

CREW PROGRESS REPORT

Lindner Center for Conservation and Research of Endangered Wildlife



Revy as adult with her kitten



Revy as kitten



Milagre as kitten

An Awful Lot of Ocelots Owe their Existence to CREW's Artificial Insemination Program

In November 2015, a young ocelot named Revy at the Santa Ana Zoo gave birth to her first kitten - another valuable addition to the small Brazilian ocelot population managed by the Ocelot Species Survival Plan (SSP). This birth was most notable because of Revy's unique history – and the fact that she (and her new baby) wouldn't be alive if not for the research efforts of CREW scientists. Revy owes her very existence to artificial insemination (AI) as do almost one fourth of the 30 Brazilian ocelots currently living in North American zoos. Revy is just the most recent AI kitten to grow up, breed naturally and give birth to her own offspring.

Brazilian ocelots (*Leopardus pardalis mitis*) are becoming increasingly endangered in their native habitat due to human population growth and related habitat loss. The Ocelot SSP's goal is to grow the ocelot population in zoos through managed breeding, but in some cases, their carefully selected pairings fail to reproduce. One solution for overcoming these reproductive barriers is AI, using laparoscopy to deposit semen directly into the uteri or oviducts of hormonally-synchronized females. In the past twenty years, five pregnancies have been produced in ocelots with laparoscopic AI, including three in the past few years through the efforts of CREW. Revy was the result of one of those AIs. Her father, Marcel, was imported from Canada by the Cincinnati Zoo in 2008 to breed with our female, Sala, but the pair was behaviorally incompatible. As an alternative, CREW scientists conducted laparoscopic AI using Marcel's semen, and Sala conceived. On New Year's Eve ('revellion' in Portuguese), she gave birth to Revy who grew up under Sala's protective care. In 2014, Revy was transferred to the Santa Ana Zoo and paired with a young male, Diego, resulting in the birth of Revy's first kitten this past year.

Two other AI offspring born to a female ocelot, Kuma, housed at Connecticut's Beardsley Zoo have experienced similar breeding success. Kuma was accidentally injured as a kitten and lost her left rear leg, making natural breeding impossible.

This physical disability did not decrease her genetic value nor presumably affect her ability to bear and raise offspring – if she only could somehow conceive. Laparoscopic AI procedures in 2010 and 2012 resulted in two pregnancies and the birth of two healthy female kittens that Kuma raised herself. Those two kittens, Milagre and Ayla, subsequently were transferred to the Dallas Zoo and Buffalo Zoo where both have bred and produced kittens of their own in the past three years. These AI successes have helped zoos to address breeding challenges and provide proof that AI can benefit conservation management of ocelots and possibly other felid species. Without the AI option, these three ocelots – Revy, Milagre and Ayla – and all of their subsequent offspring - would have never been born and the long-term sustainability of our Brazilian ocelot population would have been further diminished.

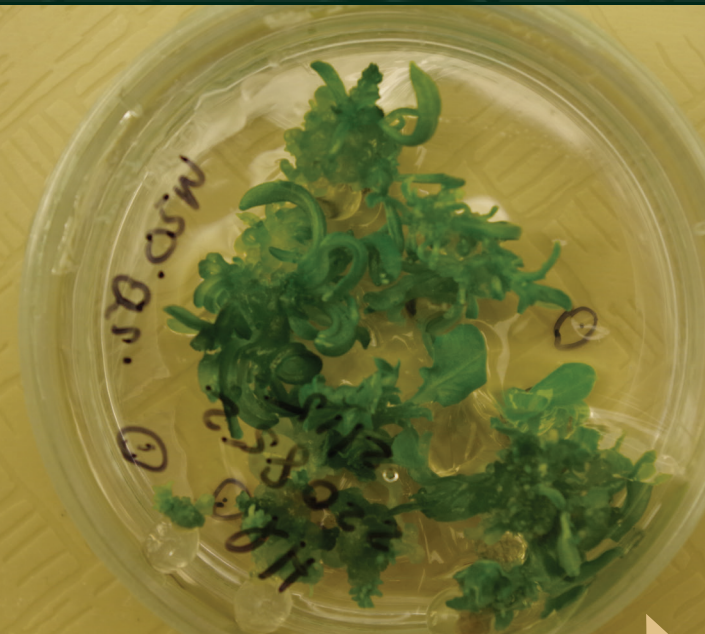


"Using Science to Learn,
Applying Knowledge to Save,
A Future for Wildlife"

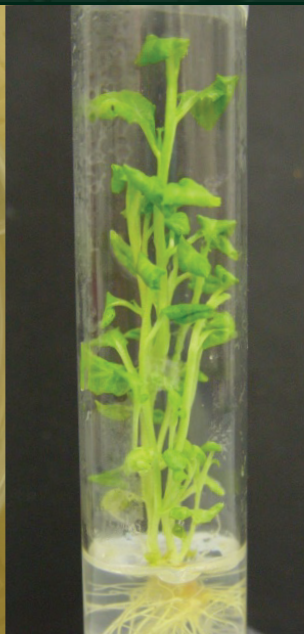
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Cultures of shoots growing from shoot tips of *Hippobroma longiflora* stored in liquid nitrogen for 18 years.



Hippobroma longiflora in culture



photo from Himeji Zoo

A Little Plant from Trinidad Comes Back to Life

It's a small plant, only about 2 feet tall, with white, star-shaped flowers—sometimes called "star of Bethlehem." Another name "madam fate" likely refers to its more sinister side, as producing toxic alkaloids. But, at CREW, *Hippobroma longiflora* has a special place as one of the first species regenerated by in vitro collecting, during the experimental IVC trips to Trinidad in the late 1990s. The first cultures came from just one half of one leaf disc, less than one cm wide, punched from a leaf in the Trinidadian rainforest—the other half of the tiny piece became contaminated and had to be cut away. The healthy tissue produced shoots, which were used to propagate plants, which were acclimatized and grew for a while in the CREW greenhouse. In addition, CREW scientist emerita, Bernadette Plair, cryopreserved shoot tips from the cultures, to demonstrate that they could be successfully cryostored. At the same time, extra samples were banked for long-term storage in CREW's Cryo-BioBank, where they have been sitting quietly in suspended animation for 18 years. Some of the samples were recently thawed and put onto a recovery medium, as part of the IMLS-funded project to evaluate the viability of samples in CREW's Frozen Garden. Within a week, growth began! Overall, survival is equivalent to that observed in the original samples (63% and 62%, respectively). While *Hippobroma longiflora* is not endangered, it demonstrates how IVC, tissue culture propagation, and shoot tip cryopreservation can provide complementary and coordinated tools for conservation, and could be particularly applied to exceptional species—those species for which seed-collecting and seed-banking are not workable options for conservation. (Post-storage research supported by grant # LG-25-12-0595, from the Institute of Museum and Library Services.)

Is Pregnancy Stressful to Polar Bears?

Ask any woman who has had a baby and she will likely tell you that her last trimester of pregnancy was a bit stressful. In fact, it is well documented that cortisol (a hormone associated with stress and/or excitement) increases in women towards the end of gestation. Because CREW's research on polar bear fecal hormone metabolites traditionally associated with pregnancy has not revealed any differences between pregnant and pseudopregnant bears, we recently collaborated with researchers at the Brookfield Zoo to determine if fecal cortisol metabolites increase during true pregnancy in polar bears. Study results indicate that pregnant polar bears do not produce more cortisol than pseudopregnant bears, nor do cortisol metabolites increase late in gestation. Perhaps the "stress" of producing tiny 2 lb cubs when you are a 700 lb female bear is not quite equivalent to what women experience during their pregnancies. (This study was funded, in part, by the Chicago Board of Trade, Endangered Species Fund.)