Racing Against Time: Reproduction In Aging Animals

By Barbara Durrant, Ph.D.,
Reproductive Physiology Division

Researchers are working on reproductive solutions to help species such as the critically endangered northern white rhino *Ceratotherium simum cottoni*.

Animals almost always live longer in zoos than they do in the wild. Balanced nutrition, lack of predators, and excellent medical care all bode well for an animal's longevity, as evidenced by the increased proportion of older animals living in zoos. From a conservation perspective, the downside is that animals lose the potential to reproduce as they age. This is especially true in females, which generally stop producing viable eggs or lose the ability to sustain pregnancy before males have stopped producing sperm. With fewer animals capable of breeding, zoo populations can also experience a decrease in genetic diversity.

The Science of Sex

Maintaining genetic diversity is particularly vital in the management of endangered species. Every effort is made to preserve as many unique genes in the gene pool as possible. Genetic specialists have designed computer programs to determine the best mate for animals in zoos, based on the animal's relatedness to others. If a particular genetic line of a species is under-represented in the population, researchers may use artificial insemination or other reproductive technologies to increase the number of offspring from these animals. Females of a prolific and over-represented family may be removed from breeding groups or put on birth control until their genes are needed again.

Waning Fertility

The effects of aging on reproduction are well known. This natural decline in fertility can be the result of changes in reproductive organs over time. Also intriguing is the fact that females who have never reproduced (called "nulliparous") have greater difficulty achieving pregnancy than mothers ("parous" females) of the same age. The reason? Researchers report changes in the ovaries of nulliparous females that are not found in parous females.

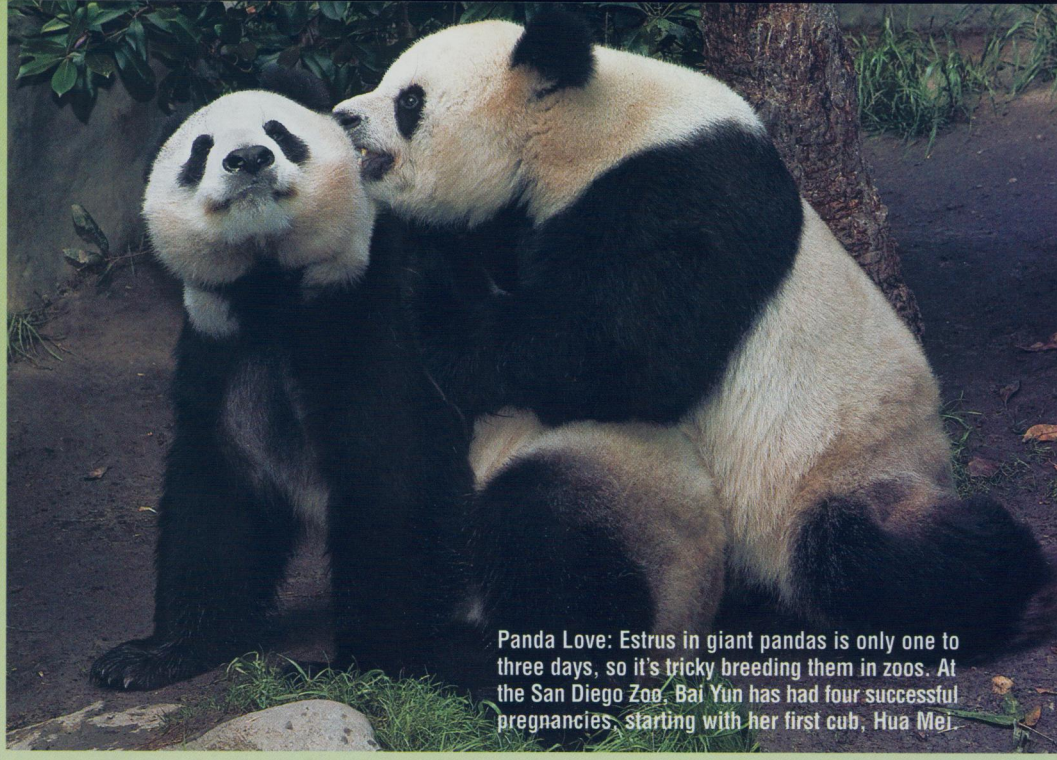
Evidently, pregnancy keeps the ovary in good working order for future pregnancies. The exact mechanisms are not clear, but most experienced animal breeders know the consequences of delayed reproduction. For example, "maiden mare syndrome" describes a common condition of nulliparous horses that experience dwindling fertility as they age.

One example of the effects of deferred motherhood is the infertility of three giant pandas at the Chapultepec Park Zoo in Mexico City. During their younger years, Xui Hua, Shuan Shuan, and Xin Xin did not have access to a breeding male. As the only living offspring of long-deceased pandas from China, their genes would be a boon to the global giant panda population. However, artificial insemination over the years has been fruitless. A multinational effort to impregnate these giant pandas was launched in 1999, when the females ranged in age from 9 to 14 years. But by then, it was too late.

In contrast, Bai Yun had her first offspring, Hua Mei, when she was 8 years old. She has successfully reproduced in the every-other-year pattern since then, experiencing no difficulty achieving and maintaining a pregnancy, even at the ripe old age of 16.

Model Dog

In our work, we do not want to risk using gametes from endangered species when a related, non-endangered animal can represent them. For carnivores, domestic dogs have proven to be a good reproductive model. We use dog ovaries and testes that are provided by local veterinary clinics following routine spaying and neutering procedures. With these tissues, we are able to develop the complex techniques of oocyte (egg) harvest, *in vitro* maturation, and *in vitro* fertilization. An interesting observation is that the ovaries of dogs older than four years often look distinctly different than the ovaries of younger dogs. We have found that significantly fewer good-quality oocytes can be recovered from the older ovaries.



Panda Love: Estrus in giant pandas is only one to three days, so it's tricky breeding them in zoos. At the San Diego Zoo, Bai Yun has had four successful pregnancies, starting with her first cub, Hua Mei.

Culture Club

Because the three giant pandas in Mexico are now in their golden years, it is highly unlikely they will reproduce without intensive assistance. Using techniques developed by our Reproductive Physiology team, we will work with researchers and veterinarians at the Chapultepec Zoo to obtain as many eggs as possible from each of these aging giant pandas. They will be given hormones to stimulate the growth and development of oocytes, similar to the treatment human women receive prior to *in vitro* fertilization.

The oocytes will be retrieved and fertilized *in vitro*, grown in culture for about five days, and then frozen in our Frozen Zoo®. When embryo transfer technology is perfected for bears, these precious embryos will be thawed and placed in the uterus of a giant panda. In this way, the irreplaceable genes of Xui Hua, Shuan Shuan, and Xin Xin may once again contribute to the genetic diversity of their species.

Rhino Romance on the Range

Coaxing reproduction in aging animals can require a variety of strategies. We experienced this with Nola and Nadi, our northern white rhinos at the Wild Animal Park. Both females were born in the wild in 1972, raised in a Czech Republic zoo, and brought to the Wild Animal Park in 1989. Neither had regular estrous cycles or had bred successfully, despite the presence of adult males. In 1994, at the age of 22, both females were treated with hormones to induce estrus and ovulation.

Both Nola and Nadi responded to treatment by coming into estrus for the first time in many years. Unfortunately, the resident male, Angalifu, did not attempt to mate with either of them. After several estrous cycles, the indifferent male was moved to another exhibit and replaced by an older male, Saut.

It was a momentous day in the East African exhibit at the Wild Animal Park when Saut and Nola bred in November 1995. All keeper activity and railway tours stopped as we watched this historic event, pleased at our efforts on the rhinos' behalf. With fewer than 20 northern white rhinos in the wild and only 10 in zoos, our two females represented a significant proportion of the genetic diversity of the species.

But our hopes for calves faded as Nola and Nadi came back into estrus month after month. Nola's repeated matings had not produced a pregnancy, and Nadi never bred at all, despite evidence of estrus and ovulation. The hormone treatments were repeated twice, with the same disappointing results. After Nadi died of old age in 2007, we attempted to rescue oocytes from her ovaries. We observed profound alterations in the form and structure of her reproductive tract, and we were not able to recover any oocytes.

There is a definite science to saving endangered animals. When possible, tissue samples are obtained from animals and then stored in the Frozen Zoo® (right) to be utilized later, when the species' gene pool can most benefit.

Resolving an Age-Old Conundrum

Our researchers and animal care staff continue to work on balancing the need for genetic diversity of species with the biological imperative to produce offspring before aging renders females infertile. The answer may lie in collecting eggs from young females and storing them in the Frozen Zoo®. That way, their genetic characteristics can be introduced into the gene pool later, when it is most advantageous—and they can make the biggest splash for the species.