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OVULATION INDUCTION AND ARTIFICIAL INSEMINATION OF A CAPTIVE POLAR BEAR (*URSUS MARITIMUS*) USING FRESH SEMEN

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Abstract: In 2008, polar bears were listed as a species threatened with extinction by the U.S. Endangered Species Act. Unfortunately, reproductive success has been poor despite breeding recommendations for almost every reproductively viable bear by the Species Survival Plan®. Assisted reproductive technologies could complement breeding efforts by overcoming the challenges of behavioral incompatibilities and deficiencies, facilitating genetic management and increasing cub production. The goal of this study was to artificially inseminate a female polar bear after inducing ovarian activity and ovulation with exogenous hormones (equine chorionic gonadotropin and porcine luteinizing hormone). Fresh semen collected from an adult male via electroejaculation/urethral catheterization was used for the insemination. Fecal steroid monitoring indicated that the female ovulated following the exogenous hormone treatment. Progestin concentrations increased in late summer, at the time implantation was expected to occur; however, no cubs were produced. To the authors' knowledge, this is the first report of ovulation induction and artificial insemination in a polar bear.

Key words: Assisted reproductive technology (ART), endangered species, noninvasive monitoring, ovarian stimulation, pseudopregnancy, wildlife.

BRIEF COMMUNICATION

Although polar bears (*Ursus maritimus*) have bred in captivity for years, reproductive success is considered low to moderate overall.[4,5] From 2008 to 2013, only four females gave birth in U.S. zoos despite the recommendations of the Species Survival Plan® (SSP) that almost every reproductively viable individual be paired for breeding. Although most pairs mate, few cubs are born, and the cause(s) of reproductive failure has not yet been identified.[11] Additionally, there have been reports of behaviorally incompatible pairs, and males that attempt but fail to copulate. The development of assisted reproductive technology (ART), such as artificial insemination (AI), may alleviate some of the challenges faced by the captive breeding program. Furthermore, ART holds potential both to increase the number of cubs produced and to provide insight into the causes of reproductive failure.

Polar bears are seasonal breeders, with most mating activity occurring between February and April, and ovulation is thought to be induced by copulation. Embryos develop to the blastocyst stage and then enter diapause until autumn. Following implantation, embryonic growth resumes and lasts approximately 60 days, with parturition occurring in November and December. If conception fails following ovulation, females often experience a pseudopregnancy, evidenced by an increase in progesterone around the time implantation would normally occur. Because performing an AI during a natural estrus is logistically challenging, the development of an ovarian stimulation and ovulation induction protocol is essential to enable the scheduling of a timed procedure. The overall goal of this project was to perform an intrauterine insemination with freshly collected semen, following ovulation induction with exogenous hormones in a captive female polar bear. To the authors' knowledge, AI has never been conducted in a polar bear and, with the exception of the giant panda (*Ailuropoda melanoleuca*), reports are scarce describing any assisted reproductive procedures in bear species.

Both polar bears involved in this study were maintained at the Seneca Park Zoo (Rochester, New York 14621, USA) and were recommended for breeding by the polar bear SSP. The female (studbook no. 955; age 22; 272.0 kg) had produced three previous litters, the most recent at the age of 12. The female was implanted with the contraceptive deslorelin at 14 yr of age, and her fecal hormone concentrations were assessed during the 4 yr leading up to the current project as part of a

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larger polar bear reproductive monitoring study conducted by the Center for Conservation and Research of Endangered Wildlife (Cincinnati, Ohio 45220, USA).[11] Fecal steroid metabolite analyses indicated that she was exhibiting ovarian activity during breeding seasons. The male (studbook no. 967; age 22; 440.9 kg) had been housed with other females previously but had never sired cubs. At the time of the procedure, no high-quality semen samples had been cryopreserved from polar bears, so it was decided that fresh semen would be collected from the male and used for the insemination procedure. The male's fecal testosterone was monitored in 2010 and 2011, and he exhibited normal seasonal increases in testosterone concentrations.[2] The pair had been together for two breeding seasons, but, despite multiple mating attempts, copulation was never observed. They were separated 6 wk prior to the insemination and remained apart for 2 wk following the procedure to ensure mating activity did not occur, which might confound the interpretation of study findings.

Exogenous gonadotropins were used to prepare the female for a scheduled AI by administering them prior to the female's expected first estrus of the season, based on previous years' records. On 24 February 2012, equine chorionic gonadotropin (eCG; 2,000 IU; ProSpec, East Brunswick, New Jersey 08816, USA) was given to promote follicular growth. Ninety hours following eCG, porcine luteinizing hormone (pLH; 20,000 IU; Sioux Biochemical, Souix Center, Iowa 51250, USA) was administered to induce ovulation. Both hormones successfully were delivered i.m. via hand injection. Approximately 44 hr post-pLH injection, the AI procedure was performed. Anesthesia was induced in both bears using tiletamine-zolazepam (Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA) at 2.2 mg/kg plus medetomidine (ZooPharm, Laramie, Wyoming 82070, USA) at 0.06 mg/kg both delivered i.m. by handheld syringe. Anesthesia was maintained via isoflurane inhalation, and atipamezole (ZooPharm) was administered i.m. at 0.24 mg/kg for reversal of medetomidine.

For semen collection, a rectal probe (3.3 cm in diameter with three 7.5 cm × 0.5 cm electrodes) was used for electroejaculation. Stimulations ranged from 4 to 10 V (125 to 320 mA). A urinary catheter (8 French) was inserted approximately 45 cm into the urethra following the second series of stimulations, and a small but concentrated volume of semen was obtained. The spermatozoa exhibited 85% forward progressive motility and 89% normal morphology. Sperm diluted in egg yolk-based extender and stored at room temperature were still motile at 72 hr postcollection.

The female was placed in a supine position, and the genital area was cleansed with dilute chlorhexidine solution. A variety of vaginal specula was evaluated in an attempt to visualize the cervix; however, the cervix was deeper than anticipated based on measurements of other bear species. A flexible endoscope (Portoscope PVS9150; 150 cm long/outer diameter of 9.2 mm) with a light source and accessory port was intended to aid in passing a catheter through the cervix, but adequate insufflation of the vaginal vault could not be maintained to visualize the cervix. Instead, a clear plastic speculum (23 cm long/outer diameter of 2 cm) was used to provide a wider, rigid structure through which the endoscope could be passed. Tubing attached to a syringe served as the semen catheter and was advanced through the accessory port of the endoscope, approximately 3.8 cm beyond the end of the speculum, until it met resistance at the cervix. The catheter was manipulated through the cervical *os* and advanced another 11.5 cm into the uterus, where 17.6×10^6 progressively motile sperm were deposited.

Fecal steroid metabolite analysis was performed to characterize the ovarian response to hormones and to monitor pregnancy status. Fecal samples were collected three to seven times per week throughout the year and frozen at −20°C. Fecal samples were processed, and hormones extracted, as previously described.[11] Samples were analyzed in duplicate for pregnanediol-3-glucuronide (PdG) and testosterone concentrations using established enzyme immunoassay methods[6] validated for monitoring polar bear ovarian activity.[11] Baseline values were established using an iterative process, as described by Brown et al.,[1] and any values greater than two standard deviations from baseline were considered to be elevated. A total of 211 fecal samples were collected and analyzed (4.1 ± 0.23 per week). Fecal testosterone concentrations were elevated following the eCG injection, returned to baseline 1 wk later, and were low for the remainder of the year (Fig. 1). Fecal PdG concentrations were higher during the 30 days following the AI (620.63 ± 59.26 ng/g dry feces), when compared with the 30 days prior (348.82 ± 28.58), suggesting luteal function. The post-estrus-to-pre-estrus PdG ratios were higher when compared with the previous two breeding seasons (Fig. 2). A lack of additional ovarian activity or mating activity the remainder of the breeding season indicated ovulation had occurred following the pLH injection. During the first week in

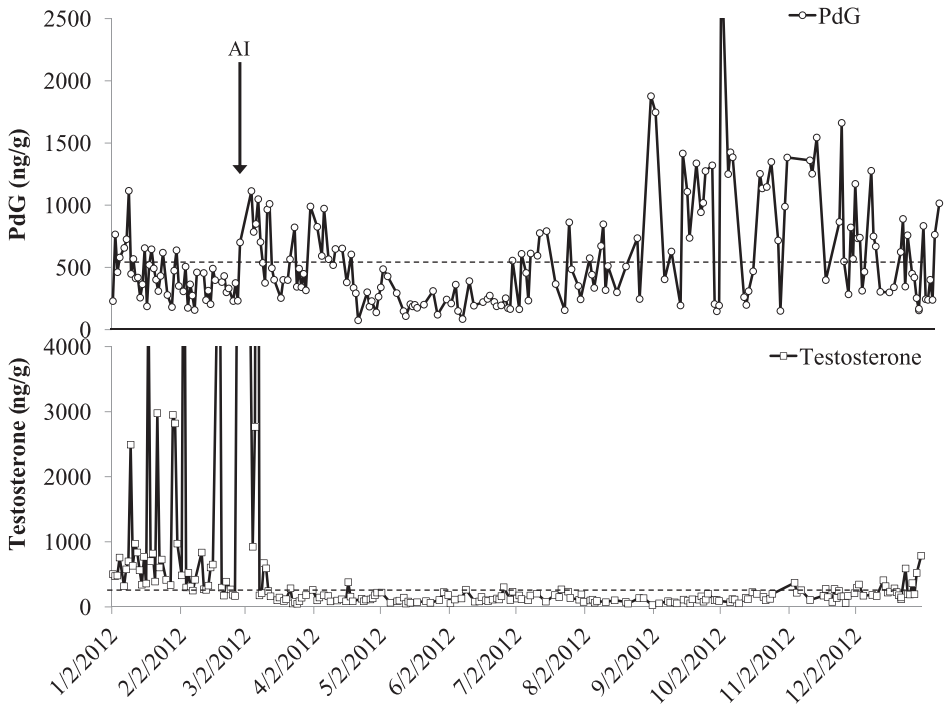

Figure 1. Fecal steroid metabolite monitoring of female polar bear studbook no. 955. Injections of equine chorionic gonadotropin and porcine luteinizing hormone were administered on 24 February and 28 February 2012, respectively. Arrow denotes day of artificial insemination. Dotted lines indicate baseline concentrations of pregnanediol-3-glucuronide (536.4 ng/g) and testosterone (202.9 ng/g).

July 2012, PdG concentrations increased accompanied by the observation of blood on the perineum, but the blood source was unclear. On 26 August 2012, PdG concentrations increased considerably (>1,000 ng/g) and remained elevated through 1 December. The female exhibited denning behaviors between 17 November and 7 December; however, no cubs were produced.

This procedure comprised the first known attempts at ovarian stimulation, ovulation induction, and AI in a polar bear. Although the female's endocrine profile revealed an increase in ovarian activity after the eCG was administered, the cycle lengths and follicular dynamics of polar bears have not been defined; therefore, it is not possible to discern whether the female responded to the exogenous hormones or if it was commencing natural estrus. Polar bears are both induced ovulators and seasonally polyestrous. Females that fail to become either pregnant or pseudopregnant generally mate multiple times into May or June, whereas females that ovulate discontinue cycling as evidenced by low fecal testosterone concentrations and cessation of mating activity.

In the present study, testosterone concentrations decreased after the pLH injection and remained low throughout the summer, indicating that ovulation had occurred. Lack of mating activity after the pair of bears was reintroduced provided further confirmation that the female had ovulated. Despite a prolonged increase in PdG initiated when embryo implantation normally occurs, the female did not give birth. The female had a history of aseasonal intermittent bleeding, but it is unknown whether the blood observed in July was vaginal in origin, suggestive of either embryo implantation or abortion, or from the perivulvar region. Reports of vulvar bleeding and blood spotting in captive female polar bears are prevalent. Although the source of the blood is often ambiguous, there have been numerous documented cases of severe ulcerative dermatitis of the genital and perineal areas, including previous cases identified in the female of this report.

In most species, including polar bears, serum progesterone (P4) increases following implantation and is higher in pregnant versus nonpregnant females.[7–9,13] However, serum P4 and fecal PdG concentrations of pseudopregnant females are

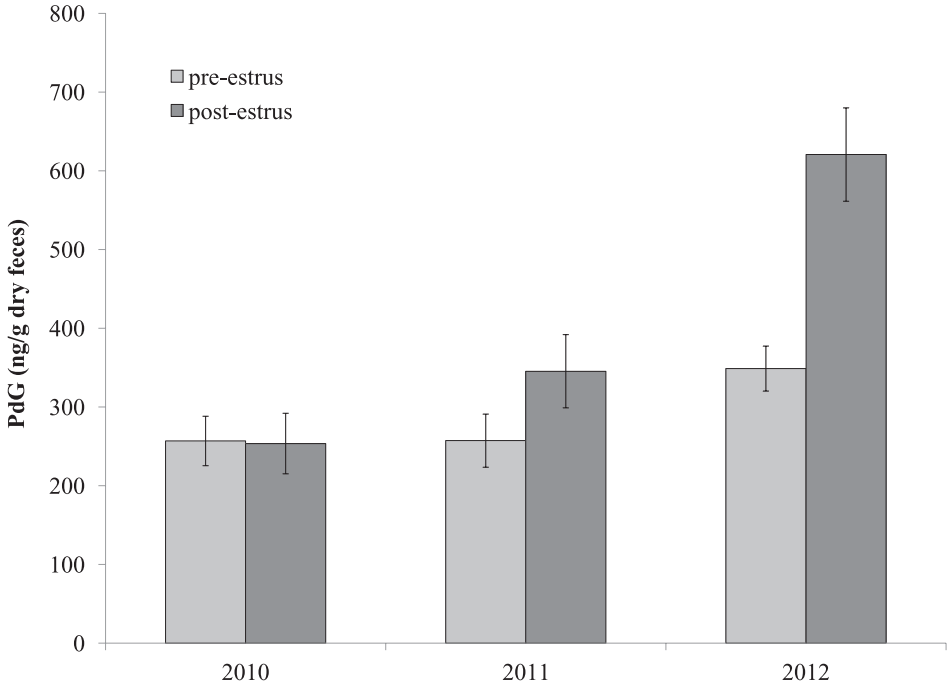

Figure 2. Fecal progesterone concentrations in samples collected 30 days pre-estrus and postestrus over 3 yr. The pre-estrus to postestrus pregnanediol-3-glucuronide ratios in 2010, 2011, and 2012 were 0.99, 1.34, and 1.78, respectively, indicating that the female exhibited luteal function following the administration of exogenous hormones and artificial insemination procedure.

similar to those of pregnant females.[11,13] This phenomenon is not unique to the polar bear, as pseudopregnancy and the inability to distinguish it from true pregnancy based on progesterone has been described in many bear species.[8–10,12] As a result, there currently is no definitive diagnostic pregnancy test for polar bears, although candidate fecal protein biomarkers have been identified.[3]

Although previous research has shown that quiescent testosterone profiles during the summer months are correlated with true pregnancy in polar bears,[11] at present, it cannot be verified whether the female conceived following the AI procedure and aborted the embryo(s) or if she experienced a pseudopregnancy. Clearly, a definitive method for noninvasively distinguishing pregnancy from pseudopregnancy would facilitate the development of ART in this species. Nevertheless, the female's endocrine response to the gonadotropin injections yielded encouraging results and demonstrated potential for controlling ovarian activity through the use of exogenous hormones in a polar bear.

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