

Supplemental Information on the Deceased Male Ocelot and the Genetic Rescue Procedure

U.S Fish and Wildlife Service and Cincinnati Zoo & Botanical Garden

In the early hours of May 18, 2021, the dreaded phone call came. An ocelot had been hit by a car and killed just outside of the Laguna Atascosa National Wildlife Refuge (LANWR), one of the only two remaining locations with ocelot populations in the United States. Ocelots are medium-sized, wild cats that are federally and state listed endangered species. Only 60-80 individuals are estimated to remain in South Texas, where they face ongoing threats of habitat loss and fragmentation, vehicle strikes, and reduced genetic diversity. Road mortality by vehicle strike is the leading cause of known ocelot deaths and unfortunately, this ocelot met the same fate that has taken many wild ocelots prematurely out of the population over the years.

Upon hanging up the phone, Dr. Hilary Swarts, Wildlife Biologist with the U.S Fish and Wildlife Service, gathered the mortality kit, filled a cooler with ice, and headed out in the night to the site of the report. Using a PIT tag reader (think of scanning a pet's microchip), she confirmed that the individual killed by the vehicle strike was an adult male ocelot, approximately nine years old, known by his identification number as OM283.

OM283 was estimated to be about one year old when he was first marked with a PIT (Passive Integrated Transponder) tag at LANWR in March of 2013. He also was fitted with a tracking collar at that moment and then two more times during his lifetime, so he could be monitored consistently through tracking as well as by remote cameras distributed across the Refuge. While he successfully made a home on the Refuge for nearly eight years, he was first detected off-Refuge in June of 2020, likely chased away by an older male who did not want to compete with him for access to female ocelots. Though OM283 briefly made his way back onto the Refuge at least once after leaving, he appeared to be relegated to the unprotected land just outside the Refuge from mid-2020 until he was killed.

While OM283's death was a tragedy, there is a chance he may live on in spirit and biology thanks to the quick reporting, retrieval, and processing of his body. Put on ice and driven to the Gladys Porter Zoo in Brownsville Texas, OM283 provided a promising test for artificial insemination (AI) efforts using wild ocelot semen recovered opportunistically from freshly deceased males.

At the Gladys Porter Zoo, Senior Veterinarian, Dr. Thomas deMaar, performed an orchiectomy (testes removal), wrapped each testicle in saline soaked gauze, and bagged them individually for overnight shipping on freezer packs to the Cincinnati Zoo & Botanical Garden. Once there (arriving within 36 hours of the male's death), Dr. Bill Swanson, Director of Animal Conservation Research at the Center for Conservation and Research of Endangered Wildlife (CREW), was ready to implement the next stage of the process. Dr. Swanson processed the chilled tissues to rescue the sperm from the testicular storage/transport tubules (the epididymides and vasa deferentes), recovering a total of 440 million sperm with 60% of the cells showing progressive motility (necessary for fertility). The sample, containing ~250 million motile sperm, was mixed with a cryoprotectant solution, transferred into 20 semen straws, slowly cooled and then frozen over liquid nitrogen vapor for long-term preservation in CREW's storage tanks.

Each frozen straw contains enough sperm to potentially conduct one AI procedure with a female ocelot.

With OM283's semen frozen for future use, plans are underway to assess its fertility by inseminating a zoo-housed female ocelot at the ABQ BioPark in Albuquerque, New Mexico later this month. For the AI, the ovarian cycle of the female ocelot will be synchronized to the timing of the AI, ensuring that the oocytes and frozen-thawed sperm have an optimal opportunity for fertilization. Dr. Swanson will conduct the AI with the assistance of Dr. Ashley Reeves, a veterinarian working with wild ocelots for her Ph.D. at the University of Tennessee, and the veterinary and keeper staff at the ABQ BioPark. Over the past 25 years, CREW scientists have developed and refined methods for semen cryopreservation and AI for the conservation management of ocelots and other endangered cat species within zoos. To date, nine pregnancies have been produced in ocelots by AI, with three of those resulting after insemination with frozen semen. Application of these AI techniques with ocelots maintained in zoos has played a key role in sustaining the genetic diversity of ocelots managed under human care by the Association of Zoos and Aquarium's Ocelot Species Survival Plan (SSP).

These efforts are one component of a broad-based conservation project involving collaborators from the U.S. Fish and Wildlife Service, Gladys Porter Zoo, Cincinnati Zoo & Botanical Garden, Caesar Kleberg Wildlife Research Institute at Texas A&M University-Kingsville, University of Tennessee and the East Foundation to develop new strategies to help the Texas ocelot to survive and thrive in the wild. This project includes the routine collection and freezing of semen of wild ocelots in the field in conjunction with biological sample collection and placement of tracking collars; sadly, this semen banking approach wasn't available the last time that OM283 received a new collar.

If the AI procedure is successful and kittens are born in October, OM283's genetics will live on and bring greater diversity to the ocelot population managed in North American zoos by the Ocelot SSP while improving our understanding of possible options for increasing genetic diversity within wild ocelot populations.

Although the genetic legacy of OM283 may be preserved thanks to these timely rescue efforts, his death still presents a great loss for the imperiled Texas ocelot population. It would be preferable to avoid the need for similar gamete rescue attempts in the future. If you are visiting or living in South Texas near ocelot habitat, please drive slowly and carefully to avoid hitting ocelots or any other wildlife. If you do see a road-killed ocelot, please take a photograph - only if you can do so safely - and call U.S. Fish and Wildlife Service Dispatch (956-784-7520) to report it with as much specific information about the location as possible. Your action could be crucial to keeping wild ocelots in South Texas for future generations.