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A Study on Diagnosis of the Fertility of one Aged Female Gorilla by using the Fecal Sex Hormone Metabolites

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Abstract : The aim of this study was to diagnose the fertility of a female western lowland gorilla kept in Seoul Zoo, in accordance with age by analyzing the fecal sex hormone metabolites. The study was conducted in two period of times, when the animal was from 35 to 37 years old and when the animal was from 40 to 42 years old. Non-invasive method by using fecal samples was used for safe and efficient fertility diagnosis. We collected the feces from the enclosure at least three times a week. Then 17β-estradiol and progesterone, which are fecal sex hormone metabolites, were measured by time-resolved fluoro-immunoassay to compare the menstruation cycle and the annual reproductive cycle. For the duration of the primary study (when the animal was 35~37 years old), irregular menstruation and high concentrations of estradiol and progesterone were observed. However, menstruation was hardly observed and the concentrations of both hormones were statistically very low in the period of secondary study (when the animal was 40~42 years old). This observed phenomenon in our study was very comparable to menopause in adult women; therefore, it was confirmed that our female gorilla has reached menopause because of the natural aging, as they become older.

Key words **:** Estradiol, Gorilla, Menopause, Progesterone.

Introduction

In general, the breeding cycle of mammals is classified into seasonal estrus cycles and regular estrus cycles. Carnivores, which are estrous at certain times of the year, have seasonal estrus cycles (4); whereas human, gorilla, orangutan, and chimpanzee, which are estrous at regular intervals regardless of season, have regular estrus cycles (1,2,7).

Gorillas have regular estrus cycles and are expected to have many breeding opportunities, but often their breeding in the wild or in zoos has not been successful. In addition, they have been designated and managed as an international endangered species because of reduction in their population, as a result of habitat destruction and poaching (12). The closest relatives of the gorillas (*Gorilla*) are Chimpanzees (*Pan*) and humans (*Homo*), thus the reproductive physiology of gorillas is very important (13). The breeding of captive gorillas is important for maintaining the species and securing genetic diversity. Although gorillas have been kept in Seoul Zoo for years, there have been no births. Scientific verification was required for reproductivity because the female gorilla was becoming old.

Gorillas have a long life span, and 25% of an aged group showed menopause in a previous study (3); therefore, clear determination of the reproductivity is very important in a breeding program. We collected feces from a female gorilla and used an indirect diagnostic technique (15) to measure estradiol and progesterone, which are the fecal hormone metabolites of gorillas.

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Case

The female gorilla was registered as International Studbook No. 1550. It was captured in the wild in 1978; therefore, we do not have an accurate date of birth. The gorilla was transferred for exhibition at the opening of Seoul Zoo in 1984. At that time, based on the body size, it was estimated that the gorilla was born in 1973. The female gorilla has lived with two male gorillas.

One male gorilla (International Studbook No. 605) was captured in the wild in 1973 according to the records. It was transferred to Seoul Zoo in 1984. Any mating behavior and breeding were not observed, and this male died in February 2011.

Afterward in February 2012, another male gorilla (International Studbook No. 1323), which was born in Port Lympne Zoo in the UK in 1994, was transferred to Seoul Zoo with the help of the European Association of Zoos and Aquaria (EAZA). Several mating behaviors with this male gorilla have been observed, but breeding has not occurred yet.

The gorillas were fed on a diet of fresh fruits, vegetables, and specially prepared food every day and could drink freely fresh water in the Great Ape House of the zoo. The outdoor enclosure was 877 m^2 and enrichment facilities were set up for their behavioral activities.

The gorillas could access freely the inner enclosure and spend the night in separate enclosures. The inner enclosures and outdoor enclosures were cleaned at 8-9 a.m. every day in

order to maintain a hygienic environment. The female gorilla could meet the male gorilla freely except in the inner enclosure at night. The animal behavior was often observed by zoo keepers and images were recorded on CCTV for observation of unusual behavior such as fighting and mating. Gorillas aged 30 or older are classified as an aged group (3); therefore, the female gorilla was included in the aged group.

For study, feces (380 samples in total) of female were collected at least three times a week from January 2008 to June 2015. Samples were put into a plastic bag (Ziploc, Korea Johnson Co., Seoul, Korea) with a label of name, number, sex, and collection date and then stored at −80ºC to avoid the possibility of environmental agents affecting the steroid metabolites (5). Collected feces were lyophilized (FDU-2100, Tokyo Rikakikai Co. Ltd., Japan) for sample management under the same conditions and stable extraction of hormone metabolites. Then, the feces were ground to a fine powder before being stored.

Sexual steroid hormone metabolites were extracted from lyophilized feces. 10 ml of ether was added to 0.2 g of lyophilized samples and 2 ml of PBS (Dulbescco's phosphate Buffered Saline, Sigma Co., USA) and the mixture was stirred in a shaker for ten minutes for homogenization (17). Then it was centrifuged at 1500 rpm and 4ºC for ten minutes before storage overnight at −80ºC in a deep freezer (Deep freezer 995, Thermo Forma Co., USA). The next day, 10 ml of ether in supernatant was poured into another Falcon tube. Ether (Diethyl Ether, SAMCHUN PURE CHEMICAL Co., Republic of Korea) was evaporated with high-pressure nitrogen gas. After evaporation, one or two residue drops were dissolved in 1 ml Tris (Trizma base, Sigma Co., USA). Next, 50 µl was taken for measurement of sex hormone metabolites by time-resolved fluoroimmunoassay (Victor 2D, PerkinElmer Co., Filand). Before measurement, estradiol and progesterone were diluted 1~2 fold and 1~10 fold, respectively, depending on the type of hormone and period of the reproductive cycle. This study only provides results from analysis of the fecal sex hormones of the female gorilla. The results for the male gorillas will be introduced later.

The menstruation cycle was calculated on the basis of changes in estradiol and progesterone concentration in order to compare changes in hormones in accordance with age. The baseline concentration and the peak concentration of both hormones were estimated. Although there were many cases of an increase in estradiol in the gorilla, these were not always accompanied by an increase in progesterone; therefore, the menstruation cycle of the gorilla was calculated on the basis of progesterone. The menstruation cycle was determined on the basis of time from an increase from the baseline to time of the peak concentration of progesterone (3).

The concentrations of steroid hormones and estrus cycle length were expressed as mean ± SD. The samples were collected for a period of six years, but they were not collected every day. The statistical analyses were performed using SPSS (version 22.0; IBM corp., Armonk, NY, USA). Data were analyzed using independent samples t-test to determine the differences of fecal estradiol and progesterone levels between primary and secondary studies. Differences between times were regarded as significant when P values were less than 0.05.

Seoul Zoo Institutional Animal Care and Use Committee (SEOUL ZOO IACUC) approved this reproductive physiology project (SEOUL 2014-001) for primates. We complied with the Guide for the Care and Use of Laboratory Animals of the Animal and Plant Quarantine Agency.

Results

The period of the study was divided into the first period from 2008 to 2010 (35~37 years old) and the second period from 2013 to 2015 (40~42 years old). The peak concentration and the menstruation cycle of fecal estradiol and progesterone were substantially different between the primary study and the secondary study.

Primary study results: aged female gorilla in breeding season

In the period of the primary study, menstruation and the peak concentration of sex hormone metabolites were observed. One menstruation cycle at a 32 day interval was observed in the sample collected from May to June in 2008 (Fig 1a). Three menstruation cycles were observed from September to November in 2009 (Fig 1b). Two menstruation cycles were observed from February to May in 2010 (Fig 1c). However, after estradiol reached the peak concentration, progesterone

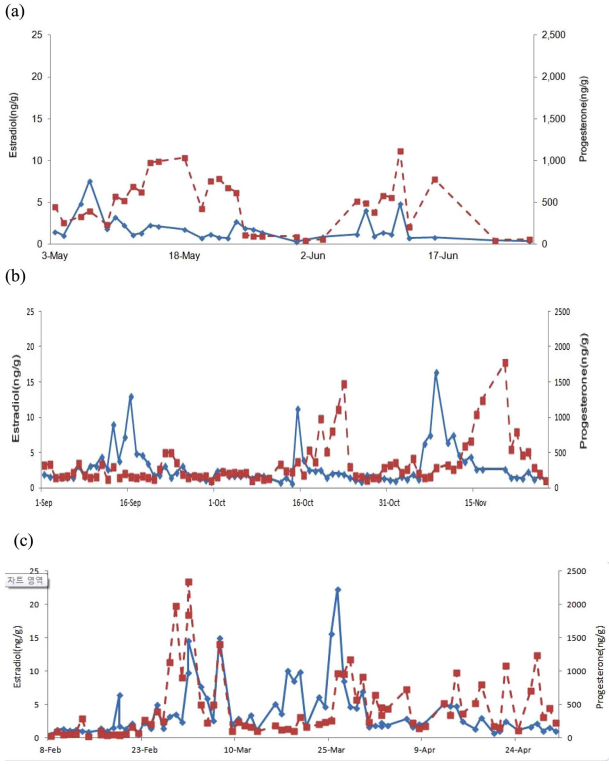

Fig 1. Fecal estradiol (blue diamond full line) and progesterone (red quadrangle dotted line) concentration in relation to age in breeding season. The menstruation cycle was irregular, but peaks for estradiol and progesterone were observed; therefore, it is suggested that the gorilla was progenitive. (a) May~June 2008, (b) September~November 2009, (c) February~April 2010; unit of measure (ng/g, hormone metabolites/feces).

Table 1. Peak concentrations of sex hormones and menstruation cycle in the aged female gorilla in breeding season

ID	Pregnancy	Estradiol peak concentration (n = 5) ng/g	Progesterone peak concentration (n = 5) ng/g	Estrus cycle (n = 6) day	Remarks
SD 1550	×	10.63 ± 4.50	1,180.95 ± 479.84	24.17 ± 3.76	

Mean ± SD.

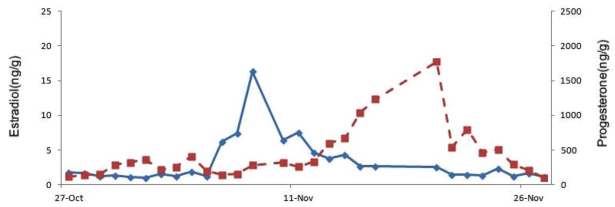

Fig 2. Menstruation cycle of the aged female gorilla. Progesterone increased five days after the peak concentration of estradiol was observed, and then the peak concentration of progesterone appeared in twelve days. The 28-day menstruation cycle was clearly observed, but this cycle was not always maintained.

did not always increase. The menstruation cycle was irregular, and this was comparable with previously reported results for progenitive aged female gorillas (3).

The menstruation cycle at these times was 26 ± 3.16 days based on the peak concentration of estradiol and progesterone (Table 1). The peak concentrations of estradiol and progesterone were 10.63 ± 4.50 and 1,180.95 ± 479.84 ng/g, respectively. These patterns suggest that fertility was maintained regardless of aging. However the menstruation cycle was irregular. At times when there was an increase in the peak concentrations of both estradiol and progesterone, the menstruation cycle was investigated in relation to changes in concentrations of the two hormones. The result (Fig 2) suggests that the female gorilla was progenitive, but no mating behavior and breeding were observed with the male gorilla SD No. 605.

The menstruation cycle and the peak concentrations of sex hormones were measured in the primary study. In this period, the gorilla was presumed to be progenitive, but an increase in progesterone was not always involved in an increase in estradiol.

Secondary study results: aged female gorilla in menopausal season

The pattern of sex steroid hormones in the secondary study was different from that in the primary study. Regular changes in estradiol and progesterone were not observed and concentrations of those two hormones were at the baseline. As a result, the menstruation cycle could not be calculated and the

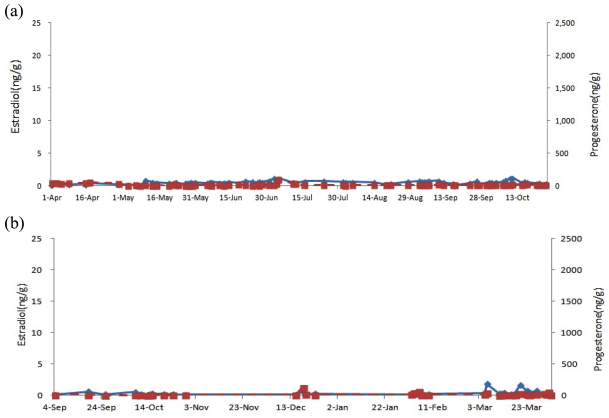

Fig 3. Fecal estradiol (blue diamond full line) and progesterone (red quadrangle dotted line) concentration in accordance with menopausal age. The menstruation cycle was not observed and the concentrations of estradiol and progesterone were at the baseline. (a) April~October 2013, (b) September 2014~April 2015; unit of measure (ng/g, hormone metabolites/feces).

dilution rate used in the primary study was not applied for the measurement of hormones (Fig 3).

The mean baseline concentrations of estradiol and progesterone in the non-menstrual period were 0.5 ± 0.3 and 18.92 ± 17.73 ng/g, respectively. Those were much lower than the baseline concentrations of estradiol and progesterone in the non-menstrual period in the primary study of 1.66 ± 0.58 and 205.58 ± 93.63 ng/g, respectively. Although mating behavior was observed with the new male (SD 1323), menstruation was not detected and pregnancy did not occur.

Comparisons of fecal estradiol and progesterone levels between primary and secondary studies

Fecal hormone levels were determined for 5 months in primary study and 10 months in secondary study. There were significant differences of sex hormone levels between primary and secondary studies (Table 2). The peak concentrations of estradiol and progesterone in the primary study were 10.63 ± 4.50 and 1,180.95 ± 479.84 ng/g, respectively. Peak concentrations of these hormone in the secondary study were significantly decreased when they were compared to those of the

Table 2. Peak concentrations and mean of sex hormones in the aged female gorilla

		Primary study (n = 5)	Secondary study (n = 10)	P value
Estradiol (ng/g)	Peak concentration	10.63 ± 4.50	0.73 ± 0.49	0.008
	Mean	2.45 ± 0.87	0.40 ± 0.22	0.006
Progesterone (ng/g)	Peak concentration	1,180.95 ± 479.84	50.85 ± 38.46	0.006
	Mean	394.15 ± 117.70	25.10 ± 14.93	0.002

Mean ± SD.

primary study ($p < 0.05$). Mean of estradiol and progesterone was significantly different between primary and secondary studies ($p < 0.05$).

Discussion

As the national competitiveness of animal resources owned by each country has received attention from around the world, studies on reproductive physiology to secure genetic diversity of species and increase populations have become more important (14,16,19). Foreign zoos and wild animal research institutes have been actively studying reproductive physiology since the late 1970s (6,8). However, these studies on wild animals in Korea have been started since 2005 with the supporting of scientific technology development (9,10,11). Our study which was conducted until 2015 has made it possible to observe changes in fertility depending on age, in this species and particular individual. It provides important, basic information about primates which are the closest species to human. Furthermore, Seoul Zoo is the only institution in Korea with gorillas. The reproductive potential of an old female gorilla (> 35 years old) is important for species management plans.

In previous studies on gorilla breeding, 25% of old gorillas aged 35 or older were menopause (3). In our primary study, irregular menstruation was observed, but there was no mating behavior or pregnancy because of probably reproductive incompetence of the male gorilla. In the secondary study, the menstruation cycle was not occurred, the fecal sex hormones were all at a lower baseline than that in the non-menstrual period reported in the primary study ($P < 0.05$). Menopause in apes is permanent and non-pathological. It arises from the reduction of ovulation rates and is dependent on age (18). In this study, it was confirmed that the female gorilla whose fecal estradiol and progesterone were at the baseline for 17 months was close to menopause; however, mating behavior was observed with a young new male in the period of the secondary study. Further behavioral and reproductive physiological studies to discover any correlation between fertility, mating, and menopause are required.

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