-generation sorting machines, which increased the sort rate from 3,000 to 8,000 sperm/second. Future AI procedures may allow us to produce rhinos of predetermined sex for improved population management.

Collaborating scientist from ST Genetics overseeing the sorting of X- and Y-enriched sperm from an African white rhino in the mobile laboratory.