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RESEARCH ARTICLES

Assessing Reproductive Cycles and Pregnancy in Cheetahs (*Acinonyx jubatus*) by Vaginal Cytology

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Vaginal cytology was used to monitor ovarian cycles, two pregnancies, and three pseudopregnancies. Vaginal smears were collected two or three times per week from three adult females; smears plus blood samples were collected once per week from a fourth, adolescent female. Mean cycle lengths, based on intervals between onset of leukocyte infusions, were 11.9 ± 4.9 days (n = 43 cycles), 10.8 ± 5.1 days (n = 49), and 12.3 ± 6.3 days (n = 7) for the three females. Weekly hormone data from the adolescent female revealed a correlation between serum estradiol and percent anuclear cells, suggesting that these cells may be indicative of estrus. The fourth female experienced two sustained, 6-week increases in serum progesterone, one spontaneous and the other following follicle-stimulating hormone (FSH) administration. Leukocyte infusions continued during these periods of increased progesterone secretion. However, leukocyte infusions ceased during the two pregnancies of one adult female and during two FSH-induced pseudopregnancies of another.

Key words: felid, cat, captive breeding, ovarian cycle

INTRODUCTION

Despite centuries of captivity and domestication [Clutton-Brock, 1981], surprisingly little is known about the reproductive cycle of the cheetah. Although births may occur at any time of the year, peaks are reported to occur from March through May and again from October through December [Manton, 1975; Thompson and Vestal, 1974] following an approximately 90-day gestation [Eaton, 1974; Asdell,

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1964]. Interestrous interval, based on behavioral observation, is reported to be at least 2 weeks [Eaton and Craig, 1973]. However, there are no published data on cycle length based on physiological parameters.

Because of their diminishing numbers in the wild and low reproductive rates in captivity [Marker and O'Brien, 1989], basic information on breeding cycles is critically needed. Equally important is the development of methods to monitor these cycles.

The vaginal epithelium is responsive to changes in circulating estrogen [D'Souza, 1978; Parakkal and Gregoire, 1972; Wied and Bibbo, 1970; deBrux, 1958; Shorr, 1940]. Under estrogen stimulation, epithelial cells enlarge, while their nuclei condense and sometimes disappear. When circulating estrogen levels wane, leukocytes may leak into the vagina. Thus changes in vaginal cytology can be used as an indirect measure of estrogen levels. Because of the need for noninvasive methods for monitoring reproductive processes of the cheetah, we evaluated the use of vaginal cytology for following ovarian cycles and pregnancy.

MATERIALS AND METHODS

Animals

Of the four females used, three were hand-reared littermates born in October, 1983 (Studbrook numbers 370, 371, 372), and the fourth (number 525), born in May, 1987, was the offspring of 370. The three older females were housed singly in outdoor enclosures (10 × 10–65 × 60 m) with access to heated dens. The zoo was open from 0800 until 1700 hr daily; animals were fed and yards cleaned daily between 1330 and 1500 hr.

A female was introduced to a male(s) for mating when subjectively judged to be in estrus (increased attention, pacing, or sniffing by or toward one of the females). Female 525 was housed at the Veterinary Hospital in an indoor/outdoor enclosure (6.2 × 3.1 m) and did not have access to a male during the study. All were fed 1.4–1.8 kg Nebraska Brand Canine Diet daily plus one beef femur bone per week, except female 372, which was fed 1.4 kg Nebraska Brand Feline Diet daily plus the weekly bone.

Sample Collection

The three hand-reared females were swabbed two or three times per week during the following periods: female 370 January 5, 1989, to March 16, 1991 (114 weeks); female 371 January 26, 1989, to March 16, 1991 (111 weeks); female 372 September 21, 1989, to January 2, 1990 (15 weeks). Female 525 was swabbed once per week from March 2 to August 16, 1989 (24 weeks).

Immobilization was necessary for sample collection from female 525, which had not been hand-reared. The following drugs were used, alone or in combination, to induce anesthesia: etorphine HCl (M99; Lemmon Co., Sellersville, PA) 0.014–0.055 mg/kg, xylazine (Rompun; Haver, Shawnee, KS) 0.024–2.15 mg/kg, ketamine HCl (Ketaset; Bristol Labs, Syracuse, NY) 13.78–15.75 mg/kg, Telazol (tiletamine and zolazepam; A.H. Robbins, Richmond, VA) 2.11–3.29 mg/kg, diazepam (Lyphomed Inc., Rosemont, IL) 0.141–0.269 mg/kg, and/or medazolam (Versed; Roche Laboratories, Nutley, NJ) 0.082–0.134 mg/kg. On some occasions, 1–2% halothane (Fluothane; Ft. Dodge Laboratories, Ft. Dodge, IA) or isoflurane (Aerrane;

Anaquest, Madison, WI) was used to maintain anesthesia after induction with Telazol or ketamine. Diprenorphine (M50/50; Lemmon Co.) 0.016–0.115 mg/kg and/or yohimbine (Yobine; Lloyd Laboratories, Shenandoah, IA) 0.041–0.123 mg/kg) were used to reverse anesthesia.

During each immobilization, in addition to vaginal smears, 10 ml of blood was drawn from the cephalic vein for analysis of serum estradiol and progesterone. Blood was allowed to clot for about 3 hr before centrifugation; serum was withdrawn and stored at −80°C until shipment for assay.

Vaginal smears were collected according to the method of Millard et al. [1988]. After cleaning of the vulval area with saline-moistened gauze pads, clean cotton swabs moistened with sterile saline were inserted into the vagina to a depth of 2.5–4 cm, rotated 360°, withdrawn, and immediately rolled on a clean glass slide.

Staining and Cytology

After air drying, slides were stained in Diff-Quik (American Scientific Products, McGaw Park, IL) and allowed to dry, and coverslips were applied. One hundred epithelial cells were counted per slide, including nucleated and anuclear superficial cells, intermediate cells, and parabasal cells, as classified by Herron [1977a,b]. Total numbers of leukocytes were counted per 100 epithelial cells.

Ovulation Induction

Ovulation was induced in two female cheetahs by daily administration, for 5 days, of 10 mg follicle-stimulating hormone (FSH-P, Schering Corp., Kenilworth, NJ) delivered by dart gun. Female 525 was treated from April 15 to April 19, 1989. On April 15 and 20, following immobilization, her reproductive tract was examined using fiberoptics, blood was drawn, and a vaginal smear was taken. Female 371 was treated with FSH as described above from November 1 to 5, 1990, and again from March 7 to 11, 1991. Her reproductive tract was examined by laparoscopy on November 1 and 6 and on March 7 and 14.

Hormone Assays

Serum estradiol-17β and progesterone were measured by radioimmunoassay according to the methods described by Plotka et al. [1975] as modified in Seal and Plotka [1983].

Behavioral Observations

Females 370 and 371 were observed from August 18 to October 25, 1990. During one-half of this period, each was housed separately and introduced to males only for breeding; during the other one-half, each was housed continuously with one (370) or two (371) males. Each focal animal (two females and three males) was observed for 1 hr each day, 7 days per week, from 0800 to 0900 hr. Interobserver reliability was >80%. The behaviors monitored are defined in Table 1. In addition, every 5 min the focal animal's proximity to other cheetahs was recorded.

To test for a lag between the appearance of behavioral changes and changes in vaginal cytology and to examine the possibly more gradual onset of behavioral changes with approaching estrus, we compared percent anuclear cells to frequencies of behaviors on each of the 5 days preceding anuclear cell peaks. In addition to behavioral observations, keepers recorded the responses of females 370 and 371 to

TABLE 1. Definitions of behaviors

Behavior	Definition
Nonlocomote	Sit, stand, crouch, recline
Walk/pace	Forward progression with no more than two feet off ground at one time
Trot/run	Faster forward progression in which more than two feet may be off ground at one time
Urine mark	Spray urine on object from standing position
Defecate	Eliminate feces
Sniff urine, feces, or other cheetah	Examination with nose in contact or within 1 inch of surface being sniffed
Lick/groom	Stroking fur or object wth tongue
Flehmen	Head raised with mouth slightly open and corners of mouth drawn back in a grimace
Head rub	Press head or cheek across an object or another cheetah
Roll	Turn over on back; need not flip completely over
Flop	Abruptly drop to reclining position
Approach/follow	One animal enters or stays within one body length of another
Chase	One animal rapidly pursues another
Attack	One animal makes aggressive body contact with another, includes biting and wrestling
Retreat	Movement away from attack
Paw swipe	Strike or attempt to strike another animal with paw
Growl	Drawn out, deep sound with mouth open and teeth usually showing; similar to domestic cat growl
Spit	Explosive burst of air, similar to spitting of domestic cat
Hiss	Slow release of air with mouth and eyes open, body tense and ears drawn back, similar to hissing of domestic cat
Eeow	Similar to domestic cat's meow, but a sharper, shorter sound
Churtling	Repetitive call; vibratory sound made in throat
Chirp	High-pitched, short sound, similar to a bird chirp

the swabbing procedure from March 27 to December 15, 1990, using the following index: 0, could not swab; 1, aggressive, but swabbing possible; 2, resistant to swabbing; 3, neutral; 4, moderately responsive; 5, most responsive.

Statistical Analysis

Percentages of cell types were compared with serum hormone concentrations (female 525) and with behavior by Pearson product-moment correlation (Number Cruncher Statistical System, Kaysville, UT). Unless otherwise indicated, all levels of significance reported are $P \leq 0.05$.

RESULTS

In nonpregnant animals, salient changes in vaginal cytology included increases in superficial (especially anuclear superficial) cells, followed by infusions of leukocytes. Because the appearance and disappearance of leukocytes were consistently more clearly delineated than changes in other cell types, cycle length was calculated based on changes in leukocyte numbers (Table 2). A new cycle was designated when leukocytes increased to $\geq$5 per 100 epithelial cells. In typical cycles (Figs. 1a,b, 2b), the percent anuclear cells often reached maximum just prior to the appearance of large

TABLE 2. Length of ovarian cycles based on vaginal cytology in adult, female cheetahs (cycle length equals the interval between appearance of leukocytes in numbers of five or less per 100 epithelial cells)

Female	Cycle length (days) (mean ± SD)	Range (days)	Number of cycles
370	11.9 ± 4.9	4–23	43
371	10.84 ± 5.1	3–27	49
372	12.3 ± 6.3	5–21	7

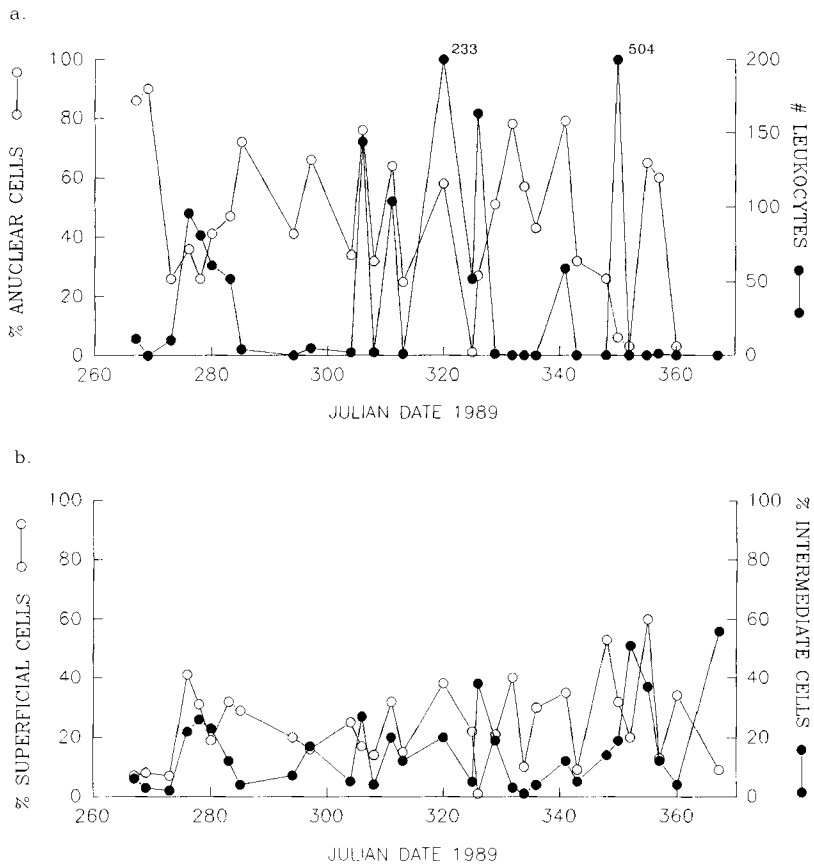

Fig. 1. Vaginal cytology pattern of female 372 showing changes in percent anuclear epithelial cells and number of leukocytes (**a**) and percent superficial and intermediate epithelial cells (**b**) during nonconceptive cycles (numbers in graphs indicate number of leukocytes when total was greater than scale).

numbers of leukocytes. After a leukocyte infusion, but before superficial cells again increased noticeably, smears often included parabasal and intermediate cells.

During the two pregnancies of female 370 (Fig. 2a,c), smears contained successive waves of superficial and anuclear cells, with no intervening leukocyte infusions. However, during the third month of gestation, occasional leukocytes (<5 per 100 epithelial cells per smear) were seen. The last peaks in anuclear cells and in

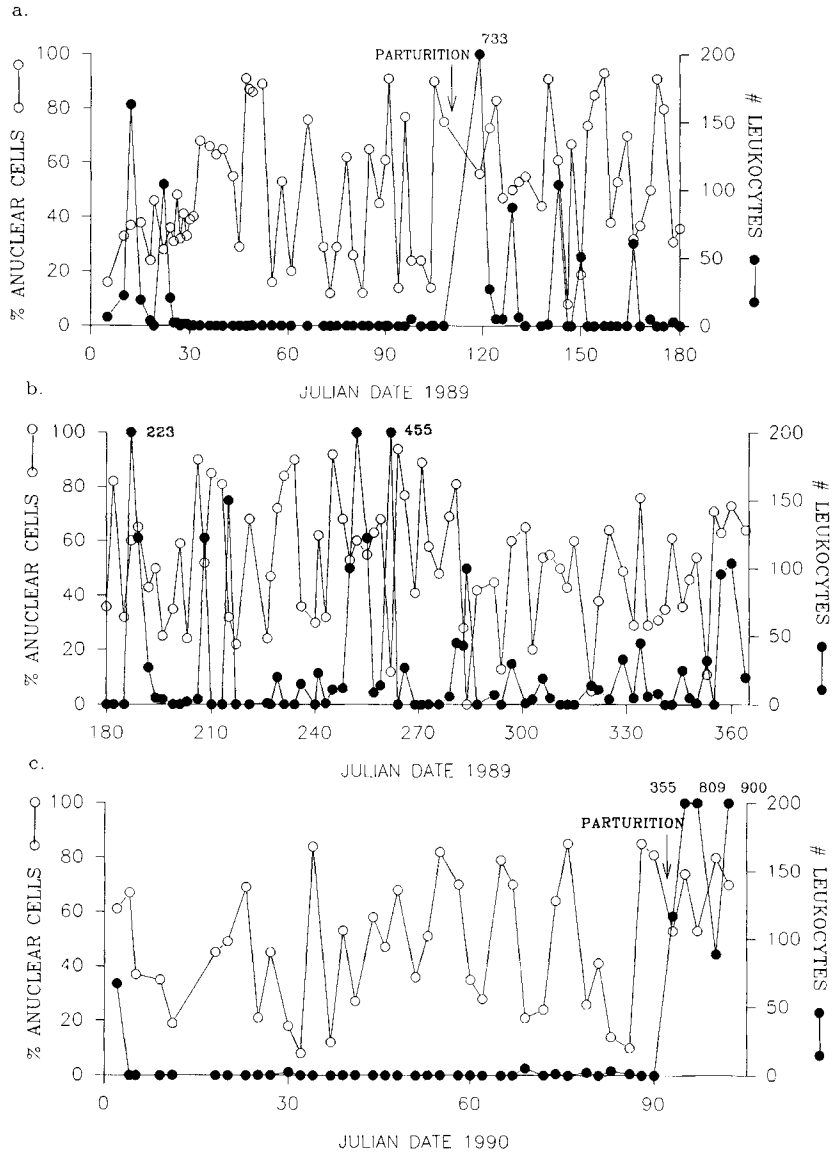

Fig. 2. Vaginal cytology pattern of female 370 during two pregnancies **(a,c)** separated by about 8 months of nonconceptive cycles **(a,b).** (Numbers in graphs indicate number of leukocytes when total was greater than scale.)

leukocytes prior to the 3-month absence of leukocytes during pregnancy were considered to be associated with conception. Thus counting forward by gestation length from these last anuclear cell and leukocyte peaks allowed us to predict the expected time of parturition. Days from this conception-associated anuclear cell peak to parturition were 90 (1989) and 92 (1990); days from the last leukocyte infusion to parturition were 87 (1989) and 89 (1990). Because Female 370's cubs were removed

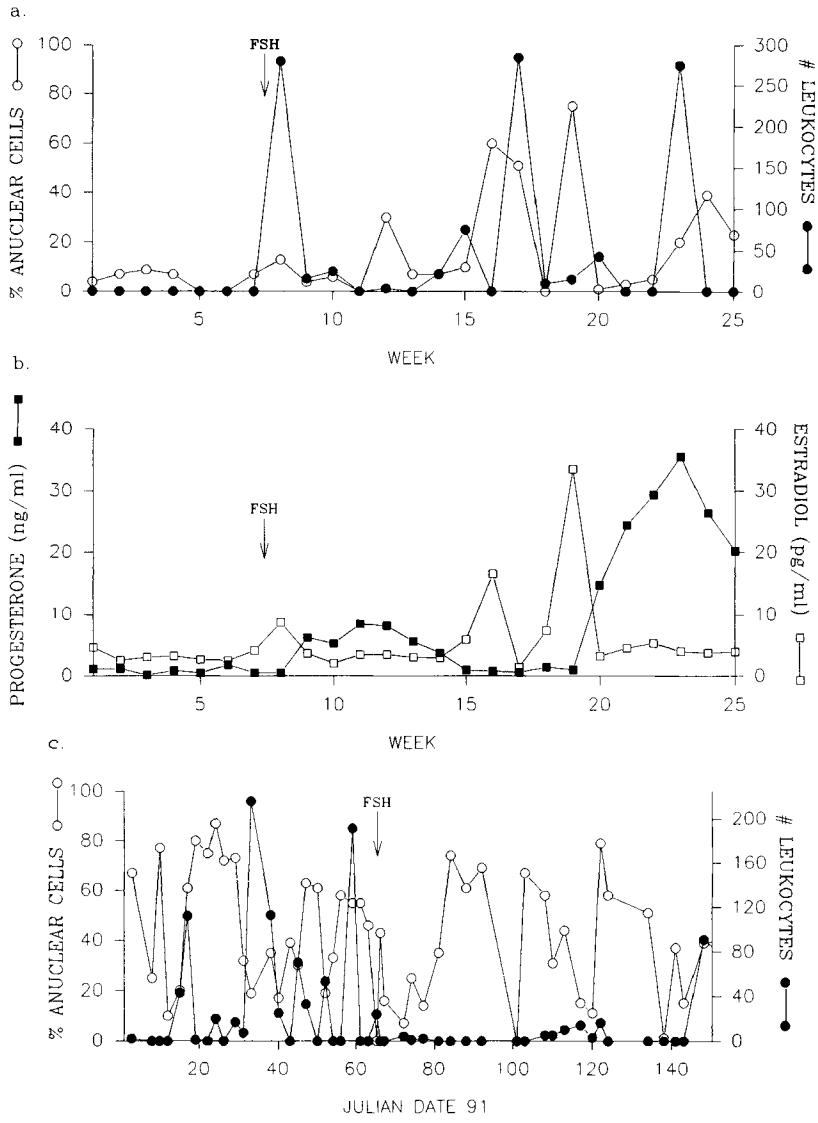

Fig. 3. Changes in percent anuclear cells and number of leukocytes (**a**) and serum progesterone and estradiol (**b**) in female 525 following FSH-induced and spontaneous ovulations, and percent anuclear cells and number of leukocytes in female 371 following the November, 1990, FSH-induced ovulation (**c**).

1 week postpartum for medical reasons, resumption of cycles was not affected by lactation.

Serum estradiol concentrations were correlated with percent anuclear cells ($r = 0.749$; $P < 0.001$), but serum progesterone concentrations were not correlated with percentages of any cell type. FSH administration to female 525 resulted in a transient estradiol increase, followed by a modest rise in progesterone, which remained above 2 ng/ml for 6 weeks; leukocyte infusions occurred during 3 of those 6 weeks (Figs.

TABLE 3. Correlations between percent anuclear cells and behavior of female cheetah 370 and male cheetah when housed together or adjacent

Day 0	Day −1	Day −2	Day −3	Day −4
		Behavior of female 370 when housed with male		
Walk/pace (r = 0.61)	Walk/pace (r = 0.68)	Walk/pace (r = 0.70)		
Growl (r = −0.64)				
		Behavior of female 370 when housed adjacent to male		
Defecate (r = 0.65)	Walk/pace (r = 0.56)		Walk/pace (r = −0.68)	
		Behavior of male housed with female 370		
Walk/pace (r = 0.65)		Trot/run (r = 0.64)		Walk/pace (r = 0.75)
Urine mark (r = 0.56)				
Sniff ground (r = 0.60)				
Chase (r = −0.64)				
		Behavior of male housed adjacent to female 370		
Sniff urine (r = 0.60)	Prox female (r = 0.56)	Head rub (r = −0.71)	Walk/pace (r = 0.56)	
Nonlocomotive (r = −0.64)			Sniff feces (r = 0.62)	
			Flop (r = 0.80)	

3a,b). A later, spontaneous surge of estradiol was followed by another period of sustained progesterone secretion of greater magnitude, which lasted at least 6 weeks; a leukocyte infusion occurred during week 4 of those 6 weeks (Figs. 3a,b). In contrast, the two FSH-induced ovulations in female 371 were followed by 9 (Fig. 3c) and 6 weeks, respectively, of smears devoid of leukocyte infusions, presumably periods of pseudopregnancy. Laparoscopy of both females confirmed ovulation; however, tertiary follicles and corpora hemorrhagica were larger and more numerous on the ovaries of the more mature female 371.

No correlations were found between female cheetah response to swabbing and percent epithelial cells. However, specific behaviors were correlated with percent anuclear cells (Tables 3 and 4).

DISCUSSION

Cheetah vaginal cell cycles were similar to published data for other felids. Cycle lengths (Table 2), which ranged from 3 to 27 days, were comparable to those reported for the domestic cat, *Felis catus* [Scott and Lloyd-Jacob, 1955; Mowrer et al., 1975; Paape et al., 1975; Wildt et al., 1978; Shille et al., 1979; Cline et al., 1980]; puma,

TABLE 4. Correlations between percent anuclear cells and behavior of female cheetah 371 and male cheetahs when housed together or adjacent

Day 0	Day −1	Day −2	Day −3	Day −4
Behavior of female 371 when housed with males				
Sniff male (r = 0.67)	Sniff male (r = 0.70)	Sniff male (r = 0.70)	Head rub (r = 0.68)	Paw swipe (r = 0.73)
Prox male (r = 0.65)	Prox male (r = 0.70)	Defecate (r = 0.70)	Lick object (r = 0.70)	Attack (r = 0.70)
Sniff ground (r = 0.75)	Anogenital groom (r = 0.70)	Urinate (r = 0.70)	Growl (r = 0.70)	Retreat (r = 0.73)
Sniff urine (r = 0.70)		Growl (r = 0.70)	Paw swipe (r = 0.76)	
Behavior of female 371 when housed adjacent to males				
Urinate (r = 0.72)			Trot/run (r = −0.65)	Walk/pace (r = 0.78)
				Flop (r = 0.71)
Behavior of male housed with female 371				
Prox female (r = 0.65)	Prox female (r = 0.70)	Walk/pace (r = 0.60)	Sniff feces (r = 0.66)	Sniff ground (r = 0.60)
Urine mark (r = 0.70)	Sniff female (r = 0.70)	Sniff ground (r = 0.62)		Sniff feces (r = 0.66)
	Sniff ground (r = 0.68)	Growl (r = 0.68)		Flehmen (r = 0.69)
				Eeow (r = 0.69)
				Chase (r = 0.73)
				Attack (r = 0.73)
				Retreat (r = 0.73)
				Paw swipe (r = 0.73)
Behavior of male housed adjacent to female 371				
				Defecate (r = −0.59)

F. concolor [Bonney et al., 1981]; lion, *Panthera leo* [Schmidt et al., 1983]; and tiger, *P. tigris* [Seal et al., 1985]. In the present study, some variation likely was due to the 2–3-day per week sampling regimen and to occasionally missed samples. Daily samples might result in less variability.

The cytology pattern, which comprised periods of increased percentages of superficial, especially anuclear superficial, cells followed by leukocyte infusions and periods with intermediate and parabasal cells (Figs. 1, 2a,b), is typical of other felid species. Percentages of superficial plus anuclear cells at estrus in the domestic cat have been reported to range from 40% to 60% [Shille et al., 1979] and from 90% to

100% [Herron, 1977b]. In this study, superficial plus anuclear cells typically reached percentages of at least 40%, and more typically >60%, between leukocyte infusions.

Leukocyte infusions were noted in some [Foster and Hisaw, 1935; Liche and Wodzicki, 1939; Scott and Lloyd-Jacob, 1955; Michael, 1958; Herron, 1977a,b; Shille et al., 1979; Cline et al., 1980], but not all, domestic cat studies [Mowrer et al., 1975]. Leukocytes were not common in smears from the lion [Schmidt et al., 1983], puma [Bonney et al., 1981], or tiger [Seal et al., 1985]. Species differences or varied staining methods may explain this disparity.

Our finding that anuclear cell counts were correlated with serum estradiol is consistent with reports for other felids [lion: Schmidt et al., 1983; domestic cat: Shille et al., 1979]. This association, which permits indirect evaluation of estrogen status by monitoring vaginal cytology, suggests that the ovaries of the cheetah, like those of the domestic cat, undergo successive waves of follicular growth. However, the usefulness of vaginal cytology in detecting ovulation is limited by the absence of effect on the vaginal epithelium of progesterone, which begins to increase at ovulation.

During both pregnancy and pseudopregnancy in one study of the domestic cat, parabasal and intermediate cells, not superficial cells, were reported to dominate vaginal smears [Herron, 1977a,b]. However, in another, periods of superficial and anuclear cell dominance accompanied by estrous behavior were regularly seen during pregnancy [Scott and Lloyd-Jacob, 1955]. During the two pregnancies in cheetah female 370, epithelial cell cycles indicative of fluctuating estrogen continued, but without the leukocyte infusions typical of follicular cycles. This continued absence of leukocytes beyond the typical cycle length may be diagnostic of pregnancy in the cheetah. In addition, counting forward by gestation length from the time of the last leukocyte infusion, assumed to be associated with the conceptive ovulation, allowed us to predict the approximate time of parturition.

Pseudopregnancy was produced in two cheetahs (370 and 525) by FSH-induced ovulation (luteal activity was confirmed by progesterone assay or by laparoscopy) (Fig. 3a–c). In addition, 525 exhibited a second, spontaneous luteal phase documented by sustained progesterone production following the induced ovulation (Figs. 3a,b). During the 2 months following ovulation, leukocyte infusions continued in smears from 525, but not in smears from 370 (Fig. 3c). This discrepancy might be related to 525's less intense ovarian response to stimulation (e.g., fewer and smaller follicles and CL) revealed by laparoscopy. During the cycle, declining levels of estrogen allow the vaginal epithelium to leak leukocytes from the systemic circulation, a phenomenon independent of ovulation or changes in progesterone. Thus, during pregnancy or pseudopregnancy, estrogen but not progesterone profiles may still determine whether leukocytes enter the vagina. However, knowledge of the hormonal dynamics of pregnancy will be required to answer this question.

Because ovulation must be induced by cervicovaginal stimulation in most felids [domestic cat: Manwell and Wickens, 1928; jaguar (*P. onca*): Wildt et al., 1979; puma: Bonney et al., 1980; tiger: Seal et al., 1985], it is not surprising that the modal cytology pattern of the cheetah is typical of induced ovulators. However, the period of sustained progesterone secretion without mating in the adolescent female indicates that spontaneous ovulation may occur under some conditions, as in the lion [Schmidt et al., 1983].

The anesthetics used to immobilize the adolescent female, 525, for blood-sampling likely reduced the chance that swabbing induced ovulation. Repeated at-

tempts to stimulate ovulation by cervicovaginal stimulation in ketamine-sedated tigers have been unsuccessful (unpublished observations), an outcome consistent with results from other species showing ketamine suppression of luteinizing hormone (LH) release [rhesus monkey (*Macaca mulatta*): Fuller et al., 1984; rat (*Rattus rattus*): Matzen et al., 1987]. Furthermore, opioid anesthetics such as etorphine are known to interfere with ovulation by suppressing LH [Cicero et al., 1977; Blank et al., 1979; Pfeiffer and Herz, 1984; Kreeger, 1988]. Although antagonists used to reverse opioid anesthesia may stimulate LH release [Blank et al., 1979], their effects on LH were expected to be minimal under the conditions and doses used in this study.

Several behaviors displayed by female cheetahs and by nearby males varied with changes in vaginal cytology (Tables 3, 4). In particular, measures of activity, olfactory communication, and proximity increased at the time of anuclear cell peaks. The diminishing level of aggression prior to anuclear cell peaks for female 371 was especially interesting. Perhaps a more powerful statistic such as sequence analysis would have revealed more associations. However, the individual differences shown by the two females suggest that the results may not be generally applicable. The array of behaviors typically observed during courtship in other felid species, such as rolling, rubbing, and vocalizations [see Ewer, 1973, for review], were not consistently observed during estrus in cheetahs in this study.

CONCLUSIONS

1. Cyclic changes in anuclear epithelial cells may be correlated with changes in circulating estradiol, whereas leukocyte infusions probably signal the end of a follicular phase associated with declining estradiol production.

2. Pregnancy may be associated with the cessation of cyclic leukocyte infusions, although changes in superficial cell proportions continue to vary and to dominate smears throughout gestation.

3. With known gestation length, the time of the final leukocyte infusion accompanying conception may be used to calculate and predict the approximate time of parturition.

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REFERENCES

Asdell, S.A. PATTERNS OF MAMMALIAN REPRODUCTION. Ithaca, NY, Cornell University Press, 1964.

Blank, M.S.; Panerai, A.E.; Friesen, H.G. Opioid peptides modulate luteinizing hormone secretion during sexual maturation. SCIENCE 203:1129–1131, 1979.

Bonney, R.C.; Moore, H.D.; Jones, D.M. Plasma concentrations of oestradiol-17β and progesterone, and laparoscopic observations of the ovary in the puma (*Felis concolor*) during oestrus, pseudopregnancy and pregnancy. JOURNAL OF REPRODUCTION AND FERTILITY 63:523–531, 1981.

Cicero, T.J.; Bell, R.D.; Meyer, E.R.; Schweitzer, J. Narcotics and the hypothala-

mic-pituitary-gonadal axis: Acute effects on luteinizing hormone, testosterone and androgen-dependent systems. JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS 201:76–83, 1977.

Cline, E.M.; Jennings, L.L.; Sojka, N.J. Analysis of feline vaginal epithelial cycle. FELINE PRACTICE 10:47–49, 1980.

Clutton-Brock, J. DOMESTICATED ANIMALS FROM EARLY TIMES. Austin, University of Texas Press, 1981.

deBrux, J. Histological criteria of estrogenic effect. ACTA CYTOLOGICA 2:357–362, 1958.

D'Souza, F. The detection of oestrous. SYMPOSIUM OF THE ZOOLOGICAL SOCIETY OF LONDON 43:175–193, 1978.

Eaton, R.L. THE CHEETAH. New York, Van Nostrand Reinhold, 1974.

Eaton, R.L.; Craig, S.J. Captive management and mating behavior of the cheetah. Pp. 217–254 in THE WORLD'S CATS I: ECOLOGY AND CONSERVATION. R.L. Eaton, ed., Winston, OR, World Wildlife Safari, 1973.

Ewer, R.F. THE CARNIVORES. Ithaca, NY, Cornell University Press, 1973.

Foster, M.A.; Hisaw, F.L. Experimental ovulation and the resulting pseudopregnancy in anoestrous cats. ANATOMICAL RECORD 62:75–93, 1935.

Fuller, G.B.; Hobson, W.C.; Reyes, F.I.; Winter, J.S.D.; Saiman, C. Influence of restraint and ketamine anesthesia on adrenal steroids, progesterone, and gonadotropins in rhesus monkeys. PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 175:87–90, 1984.

Herron, M.A. Feline vaginal cytologic examination. FELINE PRACTICE 7:36–39, 1977a.

Herron, M.A. Feline reproduction. VETERINARY CLINICS OF NORTH AMERICA 7: 715–722, 1977b.

Kreeger, T.J. The physiological, biochemical, and pathological responses of wild canids to stress. PhD Dissertation, University of Minnesota, 1988.

Liche, H.; Wodzicki, K. Vaginal smears and the oestrous cycle of the cat and lioness. NATURE 144:245–246, 1939.

Manton, V.J.A. Captive breeding of cheetahs. Pp. 337–344 in BREEDING ENDANGERED SPECIES IN CAPTIVITY. R.D. Martin, ed. London, Academic Press, 1975.

Manwell, E.J.; Wickens, P.G. The mechanism of ovulation and implantation in the domestic cat. ANATOMICAL RECORD 38:54, 1928.

Marker, L.; O'Brien, S.J. Captive breeding of the cheetah (*Acinonyx jubatus*) in North American zoos (1871–1986). ZOO BIOLOGY 8:3–16, 1989.

Matzen, S.; Kinigge, U.; Warberg, J. Effect of anesthetics on PRL and LH secretion in rats. ACTA ENDOCRINOLOGICA 115:528–536, 1987.

Michael, R.P. Sexual behavior and the vaginal cycle in the cat. NATURE 181:567–568, 1958.

Millard, S.E.; Durrant, B.S.; Yamada, J.K. Vaginal cytology as a management tool in the propagation of captive cheetahs. P. 610 in the PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ZOOLOGICAL PARKS AND AQUARIUMS, Wheeling, WV, American Association of Zoological Parks and Aquariums, 1988.

Mowrer, R.T.; Conti, P.A.; Rossow, C.F. Vaginal cytology, an approach to improvement of cat breeding. VETERINARY MEDICINE/SMALL ANIMAL CLINICIAN 70:691–696, 1975.

Paape, S.R.; Shille, V.M.; Seto, H.; Stabenfeldt, G.H. Luteal activity in the pseudopregnant cat. BIOLOGY OF REPRODUCTION 13:470–474, 1975.

Parakkal, P.F.; Gregoire, A.I. Differentiation of vaginal epithelium in the normal and hormone-treated rhesus monkey. BIOLOGY OF REPRODUCTION 6:117–130, 1972.

Pfeiffer, A.; Herz, A. Endocrine actions of opioids. HORMONES AND METABOLIC RESEARCH 16:386–397, 1984.

Plotka, E.D.; Foley, C.W.; Witherspoon, D.M.; Schmoller, G.C.; Goetsch, D.D. Periovulatory changes in peripheral plasma progesterone and estrogen concentrations in the mare. AMERICAN JOURNAL OF VETERINARY RESEARCH 36:1359–1362, 1975.

Schmidt, P.M.; Chakraborty, P.K.; Wildt, D.E. Ovarian activity, circulating hormones and sexual behavior in the cat. II. Relationships during pregnancy, parturition, lactation and the postpartum estrus. BIOLOGY OF REPRODUCTION 28:657–671, 1983.

Scott, P.P.; Lloyd-Jacob, M. Some interesting features in the reproductive cycle of the cat. PROCEEDINGS OF THE SOCIETY OF STERILITY AND FERTILITY 7:123–129, 1955.

Seal, U.S.; Plotka, E.D. Age-specific pregnancy rates in feral horses. JOURNAL OF WILDLIFE MANAGEMENT 47:422–429, 1983.

Seal, U.S.; Plotka, E.D.; Smith, J.D.; Wright, F.H.; Reindl, N.J.; Taylor, R.S.; Seal, M.F. Immunoreactive luteinizing hormone, estradiol, progesterone, testosterone, and androstenedione levels during the breeding season and anestrus in Siberian tigers. BIOLOGY OF REPRODUCTION 32:361–368, 1985.

Shille, V.; Lundstrum, K.E.; Stabenfeldt, G.H. Follicular function in the domestic cat as determined by estradiol-17β concentration in plasma: Relation to estrous behavior and cornification of exfoliated vaginal epithelium. BIOLOGY OF REPRODUCTION 21:953–963, 1979.

Shorr, E. Effect of concomitant administration of estrogens and progesterone on vaginal smear in man. PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 43:501–506, 1940.

Thompson, R.A.; Vestal, B.M. Survey of condi-

tions associated with breeding cheetah in captivity. OKLAHOMA ZOO JOURNAL 2:1–21, 1974.

Wied, G.L.; Bibbo, M. The effects of sex steroids on the vaginal epithelium. Pp. 188–213 in ADVANCES IN STEROID BIOCHEMISTRY AND PHARMACOLOGY. M.H. Briggs, ed. New York, Academics Press, 1970.

Wildt, D.E.; Guthrie, S.C.; Seager, S.W.J. Ovarian and behavioral cyclicity of the laboratory maintained cat. HORMONES AND BEHAVIOR 10:251–257, 1978.

Wildt, D.E.; Platz, C.C.; Chakraborty, P.K.; Seager, S.W.J. Estrous and ovarian activity in a female jaguar (*Panthera onca*). JOURNAL OF REPRODUCTION AND FERTILITY 56:555–558, 1979.