USE OF ARTIFICIAL INSEMINATION TO ENHANCE PROPAGATION OF GIANT PANDAS AT THE WOLONG BREEDING CENTER

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The giant panda (*Ailuropoda melanoleuca*) is an international symbol for the need to conserve biodiversity and endangered species. The species status *in situ* within China is estimated at ~1,000 individuals. However, there currently are ~130 giant pandas living in *ex situ* facilities in China, some managed under the State Forestry Administration (SFA) at the Wolong Nature Reserve and others under the authority of the Ministry of Construction's (MoC) Chinese Association of Zoological Gardens (CAZG). The three major giant panda breeding centers within China include the: 1) China Research and Conservation Center for the Giant Panda in the Wolong Nature Reserve (Wolong Breeding Center); 2) Chengdu Research Base of Giant Panda Breeding; and 3) Beijing Zoo. There are more than 30 smaller populations of giant pandas located at zoos throughout China and in a few locations internationally (USA, seven animals; Japan, six animals; Germany, two animals; Mexico, four animals). This *ex situ* population living out of the wild in zoos and breeding centers plays a crucial role in educating the public about the status of free-ranging giant pandas. This population also serves as a source of animals for basic biological studies. Recently, to increase our understanding of giant panda biology, a 'Biomedical Survey of Giant Pandas in Captivity in China' was conducted in 1998 through 2000. Teams of scientists from USA and China worked hand-in-hand to assess health, reproduction, behavior, genetics and nutrition of 61 animals (Wildt et al., 1998, 2001; Ellis et al., 1999; Janssen et al., 2000; Howard et al., 2000, 2001; Huang et al., 2000; Spindler et al., 2000, 2001). In addition, giant pandas held *ex situ* are a potential resource for future reintroduction efforts and will become even more important to the survival of the species if habitat destruction and fragmentation continues to adversely impact the wild population.

Ex Situ Breeding Program

The goal of the giant panda *ex situ* breeding program is to develop a self-sustaining, genetically diverse population. Historically, reproduction of giant pandas has been difficult due to behavioral incompatibility and aggression. Although breeding success has increased recently, only 28.6% of all male giant pandas in captivity have ever sired offspring (Lindburg et al., 1998). In the current *ex situ* population, less than 10 males living in zoos and breeding centers have mated naturally and sired young. Furthermore, the majority of these breeding males are wild-born. Because a high number of captive-born males fail to breed naturally, the potential loss of valuable genes from these non-breeding males is a major concern. Consistently successful artificial insemination (AI) would allow incorporating these genetically valuable males with

behavioral or physical anomalies into the gene pool. Since there is ample genetic diversity in the contemporary *ex situ* population, AI would serve as a critical genetic management tool.

Giant pandas have been held *ex situ* in China since 1953 when a panda was taken from Guanxian County to the Chengdu Zoo (Hu et al., 1990). Two years later, the Beijing Zoo also added giant pandas to their collection. The first *ex situ* giant panda birth, however, did not occur until 1963 when female 'Li Li' gave birth to a male cub 'Ming Ming' at the Beijing Zoo (Kan et al., 1964). In the late 1970's, both the Beijing and the Chengdu Zoos began investigating assisted reproduction techniques in the giant panda. The use of AI for giant pandas first was successful at the Beijing Zoo in 1978 (Liu, 1979, 1981; Liu et al., 1979). Males did not breed naturally, so semen was collected by electroejaculation for the first time from three non-mating males. Following insemination of four females with fresh semen, one giant panda ('Juan Juan') became pregnant and produced twins in September 1978. This was 15 years after the first birth by natural mating at the Beijing Zoo in 1963. The first successful AI with frozen semen occurred in the female panda 'Mei Mei' in 1980 at the Chengdu Zoo (Hu and Wei, 1990; Ye et al., 1991; Zhang et al., 1991). The Wolong Breeding Center, Madrid Zoo, Ueno Zoo and the Zoological Society of San Diego also have used AI to produce giant panda cubs (Moore et al., 1984; Masui et al., 1989; Huang et al., 2001).

Many zoos and breeding facilities have made continuous progress in breeding giant pandas. Animals have reproduced successfully at Beijing, Kunming, Shanghai, Hangzhou, Chengdu, Chongqing, Fuzhou, Xian and the Wolong Breeding Center. From 1963 through 2001, a total of 156 litters has been born with 240 cubs, of which 111 survived to six months of age. To increase pregnancy success, it now is common practice to combine natural mating and AI using semen from non-breeding males. Multiple births are common within litters in giant pandas (~45% of all births are twins), however, the survival of giant panda twins did not occur until 1990. By alternating maternal and human care for each infant, the Chengdu Zoo became the first institution in 1990 to successfully raise twin giant pandas. In 1992, Beijing Zoo succeeded in hand-rearing a neonate from birth that had never had the opportunity to nurse. In 1999, the Wolong Breeding Center succeeded in feeding and raising two cubs in a litter of triplets. Through practical efforts and increased scientific knowledge, propagation of giant pandas in breeding programs continues to improve.

Propagation at Wolong Breeding Center

The China Research and Conservation Center for the Giant Panda in the Wolong Nature Reserve (Wolong Breeding Center) was established in 1982, and currently is the largest such breeding facility in the world. Wolong also has the highest number of breeding males (two wild-born and two captive-born) in the *ex situ* population. Propagation continues to improve and illustrate the important role of *ex situ* breeding programs. To enhance pregnancy success, Wolong Breeding Center uses the combined practice of natural mating and AI using semen from non-breeding males. Although the number of offspring increases, some individuals still do not breed naturally. The efficient use of AI would facilitate genetic management of the population. Therefore, the goal of a

recent analysis of breeding records was to: 1) assess reproductive success in giant pandas at the Wolong Breeding Center using both natural mating and AI; and 2) determine the efficiency of AI only without natural breeding in giant pandas.

From 1998 through 2001, combined natural mating and AI were conducted on 21 females at the Wolong Breeding Center. During the same time period, seven females were anesthetized for transcervical AI without mating. For AI, semen was collected by electroejaculation from non-breeding males and assessed for ejaculate volume, pH, sperm concentration, percent sperm motility, percent live sperm, forward progression (0-5; 5 = best), percent morphologically normal sperm and percent normal intact acrosome (Howard, 1993; Howard et al., 1986, 1999, 2001; Huang et al., 2000a,b). Ejaculates were diluted in egg yolk diluent containing 0 or 4% glycerol and used for AI either fresh or after either cold-storage at 4°C (up to 48 h) or cryopreservation using the pellet freezing method (Howard et al., 1986; Huang et al., 2000a,b). Female giant pandas were monitored for natural estrus and time of AI based on behavioral signs (scent marking, vocalization, lordosis, tail-up, backward walking), vaginal cytology (increased cornified superficial cells) and urinary hormones (increased estrogen) assessed by enzyme immunoassay (Zeng et al., 1984; Huang et al., 2001; Durrant et al., 2002). The seven females (4.5-10.5 y of age) selected for transcervical AI only on 2 consecutive days were chosen due to young age (4.5 y), weak behavioral signs of estrus or behavioral incompatibility (aggression). During the same interval, the combined practice of natural mating and AI over 3-4 consecutive days was used in 21 females (6.5-16.5 y of age).

Excellent semen quality with high ratings of sperm motility, morphology and intact acrosomes was observed for all males used for AI. Mean (± SEM) ejaculate traits in the six male sperm donors used for the 'AI only' females were: ejaculate volume, 3.3 ± 0.5 ml; sperm concentration, 1,429.8 ± 235.4 x 10^6/ml; sperm motility, 81.7 ± 2.1%; progression (0-5, 5=best), 3.1 ± 0.1; and normal sperm, 79.3 ± 9.2%. For transcervical AI (n = 14) in these seven females, mean inseminate traits were: spermic volume inseminated, 2.4 ± 0.3 ml; sperm motility, 73.5 ± 2.9%; progression, 2.5 ± 0.1; and total motile sperm inseminated/AI, 684.2 ± 118.2 x 10^6. Results revealed that transcervical AI using semen from non-breeding males was effective for producing pregnancies and live offspring in the giant panda. Cold-storage of semen at 4°C maintained high sperm percent motility, percent live and forward progression for at least 48 h. Four of seven (57.1%) giant pandas inseminated with fresh, cold-stored and/or frozen semen became pregnant and produced five cubs following AI without natural mating. Mean gestation and litter size was 131.5 ± 9.7 days and 1.3 ± 0.3 cubs/litter, respectively. AI using cold-stored semen from a non-breeding male with compromised health (skin tumor, squamous cell carcinoma) was effective for producing a pregnancy. Following combined natural mating and AI, 14 of 21 (66.7%) females became pregnant and produced 24 cubs. These results indicate that AI without natural mating is effective for propagating giant pandas with similar pregnancy success as combined natural mating and AI. Using all breeding strategies in the 2000 breeding season, the highest number of cubs born (n = 12) and surviving (n = 11) in 1 year at a single facility occurred at this center. Data also revealed that cold storage of semen at 4°C is a viable method for maintaining high quality semen for AI over consecutive days.

Overall, these results demonstrate that AI is a feasible strategy for perpetuating valuable genes from non-breeding giant pandas with behavioral incompatibility problems or compromised health. Assisted reproduction can be utilized efficiently as a management tool to maintain genetic diversity and avoid inbreeding depression in the *ex situ* population.

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