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BRIEF REPORT

Sexual Behavior and Hormonal Estrus Cycles in Captive Aged Lowland Gorillas (*Gorilla gorilla*)

SYLVIA ATSALIS*, SUSAN W. MARGULIS, ASTRID BELLEM, AND NADJA WIELEBNOWSKI
Chicago Zoological Society, Chicago, Illinois

To evaluate whether observed cycles in proceptive behavior in aging lowland gorilla females (age 40+) at Brookfield Zoo were driven by ovarian activity, we compared monthly behavioral data to estradiol and progestogen cycles based on fecal hormone assessments. Progestogen peaks showed regularity and close coincidence with monthly sexual behaviors. Estradiol was more variable. Progestogen peaks varied between 22 ± 5 days for the control female (29 years old), to 24 ± 2.5 and 29 ± 8 for the two aged subjects. In the first aged female, which was housed with other females and a silverback, the high degree of cyclicity in sexual behavior, regularity of progestogen cycles, and close concordance between hormonal cycling and sexual behavior strongly compared to patterns found (in this and other studies) in gorilla females <35 years old. Cyclical progestogen peaks were longer and more variable in the second aged female—perhaps because she lacked the social mediation of other females or a male. For husbandry reasons she is not housed with the gorilla group, behavioral data were not collected from her. The value of our longitudinal study is in obtaining reproductive profiles of primate females that are approaching maximum lifespan. This pilot study is part of a larger research project on reproductive senescence that will include other captive females >35 years old, a population that is rapidly increasing in North American zoos as gorillas continue to age. Am. J. Primatol. 62:123–132, 2004.

INTRODUCTION

Primates are often discussed as possible models for the clinical study of menopause, and for research into evolutionary questions regarding the function of the menopausal phenomenon in human females [Graham, 1981; Gould et al., 1981; Bellino, 2000]. Research on the reproductive behavior and physiology of female great apes as they age may be especially relevant to our understanding of reproductive aging in human females because of the close taxonomic position of

*Correspondence to: Dr. Sylvia Atsalis, Department of Conservation Biology, Brookfield Zoo, 3300 Golf Rd., Brookfield, IL 60513. E-mail: sylviaatsalis@hotmail.com

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great apes relative to humans. Yet, with few exceptions, most work on reproductive aging in primates has focused on macaques [e.g., Graham et al., 1979; Small, 1984; Walker, 1995; Johnson & Kapsalis, 1998; Pavelka & Fedigan, 1999; Shideler et al., 2001]. Numerous cross-sectional reproductive hormonal studies based on one or two menstrual cycles in female apes have been conducted, but none of these included aged ($\geq$35 years old) gorillas as their subjects [e.g., Nadler, 1975, 1980; Graham, 1979; Lasley et al., 1982; Mitchell et al., 1982; Dahl et al., 1987; Bellem et al., 1995; Takahata et al., 1995; Jurke et al., 2001a; Miyamoto et al., 2001].

Menopause is defined as the permanent cessation of menstruation that results from the loss of ovarian follicular function [Burger et al., 1995]. From an evolutionary perspective, evidence of an extended lifespan following reproductive termination (not the result of extreme old age) is critical to determining that menopause exists in nonhuman species [Pavelka & Fedigan, 1999]. The reproductive lifespan of nonhuman female primates appears to be characterized by considerable variability. Some females have been shown to terminate reproduction several years before death [Mitsunaga et al., 1992; Sommer et al., 1992; Caro et al., 1995; Takahata et al., 1995], while others continue to cycle throughout their lives, often exhibiting increased cycle lengths and poor pregnancy outcomes [Hodgen et al., 1977; Graham, 1979; Hrdy, 1981; Borries et al., 1991].

We first became interested in reproductive aging when we noticed that a 40+ female lowland gorilla (Alpha) housed at Brookfield Zoo exhibited monthly proceptive behavior toward a male. While overt sexual solicitation on the part of female gorillas appears to be the norm [Nadler, 1975, 1980], it was unclear whether the proceptive behaviors were hormonally supported, given that a disjunct between sexual behavior and hormonal cycling has been previously reported in aging apes [Takahata et al., 1995], and that the Species Survival Plan® [Wharton & Thompson, 2000] for western lowland gorillas in North American zoos indicates a drop in age-specific fecundity to near zero above the age of 37. Thus, we were interested in evaluating whether the observed behavioral cyclicity was driven by hormonal cycles. Our primary aim was to collect daily behavioral and endocrinological data in a longitudinal study to determine, for the first time, the relationship between observed sexual behaviors and hormonal cycles in aging female gorillas. If sexual behavior was strictly hormone-driven, then our observations would suggest that the aging gorillas were still cycling. We report the results on two aged females (40+), and one 29-year-old individual housed at Brookfield Zoo. This represents the pilot phase of a study that will include a larger sample of the aging captive population in North American zoos.

MATERIALS AND METHODS

Subjects and Study Site

For the purposes of this study, we defined any female $\geq$35 years old as ''aged.'' Two wild-born females, Alpha and Beta, were estimated to be 41 years old at the onset of the study. Alpha has had seven successful pregnancies, and her last offspring was born in 1991. Beta has had two successful pregnancies—the last one in 1983. The third subject, Babs, daughter of Alpha and 29 years old at the onset of the study, served as a control (defined as a female between 12 and 30 years of age). She has given birth five times–the last a stillbirth in 1999. The main gorilla group consisted of Alpha, Babs, four other adult females, one silverback male, and a 2-year-old male. All animals are housed inside year-round. Beta, who

suffers from advanced arthritis, is housed in a separate enclosure that is easier to negotiate.

Behavioral Data Collection

Between June 2002 and May 2003, focal behavioral observations [Altmann, 1974] and proximity to other group members were recorded every 2 min for 30 min daily for the group-housed subjects. All-occurrence sampling was used for selected proceptive, reproductive, aggressive, and affiliative behaviors. The proceptive and reproductive behaviors included staring, affiliative touching, quadrupedal presenting, crouching, copulating, and masturbating. No behavioral data were collected on Beta because she lacks access to other gorillas.

Hormonal Sample Collection

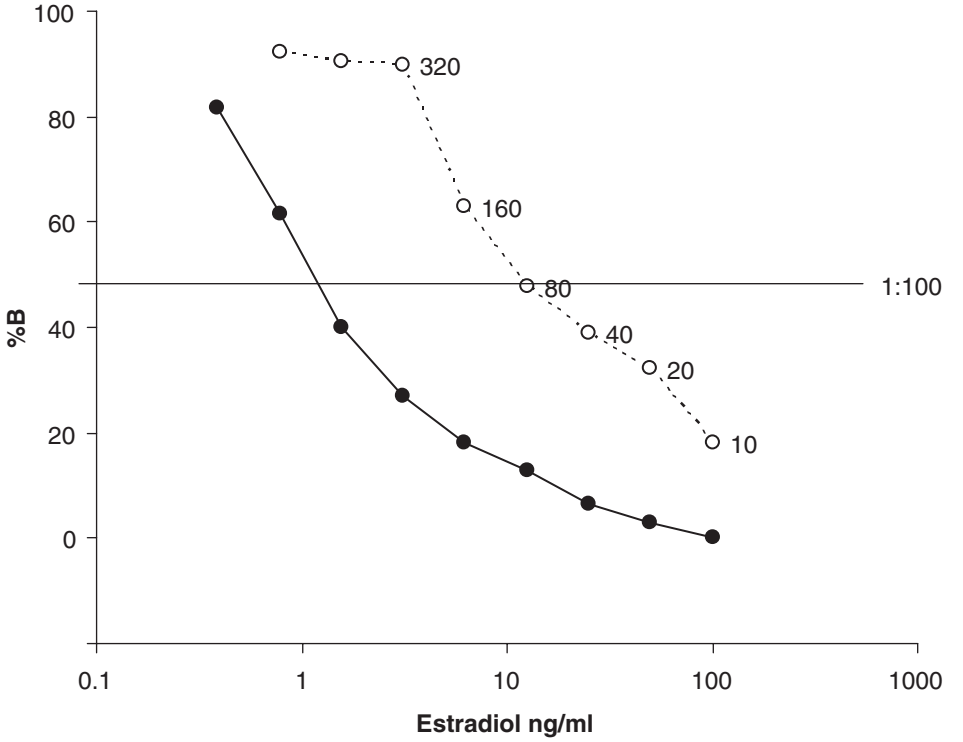
Fecal samples were collected daily from each of the three females. For Beta and Babs, samples were collected for one 3-month period. Behavioral and hormonal data collections were concurrent for Babs from July through October 2002. For Alpha, we conducted 6 months of fecal sampling (April–July 2002 and January–April 2003), four of which were concurrent with behavioral data collection.

Hormone Assays

We validated the hormone assays using serial twofold dilutions of a sample pool, which were tested for parallel displacement curves on estradiol and progesterone enzyme immunoassays (Fig. 1). We measured the recovery of exogenous hormone by adding a known amount of hormone to diluted samples.

All of the hormones and conjugates were prepared and supplied by Coralie Munro (University of California–Davis, Davis, CA). Flat-bottom 96-well microtiter plates (Nunc Maxisorb; Lab Source, Chicago, IL) were coated with 50 μl antibody in coating buffer (50 mM sodium bicarbonate, pH 9.6) overnight at 4°C. The plates were then washed five times with wash solution (0.15 M NaCl containing 0.05% Tween 20) and blocked with 50 μl assay buffer (0.1 M phosphate-buffered saline (PBS) containing 1% BSA, pH 7.0) for 2 hr at room temperature (RT). After the plates were blocked, standards, samples, and controls diluted in assay buffer were added to each well according to the plate setup, followed immediately by the addition of 50 μl per well of diluted horseradish peroxidase (HRP). The plates were covered and incubated at RT for 2 hr, washed five times to remove unbound antigen, and blotted dry. Then 100 μl of substrate solution (1.6 mM hydrogen peroxide, 0.4 mM azino-bis[3-ethylbenzthiazoline-6-sulfonic acid] in 0.05 M citrate buffer, pH 4.0) were added to each well. The plates were then incubated at RT with shaking (Lab-line plate shaker set at 2.5; Lab-line Instruments, Melrose Park, IL) for 0.5–2 hr until maximum binding was approximately 1.0. They were then read on a Dynatech MRX Revelation (Dynex Technologies, Chantilly, VA) at 405 nm.

For progesterone, the working antibody (CL425) was diluted 1:6,000. The working HRP dilution was 1:16,600, standard range was 0.05–12.5 ng/ml, sample volume was 50 μl per well, and sample dilution was 1:1,000. The assay sensitivity was 0.05 ng/ml. The intra- and interassay coefficients of variation were 6.5% and 28.0% at 30.8% binding, and 8.5% and 26.8% at 69.2% binding, respectively. The recovery of exogenous progesterone (0.39–6.25 ng/ml) was $153.6\% \pm 13.6\%$ ($y = 0.52 + 1.24x$, $r^2 = 0.98$).



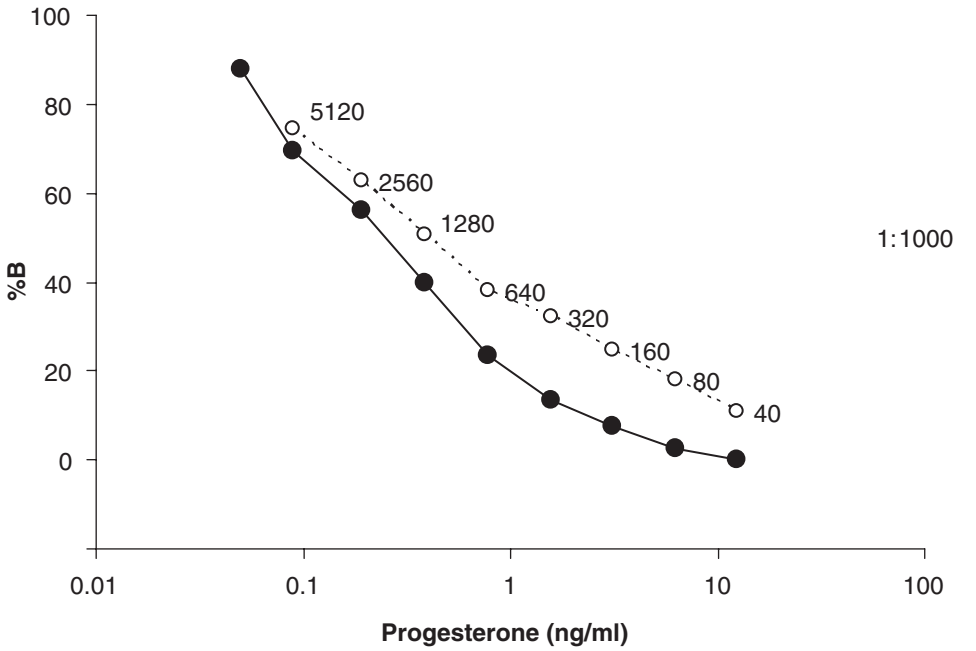


Fig. 1. Assay validations. Standards (closed circles) and samples (open circles) showing dilution linearity on estradiol and progesterone EIA assays.

For estradiol, the working antibody (R4972) was diluted 1:5,000. The working HRP dilution was 1:25,000, standard range was 0.39–100 ng/ml, sample volume was 20 μl per well, and sample dilution was 1:100. The assay sensitivity was 0.39 ng/ml. The intra- and interassay coefficients of variation were 11.5% and 18.8% at 26.6% binding, and 13.9% and 23.9% at 58.1% binding, respectively. The recovery of exogenous estradiol (3.12–50 ng/ml) was 150% ± 46.6% ($y = -0.35 + 1.81x$, $r^2 = 0.87$).

Analysis

Sexual behaviors usually lasted 1–2 days. The cycle length for behavior was calculated based on the intervals between onsets of sexual behavior. On two occasions when we were not present to record behavior, keeper notes were used to determine the onset of sexual behaviors. Isolated episodes of a small number of stares (≤ 5) were not considered to be true sexual events. Concerning hormones, we reported results from both estradiol and progestogen, but used the intervals between progestogen peaks to calculate hormonal cycle lengths because progestogens were more reliable indicators of cyclicity compared to estradiol, which was consistently variable.

RESULTS

Three-month fecal estradiol and progestogen profiles are presented for all three females (Fig. 2). Progestogen interpeak cycle lengths (mean ± SE) were 24 ± 2.5 days for Alpha (n = 8 interpeak cycles total (three are illustrated in the figure)), 29 ± 8 days for Beta (n = 3 cycles), and 22 ± 5 days for Babs (n = 4 cycles). The estradiol cycles were variable and did not correlate with progestogens or behavioral data, which suggests that the assay did not detect fecal metabolites in this species.

Sexual behavior tended to precede progestogen peaks by 5.0 ± 0.95 days when both behavioral and hormonal data were available (n = 4 cycles for Babs, n = 8 cycles for Alpha (including three cycles based on keeper assessment of sexual activity)) (Fig. 3). Cycle lengths based on intervals between episodes of sexual behavior (n = 13 cycles/female) were 26.7 ± 2.0 days for Babs (excluding one instance in which sexual behaviors were recorded only 5 days apart; inclusion of this reduces the cycle length to 24.7 ± 2.43 days), and 27.0 ± 1.7 days for Alpha.

The females demonstrated sexual and proceptive behavior in different ways (e.g., Babs masturbated, while Alpha did not; Alpha frequently touched the male, while Babs rarely did); however, staring (which accounted for 72% of Babs' and 61% of Alpha's behavior) was the predominant behavior in both.

DISCUSSION

Sexual behaviors were easily detected and were manifested largely in an all-or-none pattern (i.e., females were visibly interested or uninterested). Cycle lengths based on sexual behavior were as regular as those determined by hormonal data. For both Babs and the aged Alpha, cycle lengths based on hormonal data were consistent with previous reports for female gorillas under 35 [Nadler, 1980; Lasley et al., 1982].

Additionally, hormonal data for Alpha and Babs were consistent with previous reports of high interindividual variation in estrogen and progestogen levels [Miyamoto et al., 2001], and greater variability in fecal estrogen than in fecal progestogen hormones [Jurke et al., 2001b]. However, in both females an

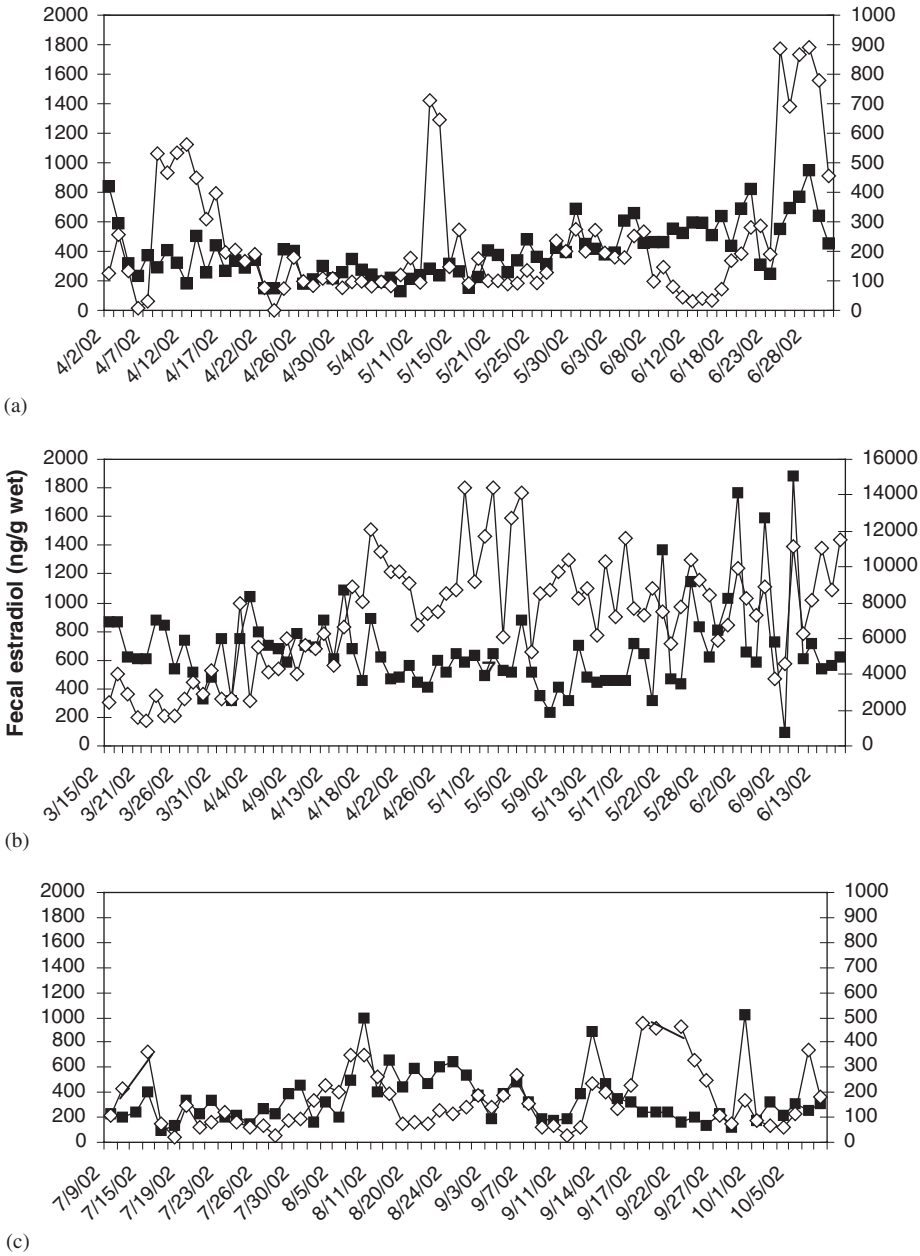


Fig. 2. Fecal progestogens (open diamonds) and estradiol (closed squares) for (**a**) Alpha, (**b**) Beta, and (**c**) Babs.

indication of biological validation of progestogen was manifested by the relative regularity of progestogen peaks, and by their close coincidence with monthly displays of sexual behaviors. However, a general trend toward increased average cycle lengths, from the 29-year-old control female Babs to the aged Alpha, and finally to the aged Beta, was apparent.

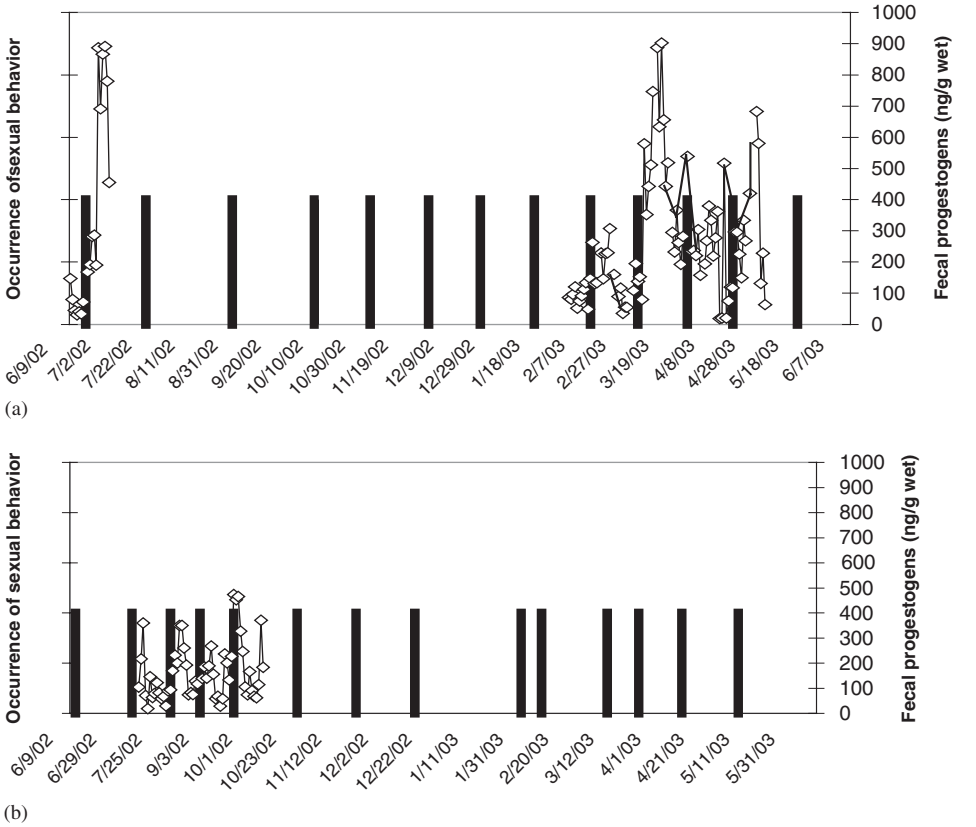


Fig. 3. Temporal patterning of sexual behavior (bars) relative to fecal progestogen measurements (diamonds) for (**a**) Alpha and (**b**) Babs. Bars indicate the observed occurrence of sexual behavior (including staring, affiliative touching, masturbating, crouching, and quadrupedal presenting) during 1 year of daily observations. Bars reflect only the presence or absence of behavior, not its frequency.

The high degree of cyclicity in sexual behavior, the regularity of progestogen cycles, and the close concordance between progestogen cycling and sexual behavior in the aged Alpha strongly compare to patterns found in females under 35 [Nadler, 1975] (this study). In the absence of behavioral indicators of sexual activity for Beta, interpretation of the hormonal data is somewhat problematic. Although cyclical progestogen peaks are evident, the length of cycles is longer and more variable, possibly consistent with early indications of reproductive failure.

The regularity in hormonal cycling exhibited by Alpha may be influenced by socially-mediated phenomena related to the presence of other cycling females in the group (as has been found in women [Stern & McClintock, 1998]), or by the presence of the male (as previously demonstrated in mice [Whitten, 1956; Vandenbergh, 1969] and cotton-top tamarins [Widowski et al., 1990, 1992]). Alternatively, the differences between Alpha and Beta may reflect variation in the onset of reproductive termination, or they may even be the result of a small age difference, if it exists.

Japanese macaque and chimpanzee females may continue to show sexual behavior years after last parturition [Takahata et al., 1995]. At Brookfield Zoo,

both the behavioral and hormonal patterns of the aged Alpha are indicative of ovulatory cycling, but can she successfully reproduce? For gorillas in North American zoos, age-specific fecundity drops to near zero above the age of 37 (Species Survival Plan® [Wharton & Thompson, 2000]). Females in their mid-thirties are characterized by reduced pregnancy rates, and, on the rare occasion when birth takes place, by poor survival of offspring (Thompson, personal communication; Wharton, personal communication). These observations are also supported by reports that females over 30 display less regular ovarian cycles [Lasley et al., 1982]. Yet, pregnancy at an advanced aged is possible: a 38-year-old female successfully gave birth at Zoo Atlanta last year (Olson, personal communication). However, based on a maximum lifespan of 51 years for females in North American Zoos, potentially up to 25% of a female's life can be nonreproductive.

Human females experience a decline in fertility associated with hormonal changes (a period termed the perimenopause) as much as 20 years prior to complete cessation of menstruation [Burger et al., 1995]. Given the combination of longevity and reduced fecundity with longer and more variable cycle lengths in some older individuals, gorillas also may experience a perimenopause prior to complete cessation of ovarian follicular function. Moreover, our planned longitudinal monitoring of interannual hormonal and behavioral changes in Brookfield's gorillas, as they approach maximum lifespan, may help to elucidate whether gorillas experience reproductive termination akin to menopause.

We realize that the small sample size of this project restricts conclusions because of the likelihood of wide interindividual responses to aging. However, the rarity of our subject species, and the possible importance of our findings in maintaining the health of aging female gorillas in captivity warrant the distribution of these preliminary results. The results from the second phase of our research, which includes more subjects from North American zoological institutions, will help determine whether the patterns found in this pilot study are supported.

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