

BIRTH OF A SIBERIAN TIGER CUB (*PANTHERA TIGRIS ALTAICA*) FOLLOWING LAPAROSCOPIC INTRAUTERINE ARTIFICIAL INSEMINATION

Ann M. Donoghue, Ph.D., Leslie A. Johnston, Ph.D., Douglas L. Armstrong, D.V.M.,
Lee G. Simmons, D.V.M., and David E. Wildt, Ph.D.

Abstract: A 7-yr-old female Siberian tiger (*Panthera tigris altaica*) was treated with equine chorionic gonadotropin followed 80 hr later by human chorionic gonadotropin (hCG) to stimulate follicular development and ovulation, respectively. Laparoscopy 46 hr after hCG revealed that the ovaries contained eight fresh corpora lutea and six preovulatory follicles. A 16-gauge catheter was inserted transabdominally into each uterine horn, and 16.8×10^6 motile spermatozoa (from a male designated by the Siberian Tiger Species Survival Plan [SSP]) were inseminated. Pregnancy was confirmed 45 days after insemination by ultrasound, and a single healthy male cub was born at 111 days of gestation. These results demonstrate for the first time that assisted reproductive technology can be used to produce genetically valuable offspring from parents identified and recommended by an SSP program.

Key words: Tiger, *Panthera tigris*, laparoscopy, artificial insemination, intrauterine.

INTRODUCTION

Assisted reproductive techniques, such as artificial insemination (AI) and in vitro fertilization (IVF)/embryo transfer, have the potential to greatly enhance captive breeding programs for wildlife species.²⁰ These strategies would be useful for circumventing cases of behavioral incompatibility and resolving certain fertility problems. When used in conjunction with spermatozoa and embryo cryopreservation, assisted reproduction would allow offspring to be produced from animals located at widely distant institutions. These approaches also would be valuable for infusing new genes from wild populations into zoo-based genetically stagnant populations. Although some assisted techniques have become routinely successful in humans^{4,12} and some domesticated and nondomestic species,^{1-3,6-9,13,19} few successes have been reported involving nondomestic felids. A litter of tiger (*Panthera*

tigris) cubs⁵ and an Indian desert cat (*Felis sylvestrus ornata*) kitten¹⁷ have been produced after IVF/embryo transfer, and AI has resulted in liveborn offspring in the puma (*Felis concolor*),¹⁵ leopard cat (*Felis bengalensis*),²¹ clouded leopard (*Neofelis nebulosa*) (Howard, unpubl.), and cheetah (*Acinonyx jubatus*).¹⁰ This report documents the birth of a Siberian tiger (*Panthera tigris altaica*) cub after laparoscopic intrauterine AI.

CASE REPORT

A prerequisite to these AI attempts was consultation with the Tiger Species Survival Plan (SSP) Coordinator and Propagation Committee, which operates under the auspices of the American Association of Zoological Parks and Aquariums (AAZPA). The SSP provided the recommendations for potential spermatozoa donor/spermatozoa recipient pairings. A 9-yr-old Siberian tiger (studbook no. 2456) served as the spermatozoa donor. The ejaculate used for insemination was collected using a standardized anesthesia and electroejaculation technique reported previously.^{5,22,23} After inducing anesthesia with ketamine hydrochloride (Vetalar®, Parke-Davis, Detroit,

From the Department of Animal Health, National Zoological Park, Smithsonian Institution, Washington, D.C. 20008, USA (Donoghue, Wildt); and the Henry Doorly Zoo, Omaha, Nebraska 68107, USA (Johnston, Armstrong, Simmons).

Michigan 48216, USA; 5.0 mg/kg i.m.), a rectal probe with three longitudinal electrodes and an AC 60-Hz sine-wave ejaculator were used to deliver 80 electrical stimuli of 3–7 V over 20 min. A 3-ml ejaculate was collected, and spermatozoa percent motility and status (forward progression) of the sperm cells based on a scale of 0 (no movement) to 5 (steady, rapid forward progression) were 90% and 4.0, respectively. Morphological analyses²² revealed that 19% of the spermatozoa from this ejaculate were pleiomorphic; the remaining cells were structurally normal. Immediately after collection, the ejaculate was transferred into a sterile 15-ml conical tube (Sarstedt, Princeton, New Jersey 08543, USA) and slowly diluted with an equal volume of Ham's F10 medium (Irvine Scientific, Santa Ana, California 92708, USA) containing 5% fetal calf serum (Irvine Scientific). Diluted semen was centrifuged (300 g, 8 min), the supernatant was discarded, and the spermatozoa pellet was resuspended gently into 500 μ l of fresh Ham's F10 medium. The spermatozoa sample was maintained at room temperature (23°C) and shielded from light until the time of AI.

A 7-yr-old female Siberian tiger (studbook no. 2645) received 1,000 IU (i.m.) equine chorionic gonadotropin (eCG, Equitech, Atlanta, Georgia 30342, USA) followed 80 hr later by 750 IU (i.m.) human chorionic gonadotropin (hCG, Sigma Chemical Co., St. Louis, Missouri 48216, USA) to stimulate follicular development and ovulation, respectively. Forty-six hours after hCG, the female was subjected to laparoscopic intrauterine AI. On this day, the female demonstrated overt signs of behavioral estrus, including vocalizing, rubbing, rolling, and lordosis.¹⁴ Anesthesia was induced with an i.m. injection of xylazine (Rompun®, Mobay Corp., Shawnee, Kansas 66205, USA; 0.5 mg/kg), diazepam (Valium®, Hoffman LaRoche, Nutley, New Jersey 08903, USA; 0.1 mg/kg), and ketamine HCl (5.0 mg/kg). The anesthesia level was maintained by supplemental i.v. injec-

tions of ketamine HCl (1.5 mg/kg). Laparoscopy was performed as described previously.¹⁶ A 180° laparoscope, 10 mm in diameter (Richard Wolf Medical Instruments, Rosemont, Illinois 61102, USA), was inserted through a 2-cm skin incision near the umbilicus and used to view the reproductive tract. Eight fresh corpora lutea (CL), 2–4 mm in diameter, and six preovulatory follicles, 2–6 mm in diameter, were observed on the ovaries. Seven other adult female tigers received the same eCG/hCG treatment; however, only three of these tigers had ovulated by 48 hr after hCG, and few CL were observed (1.3 ± 0.3 [$\bar{x} \pm \text{SEM}$] CL/ovulating female). All females were given gonadotropins without regard to a specific stage of the reproductive cycle. August, the month when this study was conducted, is considered to be the nonbreeding season for tigers, when natural ovarian activity is assumed to be minimal.¹⁸

The recipient female (no. 2645) was inseminated with spermatozoa using an intrauterine AI technique described recently for the domestic cat⁸ and cheetah.¹⁰ An accessory Palmer grasping forceps (Richard Wolf Medical Instruments) was inserted 3 cm lateral to the umbilicus and used to grasp and stabilize the uterine horn. The uterine horn was cannulated using a sterile indwelling catheter (Critikon, Tampa, Florida 33635, USA; 16-ga, 57 mm long) that was inserted percutaneously into the proximal third of the uterine lumen. The catheter stylette was removed and replaced with sterile polyethylene tubing (PE-50, Intramedic, Clay Adams, Parsippany, New Jersey 02653, USA) containing the spermatozoa suspension. The PE tubing was inserted beyond the tip of the catheter and into the uterine lumen, and the diluted spermatozoa were expelled into the lumen using ~0.4 ml of air delivered from a standard 1-ml plastic syringe. The entire procedure was repeated on the contralateral horn. A total of 16.8×10^6 motile spermatozoa was inseminated. The other female tigers also were subjected to intrauterine AI using the technique de-



Figure 1. Siberian tiger cub produced by intrauterine artificial insemination. (Photo by Jeff Bundy, Omaha World Herald.)

scribed above. Females were subjected to ultrasound 45 days after AI, and female no. 2645 was determined to be pregnant by observation of distinct heart beats from a single fetus. A healthy male cub was delivered naturally 111 days after AI (Fig. 1).

DISCUSSION

The AAZPA is developing comprehensive captive breeding programs that mandate interinstitutional cooperation to sustain genetically diverse and healthy wildlife populations.¹¹ Movement of animals among institutions is often necessary to maintain constant genetic flow. Assisted reproduction could be a particularly valuable alternative for disseminating new genetic material across time and space that would avoid the stresses of live animal transport. The birth reported here is important because it demonstrates for the first time that assisted reproduction can be used to produce ge-

netically valuable young from parents designated by a coordinated SSP program.

The ultimate utility of assisted reproductive technology will depend upon its usefulness in routinely generating offspring from genetically selected parents. Earlier AI attempts in 11 tigers using vaginal insemination resulted in no pregnancies.²³ The lack of success may have been related to improper site of spermatozoa deposition and/or timing of insemination with respect to ovulation. In addition, a preliminary study revealed decreased spermatozoa transport in the uterus of anesthetized tigers after vaginal deposition of spermatozoa, suggesting that anesthesia may reduce uterine motility and compromise the ability of spermatozoa to reach the site of fertilization.²³ Bypassing the cervical barrier by using the laparoscopic intrauterine insemination approach has improved fertilization and pregnancy rates in several species,^{1,2,9} including

some felids. Recently, liveborn offspring have resulted after intrauterine insemination in the domestic cat,⁸ puma,²¹ leopard cat,²¹ clouded leopard (Howard, unpubl.), and cheetah.¹⁰ Therefore, the technique appears to have considerable cross-species application.

The eCG/hCG treatment was effective for stimulating multiple ovulations in the tiger reported here. However, results varied widely in seven other females that received a similar hormonal regimen, indicating the need for more work in developing a consistent ovulation induction therapy for this species. Results from AI studies of the domestic cat reveal that the timing of anesthesia/insemination is critical.⁸ When laparoscopic AI is performed on preovulatory domestic cats under ketamine HCl, acepromazine, and gaseous halothane anesthesia, the pregnancy rate is low (~14%). However, ~50% of all cats become pregnant and deliver live offspring when insemination is performed 6–14 hr after ovulation has begun, suggesting that this anesthesia regimen and/or laparoscopy performed too early inhibits ovulation. Because only three of seven tigers ovulated in the current study, similar studies are in progress to increase ovulation success using exogenous gonadotropins in the tiger. However, the birth of a healthy cub using current technology is encouraging. With continued research, laparoscopic AI should become a useful tool in the more efficient management of this species.

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