

ARE CAPE GROUND SQUIRRELS (*XERUS INAURIS*) INDUCED OR SPONTANEOUS OVULATORS?

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Little is known about the ovulation patterns in ground squirrels, even though the timing of ovulation could influence mate-order effects and sperm competition, which have been documented in a number of species. The Cape ground squirrel (*Xerus inauris*) is a social, nonhibernating, semifossorial rodent with aseasonal reproduction. Evidence suggests that sperm competition is an important aspect of male reproductive success in this species. We examined whether the Cape ground squirrel is an induced or spontaneous ovulator. Fifteen reproductive adult females were removed from natal colonies and housed individually. Females were subjected to 1 of 3 trials: no male contact, chemical and visual contact but no physical contact with intact males, or direct contact with epididymectomized males. Females from each of the 3 experimental treatments exhibited similar urinary progesterone metabolite concentrations and qualitative ovarian histology. Neither the presence of males nor copulation appear necessary for ovulation to occur, and ovulation in this social African ground squirrel appears to be spontaneous.

Key words: ground squirrel, induced ovulation, progesterone, spontaneous ovulation, *Xerus*

Studies of male mating strategies have increasingly focused on the characteristics of males that help them achieve fertilization success under conditions of sperm competition (Waterman 2006). Mate-order effects (fertilization bias toward either 1st males or last males) can influence male searching strategies (whether to stay and copulate with a female or continue searching), the number and duration of copulations, and whether the male should try to guard the female or use other strategies to prevent additional matings after copulation (Lacey et al. 1997). However, the fertilization success of males also may be influenced by the behavior of the females, especially in the timing of ovulation relative to copulation (Gomendio and Roldan 1993). If the 1st copulation induces ovulation in a female, that 1st male would be able to fertilize most of the eggs because his sperm are in the reproductive tract at the right time. Thus, a male's reproductive success could then be influenced by the timing of the copulation (Lacey et al. 1997).

Ovulation in females has been described as either spontaneous or induced (Milligan 1980). Each of these mechanisms has profound effects on the reproductive strategies and overall life history of an organism. In species with spontaneous

ovulation, ovulation occurs in a predictable cycle without requiring external stimulation (Conaway 1971; Flowerdew 1987; Nelson 2000). Cycling patterns, including the length of each estrous cycle and the ability to cycle more than once in a year, can vary substantially among species (Conaway 1971; Flowerdew 1987). However, occurrence of induced ovulation requires a hormonal, behavioral, or physical stimulus (Conaway 1971; Flowerdew 1987; Hinds and Smith 1992; Nelson 2000). Considerable differences in length of estrus and types of stimuli that induce ovulation may exist among induced ovulators (Bennett et al. 2000; Flowerdew 1987; Hikim et al. 1992; Sumar 1994).

Generally speaking, induced ovulation is expected in solitary species occurring at low population densities or in species that inhabit highly seasonal environments (Conaway 1971; Flowerdew 1987; Weir and Rowlands 1973). At low densities, where opportunities to mate may be few and unpredictable, induced ovulation allows mating to occur as soon as a male becomes available. In seasonal environments, where many species hibernate, the short active period constrains females to only 1 estrous cycle for successful fertilization to occur. Therefore, ovulation should occur early in the animal's active period, but only if males are present (Landau and Holmes 1988). Induced ovulation allows females to delay ovulation if mating is delayed temporarily because of inclement weather.

Spontaneous ovulation is expected to occur in gregarious species (Flowerdew 1987). Conaway (1971) suggested that

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spontaneous ovulation may be associated with species where breeding seasons are long or continuous and estrous cycles are asynchronous. Spontaneous ovulation may facilitate a return to the estrous state in absence of pregnancy, which would be of particular advantage to species with long breeding seasons (Conaway 1971; Flowerdew 1987).

Ground-dwelling squirrels exhibit diverse social and mating systems and are found in many different ecological settings, making them ideal subjects for comparative studies. Social systems among ground-dwelling squirrels range from asocial to highly social (Armitage 1981), and their mating systems range from monogamy (e.g., *Marmota caligata*—Holmes 1984) to polygyny (e.g., *Marmota flaviventris* and *Cynomys ludovicianus*—Armitage 1998; Hoogland and Foltz 1982) to polygynandry (e.g., *Spermophilus beecheyi*—Boellstorff et al. 1994). Some species are obligate or facultative hibernators, whereas others are active year-round (Armitage 1981; Waterman 1996).

Despite the many studies of mating systems in sciurids (Armitage 1981; Becker 1993; Boellstorff et al. 1994; Lacey et al. 1997; Schwagmeyer and Foltz 1990; Waterman 1998) little is known regarding ovulation patterns of female ground-dwelling squirrels even though sperm competition and mate-order effects have been documented in a number of species. The only 2 physiological studies of ovulation in ground-dwelling squirrels were conducted on temperate, hibernating species that are solitary (*Spermophilus tridecemlineatus* and *Marmota monax*); both were found to be induced ovulators (Foster 1934; Hikim et al. 1992). Because fertilization success is associated with ovulation, studying the physiology of ovulation is a key step in understanding the reproductive strategies of both males and females. No study has examined ovulation in any social squirrel that can breed year-round.

The Cape ground squirrel (*Xerus inauris*) lives in the arid regions of southern Africa and is active throughout the year (Herzig-Straschil 1978; Waterman 1996). Sociality in this species is characterized by clusters of related females and associated bands of males. Male bands live independently of female groups, and males sleep in female clusters only during mating (Waterman 1995). Band home ranges encompass a number of female groups and males move together in small subbands from one female group to another, investigating the reproductive status of the females (Waterman 1998). Females are in estrus for about 4 h and copulate with about 4 males in this time (Waterman 1998).

Certain characteristics of the Cape ground squirrel would predict spontaneous ovulation; they are gregarious, capable of breeding year-round, and estrous cycles are asynchronous (Herzig-Straschil 1978; Waterman 1995). However, several behavioral observations would predict induced ovulation. There may be a 1st male advantage in mating, because the dominant male is generally the 1st to mate, mate guarding and copulatory plugs are rare (Waterman 1998), and rejection of copulation attempts by females occurs only early in the estrous cycle (Waterman 1998). Copulation could be the stimulus that triggers ovulation, with differential male fertilization success biased toward the 1st mate (Waterman 1998).

To better understand the reproductive strategies of the Cape ground squirrel, it is necessary to identify factors that may contribute to successful fertilization, such as the timing of ovulation. In this study we posed a simple question: is the aseasonally breeding Cape ground squirrel an induced or spontaneous ovulator?

MATERIALS AND METHODS

The research was conducted at the S. A. Lombard Nature Reserve, South Africa (28°28'S, 19°08'E), from February through July 2003. Ten adult male and 15 adult female Cape ground squirrels were caught on the reserve and 2 private farms adjacent to the reserve. Males were considered adult if the testes were completely descended, whereas females were considered adult if the nipples were swollen, indicating that they had previously produced a litter (Waterman 1996). Squirrels were captured using live traps (15 × 15 × 50 cm, Tomahawk Live Trap Co., Tomahawk, Wisconsin) baited with peanut butter and mixed birdseed. At time of capture, the mass (measured with a spring scale to ±5 g), reproductive status, and general condition (ectoparasite load, wounds, and scars) of each animal were recorded and an identification number was assigned by tagging cages where the animals were housed and by dye-marking individuals (Rodol D, Jos. H. Lowenstein & Sons, Inc., Brooklyn, New York).

Animal care protocols were approved by the University of Central Florida Institutional Animal Care and Use Committee and by the University of Pretoria Animal Ethics Committee (approval 030110-001). All procedures followed guidelines of the American Society of Mammalogists as set out in the Journal (Animal Care and Use Committee 1998). Animals were housed at S. A. Lombard Nature Reserve in galvanized wire-mesh enclosures with 25-mm mesh on the sides and top and 12-mm mesh on the bottom. Enclosures were 30 × 34 × 65 cm and were secured using aluminum J-clips. A piece of 10-cm-diameter, 22-cm-long polyvinyl chloride tubing was added for use as a hide-box, and a plastic food dish (7 × 10 × 18 cm) was attached to the side of the cage by wire or string.

Water was provided ad libitum through a standard, small-mammal water bottle. The primary diet consisted of rabbit pellets, dry corn, and sunflower seeds. Males had ad libitum access to food and females were denied access only during the daily urine collection process. Squirrels also were provided with fresh produce daily, which varied based on local availability and included sweet potato, corn, carrots, apples, pears, melons, and potato. Supplemental mechanical items and food items (newspaper, cardboard, grasses, tree branches, peanuts, raw oats, peanut butter, and corn-meal porridge) were offered as enrichment once or twice weekly to help stimulate natural behaviors.

Squirrels were maintained on a light cycle mimicking the natural cycle of the study area at the time of the project (11:13 h light : dark). Enclosures were kept in rooms heated with electric bar heaters. Average maximum and minimum daily temperatures were 25°C and 20°C, respectively. Within the natural range of the species, average maximum and minimum temperatures are 23–30°C and 6–14°C, respectively (Herzig-Straschil 1979).

Males and females were housed in separate buildings before the start of the experiment and were allowed 5 weeks to acclimate to captivity. Body mass was recorded at the beginning and end of the experimental period, and all subjects were dewormed using 0.1 ml of 1% Ivomec (Merial Limited, Iselin, New Jersey) injected subcutaneously. Urine from females was collected for 5 weeks before cohabitation with males for use as a control.

The experimental design followed that of Jackson and Bennett (2005). Females were randomly assigned to 1 of 3 treatments. The 1st

group of 5 females remained isolated from males in a separate building (female alone). The 2nd group consisted of 5 females placed in visual and chemical contact with intact males that were housed in separate cages approximately 15 cm from the female's cage (female near male). The final 5 females were allowed physical contact with epididymectomized males (female with male) through a 10 × 10 × 10-cm wire-mesh tunnel between adjacent male and female enclosures. The animals still had individual food dishes, water bottles, and polyvinyl chloride hide-boxes. The only time the pairs were separated was during urine collection. Males were dye marked on their backs to help distinguish sexes. Females near and with males were kept in separate male–female pairs.

Five males were randomly selected to undergo an epididymectomy during the acclimation period. We assumed that 3 months was ample time to ensure that the vas deferens of an epididymectomized male was clear of any semen and no pregnancies would occur. Epididymectomy was only performed on males to be placed in physical contact with a female to prevent fertilization taking place, such that any resulting corpora lutea would be of ovulation and not pregnancy.

Female enclosures included a wire-mesh divider to restrict access to approximately half the cage size (30 × 34 × 33 cm). Females were kept in this divided section for the daily urine collection process. During this time they were given their allocation of fresh produce. Once the urine sample had been collected, the dividers were removed and the squirrels were given access to the rest of their food.

The acclimation period lasted from 31 March through 4 May 2003. Urine for the initial control was collected between 5 May and 8 June 2003. The experimental urine was collected from 12 June to 20 July 2003. This urine collection period (May–July) corresponds with one of the peak breeding times for the species (Herzig-Straschil 1978; Waterman 1996). Urine was collected daily in a plastic tray placed under the cage, and all urine produced between 0700 h and 1200 h for a single squirrel was pooled. To prevent fecal contamination of the urine the tray was fitted with a sheath of 40% shade cloth, through which only urine could pass. Each animal was assigned a single tray and sheath that was washed and bleached after each urine collection. We tested this procedure using pipetted distilled water over the washed mesh used to collect urine in mole-rats and the concentration of progesterone metabolite was close to the total binding of the radioactive ligand to the antibody, indicating that progesterone was not detectable. Urine was removed from the trays using a pipette with disposable tips and the urine was stored in a –20°C freezer within 3 h of collection to await subsequent analysis in the laboratory.

Progesterone metabolite determination.—Progesterone metabolite was used to trace potential ovulations. After ovulation the granulosa cells within a follicle stop producing estradiol and start producing progesterone metabolite (Bloom and Fawcett 1994). Thus, a rise in progesterone metabolite will trace the luteal phase of the follicular cycle. Progesterone metabolite concentrations in each sample were determined using a Coat-A-Count Progesterone Kit (Diagnostic Products Corporation, Los Angeles, California) following the manufacturer's protocol. This nonextraction assay uses a solid-phase ¹²⁵I radioimmunoassay for the quantitative measurement of progesterone metabolite. The antiserum for progestin is highly specific for progesterone metabolite with cross-reactivity to all naturally occurring steroids of <0.5%, with the exception of 17α-dihydroprogesterone (3.4%), 11-oxycorticosterone (2.4%), 5β-pregnan-3,20-dione (3.2%), and 5α-pregnan-3,20-dione (9.0%). Urine samples were assayed in volumes of 100 μl and in duplicate.

The progestin kit has been validated for measurement of progesterone metabolite concentrations in the urine of several species of rodents, including mole-rats (Damaraland mole-rat [*Cryptomys*

damarensis—Bennett et al. 1994; Moltano and Bennett 2000] and the highveld mole-rat [*Cryptomys hottentotus pretoriae*—Janse van Rensburg et al. 2002]) and Tete veld rats (*Aethomys ineptus*), Namaqua rock mice (*Aethomys namaquensis*—Muteka 2003), and naked mole-rats (*Heterocephalus glaber*—Westlin et al. 1994). Two pooled urine samples (a low concentration from a male and a high concentration from a pregnant female) were double diluted from 1:1 to 1:64 and assayed as a standardization procedure for assay validation. The low pool (male) contained no progesterone metabolite (below detection limit) and the high pool (pregnant female) contained 15.9 ± 0.54 nmol/mmol creatinine. For 6 samples of urine in duplicate (dilution 1:64 from the pool with expected low concentration of progesterone metabolite), a volume of 100 μl of progesterone metabolite in increasing concentrations (0.3, 1.60, 6.40, 31.8, 63.6, and 127.20 nmol/liter) was added to the sample. The values paralleled the standard curve. Additional extracted urine samples from the 2 pools were assayed and compared with nonextracted samples from the pools. No significant differences ($P < 0.05$) were found in progesterone metabolite concentration between the extracted and nonextracted samples.

The assay was validated for the Cape ground squirrels by comparing the slope of the curve produced using a serial dilution of female Cape ground squirrel urine that had a high concentration of progestin (1:1–1:64) against the standard curve. After a log-logit transformation of the data (Chard 1978), the slopes of the 2 curves did not differ significantly (analysis of covariance: $F = 1.189$, $d.f. = 1, 9$, $P = 0.304$, $R^2 = 0.995$). The sensitivity of the assay was 0.02 nmol/liter. Intra- and interassay coefficients of variation were 4.7% and 9.3%, respectively.

Creatinine determination.—Urine concentration in all animals varies with fluid intake, hence progesterone metabolite concentrations were corrected by analyzing each sample for creatinine concentration. Creatinine concentration was assessed by using a modified Jaffé reaction (Folin 1914). Both unknown samples and standard creatinine solutions (10 μl) were combined with alkaline picrate reagent (200 μl) in the wells of a microtiter plate. These plates were incubated in the dark at room temperature for 1.5 h to allow color development. Absorbances of samples and standards were determined at an optical density of 492 nm using a microplate reader (Anthos 2001 Reader, Anthos Labtec Instruments GmbH, Wals, Austria). From the standard curve, unknown sample values were calculated. Progesterone metabolite concentrations were finally expressed as ng progestin/mg creatinine to correct for any differences in urine concentration (Bennett et al. 1994).

Histology.—Two females from each of the 3 experimental groups were euthanized with fluorothane after the final urine collection. Ovaries were removed and immediately placed in Bouin fixative for approximately 72 h. Standard histological techniques were used to dehydrate, section, mount, and stain the samples (Drury and Wallington 1967). Ovaries were transferred to 70% ethanol and subsequently dehydrated in increasing concentrations of ethanol. Entire ovaries were then embedded in paraffin wax. Using a rotary microtome, ovaries were cut into 7-μm sections and every 10th section was mounted on a slide. Sections were stained using Ehrlich acid hematoxylin and counterstained with eosin. Both ovaries from each of the 6 animals were sectioned and mounted.

Qualitative observations were made of the ovaries using a light microscope at 40× magnification. Examinations focused on the presence or absence of different stages of follicular development within the ovaries, including primordial, primary, secondary, and graafian follicles, and corpora lutea. Categorization of various stages of follicular maturity followed Bloom and Fawcett (1994).

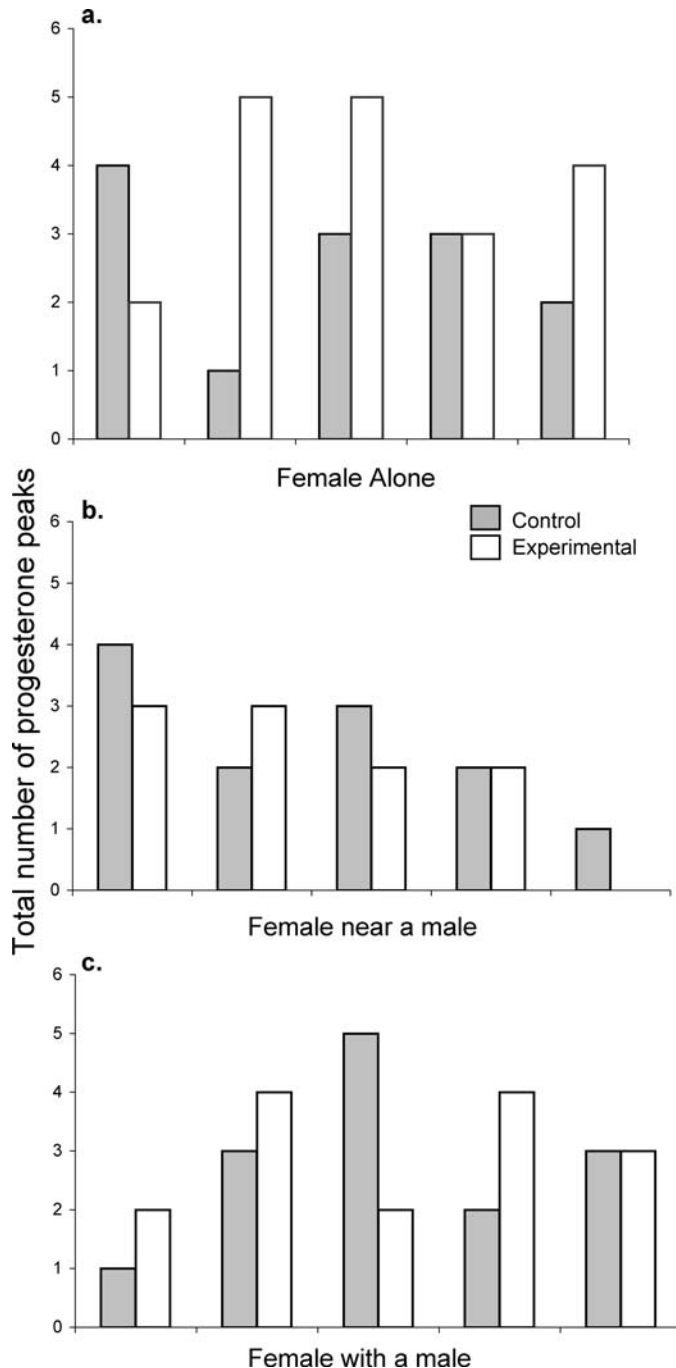


FIG. 1.—Total number of progesterone peaks for individual Cape ground squirrels (*Xerus inauris*) by treatment: a) female alone, b) female near a male, and c) female with a male (5 females per treatment). No significant effects were found in number of peaks due to treatment or time.

Vaginal smears, often used as an indicator of estrus, were not collected from females because the stimulation of the procedure has been shown to induce ovulation in both spontaneous and induced ovulators (Zarrow and Clark 1968).

Data analysis.—Progesterone metabolite profiles for each squirrel were plotted over time. The upper quartile of median progesterone metabolite concentration was determined for each animal and used as a baseline. Any progesterone metabolite concentrations that rose

above the baseline were considered peaks in progesterone metabolite. The mean numbers of peaks per treatment, the mean duration in days of the peaks, and the length of cycles were compared using Kruskal–Wallis tests (Zar 1999). A comparison of the mean number of peaks during the control versus the experimental treatments was performed using a Friedman 2-way analysis of variance (ANOVA; Zar 1999). Mean levels of progesterone metabolite were compared using a split-plot design analysis of variance (Zar 1999). The significance level was set at $\alpha = 0.1$ because of small sample sizes and the loss of statistical power associated with pooling the individual progesterone metabolite levels and analyzing the means. Statistical analyses were done in Statistix version 1.0 (Analytical Software, Tallahassee, Florida) and SPSS student version 11.0 (SPSS Inc., Chicago, Illinois). All data are presented as mean \pm SE.

Masses of all study animals were compared to those of free-ranging squirrels from the S. A. Lombard Nature Reserve at the beginning and end of the project using *t*-tests. For females, a comparison of mass at capture and mass at release was conducted using a paired *t*-test.

RESULTS

Progesterone metabolite concentrations.—Mean number of progesterone peaks per treatment for the duration of the study (control and experimental) was 6.4 ± 0.4 for females alone, 4.4 ± 1.0 for females near a male, and 5.8 ± 0.7 for females with a male. No significant difference was found in the number of progesterone metabolite peaks among the 3 treatments (Kruskal–Wallis: $H = 3.25$, $P = 0.20$, $n = 15$). Throughout the course of the study, mean duration of progesterone metabolite peaks (in days) was 3.4 ± 0.4 for females alone, 3.6 ± 0.6 for females near a male, and 3.4 ± 0.4 for females with a male. No significant difference was observed among the 3 treatments (Kruskal–Wallis: $H = 0.03$, $P = 0.98$). No effect of treatment or time period (control versus experimental) was found on the number of progesterone metabolite peaks (Friedman's 2-way ANOVA: treatment: $F = 1.74$, $d.f. = 2$, 26 , $P = 0.19$; time: $F = 0.55$, $d.f. = 1$, 26 , $P = 0.46$; Fig. 1), nor did treatment affect the length of the cycles (females alone: 29.2 ± 3.5 days; females near a male: 22.6 ± 3.0 days; and females with a male: 24.2 ± 0.9 days; Kruskal–Wallis: $H = 1.94$, $P = 0.37$, $n = 15$).

No significant differences were found in progesterone metabolite concentrations (ng progesterone/mg creatinine) among the 3 groups of females during the control period (Kruskal–Wallis: $H = 2.94$, $P = 0.23$, $n = 15$). No significant effects on total progesterone metabolite concentrations were observed due to treatment (ANOVA, split-plot design: $F = 0.41$, $d.f. = 2$, 12 , $P = 0.67$; Table 1), nor were significant differences detected in total progesterone metabolite concentrations among individuals within a treatment ($F = 1.64$, $d.f. = 2$, 12 , $P = 0.201$; Table 1). At $\alpha = 0.1$, a significant difference was observed in total progesterone metabolite concentrations as an effect of the interaction between time (control versus experimental) and treatment ($F = 3.05$, $d.f. = 2$, 12 , $P = 0.08$; Table 1). For the group where females remained alone throughout the study, progesterone metabolite concentrations were elevated during the experimental portion of the study in comparison to the control portion (control, 83.9 ± 19.9 ; experiment, 158.9 ± 35.7 ; Wilcoxon signed-rank test: $z = 1.88$, $P = 0.05$). The

TABLE 1.—Progesterone concentrations (in ng progesterone/mg creatinine) for each individual Cape ground squirrel (*Xerus inauris*; 5 females per treatment) in control and experimental periods. Nineteen samples were taken per female in the control period and 21 samples were taken per female in the experimental period.

Control ($\bar{X} \pm SE$)	Experimental ($\bar{X} \pm SE$)
Females alone	
149.32 \pm 88.6	191.75 \pm 90.4
104.68 \pm 27.8	264.01 \pm 53.1
75.63 \pm 20.9	93.36 \pm 29.1
35.59 \pm 9.2	65.71 \pm 41.6
54.52 \pm 8.5	180.13 \pm 123.8
Females near male	
159.60 \pm 43.9	105.52 \pm 19.7
66.95 \pm 39.2	45.08 \pm 13.2
147.71 \pm 60.0	128.46 \pm 13.3
505.74 \pm 363.2	231.68 \pm 90.3
432.65 \pm 196.1	33.51 \pm 3.5
Females with male	
643.81 \pm 511.7	73.29 \pm 15.6
84.29 \pm 14.5	89.89 \pm 23.1
104.77 \pm 47.1	28.24 \pm 9.8
19.58 \pm 2.3	25.45 \pm 4.4
360.59 \pm 178.9	146.99 \pm 27.0

opposite trend was seen in groups where females were near males, where progesterone metabolite decreased from 262.5 ± 86.6 to 108.8 ± 35.5 (Wilcoxon signed-rank test: $z = 1.88$, $P = 0.05$). No significant difference was found between control and experimental levels where females were with males (control 242.6 ± 115.61 and experimental 72.7 ± 22.4 ; Wilcoxon signed-rank test: $z = 1.08$, $P = 0.28$).

If the decline in progesterone metabolite concentration between control and experimental periods for females near a male was due to prolonged exposure to males, then lower levels should be apparent at the end of the experimental period. However, comparison of these periods did not support this (Mann-Whitney U -test: $U = 12$, $P = 0.55$, $n = 10$).

Histology.—Qualitative analysis of the ovaries demonstrated the presence of primordial, primary, secondary, and graafian follicles in each of 6 individuals sampled (2 individuals from each of 3 treatments). Corpora lutea were seen in only a single individual from the females near a male treatment and not in any females with males even though we observed copulation attempts (mountings) by males housed with females.

Body condition.—To determine if ovulation patterns were an artifact of body condition, we compared the body mass of our experimental animals with those of free-ranging animals. The body condition of study females at the time of capture did not differ from that of free-ranging adult females from the same time period (January–March; $t = -0.96$, $df = 40$, $P = 0.34$; Table 2). Captive females increased in body mass during the study period ($t = 51.05$, $df = 29$, $P < 0.001$), but at the end of the project the body mass of captive females was not significantly higher than that of wild females trapped in July, the last month of the project ($t = 0.65$, $df = 35$, $P = 0.52$). The body mass of captive males did not differ from that of free-ranging adult males either in June, just before experimental

TABLE 2.—Body masses (in g) of captive and free-ranging *Xerus inauris* at the beginning and end of the experimental period. Sample size is indicated in parentheses.

	Captive ($\bar{X} \pm SE$)	Free-ranging ($\bar{X} \pm SE$)
Females		
Initial	640.0 \pm 15.0 (15)	655.0 \pm 10.0 (27)
Final	715.0 \pm 15.0 (15)	700.0 \pm 15.0 (22)
Males		
Initial	720.0 \pm 25.0 (10)	730.0 \pm 10.0 (21)
Final	730.0 \pm 25.0 (10)	730.0 \pm 10.0 (21)

portion of the project ($t = 0.259$, $df = 24$, $P = 0.789$; Table 2), or in July ($t = -0.20$, $df = 24$, $P = 0.84$).

DISCUSSION

Induced ovulation has been reported for the brush-tailed bettong (*Bettongia penicillata*—Hinds and Smith 1992), the gray short-tailed opossum (*Monodelphis domestica*—Hinds et al. 1992), and the highveld mole-rat (Janse van Rensburg et al. 2002). In each of these species, a depressed progesterone metabolite profile was observed in females denied access to males, relative to females allowed access to males.

If the Cape ground squirrel is an induced ovulator, females denied access to males throughout the study should exhibit little or no change in their progesterone metabolite profiles over time. Furthermore, in females allowed access to males, progesterone metabolite concentrations should increase between the control and experimental periods. The females placed adjacent to but not in physical contact with males should show increased progesterone metabolite concentrations only if females are stimulated by some cue from males not requiring physical contact (such as pheromones). None of the hormonal patterns that would be expected for a species with induced ovulation were observed in our experiments with *X. inauris*.

Rodents that do not ovulate will not produce corpora lutea, but the developed tertiary follicle could develop into an unruptured luteinized follicle that will produce small amounts of progesterone (but not the levels recorded in the study). There were no unruptured luteinized follicles in the histological sections, but there were primordial, primary, secondary, and graafian follicles in the ovaries examined. Multiple stages of follicular development do not necessarily support either induced or spontaneous ovulation; some studies find similar folliculogenesis whereas others find decreases in secondary and tertiary follicles in anovulatory females (Bennett et al. 1994; Willingstorfer et al. 1998).

If ovulation is induced, we would expect there to be corpora lutea in the ovaries of females that had access to males for copulation, and not in the females that were alone. Furthermore we would also expect to see remnants of corpora lutea in the form of corpora albicantia. If ovulation is spontaneous and does not require the physical act of coitus we would have expected corpora lutea to be present in all 3 groups to some degree.

However, corpora lutea were only seen in 1 squirrel that was housed near a male. The lack of corpora lutea implies that the corpora lutea may have regressed or degenerated. In some rodents and primates, corpora lutea degenerate after a luteal phase of 12–16 days (Conaway 1971). With a minimum cycle length in free-ranging *X. inauris* estimated at 31 days (Waterman 1996) and a cycle length of 22–29 days in this study (possibly shorter because of a higher-quality diet—Millesi et al. 1999), the interval between estrus and the examination of ovaries could have been long enough for corpora lutea to regress. However, if corpora lutea had formed but regressed, hormone profiles should have reflected their presence. If ovulation was induced by the presence of males, the progesterone metabolite profiles would have differed among treatment groups (Bennett et al. 1994).

Luteinized unruptured follicles also can produce progesterone during the ovarian cycle without occurrence of ovulation. Luteinized unruptured follicles may act as accessory corpora lutea to maintain adequate progesterone levels during long gestation periods (Weir and Rowlands 1974). These luteinized unruptured follicles have been documented in some species of hystricomorph rodents (Weir and Rowlands 1974) and bathyergids (Bennett et al. 1994), all of which are induced ovulators. However, luteinized unruptured follicles generally produce less progesterone than corpora lutea and have a specific morphology (Bennett et al. 1994; Westfahl 1988). No luteinized unruptured follicles were seen in the ovarian cross sections in this study.

Total progesterone metabolite concentration declined between the control and experimental periods in those groups where females were near males, and a similar nonsignificant pattern occurred in females with males. This decline may reflect an adaptation in the Cape ground squirrel to avoid inbreeding. Although male-biased dispersal is the norm, with males and females generally living in separate groups, some males do not disperse from their natal burrows for up to 3 years after attaining sexual maturity (Waterman 1995). Females typically remain in their natal social group upon reaching maturity (Waterman 1995), and thus animals living in the same burrow tend to be closely related. In general, females should refuse copulation attempts by males with which they live to decrease the probability of inbreeding. The captive design of this project, in which males and females were housed together, may have induced a response in females analogous to inbreeding avoidance in free-ranging Cape ground squirrels, whereby females avoid mating with males from the same burrow cluster. This explanation has yet to be tested in the field.

Because of the highly unpredictable environment in which they live, likelihood of reproductive failure for this species is high (Waterman 1996). Within a few weeks of losing a litter, females can resume breeding and produce multiple small litters in a single year (Waterman 1996). Although there are periods of peak breeding, breeding takes place year-round (Herzig-Straschil 1978; Waterman 1996). When males are always available and the breeding season is not well defined, spontaneous ovulation would allow for a near constant cycle

of receptivity, which can maximize the chances of successful reproduction and minimize reproductive costs.

In contrast, the woodchuck (*Marmota monax*) and the 13-lined ground squirrel (*Spermophilus tridecemlineatus*) are solitary and hibernate (Armitage 1981). Under constraints of a short active period, breeding must take place very early on in the season to allow for maximum growth of the young before hibernation begins again. Thus, to maximize fertilization success, ovulation is male-induced for these solitary, hibernating species (Foster 1934; Hikim et al. 1992).

Given the uniformity in progesterone metabolite profiles and histological analyses of individual squirrels among 3 treatment groups, little evidence of induced ovulation was indicated in the Cape ground squirrel. With no support of induced ovulation, it is most likely that *X. inauris* is a spontaneous ovulator.

Postcopulatory mechanisms (i.e., sperm competition—Waterman 1998) may play an additional role in determination of reproductive timing and coordination. The multiple mating by females during estrus and the large testes size in males (Waterman 1998) are common traits when sperm competition is a factor (Kenagy and Trombulak 1986). Future studies that focus on paternity identity in free-living squirrels would provide critical insight required to address hypotheses related to sperm competition, as well as the influence of ovulatory timing and inbreeding avoidance on social systems and mating strategies of the Cape ground squirrel.

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