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## BRIEF COMMUNICATIONS

# Induction of Ovulation and Successful Artificial Insemination in a Persian Leopard (*Panthera pardus saxicolor*)

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Techniques of artificial insemination have not been readily applied to zoo animals. The nonsurgical approach has been unsuccessful in nondomestic felids until the present study where pregnancy was achieved in a Persian leopard. The demonstration of this technique should encourage renewed attempts to artificially inseminate nondomestic felids.

**Key words:** artificial insemination, *felidae*, leopards, ovulation

## INTRODUCTION

The techniques of artificial insemination, although commonly practiced in domestic animal reproduction, have not been readily applied to zoo animals. Previously reported artificial insemination efforts in nondomestic *Felidae* have utilized surgical techniques and only recently has a cub resulted [Moore et al, 1981]. Until the present project, the nonsurgical approach has been unsuccessful in the production of offspring from nondomestic *Felidae* [Dresser et al, 1981].

Small populations of the Persian leopard in zoos throughout the United States have been threatened by an increased inbreeding potential. Artificial breeding has not been achieved in these endangered felids and such knowledge is important particularly when problems in natural mating occur. Although behavioral estrus did occur, the male and female utilized in this case report were incompatible and following a 4-year exposure, no pregnancies resulted. Thus, the use of artificial insemination was warranted.

The objectives of this project were to achieve pregnancy in this Persian leopard through artificial induction of ovulation and insemination using nonsurgical techniques. It is hoped that similar information will help to develop artificial insemination techniques in wild felids and will enhance the reproductive capabilities of endangered species.

Received for publication March 7, 1982; accepted April 29, 1982.

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## METHODS

A male Persian leopard was imported from Europe in 1976 to the Cincinnati Zoo in order to introduce new bloodlines to the present population. Behavioral incompatibility prevented natural mating between this male and an unrelated female. The female was born at Cincinnati and had never produced offspring. Both animals were 7 years old when the artificial insemination was accomplished.

On the fourth day of her normal estrus in July, 2000 IU of human chorionic gonadotropin (HCG; Sigma Chemical Co.) was administered intramuscularly (im) by blowgun to the female to induce ovulation. Approximately 20 hours post-HCG the male and female were immobilized with appropriate dosages of 25 mg xylazine (Rompun; Haver-Lockhart) and 550 mg ketamine hydrochloride (Vetalar; Parke-Davis). The female was placed in dorsal recumbency with the hindquarters elevated to facilitate insemination. Semen was collected via electroejaculation into a tube surrounded by a 35°C water bath, evaluated for motility, concentration, and forward progression, then used immediately. A ram-size bipolar rectal probe with three ventral electrodes attached to a battery powered electroejaculator (Std. Precision Electronics Co.) administered 1-2 V and semen was collected after 45 seconds [Seager, 1976]. One milliliter of unextended semen was deposited within the uterine body. An 18-ga needle with bulbed tip attached to a 1.0-ml syringe was passed through the dilated cervix into the uterus. A disposable speculum (1 in diameter × 3 in length) allowed easy access to the cervix. An additional 1000 IU HCG was given im to the sedated female following the first insemination. The total procedure required 1.5 hours to complete. A second insemination was accomplished 40 hours post-HCG by repeating the exact procedure described above.

## RESULTS AND DISCUSSION

Several months prior to the estrus and procedure described in this report, an attempt had been made to induce ovulation and artificially inseminate this female. However, only one insemination was performed at 21 hours post-HCG and the female cycled 50 days later. By contrast, no further estrous cycles in this female were observed throughout gestation following the insemination procedures.

The male was immobilized for both artificial insemination attempts so that fresh semen could be used. Since there have been no previous pregnancies in nondomestic felids resulting from the use of frozen semen, it was decided to inseminate with fresh semen. Several fractions of semen were collected during each ejaculation attempt until a total volume of 2.0 ml was accumulated. Sperm concentration was  $1.5 \times 10^8$  per ml and exhibited excellent motility and forward progression. Semen in excess of that used for insemination was preserved for storage and future study.

Wildt et al [1980] studied the effects of gonadotropic hormones on ovarian activity in domestic and wild felids and through laparoscopy concluded that 95% of all domestic queens seem to be induced ovulators. The remaining 5% ovulate spontaneously and it appears genetically related. Of the wild felids studied, both induced and spontaneous ovulation was observed. Since it was important in the present project to determine when ovulation occurred in the Persian leopard so that artificial insemination could be performed, it was decided to induce ovulation with HCG. Seager and Demorest [1978] reported that ovulation in the domestic cat occurs approximately 24 hours postinduction. In some cheetah [Wildt et al, 1981] ovulation had also occurred

24 hours post HCG while in other studies on the lioness and jaguar [Sadleir, 1966; Rowlands and Sadleir, 1968; Wildt et al, 1979] ovulation had occurred by 3 or 4 days postinduction. In the puma, Moore et al [1981] achieved artificial insemination by performing the technique 20 and 40 hours post-HCG. Therefore, since the interval between coitus or hormonal treatment and ovulation in the Persian leopard was not known, two doses of HCG were administered and two inseminations were performed 20 and 40 hours later on the female in this project.

Following a 96-day gestation a stillborn male cub was delivered. The birth was breech and the ensuing dystocia was the cause of death of the cub. The female recycled approximately 65 days later, showed normal behavioral estrus and it is anticipated that the procedure will be repeated within the next several weeks.

This is the first report of successful artificial insemination in nondomestic *Felidae* using a nonsurgical procedure. It is hoped that the availability of such techniques will encourage others to renew attempts to breed *Felidae* species that heretofore have presented reproductive problems but, for various reasons, have not been artificially bred owing to the complexities of the procedure.

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