Physiology & Behavior 157 (2016) 185–195

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Endocrine assessment of ovarian cycle activity in wild female mountain gorillas (*Gorilla beringei beringei*)

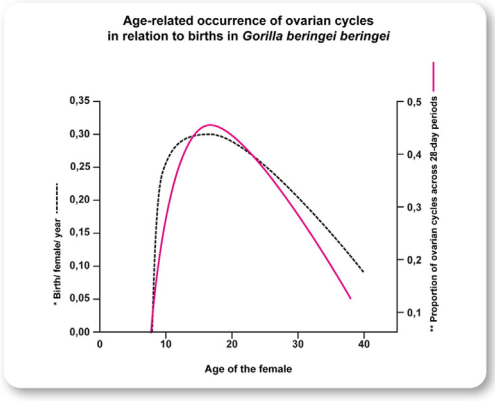
Sosthene Habumuremyi [a,b,c,*], Colleen Stephens [a], Katie A. Fawcett [b], Tobias Deschner [a,1], Martha M. Robbins [a,1]

[a] Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany
[b] Dian Fossey Gorilla Fund International, 800 Cherokee Ave SE, Atlanta, GA 30315-1440, USA
[c] Institut d'Enseignement Supérieur de Ruhengeri (INES-Ruhengeri), Musanze, North Province, Rwanda

HIGHLIGHTS

- We quantified ovarian cycles, matings and pregnancies in female mountain gorillas.
- We estimated the length of ovarian cycles and luteal phase in this species.
- Ovarian cycles were more frequent in parous than in nulliparous females.
- Proportion of mating days was comparable between parous and nulliparous females.
- Overall frequency of miscarriages was greater than previously estimated.

GRAPHICAL ABSTRACT



*Data on births were obtained from Robbins et al. "Age-related patterns of reproductive success among female mountain gorillas." Am. J. Phys. Anthropol. 131.4 (2006): 511–521.
**Data on ovarian cycles were obtained from the current study.

ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form 9 February 2016
Accepted 10 February 2016
Available online 12 February 2016

Keywords:
Fertility
Parity
Ovarian hormones
Mountain gorillas

ABSTRACT

Variability of fertility (i.e. number of births per female per year) has been reported in females of many primate species but only a few studies have explored the associated physiological and behavioral patterns. To investigate the proximate mechanisms of variability in fertility of wild female mountain gorillas (*Gorilla beringei beringei*), we quantified the occurrence of ovulation, matings, and successful pregnancies among females. We examined the profiles of immunoreactive pregnanediol-3-glucuronide (iPdG) for sixteen females (seven nulliparous and nine parous females, including one geriatric female; average sampling period for fecal sample collection and behavioral observations per female = 175 days; SD = 94 days, range = 66–358 days) monitored by the staff of the Dian Fossey Gorilla Fund's Karisoke Research Center in Parc National des Volcans, Rwanda. We quantified ovarian cycles from iPdG profiles using an algorithm that we developed by adjusting the method of Kassam et al. (1996) to the characteristics of ovarian cycle profiles based on fecal hormone measurements. The mean length of ovarian cycles was 29 ± 4 days (median: 28 days, N = 13 cycles), similar to ovarian cycle lengths of other great apes and humans. As expected, we found that female mountain gorillas exhibit longer follicular phases (mean ± SD: 21 ± 3 days, N = 13 cycles) than luteal phases (mean ± SD: 8 ± 3 days, N = 13 cycles). We also found that the frequency of ovarian cycles was greater in parous females (i.e. 20 ovarian cycles across 44 periods of 28 days; 45.5%)

* Corresponding author at: Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany.
E-mail address: sosthene_habumuremy@eva.mpg.de (S. Habumuremyi).
[1] These authors were co-principal investigators.

http://dx.doi.org/10.1016/j.physbeh.2016.02.017

than in nulliparous females (i.e. two ovarian cycles across 34 periods of 28 days; 6%). However, the frequency of days on which matings were observed did not differ significantly between parous and nulliparous females, nor between pregnant and non-pregnant females. Five pregnancies were detected with iPdG levels, but only three resulted in live births, indicating miscarriages of the other two. In sum, this study provides information on the underlying endocrine patterns of variation in fertility depending on parity, mating behavior, and pregnancy success in a critically endangered great ape.

1. Introduction

Physiological and behavioral aspects associated with variability in fertility (i.e. number of births per female per year; [2–4]) of wild female primates are important topics for scientists interested in reproductive biology and wildlife conservationists [5–9]. In most primates, female fertility is initially low, then rises, and finally declines with age [10, 11]. Components of fertility also vary among individuals as exemplified by differences in the age at first reproduction, interbirth interval, and the age at reproductive termination (carnivores [12, 13], cetaceans [14], primates [3, 10, 15–22], proboscideans [23, 24], rodents [25–27], and ungulates [28, 29]). Physiologically, this variability in fertility is likely to result from variability in the occurrence of ovulation, matings around the time of ovulation, and successful pregnancies [16, 30–32]. In this study, to investigate the proximate mechanisms of variability in fertility of wild female mountain gorillas, we quantify and compare occurrences of ovarian cycles, matings, and successful pregnancies among nulliparous females, parous females and a geriatric female.

Mountain gorillas occur in only two isolated populations, one of about 400 individuals living in Bwindi Impenetrable Park, Uganda, and the other one of about 480 individuals living in the Virunga Massif of Rwanda, Democratic Republic of Congo, and Uganda [33–35]. The average size of social groups is 10 individuals [33]. Mountain gorillas are considered to have a polygynous mating system, but 40% of groups contain more than one adult male [35–37]. In those multi-male groups, the dominant male performs the majority of matings and sires about 75% of offspring [38–40]. Gestation length is approximately 255 days and no seasonality in births has been observed [41]. About 27% of the offspring die before reaching the age of three years [21]. Less than 10% of mountain gorillas live to age 40 [35, 42, 43].

As female mountain gorillas reach maturity they undergo a period of low fertility [44], commonly referred as the period of "adolescent sterility", which also occurs in other primate species (e.g. humans (*Homo sapiens*) [45], chimpanzees (*Pan troglodytes*) [46], bonobos (*Pan paniscus*) [47], orangutans (*Pongo pygmaeus*) [48, 49], baboons (*Papio* sp.) [50, 51], rhesus macaques (*Macaca mulatta*) [52] and hanuman langurs (*Presbytis entellus*) [53]). On average, nulliparous females begin to mate when they are approximately 6–7 years of age but they generally do not have their first offspring until they are 10 years of age (range 8–13 years) [19, 54]. To further understand why there is such a long period between the onset of mating behavior and the age at first parturition, endocrine data are needed to tease apart probable causes including irregular ovarian cycles, inability to conceive, or inability to carry pregnancies to full term.

After parturition, most female mammals go through a period of lactational amenorrhea, which refers to the postpartum suppression of ovulation induced by suckling (see [55–58]). In breastfeeding women, the probability of ovulation is reduced to 31% in the first six months following delivery and remains below 67% for the subsequent six months [59]. In mountain gorillas, lactating females resume mating when dependent infants are about three years old [54], at which time the frequency of suckling is less than 0.5 bouts per hour [60]. Typically, female mountain gorillas mate on approximately one or two days every 28–30 days during the three to six months preceding conception [54, 61]. Whether the resumption of mating after parturition coincides with the resumption of ovarian cycle activity in this subspecies remains unclear. However, it is likely that sexual behavior coincides with ovulation in captive female lowland gorillas (*Gorilla gorilla gorilla*) [62, 63]. Furthermore, the extent to which ovarian cycle activity explains variability in intervals between offspring awaits further investigation.

Females are expected to resume having ovarian cycles shortly after the death of unweaned infants [60, 64, 65]. Interestingly, when a dependent infant dies, the time until the next birth is longer for primiparous than for multiparous females in mountain gorillas (i.e. mean ± SD: 21.1 ± 10.8 months versus 10.7 ± 1.3 months; [3]), and baboons (i.e. mean: 13 months versus 11 months; [66]). Therefore a difference should be observed in the pattern of ovarian cycle activity of primiparous and multiparous females, in the distribution of matings in relation to ovulation, and/or in the occurrence of miscarriages.

Female mountain gorillas appear to have a very short period of menopause (i.e. age-related cessation of giving births; [6, 10, 67, 68]) in contrast to what has been observed in various mammal species (e.g. western lowland gorillas (*Gorilla gorilla gorilla*) [69, 70], chimpanzees (*Pan troglodytes*) [6, 71], humans (*Homo sapiens*) [6, 15, 16], baboons (*Papio anubis*) [22],macaques (*Macaca mulatta*) [72, 73], killer whales (*Orcinus orca*) [14], lions (*Panthera leo*) [22], and African elephants (*Loxodonta africana*) [24]). The maximum postreproductive time for female mountain gorillas has been estimated to be 1–3% of the entire lifespan [3], while it encompasses 40% of the lifespan in humans (*Homo sapiens*) [15, 74] and as much as 25% of the lifespan in captive western lowland gorillas (*Gorilla gorilla gorilla*) [69, 70]. Additionally, the number of the offspring produced by female mountain gorillas decreases from 0.33/female/year in the first half of the reproductive life (i.e. less than 22 years) to 0.24/female/year in the second half [3, 54]. However, it remains unclear whether older female mountain gorillas experience a decrease in ovulatory function or whether they experience increased rates of miscarriages.

The timing of mating activity is another important factor influencing female fertility [75–83]. Fertility is expected to increase with both the rate of ovarian cycles and the frequency that matings coincide with ovulation [84–87]. Males of various species (e.g. primates [88]) exhibit more interest in mating with females that are more likely to conceive (i.e. parous versus nulliparous females [89, 90], and/or cycling versus lactating females [89, 91]). Female mountain gorillas mate at times other than the likely time of conception, including while pregnant [38, 54, 61, 92] but the frequency of mating behavior would be expected to decrease after conception if mountain gorillas are able to detect pregnancy-related cues and if they principally mate to achieve reproduction. Alternatively, mating behavior may continue or increase in frequency after conception if female mountain gorillas mate for other reasons such as to confuse paternity [93, 94], to maintain and strengthen friendships with males [44, 95–97], or to increase the probability of paternal care for their own offspring [98].

Another factor likely to reduce female fertility is the occurrence of miscarriages [5, 30, 32]. The frequency of miscarriages varies among primate species including humans (*Homo sapiens*) (i.e. 10–31% of total pregnancies detected after implantation; [99–102], baboons (*Papio sp.*) (i.e. 9.6–13.9% of total pregnancies detected after implantation; [103–105]) and langur monkeys (*Presbytis entellus*) (i.e. 16.5% of total pregnancies detected after implantation; [106]), but very few cases have been reported in mountain gorillas (i.e. seven miscarriages versus 214 births observed in 37 years; [3]). However, data on mountain

gorillas are based on field observations, which likely underestimate the rate of miscarriages. In contrast, in wild baboons, cessation of menstrual bleeding and sexual swellings enable visual detection of pregnancies and hence reliable detection of miscarriages [103]. Since mountain gorillas do not have those visual signals, hormone-based data are needed to better quantify the occurrence of miscarriages and assess their impact on female fertility in this species.

The overall goal of this study was to investigate proximate mechanisms of the variability in fertility of wild female mountain gorillas (*Gorilla beringei beringei*). More specifically, we aimed to address the following questions: What are the characteristics of ovarian cycles in female mountain gorillas? How often do ovarian cycles occur in nulliparous, parous and geriatric females? Following the death of a dependent infant, does the occurrence of ovarian cycles in primiparous and multiparous females mirror the longer delay in subsequent births by the former? How does the frequency of mating activity vary for females in different reproductive states (i.e. nulliparous versus parous females, pregnant versus non-pregnant females)? How often do miscarriages occur?

We used the method of Kassam et al. [1] to quantify ovarian cycles because this method allows the detection of ovulation-related changes in the profiles of progesterone metabolites. This method was initially used to ascertain anovulation in women lacking menstrual bleeding. Similarly, female mountain gorillas do not exhibit any external signs of ovarian cycles [54, 61, 92, 107]. We modified this method because 1) immunoreactive pregnanediol-3-glucuronide (iPdG) data were obtained from individuals of different reproductive status, which are expected to vary in the amplitude of post-ovulatory iPdG rises (see also [108]), 2) iPdG data were obtained from fecal samples, which usually produce more noise than hormonal data from urine or plasma samples [109], and 3) the number of collected samples varied across and within individuals (See also [110, 111]).

2. Materials and methods

2.1. Study individuals and demographic data

We collected data between April 2010 and March 2011 from seven habituated mountain gorilla groups monitored by the staff of Dian Fossey Gorilla Fund's Karisoke Research Center (KRC), in Volcanoes National Park, Rwanda. We obtained demographic information of female gorillas including the age, parity, and birthdates of offspring from the long term records of KRC. We included nulliparous females that were older than 6.5 years and had been observed mating (N = 7; Table 1). The parous females studied were those with infants that were at least 2.7 years old (N = 6) as well as one female who had miscarried and two females whose most recent infant had died [3, 54, 61]. We considered one 38 year old parous female as geriatric because she had reached the average maximum lifespan for female mountain gorillas (i.e. 35 years; [3]). We used the term 'female days' to refer to all days encompassed by the period spanning from the first day to the last day of fecal sample collection and behavioral observations of each female used in this study (Table 1).

2.2. Mating behavior

We defined mating as sexual behavior with mounting and pelvic thrusting performed by a male towards a female [38]. We used the term 'mating days' to refer to days that matings were observed [91]. We then examined if mating days occurred during the estimated time of ovulation and made comparisons of the occurrence of matings by nulliparous versus parous females, and pregnant versus non-pregnant females. Each gorilla group was observed for 3.5–4 h per day. The Rwanda Development Board regulations restrict observations to a maximum of four hours per day to reduce potential disturbance to the gorillas by human presence. We used data on matings observed during both focal sampling and ad libitum observations of the study female gorillas (as done in previous studies on mating behavior, [38, 39, 54]). The behavioral observations of each female correspond with the time period that fecal samples were collected for the hormone analysis (mean = 175 days; SD = 94 days, range = 66–358 days per female; Table 1). The data collection protocol by SH and his research assistant consisted of focal animal sampling of the study females only (2–4 h of focal time on 1–2 females per day). Additional data were collected by the staff of the Dian Fossey Gorilla Fund International as part of their long term data collection protocol. These assistants routinely conducted focal animal sampling (four different gorillas each day for 50 min each) on all adults, including nulliparous females, and the assistants regularly moved among all group members. We acknowledge that it is possible that some matings were not observed because we were not able to

Table 1
Social group, group size (number of group members), name, code, reproductive state, age of the female and her most recent infant, the sampling period, and the number of samples analyzed for the study females. Age and time period were calculated based on the starting date of fecal sample collection for hormonal analysis.

Name of the group	Group size	Name of the female	Code of the female	Reproductive state	Age of the female (years)	Age of the most recent infant (years)	Period covered by endocrine measurements and behavioral observations (consecutive days)	Number of fecal samples analyzed
INSHUTI	8	TAYINA[a,d]	TAY	Nulliparous	7.6	NA	316	125
ISABUKURU	10	BUKIMA[a,c]	BKA	Parous	16	NA	68	64
KURYAMA	15	MAHIRWE[a]	MHW	Parous	16	2.7	216	192
		MUGANGA[a,b]	MGA	Parous	14.7	NA	72	65
		UMUSATSI	UMU	Parous	26.4	3	188	163
NTAMBARA	11	KURINDA	KRN	Nulliparous	8.2	NA	163	116
		KUNGA	KUN	Nulliparous	7	NA	270	192
		TEGEREZA[a,b,d]	TEG	Parous	10.5	NA	358	191
PABLO	46	TURIBAMWE	TBA	Nulliparous	7.2	NA	66	44
		TURIMASO	TMS	Nulliparous	7.7	NA	66	45
TITUS	6	TUC	TUC	Geriatric	37.7	4.3	239	131
		UMWANA	UMW	Parous	27	3.7	148	128
UGENDA	14	UBUFATANYE	FAT	Nulliparous	6.8	NA	223	181
		IMVUNE[c]	IMN	Parous	10.9	NA	219	167
		KANAMA	KAN	Parous	15.3	3.7	92	81
		KUBANA	KNA	Nulliparous	7.3	NA	93	84

NA (not applicable).
[a] Females that pregnancy was detected.
[b] Females that miscarried.
[c] Females whose most recent infants died, the sampling periods for BKA and IMN started on the 1st and 36th day respectively after their infant died.
[d] These females were discovered to be pregnant during the observation period, so at that time we reduced the sampling regime to aim for collecting three samples per week during pregnancy.

spend all day with the gorillas. However, mountain gorillas mate approximately once every 1–2 h on days of presumed ovulation [54], so we assume that the 3.5–4 h of daily observation was sufficient time to observe at least one mating on a day that a female was sexually active. Furthermore, if some matings went unobserved, these are likely to be evenly distributed among all the females since all groups were observed on a daily basis, and all study females were sampled on a routine basis (e.g. no bias towards observing particular females).

2.3. Fecal sample collection and extraction

We collected fecal samples on an ad libitum basis for a period of at least 60 consecutive days per female with the intention of covering at least one ovarian cycle for each individual (mean collection period per female = 175 days, SD = 94 days, range = 66–358 days, N = 16 females). In total, we collected and measured 1969 fecal samples (809 from nulliparous and 1160 from parous females) that we obtained with an average rate of 0.75 ± 0.16 (median = 0.79, range = 0.4–0.94, N = 16 females) per day and per female. We placed fresh fecal samples in plastic bags, labeled them and put them in an insulated bag containing frozen ice packs [112]. On the same day of sample collection, we placed fecal samples in a freezer at −20 °C until we thawed them after a median time of 3 days (25th and 75th percentiles were 1 day and 5 days respectively) and dried them at 100 °C [113, 114]. Next, we ground dried samples with a mortar and a pestle, transferred them into labeled whirl pack bags and stored them dry at room temperature until we shipped them to Max Planck Institute for Evolutionary Anthropology in Leipzig.

We extracted steroids as described by Heistermann et al. [115]. Briefly, we mixed 0.1 g of dried fecal powder from each sample with 3 ml of methanol (80%) by using a multi-pulse vortexer for 15 min. After mixing and centrifuging, we removed a first supernatant. We added another 3 ml of methanol (80%) to the sample. After mixing and centrifuging as above, we decanted a second supernatant, mixed with the first supernatant, and stored the final volume at.

−20 °C until endocrine analysis.

2.4. Measurements of pregnanediol-3-glucuronide (PdG)

To detect the concentrations of PdG in fecal extracts, we applied an enzyme immunoassay (EIA) utilizing a polyclonal antibody R13904 (supplied by C. Munro, University of California, Davis, CA, USA) and followed EIA procedures described by Heistermann et al. [116]. Briefly, we diluted fecal extracts in assay buffer (1:10–1:50) and applied them in duplicates of 50 μl to the assay. We have previously validated this assay for mountain gorillas by demonstrating: 1) parallelism between curves obtained from the standards and serial dilutions of fecal extracts corresponding to cycling and pregnancy periods, and 2) a significant correlation with liquid chromatography mass spectrometry (LC-MS) measurements of pregnanediol in the same samples [117]. Since high performance liquid chromatography (HPLC) immunograms showed that the immunoreactivities of this PdG assay were not restricted to only pregnanediol [117], we referred to the metabolites detected by this EIA as immunoreactive PdG (iPdG). The sensitivity of the assay was 25 pg/50 μl at 80% binding. The intra- and inter-assay coefficients of variation of low and high value quality controls were 12.5% and 10.94% (N = 66) and 13.73% and 8.53% (N = 59), respectively. We expressed iPdG concentrations as ng/g dry feces.

2.5. iPdG data analysis

2.5.1. Detection of ovarian cycles

To quantify the occurrence of ovarian cycles, we developed an algorithm that was a modification of the method of Kassam et al. [1]. Originally, Kassam et al. [1] considered a nadir 5-days average of iPdG levels of a cycle as a baseline, a 3-fold or higher increase of iPdG levels above that baseline as a threshold of ovulation-related iPdG rise, and a sustained increase of iPdG levels above the threshold for at least three consecutive days as ovulation-related iPdG rise. For the present study, we used a threshold of two times the baseline for each female (excluding values when females were pregnant), which is the 10th percentile of the 5-day moving averages of iPdG values, because it allowed us to detect the maximum of visually identifiable ovarian cycles [110, 118–120]. To obtain data on follicular and luteal phases, our algorithm was optimized for performing the tasks (Fig.1).

We summarize the steps of the algorithm as follows. First, the algorithm calculated the baseline and threshold for ovulation-related iPdG rise in each female. Then, the algorithm detected the start date of each ovulation-related iPdG rise based on the criteria above and shifted it back one day to consider delayed excretion of steroid hormones in feces, which enabled us to correctly relate hormonal data to the corresponding behavioral data [121–123]. That day was marked as the presumed day of ovulation, which was considered as the end of the current follicular phase. Moreover, the algorithm detected the end date of each ovulation-related iPdG rise, shifted it back one day to consider delayed excretion of steroid hormones in feces [121–123], and marked it as the end of the current luteal phase. The algorithm constructed ovarian cycles such that a cycle is defined as a follicular phase followed by a luteal phase. The algorithm considered ovarian cycles only when the gap in sampling around ovulation-related iPdG rise was four days or less. If there was a gap, then the algorithm calculated the ovulation-related iPdG rise as the day in the middle of the gap. If the gap lasted for an even number days, the algorithm took the first day before the calculated middle of the gap as the ovulation-related iPdG rise. The algorithm considered an average follicular phase length if the luteal phase was preceded by a prolonged period of anovulation to allow for the calculation of follicular iPdG levels. To calculate the average length for either phase, we ran a preliminary version of the algorithm using data from only one female (i.e. TEG; Fig. 2), for which only the first three steps of this algorithm were applied, during which none of the detected ovarian cycles were altered using an average phase length for each phase. Based on these results, we calculated the average follicular and luteal phase lengths. We calculated a maximum acceptable follicular phase length by taking the average plus two standard deviations. We did the same for the maximum acceptable luteal phase length. If iPdG levels remained above the threshold, we considered this as a preliminary indicator of pregnancy. In this case, we considered the average luteal phase length for the luteal phase length of that conceptive cycle. The algorithm considered ovarian cycles as ovulatory if the follicular phase met the following criteria. First, for ovarian cycles whose follicular phase was less than or equal to the maximum acceptable follicular phase length, the algorithm kept the ovarian cycle if the follicular phase has no more than one consecutive sample above the threshold and the cycle is at least 10 days long. Second, for ovarian cycles with a follicular phase of greater than the maximum acceptable follicular phase length, the algorithm checked the characteristics of each of detected ovarian cycles as described above. If all criteria were met, the algorithm kept the ovarian cycles and considered the average follicular phase length for that ovarian cycle.

2.5.2. Definition of presumed fertile period, pregnancy and miscarriages

To examine the occurrence of matings when females were potentially able to conceive, we defined the 'presumed fertile period' as a five-day period including the day of ovulation, three days preceding the day of ovulation to account for viability of male gametes in female reproductive tracts [124], and one day after the day of ovulation to account for error in estimating ovulation [125].

We confirmed that females were pregnant when they exhibited persistently elevated iPdG levels above the threshold for ovulation-related iPdG rise for more than three weeks [5]. To characterize variation in iPdG levels during pregnancy, we calculated and reported the averages of iPdG levels in the first week after conceptive ovulation (estimating

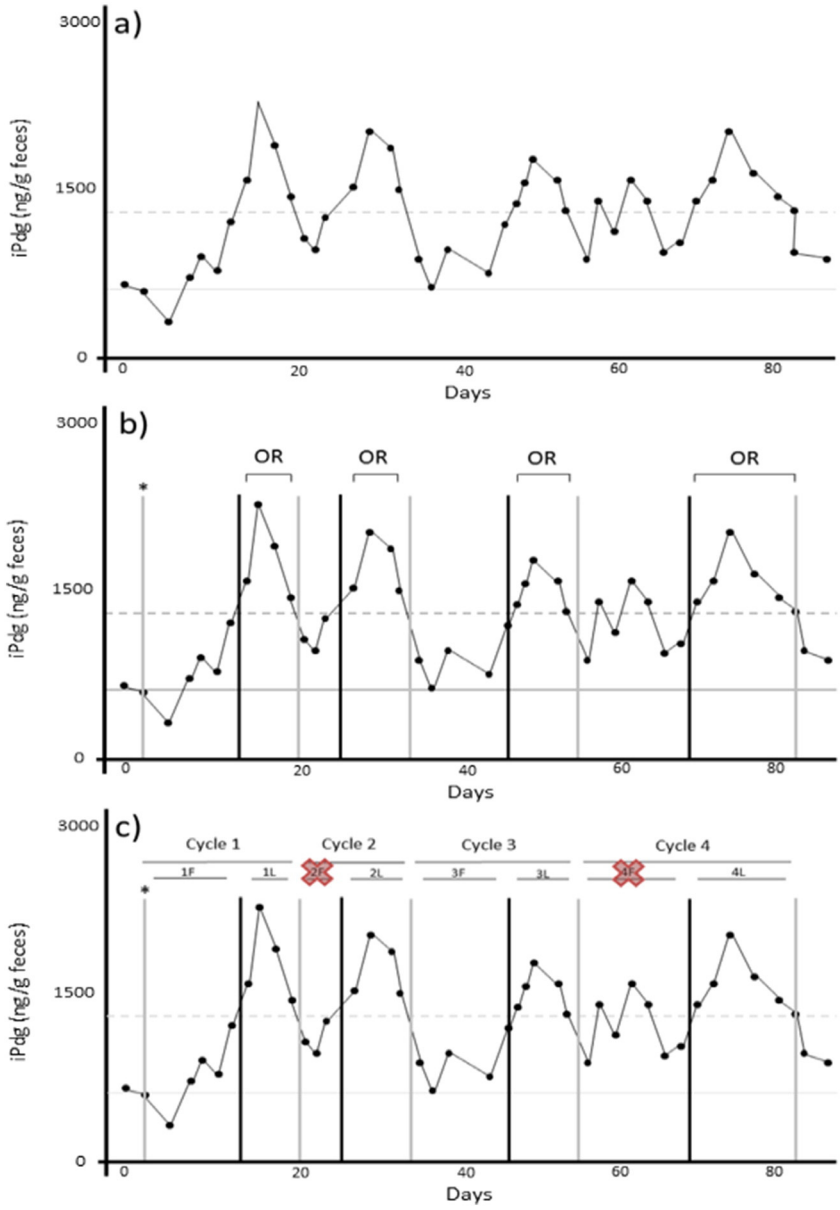


Fig. 1. Key-steps of the modification to the method of Kassam and colleagues [1] to detect ovulatory cycles using simulated data. a) The algorithm calculated the baseline and the threshold for ovulation-related iPdGs rise (i.e. the lowest and the highest horizontal lines respectively). b) The algorithm detected the start and end dates of ovulation-related iPdGs rise. Dark vertical lines indicate the beginnings of luteal phases. Light vertical lines indicate the start of follicular phases. c) The algorithm constructed all potential cycles and kept those that were not altered in their luteal or follicular phase. Illustrative scales are given for both x- and y-axes.

iPdG values at the start of implantation; [124]), in the second week after conceptive ovulation (estimating iPdG values towards the end of implantation; [124]), and in the third week after conceptive ovulation (estimating values of the beginning of embryonic period). For the females for which samples at the beginning of the pregnancies were not available (censored pregnancies, see [126, 127]), we calculated averages of iPdG for the periods for which samples were obtained. We calculated pregnancy length as the time between the presumed day of conceptive ovulation and the birth date of a live offspring. We considered miscarriages to have occurred when the detected pregnancies did not result in live births.

2.6. Statistical analysis

To test whether parous females were observed to mate on more days than nulliparous females, we used a Generalized Linear Mixed Model (GLMM) with Poisson error structure and log link function [128]. The response variable was the number of mating days during non-pregnancy periods (N = 15 females). The independent variable was female parity (i.e. nulliparous or parous). We did not include the geriatric female because we had only one female for that category. We considered the log-transformed number of female days as an offset term to control for the number of mating days per female days for each female (see [129]). We included the females' group identity as a random effect. To determine the statistical significance of the test predictor, we compared the fit of the full model with that of the null model (including only the random effect) using a likelihood ratio test [130, 131].

To test whether there was a difference in the proportion of days that we observed matings by pregnant and non-pregnant females, we ran a second GLMM with Poisson error structure and log link function [128]. We considered the number of mating days as the response variable and the log-transformed total number of female days as an offset term. We considered the physiological state of female (i.e. pregnant or

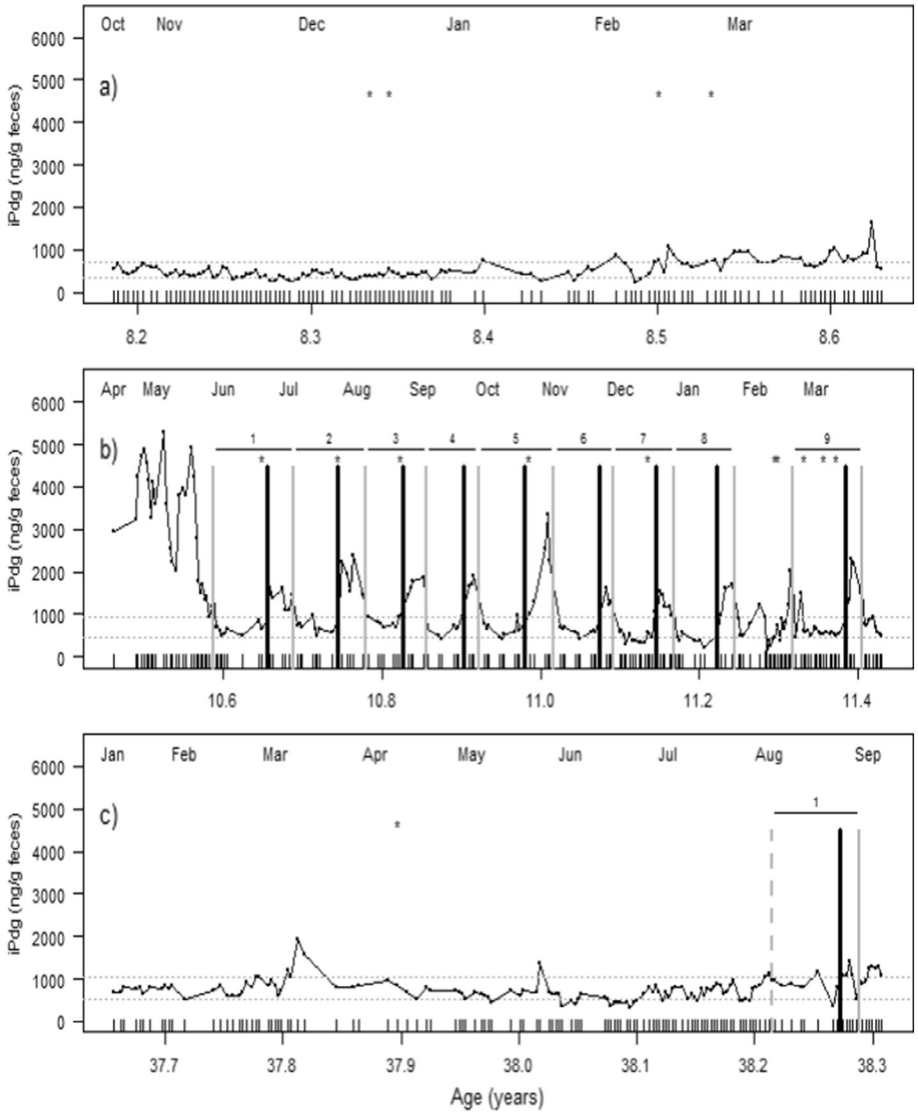


Fig. 2. Examples of iPdGs profiles for a) a nulliparous female (KRN), b) a parous female (TEG), and c) the geriatric female (TUC). y-Axes represent the concentrations of fecal iPdGs. Stars indicate mating days. For each female, the lowest and the highest horizontal lines represent the baseline and the threshold for ovulation-related iPdGs rises respectively. Dark vertical lines indicate the beginnings of luteal phases. Light vertical lines indicate the start of follicular phases. Small bars on x-axis show the density of samples collection. The months of samples collection are indicated by their initials on the top of the figure.

not) as the independent variable. We included female identity and group identity as random effects. The statistical significance of the full model was established as in the first model.

The assumptions of both models were met. We found that the dispersion parameters for the first and second model were 1.44 and 0.76, respectively, which is close to the ideal value of one, indicating a lack of overdispersion. We evaluated the stability of each model by removing each level of each random effect (i.e. each female and each group) one by one, rerunning the model, and comparing the coefficients to those from the original model using all data. We found both models to be stable.

3. Results

3.1. Occurrence of ovulations and ovarian cycles parameters

We detected a total of 23 ovarian cycles in nine of sixteen females (two of seven nulliparous females, and seven out of nine parous females, including the geriatric female). We calculated cycle lengths for only 13 ovarian cycles obtained from four parous females because we did not detect consecutive cycles in the other females. On average, we found that ovarian cycles lasted 29 ± SD 4 days (median: 28 days), with follicular phases being longer than luteal phases (i.e. 21 ± SD 3 days versus 8 ± 3 days). Expecting a maximum of one ovulation in every 28-day period, as this was the median cycle duration, for each female we took the observation period when she was not pregnant and divided it by 28 to calculate the occurrence of ovarian cycles across 28-day periods. We found that the occurrence of ovarian cycles was low in nulliparous females (i.e. two ovarian cycles across thirty-four 28-day periods; 6% of all 28-day periods), then increased in parous females (i.e. twenty ovarian cycles across forty-four 28-day periods; 45.5%) and decreased again in geriatric female (i.e. one ovarian cycle across eight 28-day periods; 12.5%) (Fig. 2). The mean ratio of follicular to luteal iPdG levels was 0.58 ± SD 0.13, with nulliparous and parous females exhibiting comparable mean ratios (i.e. 0.58 ± SD 0.05 and 0.58 ± SD 0.12 respectively). The ratio obtained from the geriatric female was elevated (i.e. 0.82), indicating a smaller postovulatory rise in iPdG levels as compared to other females.

A multiparous female (i.e. BKA) whose infant died at the age of 18 days showed the first ovarian cycle after nearly one month following the death of the infant and that ovarian cycle resulted in successful pregnancy (Fig. 3). A primiparous female (i.e. IMN) showed the first

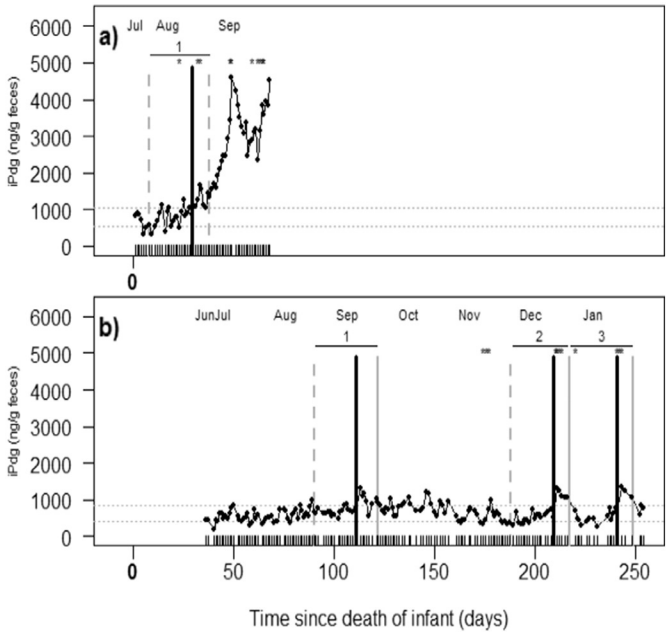


Fig. 3. iPdGs profiles for females who resumed cycling following the death of an unweaned infant: a) BKA (multiparous) and b) IMN (primiparous). x-Axes represent the time since the death of unweaned infant (Day 0). y-Axes represent the concentrations of fecal iPdGs. Stars indicate mating days. For each female, the lowest and the highest horizontal lines represent the baseline and the threshold for ovulation-related iPdGs rises respectively. Dark vertical lines indicate the beginnings of luteal phases. Light vertical lines indicate the start of follicular phases. Small bars on x-axis show the density of samples collection. The months of samples collection are indicated by their initials on the top of the figure.

ovarian cycle three and a half months after her infant died (at age 5.6 months) and regular ovarian cycles after seven months (Fig. 3).

3.2. Mating activities in relation to reproductive stages of the females

Overall, matings were observed on a total of 102 of 2797 female days (i.e. 4% of female days). All females mated, but matings during the presumed fertile period were observed during only five ovarian cycles of two parous females. We found no difference in the proportion of mating days between nulliparous and parous females that were not pregnant (likelihood ratio test comparing full and null model: $\chi^2 = 2.01$, df = 1, $p = 0.16$). We also did not find a difference in the proportion of days that matings were observed between non-pregnant females and pregnant females (likelihood ratio test comparing full and null model: $\chi^2 = 1.24$, df = 1, $p = 0.27$).

3.3. Occurrence of pregnancies and miscarriages

We detected pregnancies in iPdG profiles of five of the sixteen females. Three of the five pregnancies resulted in live births and the other two in miscarriages. We obtained samples around the conceptive periods only from one unsuccessful pregnancy and two successful pregnancies. Live births occurred after 248 days and 256 days following the presumed days of ovulation. Averages of iPdG levels measured in the two successful pregnancies showed a steady increase in the weeks following conceptive ovulation, with a similar increase observed from the first to the second week (1.5 times) and from the second week to the third week (1.4 times). We observed a comparable pattern in the unsuccessful pregnancy for which iPdG levels in the second week and the third week were 1.6 and 0.9 times the iPdG levels in the first week and the second week respectively.

For the female (TAY) whose hormonal data covered the entire period of her pregnancy and one month after, the averages of iPdG levels in the first, second, and third trimester of pregnancy increased 3.6, 8.3, and 15.3 times respectively above the threshold for her ovulation-related iPdG rise. The iPdG levels fell abruptly after the birth.

For one of the two miscarriages, the iPdG levels were above the typical level for the first trimester of pregnancy (i.e. 4 times the ovulation threshold), but then declined sharply, indicating that the miscarriage occurred at the beginning of the second trimester. We could not estimate when the other miscarriage occurred because that female exhibited elevated levels of iPdG until the end of sampling period but she did not give birth in expected period of normal pregnancy (8.5 months).

4. Discussion

In this study, we investigated the occurrence of ovarian cycles, matings, and pregnancies in nulliparous, parous and geriatric female mountain gorillas. Despite the sampling effort covering a total of eighty-six 28-day periods for 16 females over the course of a year, when broken down to individual research components/questions, our results are based on relatively small sample sizes. Nonetheless, data obtained from 23 ovarian cycles allowed us to establish that: 1) the average length of ovarian cycles of female mountain gorillas was in the range of cycle lengths previously estimated from field observations of matings in this species (i.e. median of 28 days; [54]), 2) ovarian cycles were more frequent in parous females than both nulliparous females and the geriatric female despite no difference in the proportion of days that matings by nulliparous and parous were observed, 3) and following the death of their unweaned infants, there was a longer delay in the resumption of ovarian cycle activity in a primiparous female than a multiparous female, which corresponds to the difference in time until conception. Furthermore, this study demonstrates that the period prior to first parturition (for nulliparous females) and the interval between births (for parous females) include miscarriages. The rate of miscarriages is higher than previously estimated, but is based on a small sample size (i.e. two miscarriages out of five detected pregnancies versus seven miscarriages for 214 births calculated from field observations; [3]).

Methods to detect ovarian cycles vary widely depending on the types of data and definitions of thresholds used to estimate ovulation, but they are expected to produce comparable results when applied in the same species or closest related species. For instance, using hormonal data our algorithm detected a similar median length of ovarian cycles (i.e. 28 days) as estimated from field observations of matings in the same population of mountain gorillas [54]. However, we detected longer follicular phases (i.e. average = 21 days) than those estimated from two ovarian cycles (i.e. 13–17 days) based on the detection of menstruation-related blood cells and ovulation-related peaks of estrogens in urine from this same population [92]. This average length of follicular phases was similar to the one determined in captive western lowland gorillas from fecal progesterone metabolites measurements (i.e. 18 days), and by using different definitions of thresholds for the ovulation-related iPdG rise (i.e. fixed value above the mean + 2.5SD obtained after iterative process of eliminating very high and low hormonal levels: [69]; mean + 2SD: [132]). Additionally, the mean length of luteal phase estimated here (eight days) was comparable to the mean length of luteal phase determined in western lowland gorillas (11 days; [69, 132]). In general, a practical advantage of our algorithm is that it produces reliable results using only one type of hormonal measurement. Overall, our algorithm is a useful method for field researchers interested in long-term monitoring of female fertility in the wild, especially in primate species that do not show any external signs of ovarian cycles.

van Schaik et al. [133, 134] proposed that lengthy follicular phases may have evolved in species with a multi-male-multi-female social structure as a strategy enabling females to mate over a long proportion of the cycle, avoid monopolization by males, and mate with multiple males to confuse paternity and therefore reduce the risk of infanticide. The follicular phase length measured in this study (i.e. mean: 21 days) is longer than in western lowland gorillas (i.e. 18 days; [69, 132]) and

falls into the range of chimpanzees and bonobos (i.e. median: 20 days; [135] and, mean: 20 days; [116] respectively). Therefore our results provide some support for van Schaik et al's predictions because although gorillas have a predominantly one-male mating system, other characteristics such as multimale groups, mating with more than one male, and infanticide occur in the mountain gorilla subspecies [36, 38]. However, our estimates of the length of follicular phase need to be taken with caution because high noise levels (which can be expected in hormone measurements of fecal samples; [109]) might lead to a delayed detection of a significant rise of iPdG levels and therefore to a slight overestimate of the follicular phase length. Indeed, urine-based measurements performed by Czekala and Sicotte [92] produced slightly shorter follicular phase length (i.e.13–17 days) which falls closer to the lengths calculated for captive western gorillas.

Our results suggest that female mountain gorillas experience an age-related pattern in ovarian function as found in humans (*Homo sapiens*) [108, 136], chimpanzees (*Pan troglodytes*) [113], lion-tailed macaques (*Macaca silenus*) [7], tamarins (*Saguinus sp.*) [137], eastern black rhinoceros (*Diceros bicornis michaeli*) [86], and spotted seals (*Phoca largha*) [138]. This pattern indicates that nulliparous female mountain gorillas experience a physiological transition to the full reproductive state, in which the probabilities of ovulation, normal luteal phases, and successful pregnancies increase over time. Although all seven nulliparous females were observed mating, ovarian cycles were detected in only two of them. This is informative as they were all around seven years of age, which is two years earlier than the average age of conception (average age of first birth is 10 years; [15, 19]), indicating that mating in nulliparous females starts before the occurrence of regular ovarian cycles.

Few studies have compared rates of ovulation in nulliparous and parous female mammals. Ovulations were more frequent in parous females (i.e. 45% of 28-day periods) but seem to occur at lower rates than in humans (e.g. 63–74% of potential ovarian cycles; [139]). This frequency of ovulation may be what really occurs or our algorithm missed some true ovarian cycles in cases where post-ovulatory iPdG rises were not high enough and sustained. This is less likely because our algorithm detected all ovarian cycles that were identifiable visually.

In the geriatric female, only one ovulation was detected in eight months. Physiological changes that influence ovarian function in aging females include problems in the secretion of gonadotrophins [140–142], failures in developing preovulatory follicles [143], and depletion in ovarian follicles [144–147]. It is unknown which of these occur in geriatric female mountain gorillas.

Our results suggest that the resumption of ovarian cycle activity in mountain gorillas not only depends on the suppression of lactation [148, 149], but also may be influenced by energetic balance of the female [150]. For example, primiparous females may have delays in resuming ovarian cycle activity because they are likely allocating energy to their own growth [151]. They might need more time than multiparous females to recover from their recent pregnancy, lactation, and to build up new reserves for the next pregnancy. Additionally, the age of the infant at death may potentially influence the resumption of ovarian cycles. However, this is unlikely to explain differences in interbirth intervals following the death of an infant between primiparous and multiparous females in mountain gorillas [3] and baboons [66], for which the age at death of the infants varied considerably.

Matings were observed outside the presumed fertile period as commonly occurs in other hominoid species (i.e. western lowland gorillas (*Gorilla gorilla gorilla*) [98], humans (*Homo sapiens*) [152, 153], chimpanzees (*Pan troglodytes*) [154], bonobos (*Pan paniscus*) [155], orangutans (*Pongo pygmaeus*) [156]), and in other mammals (e.g. giraffe (*Giraffa camelopardalis*) [84], and eastern black rhinoceros (*Diceros bicornis michaeli*) [86]). The absence of clear advertisement of ovulation and pregnancy in female mountain gorillas may explain why some matings occur out of the presumed fertile period in this species (See [87, 157]). It is also possible that female mountain gorillas are receptive outside the presumed fertile period so they can mate with multiple males and confuse paternity [156–158]. Additionally, matings after conception might be induced by internal stimuli associated with elevated ratios of estrogens and progesterone levels in pregnant females [159, 160].

Moreover, we have been able to detect and characterize pregnancies. First, we found a similar duration of pregnancy as Czekala and Sicotte [92] (i.e. 255 days), who used the peak of urinary estrogens to pinpoint the start of pregnancy. Second, similar gestation lengths have been estimated in captive western lowland gorillas (*Gorilla gorilla gorilla*) (i.e. 237–255 days; [132, 161]). Third, we found that female mountain gorillas exhibit a gradual increase of iPdG levels over the course of pregnancy as reported in several mammal species [103, 115, 162–167]. A steady increase of progesterone secretion during pregnancy is important because it induces uterine receptivity for the conceptus [168], and stimulates further development of the embryo [169].

Our study emphasizes that an accurate assessment of miscarriages in mountain gorillas can be detected only through regular endocrine monitoring. The patterns of iPdG levels measured did not reveal any predictive signal of the fate of pregnancy in mountain gorillas since the ratios between iPdG levels and respective thresholds were comparable between successful and miscarried pregnancies. Similar observations have been made in humans (*Homo sapiens*) [170], baboons (*Papio* sp.) [5, 171], and marmosets (*Callithrix geoffroyi*) [172]. Causes of miscarriages are rarely identified in wild female primates, but they might include chromosomal abnormalities of the fetus, uterine infections, endocrine dysfunctions and immune disorders of the mother [173]. Based on two miscarriages out of five detected pregnancies, our results suggest that female mountain gorillas could experience pregnancy failures at a higher rate than monkeys (e.g. baboons (*Papio* sp.): 9.6–13.9% of pregnancies detected after implantation; [103–105], langur monkeys (*Presbytis entellus*):16.5% of pregnancies detected after implantation; [106]), but at a comparable rate to humans (i.e.31% of pregnancies detected after implantation; [102]). Furthermore, this study confirms that both the age at first birth and interval between births may be lengthened by miscarriages [3, 21, 54].

5. Conclusion

In sum, our study demonstrates that the variability of fertility among females of different reproductive states is linked to the occurrence of ovarian cycles and mating during the presumed fertile period. To further our understanding of the variability in fertility of mountain gorillas, future studies should examine factors underlying differences in ovarian cycle activity and mating behavior among females of comparable reproductive status. Furthermore, understanding any underlying ecological, social and life history factors linked to the evolution of the lengthy period of adolescent subfertility and post reproductive lifespan would be a useful avenue of future research.

Acknowledgements

We are indebted to Max Planck Society, Deutsche Akademische Austauschdienst, the Leakey Foundation and the Dian Fossey Gorilla Fund International for supporting this research. We thank Thomas Lutz and anonymous reviewers for their valuable comments on this manuscript. We thank Glenn K Bush, Tony Mudakikwa, Felix Ndagijimana, and Veronica Vecellio who helped to implement this study. Christophe Boesch and Tara Stoinski are acknowledged for endorsing this study and providing helpful comments on an earlier version of this manuscript. We thank Roger Mundry for his help with statistical analysis. We thank Vera Schmeling and Róisín Murtagh for their assistance with hormonal analysis. We are grateful to Rwanda Development Board and Karisoke Research Center staff, especially Marie Rose Umuhoza and Joseph Mutabazi for their technical support in the field.

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