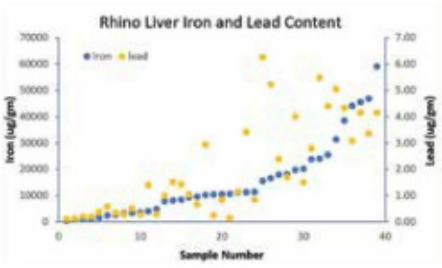
Key to cover

How did you do? Yes, we threw you a couple of curve balls with our new CREW Charlotte R. Schmidlapp Scholars, Claire and Kendra.

Rhinoceros *SIGNATURE* PROJECT UPDATES

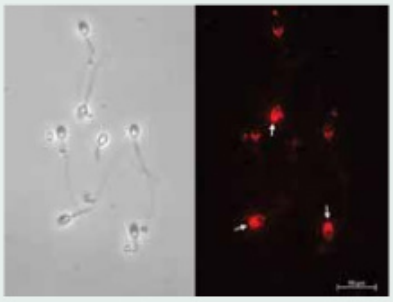
Mining for Answers to Iron Overload in Rhinos



As CREW's research on iron overload disorder (IOD) in rhinos has progressed, it has become clear that the etiology of the disease differs between black rhinos and Sumatran rhinos. For black rhinos, which rarely die from liver failure due to excessive iron stores, we have long questioned just how much IOD compromises their health and liver function. Answering that question requires direct liver tissue evaluation, which is simply not possible in living rhinos. The next best thing is to analyze liver tissue post-mortem, so that is exactly what has been happening over the past two years with the help and much-needed expertise of our colleagues at Michigan State University (MSU), St. Louis Zoo and Stellenbosch University. In 2019, Hailee Butler, an MSU veterinary student keenly interested in the field of pathology, was provided a stipend by CREW to work with expert MSU pathologist Dr. Dalen Agnew to prepare slides and tissues for analysis. To-date, 45 livers have been analyzed for mineral content, and now a subset of those are being evaluated for pathological changes that may reveal the impact on rhino health. However, the mineral data already are yielding some interesting results. For example, an unexpected positive correlation was found between iron and lead concentrations in the livers, suggesting black rhinos may be accumulating minerals more toxic than iron. Is this finding just incidental or is it an important piece of the puzzle? Time (and more research) will tell. (*This project was supported by a very generous anonymous CREW donor.*)

Painting the Rhino Sperm Red

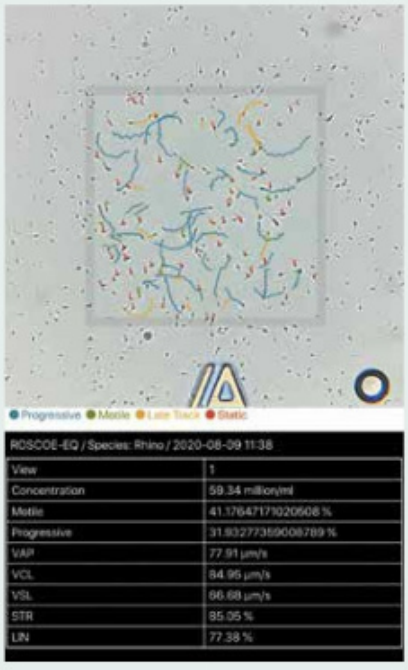
Sex ratio management is essential to building a sustainable and thriving population of rhinos. Preselecting the gender of offspring is possible by utilizing sex sorted sperm, i.e., sperm that has been separated into X-bearing (female producing) and Y-bearing (male producing) populations. The current technology for sorting has successfully been customized for rhino sperm by CREW's colleagues at SeaWorld San Diego. However, there are several drawbacks to the methodology, including high instrument cost (>$100K), the need for specialized expertise, long hours of labor, and the loss of up to 50% of sperm cells during the process. There may be a more user-friendly and cost-effective alternative that relies on targeting specific proteins (TLR7/8) on the sperm to reduce the swimming speed of the X-bearing sperm without affecting the mobility of the Y-bearing sperm. The first step in determining if this technology can be applied to rhinos involves evaluating sperm for the presence of the specific proteins. Antibodies labeled with a red fluorescent dye allow us to look for the presence of TLR7/8 on sperm cells through the microscope. This painting of sperm recently revealed that TLR7/8 are indeed on rhino sperm. Interestingly, ~50% of the sperm has staining on the top of their heads (white arrows), and ~50% do not. Now the question is, can this difference be used to separate the female producing sperm from the male producing sperm? *(Special thanks to Jackie Dieckman and Mike Camery for their gift that supported the fluorescent scope and camera upgrade to improve CREW's ability to see rhino sperm painted red.)*



Same slide shown under phase contrast (left) and fluorescence (right).

Spy with the iSperm

Before freezing semen to become part of the CryoBioBank® at CREW, it is essential to evaluate the swimming skills of sperm within the sample. Specifically, how many of the sperm are swimming (i.e., motile) and in a mostly straight path (i.e., progressively motile)? Sperm that swim slowly or in circles tend to be unsuccessful at fertilization, especially after the cryopreservation process. However, accurate assessment of sperm motility can be challenging for humans. When watching sperm on a microscope, we tend to focus on those swimming, and the non-motile sperm kind of fade into the background. And because each person sees things a little differently, motility values from the same sample often vary from person to person even after extensive training. Technology adopted by human IVF clinics and livestock industries overcomes this bias. Computer assisted sperm analysis (CASA) uses recorded images and software to accurately measure sperm movement, marking each sperm as non-motile or motile. With CASA, after less than a minute, we know not only how many sperm are motile, but also their swimming speed and the concentration of the sperm. CREW currently is beta testing rhino specific software for a portable sperm analyzer, the iSperm. Aidmics Biotechnology, the inventor of the microscope system that mounts on an iPad mini, has generously provided the setup free of charge to CREW. Once validated, iSperm for rhinos could be used to standardize evaluations across the many facilities with staff now trained to collect and bank rhino semen. It would remove the human bias and provide quality control assurance to those requesting samples for rhino ART procedures in the future.



Rhino sperm tracking by iSperm