NOTE *Theriogenology*

Ovarian Activity and Pregnancy in the Siberian Tiger, *Panthera tigris altaica*, Assessed by Fecal Gonadal Steroid Hormones Analyses

Heri Dwi PUTRANTO[1,2], Satoshi KUSUDA[1,3], Kayo INAGAKI[4], Gaku KUMAGAI[5], Rie ISHII-TAMURA[6], Yoko UZIIE[6] and Osamu DOI[4]*

[1]*Laboratory of Animal Reproduction, United Graduate School of Agricultural Science, Gifu University, Yanagido, Gifu 501–1193, Japan,* [2]*Department of Animal Science, Faculty of Agriculture, University of Bengkulu, Jln. Raya Kandang Limun, Bengkulu 38371, Indonesia,* [3]*JSPS Research Fellow,* [4]*Laboratory of Animal Reproduction, Faculty of Applied Biological Sciences, Gifu University, Yanagido, Gifu 501–1193,* [5]*Tama Zoological Park, Hodokubo Hino, Tokyo 191–0042 and* [6]*Yokohama Zoological Gardens ZOORASIA, Kamishiranecho, Asahi-ku, Yokohama, Kanagawa 241–0001, Japan*

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ABSTRACT. Feces were collected from two female and one male Siberian tigers, *Panthera tigris altaica*. Steroid hormones were extracted from lyophilized feces and quantified by enzyme immunoassay. The fecal contents of estradiol-17β (E_2) and testosterone in the females and male, respectively, changed markedly throughout the year. The fecal E_2 contents of females Nos. 179 and 238 increased at 26.4 ± 8.0 and 28.0 ± 14.2 day intervals, respectively. However, the fecal contents of progesterone (P_4) in the female kept alone did not change. In contrast, the other female, which was kept with a male, had increased fecal P_4 contents after copulation. The fecal progesterone levels of the pregnant female remained high during her 106-day pregnancy.
KEY WORDS: fecal estradiol, progesterone, Siberian tiger.

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According to the 2006 IUCN Red List of Threatened Species [1], all living tigers (*Panthera tigris*) are categorized as endangered. The Siberian tiger (*P. t. altaica*) is an endangered tiger subspecies, and few are left in the wild.

Reproduction in Siberian tigers, as in other Felidae, remains poor and unclear. With its restricted population, it would be a challenge for scientists to learn and familiarize themselves with the reproductive potential of Siberian tigers to improve the breeding potential of captive tigers. Gonadal steroid hormone analysis is a major point in reproduction, and assessing its changes could allow the enhancement of captive breeding programs. In the last two decades, there have been 3 reports on the endocrinology of the Siberian tiger [5, 6, 9].

Serum hormones are the most accurate reflection of gonadal activity. Lately, some studies have shown similar patterns in serum and fecal hormones, and utilization of fecal samples as a noninvasive tool is widely used to monitor gonadal activity in farm, wild and zoo mammals [5, 8]. Most steroid hormones are metabolized in the liver, discarded into urine and waste material in feces, and finally removed from the body through the defecation mechanism. A previous report indicated that in domestic cats, > 85% of progesterone (P_4), estradiol-17β (E_2), and testosterone (T) are excreted in feces [3]. It has also been reported that monitoring fecal gonadal steroid hormone changes could indicate the characteristics of gonadal activity in some Felidae species, such as cheetahs (*Acinonyx jubatus*), clouded leopards (*Neofelis nebulosa*), snow leopards (*Uncia uncia*), leopard cats (*Felis bengalensis*), and tigers [2–6, 9].

* CORRESPONDENCE TO: DOI, O., Laboratory of Animal Reproduction, Faculty of Applied Biological Sciences, Gifu University, 1–1 Yanagido, Gifu 501–1193, Japan.

The present study was undertaken to illuminate the reproductive status of Siberian tigers by assessing the changes in the fecal contents of P_4 and E_2 in female tigers and T in a male tiger.

The animals monitored included a single female (Japanese Studbook No. 179, 13 years of age at the beginning of this study) housed at Yokohama Zoological Gardens, Kanagawa, Japan, and a female (No. 238, 6 years of age) and male (No. 239, 8 years of age) housed at Tama Zoological Park, Tokyo, Japan. All tigers were sexually mature based on their individual ages when this study was conducted. They were fed a diet consisting of horse meat and chicken heads.

Fecal samples were collected within 18 hr after excretion once or twice a week from November 2004 to October 2005 for No. 179 and from March 2004 to June 2005 for Nos. 238 and 239. They were stored at –20°C immediately after collection. The fecal extraction procedure was a modified version of that described in a previous report [7]. Briefly, the fecal samples were lyophilized for 48 hr and pulverized prior to extraction in order to separate the digested fecal material from the indigested bones and hair. Fecal powder (0.1 g) was solubilized in 5 m*l* of 80% methanol in water, mixed and shaken for 30 min. Each aliquot of solution was diluted 10 times with assay buffer.

The fecal contents of P_4, E_2, and T were determined by the enzyme immunoassay method [7]. The P_4 antiserum mainly cross-reacts with P_4 (100%), 5α-pregnanedione (62.2%), pregnenolone (6.3%), 11-deoxycorticosterone (3.9%), 17α-hydroxyprogesterone (2.3%), and 11α-hydroxyprogesterone (1.2%). The E_2 antiserum mainly cross-reacts with E_2 (100%), estrone-3-sulfate (8.0%), 16-epiestriol (5.3%), estrone (3.2%), and estriol (1.8%). The T

antiserum mainly cross-reacts with T (100%), 5α-dihydrotestosterone (41.3%), and androstenedione (2.1%).

All data are shown as the mean ± SD. The peak fecal E_2 contents were defined as those values that were greater than the mean of all values from an individual tiger. The length of the ovarian cycle was calculated as the number of days of the peak fecal E_2 interval for periods not exceeding 50 days (that is, longer than about twice the estimated cycle length).

In female Siberian tiger No. 179, which was kept alone, the fecal P_4 contents were an average of 0.78 ± 0.96 μg/g (n=100). Although only two changes were recorded on February and March 2005, no distinct cycles were shown in fecal P_4 contents (Fig. 1). On the other hand, the fecal contents of female No. 238, which was kept with male No. 239, increased remarkably after copulation on March 3 and April 24, 2004 (Fig. 2). The P_4 contents were approximately 2- to 6-fold higher than the mean value (5.20 ± 6.13 μg/g, n=107), but the first copulation did not result in pregnancy. The second copulation resulted in pregnancy, and the female gave birth on August 8, 2004. The length of the pregnancy was 106 days. During pregnancy, the fecal P_4 contents varied from 0.94 to 24.29 μg/g. From the beginning of pregnancy, the fecal P_4 contents increased and then decreased to baseline after parturition (Fig. 2). The duration of the increased P_4 contents after copulation differed between non-pregnancy and pregnancy. In leopard cats, clouded leopards, snow leopards, and cheetahs, it has been reported that the duration of increased fecal P_4 contents during presumed pseudopregnancy is about half the pregnancy length [4]. The main indicator to distinguish between pregnant and pseudopregnant conditions is the duration of increased P_4 content. Thus, P_4 monitoring can be used to confirm pregnancy in Siberian tigers.

The fecal E_2 contents in female Nos. 179 and 238 were an average of 0.39 ± 0.55 μg/g (n=100) with a peak of 1.37 ± 0.93 μg/g (n=14 peaks, Fig. 1) and 0.49 ± 0.61 μg/g (n=107) with a peak of 1.69 ± 1.04 μg/g (n=12 peaks, Fig. 2), respectively. They showed cyclic changes at 26.4 ± 8.0 day (n=13 cycles) and 28.0 ± 14.2 day (n=9 cycles) intervals, respectively. A similar finding was made in a previous report, which showed cyclic changes at 29.3 ± 4.4 day intervals in crossbreed Bengal-Siberian tigers [6]. Our findings suggest that fecal E_2 was probably excreted in parallel with follicular growth and that the remarkable changes in the fecal E_2 contents indicate a regular ovarian cycle.

The fecal E_2 contents of female No. 238 began to change remarkably from November 2004. The female's cub began to eat horse meat from about October 2004, and female No. 238 and the cub were released to the outdoor paddock on October 2, 2004. Weaning and natural light stimulation might affect the ovarian cycle, and the remarkable changes in fecal E_2 contents probably reflect recurrence of estrus. In male Siberian tiger No. 239, the fecal T contents varied from 0.02 to 2.45 μg/g (0.70 ± 0.05 μg/g, n=102 samples). The changes in the fecal T contents did not have any clear relevance to the periods of mounting behavior or estrus in female No. 238.

We conclude that it is possible to examine natural ovarian activity, such as functional luteal activity and follicular maturation, and to confirm pregnancy in female Siberian tigers by continuous fecal P_4 and E_2 analyses.

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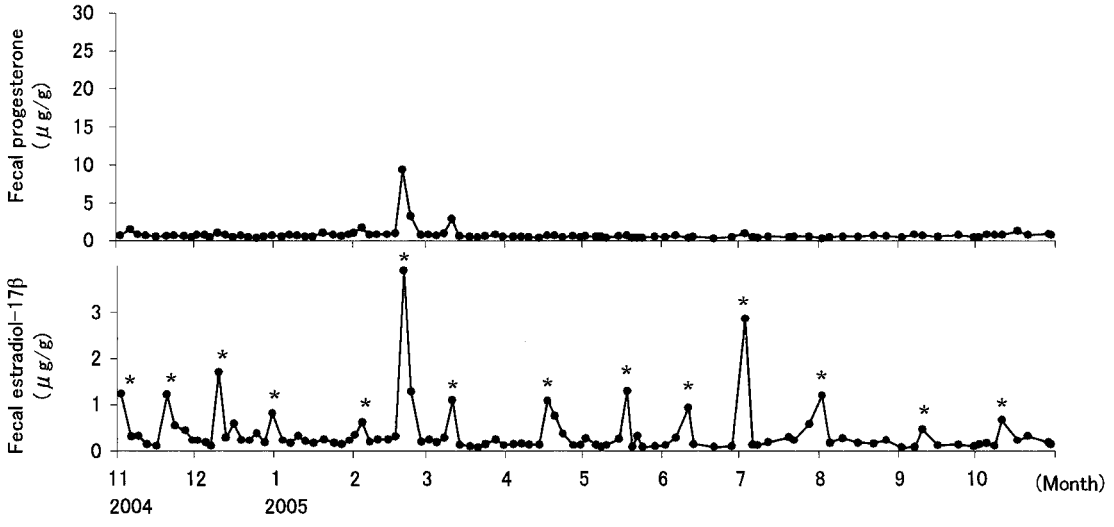

Fig. 1. Changes in the fecal contents of progesterone and estradiol-17β in female Siberian tiger No. 179, which was kept alone at Yokohama Zoological Gardens. The 14 asterisks indicate the peaks of fecal estradiol-17β.

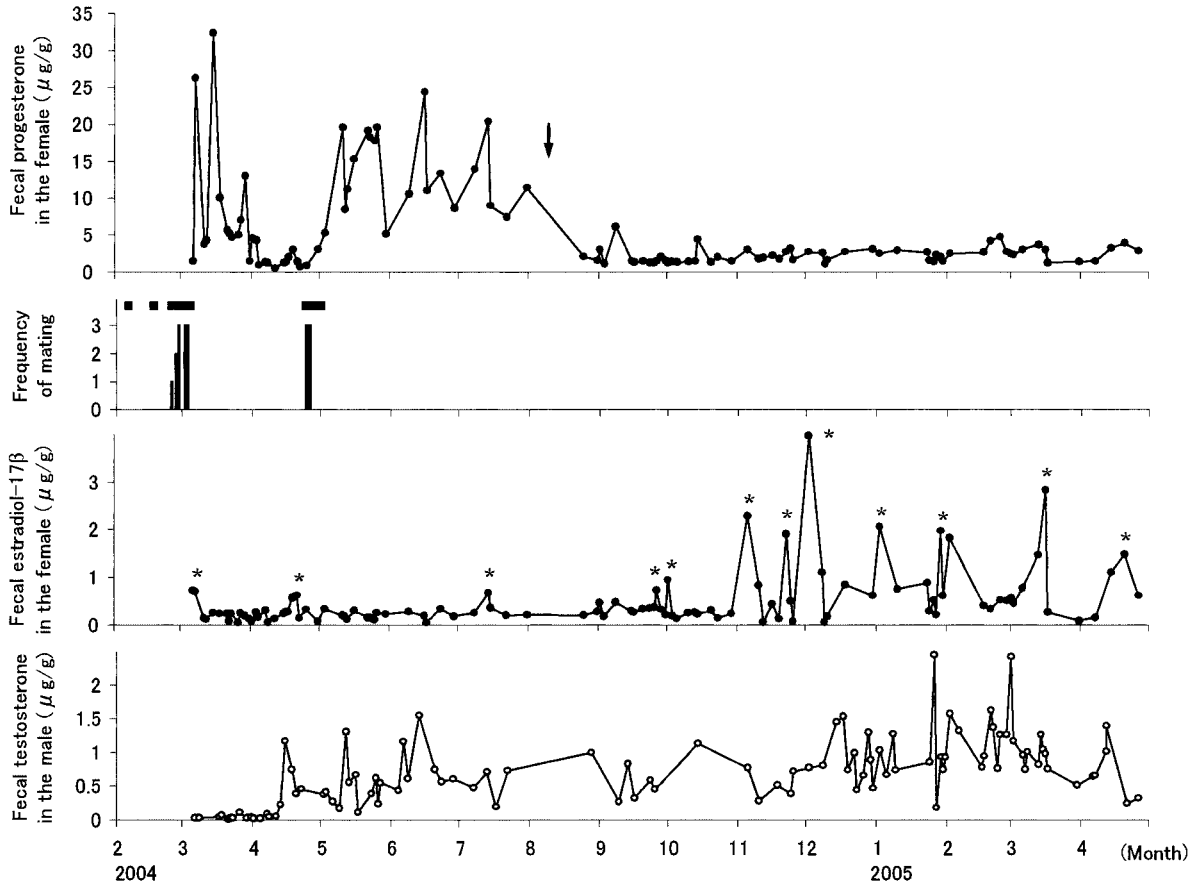

Fig. 2. Changes in fecal contents of progesterone and estradiol-17β in female Siberian tiger No. 238 (●) and testosterone in male No. 239 (○), which were both kept at Tama Zoological Park. The arrow indicates parturition. A higher score for copulation means higher frequency. Horizontal bars indicate the periods when the female was housed together the male. The 12 asterisks indicate the peaks of fecal estradiol-17β.

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