

CREW PROGRESS REPORT

Lindner Center for Conservation and Research of Endangered Wildlife



Glass Glass Baby: Birth of Healthy Kittens following Sperm Vitrification for Artificial Insemination

Cryopreservation of cat semen for assisted reproduction can be challenging, requiring technical expertise and specialized equipment for semen collection, processing and freezing. As a simplified alternative to standard semen cryopreservation methods, CREW scientists have been investigating the use of vitrification – the ultra-rapid cooling of liquids to form a solid without ice crystal formation. This approach essentially preserves semen as glass instead of ice. For vitrification, cat semen is diluted in a chemically-defined medium containing soy lecithin and sucrose as cryoprotectants and, after a five minute equilibration period, pipetted in small volumes (~30 microliters) directly into liquid nitrogen to form tiny glass marbles of frozen sperm. In initial studies, we found that cat sperm survived vitrification as successfully as that frozen using standard straw freezing methods and that vitrified sperm were capable of fertilizing cat oocytes in vitro. In our first assessment of in vivo viability, eight of these embryos were transferred into three synchronized females. Although one female appeared to have two early implantations, no offspring were produced. In a follow-up study, artificial insemination (AI) with vitrified sperm was assessed in three additional females. With our laparoscopic oviductal AI technique (LO-AI), only a couple million sperm are required per insemination, allowing the use of the relatively low sperm numbers that are preserved in vitrified semen pellets. Following LO-AI with vitrified sperm, all three females conceived, with two of the pregnancies progressing to term and culminating in the birth of two healthy kittens in early April. These kittens, a male named Vito (short for vitrification) and a female named Elsa (after the character in the movie Frozen), are the first non-human offspring – of any species – produced with vitrified sperm (although three human babies have been born from earlier research). Our preliminary results with semen from fishing cats and ocelots indicate that vitrification is effective for preserving post-thaw sperm viability and function across cat species. These findings suggest that this fast and simple cryopreservation method may have broad applicability for semen banking of endangered felids housed in zoos and possibly living in the wild. The study's lead author, veterinary student Jaci Johnson, has been selected to present these findings at the upcoming American Association of Zoo Veterinarian's Annual Meeting in Portland, Oregon. (Funded, in part, by the Procter & Gamble Wildlife Conservation Scholarship program in collaboration with Ohio State University's College of Veterinary Medicine)



"Using Science to Learn,
Applying Knowledge to Save,
A Future for Wildlife"

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That's Nuts!

Nuts from the endangered American chestnut tree are always special, but some are particularly important. This magnificent tree was almost entirely obliterated by the chestnut blight by the mid-twentieth century, but breeders have been working to develop a resistant tree. One of their key tools is pollen, but American chestnut pollen viability declines rapidly, so maintaining important lines of pollen from year to year is difficult. In 1993, CREW post-doc, Dr. Leslie Leverone, received a grant from the American Chestnut Foundation to study the cryopreservation of American chestnut pollen, and as part of that work, she stored pollen at CREW in liquid nitrogen. As part of the current IMLS-funded study on the viability of samples stored in CREW's Cryo-BioBank, pollen was removed, and tests in the laboratory showed that it was still alive. But, in order to demonstrate its ability to pollinate, CREW post-doc, Dr. Dani Ballesteros, travelled to the ACF's farm in Virginia last spring and worked with them to pollinate trees, carefully bagging the flowers to eliminate pollen from any other source. After several months of waiting, the news arrived that the pollinated trees had indeed formed nuts! Paternity testing is currently being done at CREW, but the indications are very good that cryopreservation can indeed maintain pollen viability for at least 20 years—a fact that should provide a new tool for those working to save this majestic American tree.



CREW Supports Sumatra's Rhino Breeding Program

This past winter, Dr. Terri Roth traveled to Indonesia for an extended visit (~5 weeks) to assist the new veterinary team at the Sumatran Rhino Sanctuary (SRS) with their reproductive assessments and breeding efforts. The goal was to share all that has been learned at the Cincinnati Zoo, the most successful breeding center for this species, and to guide the team through a couple of rhino breeding efforts. The visit was extremely productive. The new veterinarians proved to be highly skilled and were trained on a new ultrasound machine donated by SOS Rhino that will enhance their ability to monitor their rhinos closely. The team effort resulted in a successful rhino mating - the first at the SRS in four years! Hopefully, the female conceived, but if not, the new vets have the knowledge and skills necessary to breed the pair again. Training others and building capacity are two of CREW's most important roles, especially with regard to saving the Sumatran rhino since the breeding program on the island of Sumatra is the last of its kind for this critically endangered species.