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GUEST EDITORIAL

Birth of a Giant Panda Cub After Artificial Insemination with Frozen–Thawed Semen: A Powerful Reminder About the Key Role of Biopreservation and Biobanking for Wildlife Conservation

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The giant panda (*Ailuropoda melanoleuca*) is a unique species within the bear family that has evolved separately and became highly adapted to the bamboo forest in central China. Because of its charisma and dependence on a very fragile ecosystem, it has been considered as a symbol of biodiversity conservation efforts for >50 years. The wild population in central China now counts >1600 individuals. Fortunately, sustained protection efforts and restoration of natural habitats have recently resulted in the reclassification of the species as ''vulnerable'' instead of ''endangered'' by the International Union for the Conservation of Nature. In addition to the wild population, ~500 giant pandas live in ex situ facilities in China and zoos around the world. This ex situ population is an invaluable asset to study the biology of that species and to raise awareness about saving the giant panda as well as other wild species. Importantly, giant pandas held ex situ are managed as a metapopulation and represent a resource for ongoing reintroduction efforts. In conservation centers, breeding pairs of giant pandas are formed based on their genetic background. This ''matching game'' is essential to avoid inbreeding and maintain good genetic diversity in the ex situ population. This ensures the sustainability of the populations and production of healthy offspring that could potentially be reintroduced in their natural habitat.

The recent birth of a giant panda cub after artificial insemination with frozen–thawed semen at the Smithsonian's National Zoo and Conservation Biology Institute in Washington, DC, is another milestone in the conservation breeding of that species (https://nationalzoo.si.edu/news/giant-panda-cub-born-smithsonians-national-zoo-0). Giant pandas have been raised at the National Zoo since 1972 and have been part of long-term studies on husbandry, nutrition, behavior, genetics, veterinary medicine, and reproduction. For more than three decades of joint efforts with Chinese partners and other zoos, knowledge about the reproductive biology has considerably increased. Specifically, major advances have been made possible by noninvasively tracking metabolites of hormones and sex steroids in fecal or urine samples.[1,2] This approach then has been used to detect estrus periods, determine optimal timing for natural mating, and monitor pregnancies. However, conservation breeding of giant pandas is not trivial since females only have one ovulation per year in the spring. Gestation length then is variable because of embryonic diapause. Unfortunately, some giant panda pairs are not able to breed naturally because of behavioral incompatibility or ''lack of chemistry'' between individuals. The potential loss of valuable genes from these nonbreeding pairs is a major concern for the survival of the species.

Although many mysteries of panda reproduction remain to be unlocked, assisted reproductive techniques such as semen collection and artificial insemination have been developed early on to overcome breeding issues mentioned earlier. The first panda birth from artificial insemination with fresh semen occurred in 1978 in China. Since then, the technique has been successfully implemented in many conservation centers and zoos.[3] Importantly, semen cryopreservation and banking have been added to the conservation toolbox in tight association with the development of reproductive biotechnologies. The use of artificial insemination with frozen–thawed semen can facilitate genetic management through the distribution of genes among geographically dispersed panda populations, including those living in remote breeding centers and zoos. Availability of frozen semen also permits international transport of genetic material without compromising political and legal restrictions. Finally, effective sperm cryopreservation could theoretically allow for males that died long time ago to still be part of the genetic management plans.

Pioneering work at the National Zoo on semen collection and first attempts in cryopreservation showed that giant panda sperm is resilient to cold storage and cryopreservation.[4] However, successful births after artificial insemination with frozen–thawed semen are not common in giant pandas and have only been reported in China (https://phys.org/news/2009-07-china-panda-frozen-sperm.html). The semen that was used during the last artificial insemination at the National Zoo on March 22, 2020 was collected from our own male and frozen back in February 2015. Yearly semen collections have been performed to check the male fertility and also to produce insemination doses since the pair had

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difficulties to breed naturally. Semen from that same male also has been systematically cryopreserved since 2005 for later use at the National Zoo and in other centers breeding pandas.

The 1.5-mL sample collected by electroejaculation in February 2015 contained several billions of motile sperm, which is within the normal range for male pandas. Semen was then extended in TEST-egg yolk containing 5% of glycerol before freezing in liquid nitrogen following a standard protocol.[5] On March 22, 2020, several straws from that frozen batch were thawed to prepare the dose for transcervical artificial insemination (performed under anesthesia). About 800 million of motile sperm were deposited into the uterus of the female. After almost 5 months of pregnancy, one healthy cub was born on August 21, 2020. This is the first birth outside of China from artificial insemination with frozen–thawed semen only.

Beyond the joy of welcoming a new giant panda on our planet, what does it represent? This success reminds us of the value and key role of systematic biobanking in species conservation. Genome Resource Banks are invaluable conservation tools for understanding and sustaining reproduction as well as biodiversity at large.[6] For many years, our conservation community has been advocating for the integration of assisted reproduction and biobanking into the genetic management of rare and endangered.[7] So far, few species have really benefited from those integrative approaches, the giant panda being one of the few examples. Importantly, creating and maintaining biobanks for wildlife preservation requires solid programmatic and financial supports. Not all conservation centers and zoos over the world can afford it. Therefore, cooperation between institutions are key and creation of large biorepositories are necessary to offer long-term storage of valuable biomaterials to other partners. The Pan-Smithsonian Cryo-Initiative actually is a good example of a collaborative effort in conservation biology (https://www.si.edu/psci).

Finally, although we are celebrating the birth of new giant panda cub and recognizing the major impact of biobanking, we need to keep in mind the value of multidisciplinary efforts in conservation centers, zoos, as well as in natural habitats. From ecology to technology, everything counts to really make an impact on the protection and restoration of wild animal species.

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