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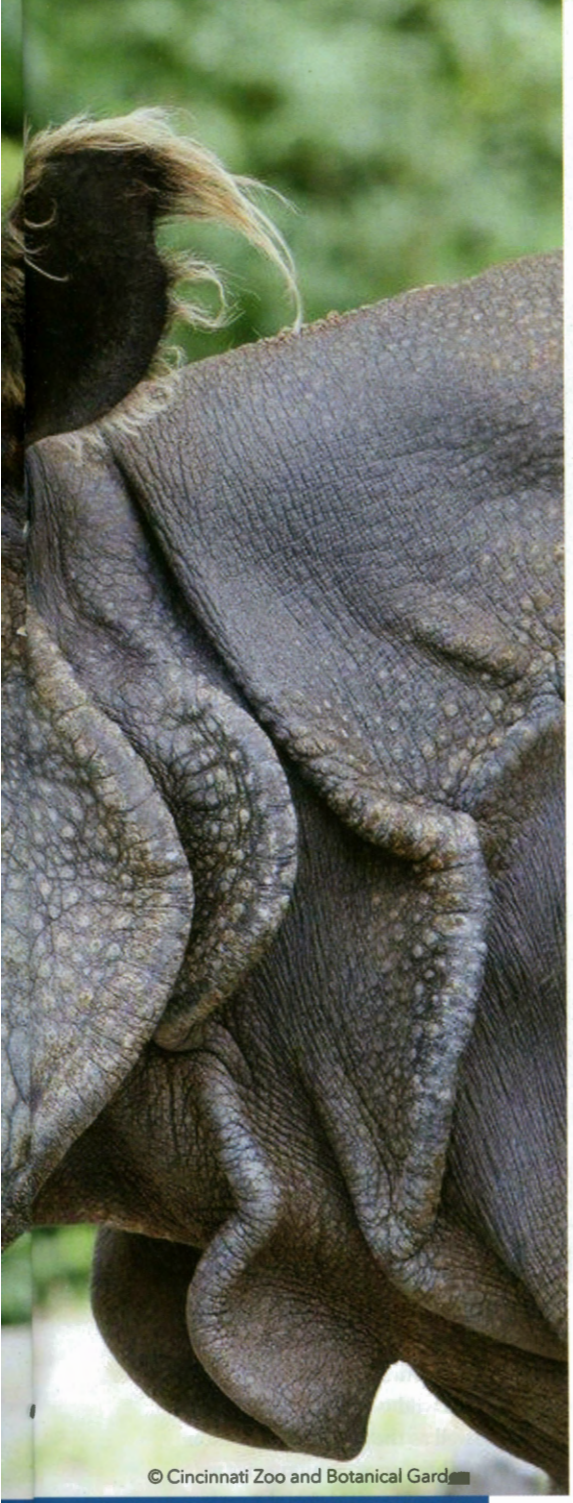
**Features**

Greater One-horned Rhino

**For decades, zoos and aquariums have been using artificial insemination to diversify gene pools and strengthen populations. Here's a look at some of the success stories.**

By Kate Silver




# BANKING ON THE FUTURE OF CONSERVATION

Dozens of ultra-cold freezers and liquid nitrogen storage tanks fill a warehouse-like building at the Smithsonian's National Zoo and Conservation Biology Institute (SCBI) in Front Royal, Va.

Within the freezers and tanks are thousands of biological samples and cells from more than 124 species—including sperm dating back to the 1980s. That's when scientists here started banking semen and honing the artificial insemination techniques and technologies used today in assisted reproduction. The semen, if collected and stored properly, has no expiration date. That's how a biological specimen collected from a male black-footed ferret, for example, was able to impregnate a female black-footed ferret decades after it was frozen, and many years after the originator died.

"That's a huge extension of their reproductive output," said Dr. Budhan Pukazhenthi, a reproductive physiologist at SCBI. "We're bringing those genes back into the population."

Pukazhenthi ticks off the pros of artificial insemination: it serves as an "insurance policy" for an animal, preserving its genes in case it's unable to reproduce on its own; it allows for more genetic diversity in a population; and it eliminates the need to move large animals from one place to another to reproduce naturally.

Artificial insemination isn't new in zoos and aquariums. But the process is continually being fine-tuned, and scientists are finding new successes in different species.

Pukazhenthi, for example, is a part of a team that began studying artificial insemination in the scimitar-horned oryx—a species that is extinct in the wild—in the 1990s, and published a report on the first successful birth in 2000. Over the years, he and his colleagues have refined the procedure and, in 2018, they performed it successfully for the first time without using anesthesia. In the future, they hope to see even more births, and will continue to tweak different aspects of their approach as they work to improve the success rate.

In zoos and aquariums across the country—and around the world—scientists are using artificial insemination as a tool to supplement natural breeding. With black-footed ferrets, they've used assisted reproduction to actually bring a species back from the brink of extinction; with greater one-horned rhinos, a female born from artificial insemination is about to welcome her first baby, conceived naturally; and with coral, scientists are hedging their bets and collecting sperm and specimens to preserve them for the future. Here are their stories.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## BLACK-FOOTED FERRETS

Black-footed ferrets are the only ferret species native to this continent, and at one time, they were thought to be extinct. That is, until 1981, when a small number of the small carnivores were found on private land in Meeteetse, Wyo. The U.S. Fish and Wildlife Service (USFW) and the Wyoming Game and Fish Department moved the ferrets into a managed breeding program with the goal of reintroducing them to the wild one day. Many partner organizations joined in, including SCBI, and the program has become one of the Zoo's most successful conservation projects.

"We've brought back males that have been dead for years, and it has reintroduced those genes into the contemporary population," said Dr. Adrienne Crosier, a biologist with SCBI. "From a genetic standpoint, that's a huge boost."

Since the program began in the late 1980s, SCBI has welcomed the birth of 1,146 ferrets, 142 of which have been by artificial insemination. Many of those ferrets are then moved to the National Black-Footed Ferret Conservation Center in Carr, Colo., which is managed by USFW, where they're prepared to survive in the wilderness before being released. There, Crosier works with a team to artificially inseminate select ferrets that haven't bred naturally. "They like to target males that maybe show too much aggression towards females when being paired," she said. "Or vice versa, if there's a male that just doesn't have enough confidence to breed with the females, and they want him to be genetically represented, we can collect sperm from him to use for artificial insemination."

The reintroduction of a near extinct species into the wild is a huge point of pride for SCBI, as well as the entire conservation community. "There's not many species we can say we've done that with," said Crosier. And, starting with just 18 ferrets, it's been possible thanks to assisted breeding.

## GREATER ONE-HORNED RHINOS

In the early 2000s, the greater one-horned rhino population was breeding in a "skewed" manner in zoos, recalls Dr. Monica Stoops.

"A few individuals were breeding regularly, but a lot of the population wasn't," said Stoops, who started working at Cincinnati Zoo and Botanical Garden in Cincinnati, Ohio, in 2002 as a

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postdoctoral scientist and then was hired on as a reproductive physiologist. To try and balance the breeding, a team began collecting and storing semen.

To date, they've welcomed more than ten calves born using artificial insemination. "We've come a long way," said Stoops, who now works at Omaha's Henry Doorly Zoo and Aquarium in Omaha, Neb., as director of reproductive sciences; she's also the reproductive advisor for the Rhino Taxon Advisory Group (TAG).

One rhino, in particular, is a point of pride: Monica. Monica was born at Buffalo Zoo in Buffalo, N.Y., in 2014. Her father, Jimmy, had a hip injury and wasn't able to breed naturally safely, so his semen was collected and frozen prior to his death in 2004. About ten years later, it was deemed a good match for a female rhino in Buffalo, and the birth was a success. Monica—whose keeper, in fact, named her after Stoops—who now lives at Tanganyika Wildlife Park in Wichita, Kansas, was the first greater one-horned rhino conceived by artificial insemination to survive past early infancy. Now, to Stoops' delight, Monica is pregnant by natural means and due this spring.

"This is what we want to demonstrate in terms of science: that these animals are normal, they can be rhinos and do their thing," said Stoops. "We're using artificial insemination as a tool, along with our natural breeding, to get the population and individuals represented. It's full-circle."

Today, the greater one-horned rhino population stands at 78 animals, including 35 males and 43 females at 19 AZA facilities. Stoops said her team's reproductive priority, right now, is to welcome in more female rhinos, because males have to be managed separately, and a limited number of zoos are equipped to do so. They're hoping to accomplish that through "sperm sorting," which is a gender-selection technique.

"We're trying to do better by the animals, themselves," said Stoops. "But also for the population in general."

## CORAL

It's no secret that coral reefs around the world are in danger. The United Nations Environment Programme (UNEP) estimates that up to half of the world's coral reefs have been destroyed, and 60 percent are threatened. Dr. Mary Hagedorn, who is a senior research scientist with SCBI/Hawaii Institute of Marine Biology, is working with a team to pioneer techniques to preserve and reproduce coral, collecting what she calls a "treasure trove of genetic information and live cells and live fragments," so that one day they can reseed some areas of the ocean. "I don't think in the wild coral are going to last much beyond the mid-2030s, so we don't have much time," she said.

Hagedorn began studying coral cryopreservation in 2004, applying knowledge gleaned from mammals to the invertebrate world. By their nature, corals present some unique challenges: some spawn for only two nights a year, and they often live in remote locations that can be impacted by hurricanes.

She and her team have devised techniques to collect and freeze sperm, and to date they have cryopreserved genetic material from 50 different coral species around the world. They've also been testing ways to cryopreserve coral larvae using a mesh technology, and they're also working on cryopreserving small fragments of coral. In her work, Hagedorn is dedicated to coming up with inexpensive and accessible techniques to encourage scientists all over the world to help in these conservation efforts. "We always want to democratize our science," she said.

To that end, SCBI offers an online training course to educate professionals on how to collect and cryopreserve coral sperm. And SCBI, along with AZA Florida Reef Tract Rescue Project, AZA SAFE coral, and other organizations are partners in the Coral Biobank Alliance, which seeks to collect and preserve coral for the future.

All of this work gives Hagedorn hope for coral. "I'm not going to say that we're going to completely revitalize every reef on earth," said Hagedorn. "But we will provide the seeds of hope for many of the reefs that are around the world in the foreseeable future."

Stories of success in reproductive assistance give hope to people like Dr. Erin Curry, who is a reproductive physiologist with The Lindner Center for Conservation and Research of Endangered Wildlife (CREW) at Cincinnati Zoo and Botanical Garden. Her team has been banking polar bear semen since 2010, and performed about 15 artificial insemination procedures.

They haven't yet had success. But they're not done trying. "It's still in its infancy," said Curry. "We still have a lot to learn."

Fortunately, there's time. The semen, after all, has no expiration date, so it can wait for the science to catch up.

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