TIME: IS IT RUNNING OUT

the clock is ticking for endangered and threatened species around the world.

Do we have the time to save the Indian rhino? Do we have the money to ensure the survival of the African hunting dog? Do we have the expertise to save the Spanish Imperial eagle? These are some of the questions — and challenges — that face reproductive scientists at some of the leading research institutions in the zoo industry today.

Many of the tools they bring to the race against extinction are as well-known as they are impressive: non-invasive hormone monitoring; artificial insemination; in-vitro fertilization; embryo transfer; genome resource banking; and cloning, among others. A big success with an endangered species using these high-tech reproductive methods can bring recognition and plaudits. If you would like to make national headlines for your institution, a successful giant panda birth using assisted reproductive technology is sure to garner the media's attention and the public's adoration.

However, is high-tech reproductive technology, and all the fanfare that accompanies those high-profile successes, really the panacea for endangered and threatened wildlife that the press often makes it out to be?



A MEASURED APPROACH

Dr. Terri Roth, Ph.D., Vice President of Animal Sciences and Director of the Lindner Center for Conservation and Research of Endangered Wildlife (CREW) at the Cincinnati Zoo and Botanical Garden, realizes the problems that face many endangered species are complex, often little understood, and don't necessarily render themselves easily to a technology-driven solution.

"The research goes beyond just reproduction, we are looking at the species and looking at what the issues are that the animal faces. What are the challenges to the survival of these species and what type of research is going to help with that effort?" asks Roth. "We find that it is not always assisted reproduction and it is not always embryo transfer - sometimes it is something else."

CREW is involved with a wide range of conservation projects around the world, but their most well known recent success has been the captive breeding of the highly endangered Sumatran rhino. For over a century, efforts to breed the Sumatran rhino in captivity had been an abject failure. Then in 2001, using hormone and ultrasound monitoring, CREW scientists were able to achieve success when the Zoo's "Emi" became the first captive Sumatran rhino to reproduce successfully in 112 years. On 30 July 2004, a second calf was born to Emi - the first time in history that one captive Sumatran rhino has given birth to two calves.

"All the research and science we put into that project was just to get the species to breed naturally," said Roth. "It was none of the really high-tech procedures like embryo transfer or in-vitro fertilization."

Roth believes the research with some of the greatest value involves learning the basic biology of different species that are candidates for reproductive intervention.

"The Sumatran rhino project relied on two things: monitoring hormone levels and using serial ultrasound exams so that we could determine the reproductive cycle of the rhino."

Prior to the project, it wasn't known that the Sumatran rhino is an induced ovulator - an animal that ovulates after mating. The study also helped CREW staff understand behavioral issues that had been observed over the years. Now they could pair the rhinos at the appropriate time and avoid the serious fighting that had taken place in the past.

"If there was no science involved, we could have gone years and

years breeding without knowing why she never had a calf."

COMING **TOGETHER TO MEET A GROWING CHALLENGE**

While Dr. Barbara Durrant, Ph.D., Division Head of Reproductive Physiology for the San Diego Zoo's Department of Conservation and Research for Endangered Species (CRES), sees the need to focus on maintaining viable wild populations, she also knows that reproductive technology has a role to play in the larger conservation picture. This will be especially true as time goes



Researchers at the Smithsonian's National Zoological Park believe in natural breeding. Their most recent success is with clouded leopard breeding in Thailand. By improving housing conditions, nutrition and management practices, they have produced ten cubs within a span of two years.



Head of the Animal Conservation Division and other scientists at **CREW** achieved a conservation milestone when they were the first to breed the endangered ocelot from embryo transfer. It was also the first endangered cat to be produced from the transfer of frozenthawed embryos.

on and populations of wild animals become increasingly fragmented and isolated, limiting the natural exchange of

genetic diversity that would otherwise occur.

"There is no substitute for natural breeding. I would hate for conservation organizations or zoos to rely too heavily on artificial reproduction and reproductive technology. Certainly reproductive technology is the key for maximizing genetic diversity and capturing that genetic diversity in all the species that are living now," says Durrant. "This has to be a coordinated effort with behaviorists, with habitat restoration and preservation scientists, and field biologists to make sure this is part of the bigger picture that includes captive breeders, husbandry people, and nutritionists, to ensure that we have healthy viable populations that are capable of reproduction."

Researchers at the Smithsonian's National Zoological Park in Washington, D.C., believe in natural breeding, and promote it by examining the impact of sub-optimal husbandry practices and developing ways to reduce stress in animals. The most recent example is their success with clouded leopard breeding in Thailand. By improving housing conditions, nutrition and management practices, they have produced ten cubs within a span of two years.

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"None of this would have been possible without the scientific understanding of the effects of stress, nutrition and husbandry on reproduction," said Budhan Pukazhenthi, B.V.Sc, Ph.D., Gamete Biologist at the National Zoo, "Technology is not always the answer for species conservation."

> The task might seem overwhelming at times, especially when the number of species that are endangered and in need of assistance are compared with the limited resources available. Durrant points out that there are many basic studies that take years to unravel in part because of the limited number of animals that scientist have to work with and study.



San Diego Zoo's Reproductive Physiologist Barbara Durrant, Ph.D., performs an artificial insemination procedure on giant panda, Bai Yun, with the help of Zoo researchers and veterinarians.

"When you have two or three animals — or if you are lucky, half a dozen — it is very difficult to make general statements and to design protocols that would be useful for the entire species."

Roth agrees that the science will take more time with a limited number of animals available to researchers working with endangered wildlife.

"When you don't have a large population to work with, science is always going to be slower. It takes us longer to say whether something is statistically significant one way or another. But one of the real positives about working within the AZA is that institutions do collaborate. We have had wonderful success working with institutions that have additional animals."

Other challenges include prioritizing species and projects that need to be worked on. At the National Zoo these choices are driven by a number of factors. The Zoo works closely with AZA Species Survival Plans (SSP), US Fish and Wildlife Service and other global conservation NGOs such as the IUCN - Conservation Breeding Specialist Group to identify species to focus on. Although the National Zoo has a long-standing interest in breeding endangered cats including cheetahs and clouded leopards, they have also been working for two decades to help save the black-footed ferret. Using artificial insemination, scientists at the National Zoo have produced over 135 black footed ferret kits to support their reintroduction effort, as well as increase the genetic representation of individuals within the SSP population. Furthermore, they have implemented a successful program to save several birds of prey species including the critically endangered Spanish Imperial eagle. In general, global issues and the Zoo's focus on certain species or regions of the world drive choices, but individual researchers can influence decisions.

"If I am drawn to a certain species, if I can make a fairly good case for why we should be studying that particular species the challenge falls on me to work with our institution to develop a strong science-based program, as well as raise money," says Pukazhenthi. "There are certain species, like the giant panda, that you are able to raise a lot more money for. On the other hand, if I am working with a toad from some obscure place — it could be the most endangered species — but it is going to be hard to raise the money."

A FOCUS ON REPRODUCTIVE TECHNOLOGY

As science is one of the missions at the Audubon Nature Institute in New Orleans, La., building the Audubon Center for Research of Endangered Species (ACRES), allowed the institution to focus one aspect of science with which they believed they could make headway — advancing the science of assisted reproductive technology.

Dr. Betsy Dresser, Ph.D., Senior Vice President/Research, Audubon Institute, and Director of ACRES, sees a need to focus on developing the reproductive technology if the industry is going to be able to save endangered and threatened species in years to come.

"If you have a \$20 or 30 million dollar budget you can build a big scientific institute like a university might, but because zoo budgets are quite low and research programs have to raise their own dollars we thought it would be best to focus on this one area of science — assisted reproductive technology and learning more about breeding animals in captivity naturally," says Dresser.

ACRES scientists are involved in basic research on the animals they work with and they employ all the current reproductive technologies.

"We collect sperm and eggs, we make embryos, we do in-vitro fertilization to make embryos, we transfer embryos, we freeze those embryos, we have begun to develop cloning technology, and we do artificial insemination. We have begun to apply all these technologies to exotic species after doing the basic research," says Dresser.

Dresser's focus is, however, on the assisted reproductive technology and how it might be applied in the future.

"There are different groups around the country focusing primarily on basic research — that being looking at hormone profiles, nutritional differences, and behavioral differences. We take those into consideration and we work with some of it, but our major focus is to develop the technology to assist reproduction."

Dresser believes that the industry needs to develop the technology while there are still animals available, so as to provide a safety net for a wide range of species in years to come. She sees a future where man-made pressure on wild habitats and wild animals will continue to grow; a future where reproductive technology may provide a buffer against extinction for many species.

"We don't know what the technologies of the future are going to allow us to do in 50 or 100 years, but I think the work we are doing now is going to allow us to address helping both wild populations of animals and those populations in captivity."

THE DEBATE OVER CLONING

One high-profile reproductive technology that inspires industry debate is cloning. Is it a hedge against extinction or a technology that, given its depiction in the mass media, can lead to a false sense of security when the public looks at the issue of conserving endangered species and the habitats in which they thrive?

"Cloning got a bad rap when it first came out. Now it's beginning to be looked at for other reasons," says Dresser. "I think that it will be viewed just like in-vitro fertilization was. At first people were horrified thinking about test-tube babies. Today, it is so common that millions of babies have been born using the technology ... Someday, not in humans, but in animals, it will be just another tool."

Audubon is the first institute to successfully clone both male and female African wildcats. When the unrelated male and female kittens reach maturity they will be bred naturally to show that clones can contribute to the genetic diversity of endangered species. To date Audubon has produced eight cloned African wildcats.

Roth sees that cloning may have it's applications in the future in terms of conserving some highly endangered species, but wonders about the public's perception of how the technology is presented in the media.

"There are certainly some specific cases where you have a very small population of a particular species, a very important founder is dead, and all you have is a cell line — that might be a case where you have to turn to cloning to get some genetic diversity into the population," says Roth.

"The way it gets promoted in the popular press is that people can sit back and say that we don't have to save this habitat or these wild animals because we can always bring them back by cloning. That is such a very scary message to be sending.

"Yes, we should be working on developing the tools — we try to have as many tools as possible — but where do we put the emphasis? And we have to be careful of what message we are sending out."

THE FUTURE

There is little doubt that reproductive technology has a role to play in the conservation of endangered and threatened animals, both in the wild and in captivity. There is also little doubt that AZA institutions are playing a leading role in the basic research about a wide range of species that are in need of reproductive intervention and development of the technology that will be used to help them now and in the future. A healthy debate is also taking place on which areas of science institutions should be focusing in order to make the most effective use of limited resources in terms of conserving both wild populations of animals and better managing captive populations.

"I think, as the wild gets smaller and smaller, we are going to depend on captive populations as being supplementary to wild populations. More and more facilities are going to work closer and closer together. And I think that is how it should be," says Dresser.

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The clock is ticking.



ACRES' FIRST FEMALE WILDCAT CLONES © AUDUBON NATURE INSTITUTE

Tim Lewthwaite is Publications Manager at AZA.

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