RHINOCEROS *SIGNATURE* PROJECT UPDATES

Southern Black Rhino Fertility Round-up


Kendi

Several years ago, AZA's Rhinoceros Taxon Advisory Group decided to focus on managing just one black rhino sub-species, the Eastern black rhino (*Diceros bicornis michaeli*). Our Cincinnati Zoo recently contributed to that important program by producing "Kendi", our new male black rhino calf, who was conceived via natural mating in the spring of 2016 and born in July 2017. He is a significant addition to the small (~60) population of Eastern black rhinos in the Species Survival Plan. However, the 28 Southern black rhinos (*Diceros bicornis minor*) in the U.S. also need assistance. The International Rhino Foundation offered to oversee the management of this population and immediately took action moving animals among facilities to pair those most genetically suitable for breeding. Unfortunately, after three years, only three calves have been born and only one of those three survived. Therefore, CREW's rhino team was called in to conduct fertility exams on thirteen Southern black rhinos at seven facilities in Kansas, Texas, Georgia and Florida. Assessments revealed that three of the thirteen rhinos (2 female, 1 male) appeared to be infertile based on reproductive pathologies identified during ultrasound exams, and failure to produce sperm in the case of the male. However, the other ten rhinos appeared to be fertile. Efforts to breed these ten have been renewed, and hopefully, some of them will soon contribute to this small, endangered population of Southern black rhinos.


Rhino Fertility Team, Austin Savanah, TX

(*Funded by a grant from Mr. and Mrs. Jeremy S. Hilton and Family with supplemental support from Dr. Thomas and Rita Bell.*)

Rhino Sperm Don't Like Piña Coladas (or Getting Caught in the Soy)

Semen cryopreservation is an important tool for the genetic management of endangered species, allowing for long-term storage, easy sample transport and on-demand artificial insemination. To survive the ordeal of being frozen to temperatures lower than -300°F, sperm need more than a warm blanket. Scientists have worked for decades to develop cryopreservation media that can increase the sperm's chance of survival. As all species are unique, even down to the make-up of their sperm, every extender needs to be tailor-made. Egg-yolk has become a gold-standard ingredient for extenders, providing a source of energy and protecting the sperm cell's membrane. Recently, egg-yolk has come under scrutiny: composition varies from egg to egg and it may carry disease. To address these issues in rhinos, CREW tested four vegan alternatives to egg yolk: coconut water, coconut milk and two concentrations of soy lecithin (from soybeans). Unfortunately, our hopes that rhino sperm may respond favorably, like felid sperm to vegan extenders, were quickly dashed. In the coconut extenders, rhino sperm lost their acrosomes (sperm head caps containing enzymes), rendering them incapable of fertilization and causing the loss of many a sperm life. The soy extenders didn't out-right kill the rhino sperm, but created a sticky situation. Large sticky masses formed post-thaw and the sperm were no longer able to move progressively, which is not ideal when there's still a long way to travel to any awaiting egg. Though these options proved inferior to egg-yolk, this research has provided some insight into what rhino sperm may prefer, and CREW is currently testing some promising new options. (*Supported by the Coombe Family Fund of the Greater Cincinnati Foundation.*)

SAVING SPECIES WITH SCIENCE®

Love Potion No. 9

Several captive Eastern black rhinos are not in breeding situations because genetically compatible mates are not available, they have health issues, or the facility lacks space for new individuals. Long, non-breeding periods are associated with changes in reproductive hormone production in both male and female rhinos. CREW scientists are currently investigating whether olfactory stimulation (i.e., exposure to feces from the opposite sex) can maintain or improve reproductive health during these non-breeding periods. For this study, six individuals from four U.S. institutions have been "sniffing" olfactory stimuli via custom made olfactory tubes. Fecal samples were collected to monitor any changes in progesterone, testosterone, cortisol, and corticosterone metabolite concentrations and over 100 hours of olfactory behaviors were recorded using GoPro cameras. Preliminary results indicate that during olfactory exposure, females that were previously experiencing normal estrous cycles continued with this pattern, whereas changes were observed in a female with an abnormal cycling profile. Specifically, progesterone metabolite concentrations increased (80.87 to 173.63 ng/g feces) and estrous cycle length normalized (from 17 to 25 days; species' average is 26 days). Corticosterone levels in all females also increased while "sniffing" feces. Furthermore, exposure to olfactory stimuli elicited a series of olfactory behaviors, some of which are associated with breeding. It is well established that animals respond to chemical cues in feces. Our results suggest these chemical cues may facilitate reproduction and/or be used to maintain or stimulate cyclicity in female rhinos during non-breeding conditions. (*Supported by a grant from the Institute of Museum and Library Services.*)

What's in the Water?

When it comes to aquatic salamanders, a plethora of information is contained in the water in which they live. This past year, CREW research intern Andrew Nagel sought to determine if a novel waterborne hormone analysis technique developed for smaller amphibians and fishes could be adapted for measurement of hormones from large fully aquatic salamanders called waterdogs. Endocrine studies involving amphibians commonly rely on the use of blood or tissue samples, which are limited in many cases to a single measure and their interpretation can be confounded by the physical restraint needed to obtain samples. CREW scientists previously confirmed fecal hormone analysis could be used to assess reproductive activity non-invasively in waterdogs, but the frequency of sample collection required from group-housed individuals was challenging. Quantifying secreted/excreted hormones in water could be an alternative for monitoring their endocrine profiles. To biologically validate this method, a male waterdog was administered adrenocorticotrophic hormone (ACTH) which stimulates endogenous release of the stress hormone corticosterone. Andrew found peak waterborne corticosterone concentrations were achieved 4 hrs post-ACTH-injection in waterdogs and increased 44-fold over baseline values. However, achieving these results required the collection and extraction of 10-25 times the volume of water utilized in similar studies of smaller amphibians. Now that waterborne hormone analysis has been validated in waterdogs, it should lead to a greater understanding of their physiological function including stress responses, which is especially important as climate change and human disturbance may lead to the decline of wild populations. (*Supported by private donations.*)