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TECHNICAL REPORT

Use of Positive Reinforcement Conditioning to Monitor Pregnancy in an Unanesthetized Snow Leopard (*Uncia uncia*) via Transabdominal Ultrasound

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Closely monitoring snow leopard (*Uncia uncia*) fetal developments via transabdominal ultrasound, with minimal stress to the animal, was the goal of this project. The staff at Potter Park Zoo has used the principles of habituation, desensitization, and positive reinforcement to train a female snow leopard (*U. uncia*). Ultrasound examinations were preformed on an unanesthetized feline at 63 and 84 days. The animal remained calm and compliant throughout both procedures. Fetuses were observed and measured on both occasions. The absence of anesthesia eliminated components of psychologic and physiologic stress associated with sedation. This was the first recorded instance of transabdominal ultrasound being carried out on an unanesthetized snow leopard. It documents the feasibility of detecting pregnancy and monitoring fetal development via ultrasound. Zoo Biol 27:78–85, 2008. © 2007 Wiley-Liss, Inc.

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INTRODUCTION

The establishment of controlled breeding programs and improvements in veterinary and reproductive techniques have enhanced sustainable captive populations of many rare and endangered species. This includes the snow leopard (*Uncia uncia*), a native of some of the most harsh and desolate climates in Central Asia. In the wild, there are fewer than 2,500 individuals of breeding age and the number continues to decline [McCarthy and Chapron, 2003]. Although it is known for its reclusive nature in the wild, it is typically timorous and docile in captivity. This species has fared reasonably well through captive breeding programs such as the Species Survival Plan. The population of animals was 452 at 109 institutions in 2005.

Unfortunately, very little is known of the reproductive biology of this species. Snow leopards are seasonal breeders, females coming into estrus between January and mid-March. Gestation is approximately 93–110 days. A typical litter size ranges from one to five cubs. Behavioral indicators of pregnancy are minimal, including absence of estrus behaviors such as vocalizations, rubbing, and rolling [Freeman, 1983]. Typical physical indicators of pregnancy, such as nipple enlargement, are difficult to evaluate owing to their thick hair coat. Monitoring of ovarian function through use of fecal assays of estradiol-17B and progestin has been accomplished, but there is some evidence that large felids have relatively frequent pseudopregnancies [Graham et al., 1995]; hence, determining true pregnancies may be difficult. Fecal assays are not necessarily cost-effective for most facilities because of the high cost of laboratory fees. Information regarding ultrasonography has not been forthcoming. Therefore, additional information relating to ultrasonography and monitoring of pregnancy and fetal development is urgently needed to further contribute to maintaining the captive breeding population of snow leopards.

Pregnancy detection via real time ultrasound has been successful on unanesthetized giant pandas [Sutherland-Smith et al., 2004] and babirusa [Houston et al., 2001]. Although various training techniques have been documented for use in many other contexts such as in laboratory primates [Phillippi-Falkenstein and Clarke, 1992; Laule et al., 1996; Bloomsmith et al., 1998] and hoofed animals [Grandin et al., 1995; Phillips et al., 1998], use in a zoo setting is only now gaining increasing popularity. Through the principles of habituation, desensitization, and positive reinforcement, transabdominal ultrasound can be accomplished with minimal stress to the animal. This reduces the risk to both mother and cubs that is associated with anesthesia. Anesthetic agents can induce susceptibility to airway obstruction, hypoventilation, hypoxemia, and hypotension in both the dam and offspring [Gilroy and DeYoung, 1986]. This report documents the process of conditioning a female snow leopard to allow ultrasound examination and shows the feasibility of using this technique in this species.

MATERIALS AND METHODS

Subject Information

The subject of this report was a primiparous female snow leopard, 7 years old at the start of training. She was born and was housed at Potter Park Zoo her entire life. Before the start of training, she had no formal experience with operant conditioning. She was introduced to the male for breeding January 3, 2006 (day 0).

Mating was observed to occur immediately after introduction and for the following 3 days.

Facility Description

Training took place in several locations throughout the snow leopard exhibit and holding areas. The indoor exhibit is 5×10 m with a glass front for viewing. The keeper access door is 5×10 cm steel mesh. The outdoor exhibit is $17 \times 13 \times 5$ m and is enclosed entirely with 5×5 cm mesh. The holding consists of nine 2.5 m $\times$ 4 m enclosures placed adjacent to one another along a long corridor. The enclosures are each connected by steel guillotine doors. Enclosure fronts are again 5×10 cm steel mesh. The holding area adjoins the lion and tiger exhibits and is used regularly for these animals. The holding area also connects to a restraint device that was manufactured by Animal Care Products, Inc of Bryan, Texas. The restraint device is $2.2 \times 1.1 \times 0.86$ m. It has a guillotine door at each end. One side of the device can be moved by manual crank to immobilize an animal. The snow leopard exhibit area and the restraint device are at opposite ends of the holding corridor.

Conditioning Methods and Food Rewards

Traditional operant conditioning methods were applied to the training of this animal. A stimulus cue would be given to perform a certain behavior or to submit to a certain procedure, followed by a bridging stimulus (in this case a clicker) if the behavior was carried out accurately. In the earliest stages of conditioning a specific behavior, the animal was rewarded with food on a continuous reinforcement schedule, progressing to a variable schedule as the behaviors improved. The reward was delivered with 12-in long medical tweezers for the safety of the trainer. Conditioning began formally in late October 2005, with sessions occurring three to four times per week. There were several behaviors being trained simultaneously, each meant for husbandry and veterinary purposes. Detailed logs of each training session were maintained to track progress and expedite communication between trainers.

At the start of training this animal was uninterested in participating in sessions using her regular horsemeat diet (Dallas Crown 90–10 carnivore diet) as the reward. However, she was highly motivated to work for chicken; hence, this was used most often for training. As training progressed, she came to accept meatballs of Dallas Crown as a reward on occasion. All meat used for training is considered part of the daily diet of the animal.

Habituation to Holding and the Restraint Device

Before the initiation of training, the snow leopard was never given access to any holding enclosures except the two at the far end of the corridor. As the restraint device is at the opposite end of the corridor, the animal had to become comfortable moving down the row of enclosures and into the restraint device. To accomplish this, she was given access as often as possible to the entire holding area. This was not always feasible because of other animals being kept in the holding. Access was allowed for 3–4 hr twice a week. Portions of meat or enrichment items were placed in various enclosures to encourage exploration. This process continued for several weeks, even after the animal would enter the restraint device on command.

Desensitization and Operant Conditioning

The initial goal at the outset of training was to get the animal to walk through the holding enclosures and enter the restraint device. To accomplish this, the animal was asked to follow the trainer and enter less familiar areas, receiving a reward for doing so. Then she was led into the restraint device, being rewarded for going further in each time. After approximately 4 weeks, she would willingly come all the way down the holding and enter the restraint device when commanded.

At this point, we began introducing capture in the restraint device. The guillotine door would be lowered, the animal was rewarded, and then she was released. Increasing intervals of capture time were used to desensitize her until she became comfortable being in the device for longer periods with the door closed. The behavior was established reliably by mid-December 2005. Using this same method, we introduced and desensitized the snow leopard to having the mechanism cranked to compress the sides of the device together. This process began in mid-January 2006 and she was no longer reactive to the noise or compression by mid-February 2006. The animal has never been completely immobilized in the device, as this has not proved necessary.

We first incorporated touch desensitization into training sessions outside of the restraint device. A small wooden dowel with the end wrapped in duct tape was introduced at the end of November 2005. Previous experience with this animal indicated she was not likely to ingest this object. Various body parts were touched, then the animal was given the bridging stimulus and rewarded for allowing this. This included desensitization to having her abdomen touched. Once she became accustomed to this, we added a set of inexpensive battery operated clippers to the routine. The clippers were turned on and run during three training sessions at the end of December 2005, then taped to the dowel and used to touch the snow leopard. The cutting mechanism was dismantled so the blades would not cut or pull hair. Once the animal became acclimated to this, which occurred at the end of January 2006, we were able to work in the restraint device with a functioning set of clippers and shave her caudal ventral abdomen. We shaved a 10×10 cm area on the ventral abdomen, just lateral to midline bilaterally. The location was just cranial to her hind limbs while she was in ventral recumbency. Finally, we began to apply ultrasound gel to the shaved areas in small amounts. The animal had little reaction to the gel in any quantity applied.

Safety Protocols for Direct Contact in Restraint Device

The restraint device's mechanism was cranked to compress the sides to the point at which the animal cannot turn around easily, and she was required to maintain ventral recumbency, making it safe for a trainer to touch the animal without risk of injury. Before coming into physical contact with the cat, the secondary personnel had to receive permission to proceed from the main trainer (at the head). This ensured that the animal's attention was focused and decreased the risk of any accident occurring. A warning word for cessation of contact ("hands") was also used if the animal's attention began to turn toward the secondary personnel rather than the main trainer.

RESULTS

A brief ultrasound examination was done at 63 days postbreeding. Pregnancy was verified at this examination. An anechoic gestational sac containing an elongate echogenic fetus was seen in the left uterine horn. A heartbeat was evident. The placenta was also visible. The fetal head circumference was measured and was 1.94 cm and the fetal length was 3.78 cm. Movement was visualized. Owing to the cooperation of the subject, the exact number of fetuses could not be determined at that time. The information gathered during the procedure led the veterinarian to suspect the presence of a second fetus (Fig. 1).

A second ultrasound examination was carried out at 84 days of gestation. Two fetuses were visualized in the left uterine horn. A heartbeat was evident in both fetuses. The fetuses were located at a depth of 3–6 cm within the abdomen (Figs. 2 and 3). The complete fetal length could not be measured at the second ultrasound examination owing to the large length of the fetuses. Measurements of the femur, skull circumference, and heart were carried out. The skull measurement was 3.60×2.63 cm. The femur was 2.43 cm long. The heart was 1.87 cm in diameter, with the left ventricle being 0.5 cm in width. The fetal heart rate was measured at 194 beats per minute.

Parturition began on day 98. An ultrasound was attempted 20 hr after she had delivered one fetus and developed a bloody vaginal discharge. The ultrasound machine was nonfunctional; hence, an ultrasound could not be carried out, although the leopard would have tolerated it. Palpation of her abdomen through a method similar to the ultrasound, without anesthesia, confirmed that there was still a fetus present. In addition to the previous ultrasound imaging of two fetuses, it was determined that one fetus was still in utero. Viability could not be assessed. She was anesthetized using ketamine hydrochloride and medetomidine and maintained under isoflurane. A cesarean section was carried out and a live fetus was delivered.

Fig. 1. A fetal head at 63 days with a circumference of 1.94 cm.

Fig. 2. A fetal head at 84 days with dimensions 3.60×2.63 cm.

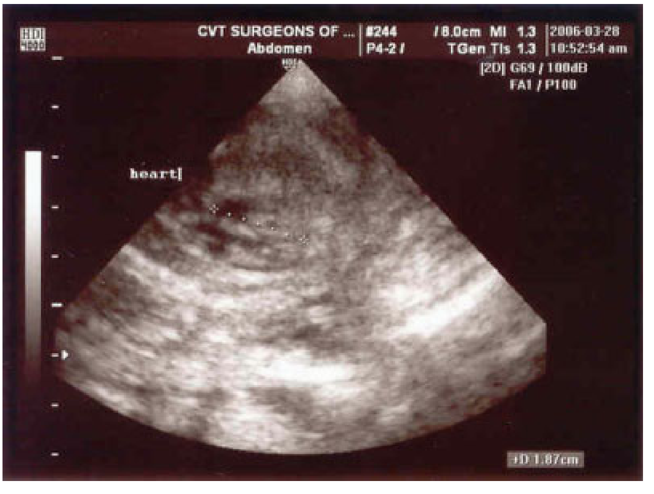

Fig. 3. A fetal heart at 84 days with a 1.87 cm diameter and a 0.5 cm left ventricle width.

DISCUSSION

Before the initiation of conditioning, there was an established, trusting relationship between the trainers and the snow leopard. This made it possible to assess the disposition of the animal as well as her willingness to cooperate during shaping of behaviors. During this time we found her to be docile in nature and relaxed in her environment, which is likely attributed to the animal having been born at this facility. Determination of an appropriately motivating reward was also vital to progress. The animal proved to be uninterested in the use of her regular diet as reinforcement, but chicken invoked a stronger response. Accurate record keeping and ongoing dialogue between trainers was instrumental in maintaining consistency. These factors permitted training to progress expediently.

The conditioning process proved to be successful and two ultrasounds and abdominal palpations were completed without the use of anesthesia. The animal remained calm and compliant throughout the procedures, which lasted approximately 15–20 min each for the ultrasounds and up to 5 min for the abdominal palpations. Anesthesia would have introduced a component of psychologic and physiologic stress that could have produced side effects including hypoventilation, hypoxemia, and hypotension [Gilroy and DeYoung, 1986] or possibly abortion or death of the dam.

The targeted, shaved area on the caudal ventral sides of her abdomen proved to be an acceptable size and location for performing ultrasound on an animal in ventral recumbency. This is significant for it can be difficult to train an animal to expose its abdomen because of an innate feeling of vulnerability in this position. In addition to preparing for ultrasounds, training allowed the veterinarian to palpate the animal's abdomen for mammary and fetal development.

Frequent training had additional benefits. Along with monitoring the physical health and condition of the animal during pregnancy, it provided an opportunity for the observation of behavioral indications of impending parturition. The day before parturition initiated, the animal appeared nervous and was less cooperative than usual. The trainers' knowledge of the animal's typical attitude during training sessions alerted them to this subtle change in her interest and mannerisms.

The most significant accomplishment of this effort was the determination of fetal number. During both ultrasounds, two fetuses were observed. During labor, vaginal bleeding started after an extended period of time had passed subsequent to the birth of the first cub. At that time, based on the knowledge that a second cub remained in utero, the veterinarian diagnosed dystocia and elected to anesthetize the animal and perform a caesarian section. Uterine dystocia is a significant cause of fetal loss in other carnivore species such as the domestic dog and cat [Johnston and Raksil, 1987]. The second cub was extracted 29 hr after the birth of the first and was revived. The source of the bleeding was determined to be placenta previa (partial detachment of the placenta from the uterine wall). Without foreknowledge of the fetal number, the complications during birth might not have been detected early enough to avoid further complications.

In retrospect, it would have been ideal to have applicable behaviors established before breeding. This would have allowed for additional ultrasounds earlier in pregnancy. Having limited access to an ultrasound machine impeded frequent measurements of fetal growth. Additional data would allow the estimation of fetal age based on fetal measurements, which could be useful determining the date of parturition in animals where the date of conception is unknown. Previous studies in hyenas and domestic cats have indicated that fetal growth curves can be created and used as an accurate estimation of gestational age [Place et al., 2002; Zambelli et al., 2004].

It would be of interest to future studies to collect additional physiologic data corresponding to pregnancy. Hormone levels could be monitored via blood, feces, and urine. Fluctuations in hormones could be used as markers to indicate changes in uterine and fetal development.

CONCLUSIONS

1. This was the first recorded instance of transabdominal ultrasound being carried out on an unanesthetized snow leopard. It documents the feasibility of detecting pregnancy and monitoring fetal development via ultrasound.
2. Snow leopards can be conditioned to perform a variety of behaviors, facilitating veterinary and husbandry procedures. Desensitization and operant conditioning allowed ultrasound and abdominal palpation without anesthesia, therefore reducing risk of stress and drug-induced injury to the dam and cubs. Although it may not be feasible in every situation because of time and staff constraints, in this instance it proved to be a valuable tool for the management and diagnosis of pregnancy and dystocia.

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