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New technologies for the study of carnivore reproduction

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Abstract

Routine analysis of urinary metabolites of estrogen and progesterone provided substantial information about the estrous cycle of bears. However, these data alone were not adequate to determine the precise timing of ovulation needed to maximize AI success rates, or to distinguish between pregnancy and pseudopregnancy. Therefore, there is a critical need to develop technologies that will enhance understanding of the reproductive mechanisms of ursids. Using the domestic dog as a model, three techniques were investigated for potential application to the propagation of captive endangered bears. In a modification of standard staining of bitch vaginal cells, trichrome staining of giant panda cells revealed two consistent chromic shifts 9 and 2 days prior to the periovulatory decrease in urinary estrone sulfate, enhancing the ability to predict ovarian events preceding ovulation. To further define the relationship between the decrease in estrogen and ovulation, the utility of a rapid immunochromatographic LH assay was investigated for giant pandas using a commercial LH kit canine serum. Serum collected during estrus exhibited positive test results, indicating the cross-reactivity of giant panda LH with canine LH antibodies, and preliminary data supported further development of the LH kit for the detection of LH in bear urine. Due to the limitations of hormone analysis for distinguishing pregnancy from pseudopregnancy in canids and ursids, forward-looking infrared thermography was evaluated as a method to visualize proliferating placental tissue, fetuses, or both. While further investigation is needed to confirm the utility of thermal imaging for pregnancy diagnosis in the domestic bitch, pregnancy and pseudopregnancy were successfully detected in two giant pandas.

Keywords: Vaginal cytology; Infrared thermography; LH; Carnivore; Ursid

1. Introduction

At least six of the eight extant ursid species are in danger of extinction [1]. Perhaps the most charismatic of all bears is the giant panda (*Ailuropoda melanoleuca*), whose combined free-ranging and captive populations number less than 2000 individuals [2]. Pandas in the wild are confined to five mountainous regions in the provinces of Shaanzi, Gansu and Sichuan in China [3] and the extent to which genetic diversity has been affected by this restriction is unknown. Although natural breeding produces the majority of giant panda cubs in captivity, the number of breeding males is insufficient for the creation or maintenance of a diverse population. Inclusion of behaviorally incompetent but genetically valuable males in the effective breeding population is possible through artificial insemination (AI) with fresh or frozen semen, but the current AI success rate is only 25% worldwide [4]. Imprecise estimation of the time of ovulation is the most likely cause of AI failure, and improved methods of monitoring the estrous cycle to pinpoint the time of ovulation are critical for improving the success rate.

The close taxonomic relationship between canids and ursids [5–7] supports the use of the domestic dog as a model for the development of reproductive techniques in bears. Because exfoliated vaginal cells are routinely

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analyzed to monitor the estrous cycles of domestic carnivores (dogs and cats), it was reasonable to expect a similar association of vaginal cytology with estrous events in ursids. However, the monochrome cytology stain normally used in this simple, rapid technique provides only a profile of increasing superficial (anucleate) or keratinazed cells, which was not sufficiently detailed for the prediction of ovulation. The application of the trichrome Papanicoloau stain to domestic bitch and giant panda vaginal cells has added substantial detail to the cytology profile. In addition, refined vaginal cytology analysis has introduced an element of predictability to the timing of the precipitous decrease in estrogen associated with ovulation in the giant panda [8,9].

Despite these advances in the understanding of estrous events, temporal relationships between vaginal cytology or the decrease in estrogen with the LH surge in ursids have not been well-documented and the exact time of ovulation remains unknown. Frequent hormone analysis is required to characterize the transitory ovulatory LH peak in mammals. However, multiple serum collections per day during estrus are rarely feasible for exotic species, necessitating the development of a urinary LH assay. Because the structure of LH is highly conserved among mammalian species [10] perhaps a commercial canine serum LH assay kit may be employed to detect LH in bears. The addition of LH data to endocrine profiles, behavioral analysis and vaginal cytology will further define estrous cycle events in exotic carnivores and will prevent premature AI or pairing for natural mating.

To avoid late inseminations, frequent direct visualization of the ovary by ultrasound is necessary to determine the exact interval between the LH peak and ovulation. However, current ultrasound techniques are not sufficiently developed to assess follicle growth or ovulation and CL formation in bears.

Following insemination, it is not possible to distinguish pregnancy from pseudopregnancy in bears by endocrine profiling or behavioral analysis. Ultrasound has been used successfully to detect fetuses late in gestation in the giant panda [11] and the brown bear [12], but early pregnancy diagnosis in ursids is confounded by long, variable periods of delayed implantation during which time preimplantation embryos or placental membranes have not been visualized by ultrasound [13]. Infrared thermography, on the other hand, generates images created by measuring the temperature of the skin surface, which reflects underlying heat variations. Because proliferation of placental and fetal tissues involves angiogenesis, a heat-producing activity, a forward-looking infrared thermography camera was used to identify products of conception in giant pandas near the time of implantation.

The studies presented here are preliminary in nature due to the opportunistic nature of sample collection and the small number of animals of each species. However, the results were intended to illustrate the potential of vaginal cytology, rapid LH measurement and thermography to enhance understanding of the physiology of reproduction in endangered carnivore species.

2. Materials and methods

2.1. Animals

A primiparous giant panda (*A. melanoleuca*, SB#371, born in 1991) was housed individually at the San Diego Zoo (San Diego, CA, USA). Although the female experienced occasional olfactory and auditory exposure to an adult male, visual and direct contact was restricted to brief introductions during estrus for mating. A nulliparous giant panda (SB#452, born in 1997) was housed at Zoo Atlanta (Atlanta, GA, USA) with frequent, lengthy periods of cohabitation with an adult male throughout the year. Two nulliparous giant pandas (SB#291, born in 1985, and SB#360, born in 1990) were housed individually at the Chapultepec Park Zoo in Mexico City, Mexico, each within olfactory, visual and auditory proximity to two other adult females. All female pandas were maintained on diets comprised primarily (50–95%) of fresh bamboo and supplemented with folivore biscuits, raw vegetables and cooked rice (SB#291 and #360 only). Water was available ad libitum for each female on public exhibit during the day and at night indoors in private enclosures.

Four multiparous domestic bitches (three Labrador retrievers and one pointer) ranging in age from 3 to 7 years were swabbed for vaginal cytology. An additional six primiparous and four multiparous Labrador retrievers ranging in age from 2 to 6 years were imaged by infrared thermography. All Labrador retrievers were housed at Guiding Eyes for the Blind (Yorktown Heights, NY, USA). The pointer was kenneled in a private breeding facility (San Diego, CA, USA). All bitches were maintained on commercial dog food with water available ad libitum.

2.2. Vaginal cytology

Vaginal cells collected daily during proestrus and estrus from three giant pandas and four domestic bitches were stained with a modified Papanicolaou (PAP)