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The birth of a giant panda: Tracking the biological factors that successfully contribute to conception through to postnatal development

David C. Kersey [a,*], Copper Aitken-Palmer [b], Sam Rivera [c], Erin L. Willis [d,1], Liu Yu Liang [e], Rebecca J. Snyder [c,f]

[a] College of Veterinary Medicine, Western University of Health Sciences, Pomona, California, USA
[b] Department of Conservation Medicine, Smithsonian Conservation Biology Institute, Front Royal, Virginia, USA
[c] Department of Animal Health, Zoo Atlanta, Atlanta, Georgia, USA
[d] Department of Conservation and Research, Memphis Zoological Society, Memphis, Tennessee, USA
[e] Chengdu Research Base of Giant Panda Breeding, Chengdu, People's Republic of China
[f] Department of Conservation and Science, Oklahoma City Zoo and Botanical Gardens, Oklahoma City, Oklahoma, USA

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ABSTRACT

Reproducing giant pandas (*Ailuropoda melanoleuca*) remains the most challenging aspect of managed care of this species. However, advancement in knowledge stemming from basic science research on the giant panda has facilitated a growth in the population. Here, we report the successful application of reproductive technologies, including noninvasive hormone monitoring, behavioral/morphometric observations, ultrasonographic evaluations, and acute phase protein assessment, in an individual female. By applying these approaches to one female, we report the practicality and usefulness of a multidisciplinary approach to reproductive care of the species. In addition, the utilization of various technologies across multiple physiological states also provided us an opportunity to record previously understudied events, such as maternal response to weaning and growth of a conceptus.

1. Introduction

Reproduction is one of the most challenging aspects of maintaining giant pandas (*Ailuropoda melanoleuca*) in zoos and breeding centers. Advances in knowledge of the species' reproductive physiology and behavior have permitted the development of assisted reproductive technologies that have improved reproductive success rates ex situ. More precisely, substantial progress has been made in utilizing hormones and behaviors to determine optimal time for breeding or artificial insemination (AI) [1–3], semen evaluation and handling for fresh insemination [4,5], tracking trends on gestational hormones [6], mid-term pregnancy diagnosis [7], and state-of-the-art ultrasonographic monitoring of fetal development [8,9]. Although this knowledge and these techniques are broadly applied in giant panda management, the utility of this collective information is yet to be found in a single animal. To this end, we applied an array of these technologies to aid in the breeding/AI, pregnancy monitoring, and dam endocrine activity during nursing and weaning.

Giant panda reproduction is restricted to a 4-month breeding season, during which a female will have only one estrus [10]. With such a confined period of fertility, the

* Corresponding author. Tel.: 909-706-3534; fax: 909-709-3756.
E-mail address: dkersey@westernu.edu (D.C. Kersey).
[1] Present address: Department of Physiological Sciences, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, Oklahoma, USA.

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importance of identifying ovulation cannot be understated. As a result, approaches to define the peak in fertility have emerged as instrumental in ensuring successful propagation of the species. Among the investigated approaches, the pairing of urinary steroids measures with behavior/morphology observations has produced the most consistent results in pinpointing the ~48-hour fertility window [1–3]. Mate pairing is preferred periovulation; however, in the absence of successful copulation, transcervical AI can be used to introduce sperm to the female reproductive tract [3]. The ensuing biphasic luteal phase is indistinguishable between pregnant and nonpregnant females from a progestagen trend perspective [6]; however, a new technology in ceruloplasmin evaluation holds promise for early pregnancy detection [7]. In addition, pregnancy status can be confirmed during late gestation via ultrasonography [8,9]. After parturition, females have a period of acyclicity likely tied to lactation, only to resume reproductive cyclicity after offspring have been weaned [11].

By using all the aforementioned approaches to managing giant panda reproduction in one female, we sought to report the most comprehensive description of a complete, successful reproductive cycle.

2. Materials and methods

2.1. Animals, facilities, and management

The events detailed in this report occurred in 2010, during which Zoo Atlanta maintained an adult breeding pair of giant pandas (female: studbook [SB] 452, year of birth [YOB] 1997; male: SB461, YOB 1997), and one subadult male giant panda (SB731; YOB 2008), an offspring of the breeding pair. The multiparous female experienced lactational anestrus the previous breeding season after giving birth on August 30, 2008 to a single cub, with the conclusion of managed weaning in February 2010. With an exception for breeding purposes (June 11, 12, 13, 2010), the adult male and female were physically separated but were housed in close enough proximity to allow constant aural contact. In addition, they exchanged enclosures at least once a day to provide daily olfactory contact. All animals were healthy through the described period, provided with *ad-libitum* water, and a diet primarily comprised fresh-cut bamboo (~90%) supplemented with carrots and a high-fiber biscuit, that included amino acids, minerals, and vitamins. All animals were housed indoors (30–90 m^2) at and had access to indoor exhibit rooms (63 m^2) or outdoor yards (225–325 m^2) during daylight.

2.2. Behavioral and morphometric observations

Selected animal behaviors and morphometrics were determined from previous studies [12–14]. Behavioral and morphometric measures were recorded daily opportunistically by animal care staff from 1 January through 13 June (periestrus), and 27 September through 3 November (prepartum). Periestrual behaviors included: urination, scent marking, depressed appetite, olfactory investigation, genital manipulation, tail up, lordosis, and vocalizations (chirp; bleat). Morphometric observations during periestrus focused on vulval changes that included redness, swelling, and dilation. Behaviors observed during periparturition included: vulval licking, water breaks, nest building, restlessness, urination, and depressed appetite. In addition to vulval morphologic changes during periparturition, development and growth of the mammae were recorded. All behavioral measures were recorded at the end of each day by the animal care staff using a rating scale ranging from −3 to 3. Morphometric measures were recorded once a day using the same −3 to 3 rating scale when the female was in a position, which allowed animal care staff to closely examine its vulva and mammae.

2.3. Urine collection and processing

Freshly voided urine was collected daily from the adult female throughout the reported period, with increased sampling (4–6 samples/d) during periestrus and periparturition. All urine were collected, stored, and quantified for creatinine (Cr) as previously described [10]. Urinary hormone concentrations (mg/mL) and ceruloplasmin values (change in absorbance/enzyme [u/mL enzyme]) were indexed by Cr value and expressed as hormone mass (ng/mg Cr) and ceruloplasmin concentration (u/ml enzyme × mg Cr) per unit of Cr, respectively.

2.4. Enzyme immunoassays

All urine were quantified for excreted estrogen, progestagen and glucocorticoid (GC) metabolites via group-specific estrogen glucuronide (E1G; R522–2; C. Munro, University of California, Davis, CA, USA; [15]), progesterone (P4; CL425; C. Munro; [16]), and cortisol (R4866; C. Munro; [17]) enzyme immunoassays, respectively. Methods of sample analysis for each assay were performed as previously described (E1G: [15]; progesterone: [10]; cortisol: [18]). Interassay coefficient of variation for the high and low controls were 16% (mean binding 21.1%) and 17% (mean binding 52.7%) for E1G ($n = 16$), 17% (mean binding 18.5%) and 14% (mean binding 60.4%) for P4 ($n = 17$), and 6% (mean binding 29.9%) and 3% (mean binding 66.6%) for cortisol ($n = 14$).

2.5. Ceruloplasmin

Ceruloplasmin was quantified from urine via oxidasic activity as per Sunderman and Nomoto [19] and modified for sample analysis by Willis et al. [7]. Interassay coefficient of variation was 13.8%.

2.6. Artificial insemination

After several breeding introductions were attempted, copulation was not achieved with the female rebuking the male, two AI procedures were conducted, with the second following the first by approximately 7 hours on Day 0 (June 13). For the first AI, the female giant panda was immobilized with ketamine and xylazine, with anesthetic plane maintained via isoflurane. The female was placed in dorsal recumbency, vulva was rinsed with sterile saline, and a vulvar drape was placed over the vulvar opening. A glass