

Induction of Ovarian Activity in the Cheetah (*Acinonyx jubatus*)

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ABSTRACT

Serial injections (i.m.) of 10 mg follicle stimulating hormone (FSH-P) to cheetahs for 5 days followed by human chorionic gonadotropin (hCG, 500 IU) administered on both Days 6 and 7 resulted in ovarian follicle development and/or ovulation in six of seven attempts. One additional female given the same gonadotropin regimen when distinct corpora lutea (CL) were visible on the ovaries failed to respond with new follicular growth. No estrous behavior was observed following hormonal treatment.

INTRODUCTION

Increased interest in obtaining baseline ovarian-endocrine information on captive wild felids has stimulated recent reports on the jaguar (Wildt et al., 1979) and lion (Schmidt et al., 1979). Investigations involving hormonal induction of ovarian activity in wild felids are rare. In the one reported study, Rowlands and Sadleir (1968) successfully induced ovulation in two of three lionesses using a single injection of pregnant mares serum gonadotropin (PMSG) followed by an hCG injection 3 days later. A more comprehensive examination of the effects of exogenous gonadotropins has been conducted in the domestic cat (Wildt et al., 1978; Wildt and Seager, 1978). In these studies, follicle growth, estrous behavior and ovulation were stimulated with daily injections for 5 days of follicle stimulating hormone (FSH-P, 2 mg) followed by injections (500 IU) for 1 or 2 days of human chorionic gonadotropin (hCG).

A review by Seager and Demorest (1978) has summarized the baseline reproductive factors

known for the cheetah (*Acinonyx jubatus*), an endangered species. Field studies in the wild have indicated that the cheetah is primarily a solitary animal with male and female interaction generally occurring only during the breeding season. During the latter 3-4-month period, which varies considerably with geographic location, the female periodically shows sexual receptivity for about 10 to 14 days. Female behavior and posture during copulation are similar to that of the domestic cat, and following a gestation of 90 to 95 days three to four cubs are normally produced. In the present study a hormonal regimen originally developed and utilized in the domestic queen was modified and tested for its effectiveness in stimulating ovarian activity in the cheetah.

MATERIALS AND METHODS

Six adult female cheetahs ranging from 2 to 12 years of age and 30-38 kg BW were studied. Four of the animals were members of the breeding population of either the National Zoological Park, Washington, D.C., or the Gladys Porter Zoo, Brownsville, TX, and the remaining two females were privately owned. Although mature, none had previously displayed signs of behavioral estrus (Eaton, 1973, 1974), mated, or produced offspring. Prior to gonadotropin treatment, all animals were maintained in large outdoor pens of various sizes, fed a commercial zoo diet, and provided water ad libitum. During the treatment period, they were restricted to indoor runs.

The gonadotropins used were FSH-P (Burns-Biotec Labs; potency, 1 Armour unit/mg) and hCG (Pregnyl: Organon, Inc.). For injection the animals were restrained manually or in a squeeze cage. The first day

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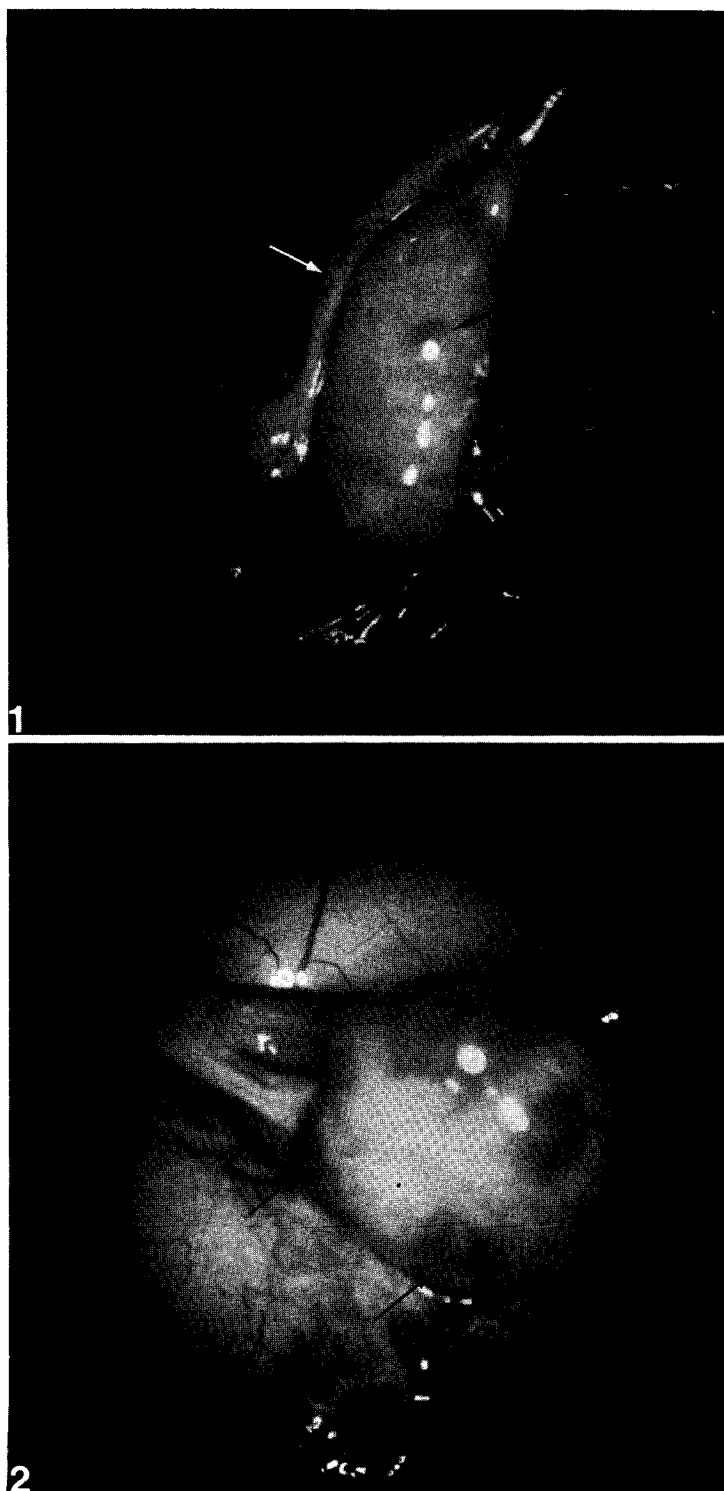


FIG. 1. Quiescent left ovary (prior to gonadotropin treatment) containing a small (~ 2 mm) indistinct follicle (black arrow). White arrow denotes fimbria lying adjacent to ovary.

FIG. 2. Right ovary containing four vesicular follicles (arrows) on the day following the fifth injection of FSH-P.

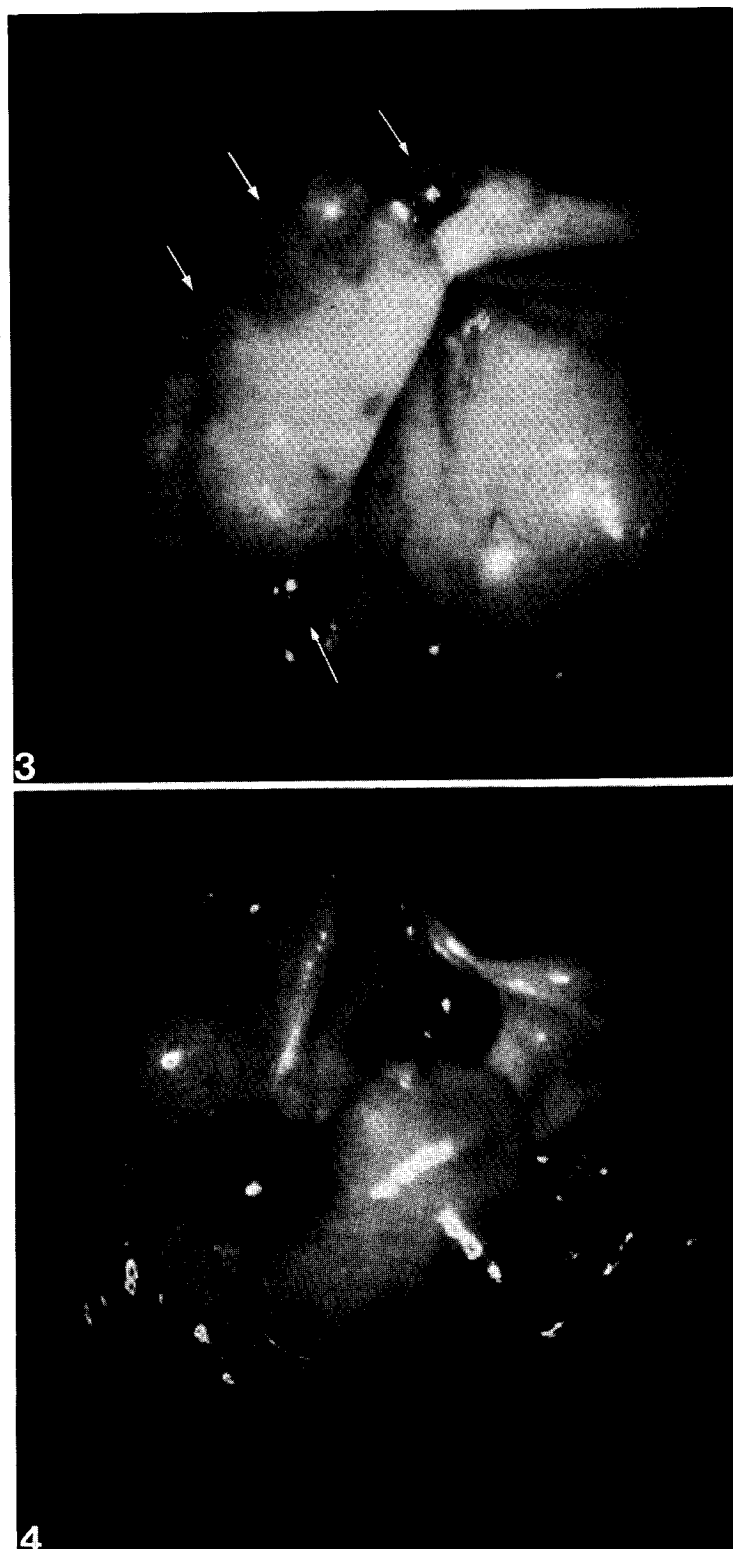


FIG. 3. Left ovary containing four CH (white arrows) five days following the first hCG injection. Black arrow denotes gelatinous appearing stigma on one of the CH.

FIG. 4. Left ovary containing three prominent CH ~24 h following the first hCG injection.

of FSH-P administration was designated Day 1 of the study. A dosage of 10 mg FSP-P in 1 ml of saline was injected (i.m.) daily for 5 days. This dosage was based on earlier studies which demonstrated that 2 mg FSH-P daily for 5 days was the optimum regimen for inducing ovarian activity in the domestic cat (Wildt et al., 1978). During treatment each cheetah was examined daily for visual evidence of sexual behavior including the lordosis posture, vocalization, and increased restlessness and pacing activity (Eaton, 1973, 1974). Males were not available to test for sexual receptivity. On Day 6 (~1600 h) and again on Day 7 (~0800 h) hCG was administered (500 IU, i.m.). This dosage previously had been used successfully in the jaguar, causing ovulation of follicles developing during natural estrus (Wildt et al., 1979).

Laparoscopy was used to monitor ovarian response to treatment. For this procedure each cheetah was anesthetized by an i.m. injection of ketamine hydrochloride (Ketaset: Bristol Labs., 7.5 mg/kg) and xylazine (Rompun: Haver Lockhart, 1 mg/kg) and, if necessary, supplemented with inhalation anesthesia (Fluothane: Ayerst Labs.). Details for laparoscopy, laparoscopic photography, and the effectiveness of this technique for documenting ovarian activity in this and similar felids have been published (Bush et al., 1978; Bush et al., 1980). Because of the rarity and endangered status of this species, it was feasible to induce anesthesia and perform laparoscopy immediately prior to hormone treatment (i.e., Day 0) in only one-half of the available females. However, laparoscopy was performed on all cheetahs on either Days 6 and 10 (two animals) or Day 7 (six animals) to assess follicle development and the presence of corpora hemorrhagica (CH). Two of the six cheetahs, animal nos. 1 and 2, were studied twice.

RESULTS

Variations in ovarian morphology as affected by gonadotropin treatment were distinctly visible using laparoscopy (Figs. 1–4). The

ovoid-shaped ovary was located at the terminus of a bicornuate uterine horn. A translucent retractable fimbria completely covered each ovary or lay free and adjacent to the ovarian surface (Fig. 1). Prior to gonadotropin treatment the ovaries were smooth and sometimes contained small (≤ 2 mm in diameter) follicles (Fig. 1). Follicles observed 1–2 days following the last FSH-P treatment were translucent and spherical (4–6 mm in diameter), and were generally flattened or protruded slightly above the ovarian surface (Fig. 2). CH had a dark, hemorrhagic appearance and were characterized by their diameter (6–8 mm) and prominence (4–6 mm) above the ovarian surface (Figs. 3–4). CH often possessed a flattened or cratered dome and sometimes a gelatinous appearing stigma (Fig. 3). The hemorrhagic appearance of these luteal structures was still evident in the animal laparoscopically examined on Day 10.

The effect of the gonadotropin regimen in the six cheetahs is illustrated in Table 1. No estrous behavior was observed during the study. Overall, of the eight cases in which gonadotropins were administered, six resulted in mature follicle development and four in CH formation. Two animals (cheetah no. 1 during the second series and cheetah no. 3) treated during December failed to respond. The former animal produced five CH following its first induction regimen begun in October; however, the second treatment series given ~50 days later was ineffective in stimulating new follicle development. During the latter period, aged CL

TABLE 1. Information and results following FSH-P and hCG administration to a group of adult cheetahs.

Cheetah no. ^a	Age (years)	Month treatment began	Day(s) subjected to laparoscopy	Ovarian activity prior to hormone treatment	Mature follicles/fresh CH following hormone treatment (n)
1 (1st series)	12	October	0, 6, 10	No mature follicles or CL	5 CH
1 (2nd series) ^b	12	December	0, 6, 10	5 CL	0
2 (1st series)	12	December	0, 7	No mature follicles or CL	6 CH
2 (2nd series)	12	July	0, 7	No mature follicles or CL	6 CH
3	4	December	7	...	0
4	12	December	7	...	6 Follicles
5	4	July	7	...	5 Follicles
6	2	July	7	...	6 Follicles, 5 CH

^aGeographic location: Cheetah nos. 1, 2, U.S. National Zoological Park, Washington, D.C.; Cheetah nos. 3, 4, Beaver Dam, WI; Cheetah nos. 5, 6, Gladys Porter Zoo, Brownsville, TX.

^bThe second series treatment began 50 days following the first series treatment (i.e., during the existing luteal phase).

induced from the first hormonal series were still visibly prominent on the ovaries. The ovaries of cheetah no. 3, although void of any visible luteal tissue from previous cycles, also failed to produce any detectable follicular growth.

Some animals had not initiated or completed ovulation at the post-treatment laparoscopy. In six instances laparoscopy was performed on the afternoon of Day 7, which was approximately 24 h following the first hCG injection. At this time, ovarian activity was observed in five cases. On three of the latter occasions (cheetah no. 2 during series 1 and 2 and cheetah no. 6) ovulation had already occurred as confirmed by the presence of prominent CH. None of the females superovulated; cheetahs no. 1 (first series), no. 2 (first series), no. 2 (second series), and no. 6 produced five, six, six, and five CH, respectively. The ovaries of the latter animal, in addition to animals no. 4 and no. 5, also contained vesicular follicles which appeared morphologically normal and capable of rupturing. Abnormally enlarged, cystic appearing follicles were not noted in any animal.

DISCUSSION

Sequential laparoscopic observation of ovarian anatomy has demonstrated a comparative similarity among three species of felids. The stages of ovarian morphology in the gonadotropin-induced cheetah were consistent with previous descriptions of the cycling jaguar (Wildt et al., 1979) or the domestic cat in natural (Wildt and Seager, 1980) or hormonally induced estrus (Wildt et al., 1978). The cheetah, however, appeared to produce no cystic follicles and less variation in follicle numbers and ovulation rate when treated with the present regimen in contrast to the domestic cat receiving the same number of FSH-P injections (Wildt et al., 1978). It was of particular interest that no cheetah produced an excessive ovulatory response, even though one animal had produced five CH and six mature preovulatory follicles at the post-treatment examination. Ovulation of these latter follicles could still not have necessarily been considered a superovulatory response since litters consisting of as many as eight offspring have been reported in the cheetah following natural breeding (Eaton, 1970).

The precise reason that two females did not produce follicular activity is unknown. The inability of one of the two females to respond

to gonadotropins may likely be attributed to the presence of distinct CL at the time of treatment. A similar lack of response has been noted in luteal phase domestic cats treated with gonadotropins (Wildt et al., 1978).

Because a daily laparoscopic examination schedule was not used in the present study, it was not possible to make definitive conclusions on the precise time of ovulation in relation to hCG administration. However, three of five responding cheetahs had fresh ovarian CH by 24 h following the first hCG injection. This may suggest that hCG-induced ovulation in the cheetah is generally initiated at a time similar to that reported for hCG-treated domestic cats (i.e., 26–36 h following hormone injection) (Scott, 1970; Sojka et al., 1970; Platz et al., 1979).

It was of interest that no behavioral signs of estrus were observed in any animal. In both the wild and captive state, cheetahs are primarily solitary, preferring large territorial ranges (Eaton, 1973, 1974). Recently, this behavioral predisposition to isolationism and territorial size has been directly correlated to estrual displays in females and subsequent successful breeding of certain compatible pairs (Seager and Demorest, 1978). In the present study, close confinement of individual females during the gonadotropin treatment was probably not conducive to the formation of behavioral displays of sexual receptivity. Regardless, because of the rarity of the species, future breeding may be necessary using hormonal induction of ovarian activity, semen collection (Seager and Platz, 1977), and artificial insemination techniques. The present results suggest that hormonal induction of follicular development and ovulation for such an artificial breeding program can be achieved with serial injections of FSH-P and hCG.

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