

# CREW PROGRESS REPORT

Lindner Center for Conservation and Research of Endangered Wildlife



## CREW Achieves First AI Success in the Last Big Cat Species

Over the past forty years, reproductive biologists have been striving to develop artificial insemination (AI) as a conservation management tool for the world's seven largest cat species. During that time frame, at least one pregnancy and offspring have been produced by AI in each of these species with one glaring exception: the jaguar. CREW scientists have now eliminated that deficit with an approach that may improve the prospects of using AI more effectively in all large cats. Working with colleagues from Associação Mata Ciliar and the Federal University of Mato Grosso in Brazil, CREW initiated a three-year study in 2016 to develop assisted reproductive technologies in the jaguar. One of the major challenges for AI in any felid species is ensuring the procedure is performed during the appropriate stage of the female's estrous cycle. One strategy to more precisely time ovulation induction is to administer an oral progestin (synthetic progesterone) beforehand to temporarily suppress ovarian activity. As a first step in this process, CREW used fecal hormone analysis to non-invasively monitor responses of female jaguars to two different oral progestin doses and more finely tune ovarian suppression in preparation for AI. Their next step was to refine their gonadotropin treatment regimen to yield a more reliable ovarian response. In initial AI attempts in 2017, multiple follicles formed on the jaguars' ovaries following gonadotropin injections, but few follicles ovulated.

In the latest AI procedures, alterations in injection timing and gonadotropin dosage produced ovulation of multiple follicles in each of the five jaguars. One of these females, Bianca, conceived and gave birth to a single viable jaguar cub on February 16, 2019. Remote video monitoring showed excellent maternal care and nursing by a vigorous cub. Unfortunately, the cub disappeared from the maternity den two days after birth, presumably consumed by the mother. While the cub's loss was disappointing, it's not uncommon for carnivores, especially first-time mothers, to behave this way with their offspring. Nonetheless, this study resulted in the first jaguar cub ever born from AI and represents a major scientific advancement for the conservation of this species. The birth of this cub heightens the possibility of using CREW's novel AI approach as a management tool to conserve this iconic cat as well as other endangered large cat species.



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

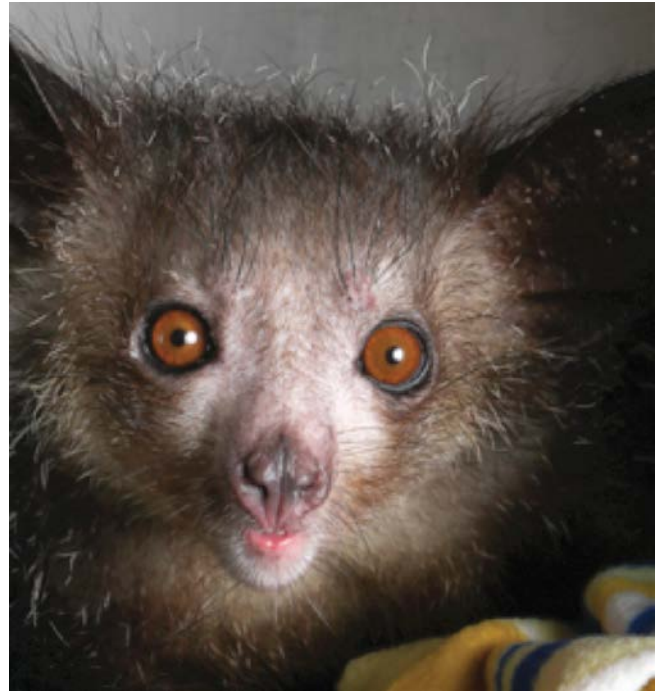
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## Shedding New Light on a Mysterious Nocturnal Species

The Cincinnati Zoo and Botanical Garden is fortunate to be caring for 2 of only 27 aye-ayes (pronounced “eye-eye”) in North America. Though this pair has an SSP breeding recommendation, they have not produced any offspring. Therefore, the animal staff implemented exhibitry changes in hopes of promoting successful reproduction in this nocturnal species. Changing from blue to red lights resulted in increased mating behaviors but no pregnancy. CREW scientists offered to help by assessing fecal reproductive hormone metabolites of female, Medea. After a year of testing, CREW scientists were able to confirm that Medea’s reproductive cycle could be tracked via estradiol (E2) levels. However, progesterone (P4) was ambiguous with no elevations occurring to indicate ovulation after reported breeding activity. To address the question about the efficacy of P4 for monitoring reproduction in this species, CREW collaborated with Duke Lemur Center and Denver Zoo to collect fecal samples from females that did breed regularly and became pregnant. Data from these samples proved that P4 can be used to detect pregnancy in mid- to late-gestation, but does not exhibit the initial rise expected with ovulation. Through this project we were able to validate E2 and P4 assays for monitoring reproduction in aye-ayes. Knowing now that our female appears physiologically normal, the unusually brief mating encounters observed in our pair seem more likely responsible for reproductive failure.



Cincinnati Zoo’s female aye-aye, Medea.

## From Tropical Hawaii to the Frozen Garden



Ha’iwale (*Cyrtandra kaulantha*)

Scientists at CREW have been hard at work developing cryopreservation protocols for some of our new herbaceous residents in the Plant Research Division. In collaboration with the Lyon Arboretum in Honolulu, Hawaii, CREW researchers are working to protect endangered exceptional Hawaiian plants right here in Cincinnati. To date, CREW has received 14 new species of plants from Lyon, and has begun testing cryopreservation protocols on seven of those. While some species, like nānū (*Gardenia brighamii*), are not big fans of the cryopreservation process, we’ve seen survival after liquid nitrogen (LN) storage in six other species so far! CREW is using a newer cryopreservation technique called droplet vitrification to bank these species, which involves freezing tiny bits of tissue like shoot tips in a single drop of vitrification solution. So far, we have banked three species in the CryoBioBank®: alani (*Melicope mucronulata*), and two species of ha’iwale (*Cyrtandra gracilis* and *C. kaulantha*). Alani is found in one single population in the wild on Moloka’i, which consists of only three plants. Luckily for this extremely endangered species, we’ve seen survival of up to 70% after LN storage. These are just the first of many new tropical species that will be taking up residence in our Frozen Garden.