

Reproductive Pattern of the Sun Bear (*Helarctos malayanus*) in Sarawak, Malaysia

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ABSTRACT. Fecal progesterone assays were conducted in 3 captive female sun bears (*Helarctos malayanus*) segregated from males to characterize the species-specific reproductive pattern in their original distribution area in Sarawak, Malaysia. Peaks of fecal progesterone concentrations were observed once annually, and lactation was observed after increasing progesterone concentrations in all females without mating stimulus. These results suggest that sun bears in Sarawak, Malaysia, may have a seasonal reproductive pattern and ovulation was noted to occur spontaneously, followed by pseudopregnancy.

KEY WORDS: *Helarctos malayanus*, ovulation, pseudopregnancy, seasonality, sun bear.

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The sun bear (*Helarctos malayanus*) is the smallest bear among the Ursidae and distributing in Southeast Asia, including Borneo and Sumatra [11, 14, 16]. Although this bear is sometimes kept as pets illegally in this region, information on their population status is known little. Therefore, The World Conservation Union (IUCN) categorized this species as “Data Deficient” on the 1996 IUCN Red List of Threatened Animals because there is inadequate information to make a direct or indirect assessment of an extinction risk based on its distribution and/or population status [10]. However, it is believed that the population is in decline due to their habitat loss and being hunted for food and for use as traditional medicine [23]. In addition, little is known about the sun bear's behavior and ecology in the wild [11, 14, 16]. In captive populations, the effect of dissociative anesthetics and their reproductive pattern were studied [3, 5, 6, 12, 22]. The reproductive characteristics of female sun bears in captivity have been reported to include no seasonal reproductive pattern; parturition occurred throughout a year; the gestation period was from 95 to 230 days; delayed implantation was suspected; and their average estrus cycle length, as determined by fecal progesterone metabolite analyses, was from 5 to 7 months [2, 20]. However, the previous reproductive studies of female bears were conducted mainly in the Temperate Zone [2, 5, 6, 12]; thus, very little is known about the bears' reproductive pattern in their original distribution area.

Since 1997, we have observed two cases of lactation in non-pregnant sun bears at Semenggoh Wildlife Rehabilitation Center (SWRC) in Sarawak, Malaysia. The bears had been segregated from males since their arrival in 1996. Thus the lactation suggested that the sun bear may have a pseudopregnancy period in its reproductive cycle. The reproductive status can be monitored by observing ovarian activity. Progesterone (P₄) level is a reliable index for monitoring ovarian activity. Therefore, we conducted fecal P₄ analysis, which become a common method because it is a noninvasive sampling method. This report describes the

result of this analysis.

MATERIALS AND METHODS

Study area: Sarawak is located in the northwest sector of the island of Borneo, and it covers 124,450 km². The area lies between 0°50'N and 5°00'N, and between 109°30'E and 115°40'E. Because of its location, Sarawak's photoperiod varies little annually. The mean annual temperature at sea level is 27.0°C, and the annual variation in mean temperature is 1.0–2.0°C. The annual precipitation varies among recording sites, ranging between 2,571 mm and 6,661 mm. Prolonged rainy periods occur from November to February, and dry periods occur from June to August. [4]

Animals: Fecal samples were collected from three females (5, 7, and 11 years old) housed at SWRC. All of these animals were confiscated by the enforcement unit of the Sarawak forestry department from the general public for illegal captivity. During the study period (from August 1998 to July 1999), each bear was kept in a metal cage with a cement floor (4 m × 5 m × 5 m). There was a water bath (1.1 m × 0.8 m × 0.5 m) in each cage. Bananas, papayas, watermelon, sugar cane, and commercial dog food, along with multivitamin syrup and a calcium tablet, were given two times a day. Fenbendazole was given every 3 months to control parasite infection. These bears appeared healthy, and no abnormalities were observed during the study period. The bears were segregated from male bears during the study period.

Steroid assay methods: Fecal sample preparation was conducted with a modified version of the procedure described by Goeritz *et al.* [8]. In brief, 5 ml of 100% methanol was added to 0.5 g of wet fecal sample, agitated for 30 min and centrifuged (15 min, 112 × g), then 1.0 ml of the supernatant was submitted to the Advanced Diagnostic Laboratories (ADL) (Kuching, Sarawak, Malaysia). Laboratory technicians in the ADL then conducted further dilution and processing. P₄ was quantified with Enzymum-Test® Sys-

tems (Roche Diagnostics, Germany), that is a specific enzyme immunoassay kit for the Boehringer Mannheim ES300 system (Roche Diagnostics, Germany). This procedure can extract and measure an unconjugated P_4 form in feces and the concentrations were expressed as ng/g. Two fecal samples (mean P_4 values were 15.0 ng/g and 55.7 ng/g) gave a coefficient variations of 4.8% and 5.0% for intra-assay ($n=6$), and 20.8% and 16.8% for inter-assay ($n=6$), respectively.

Investigation for the correlation between serum and fecal P_4 : Prior to collecting fecal samples, an investigation for the correlation between serum and fecal P_4 was conducted. Blood samples by jugular venipuncture with a disposable syringe were obtained from the three female sun bears under immobilization with a mixture of 50 μ g/kg of medetomidine solution (Domitor[®], 1 mg/ml, Orion Corp. FARMOS, Turku, Finland) and 2 mg/kg of zolazepam-tiletamine solution (Zoletil 100[®] 100 mg/kg Laboratories Virbac B.P., France) (MZT mixture). The mixture was administered by a CO₂-powered rifle (Telinject[®] Vario 1V., Telinject U.S.A., Inc., California, U.S.A.). Three to four samples were collected from each bear, for a total of ten blood samples. To separate the serum, the blood samples were transferred to test tubes and centrifuged for 30 min at $1,008 \times g$. Fecal samples were collected from the rectum at the time of blood sampling or after recovery from immobilization on the same day. Sera and fecal extractions were presented to the ADL immediately, and the same immunoassay kit and the system mentioned above were utilized for analyzing both types of sample materials.

Fecal sample collection and the examination of lactation: Fecal sampling was conducted every 10 to 14 days from 15 August 1998 to 9 July 1999. Samples were frozen (-20°C) until extraction was performed. Twenty-five fecal samples each were collected from the 5- and the 7-year-old female, and twenty-two from the 11-year-old female. The 11-year-old female was immobilized with MZT mixture in February 1999 and the other females were immobilized in March 1999, to check for the occurrence of lactation by squeezing the nipples.

Statistical Analysis: The Pearson correlation coefficient with p -value is given for the correlation between fecal hormone excretion and serum hormone levels performed with Microsoft EXCEL97 for Windows.

RESULTS

Correlation between serum and fecal progesterone concentrations: The progesterone concentrations showed a significant positive correlation with the fecal extraction and serum samples ($r=0.784$, $p<0.01$, $n=10$) (Fig. 1).

Annual changes in P_4 concentrations in feces: The three bears showed similar changes in fecal P_4 concentrations during the study period (Fig. 2). The P_4 concentrations ranged from 32.2 ng/g to 157.3 ng/g. The concentration showed a peak value in November 1998 (152.1 ng/g in the 5-year-old female and 102.7 ng/g in the 11-year-old female) or January

1999 (157.3 ng/g in the 7-year-old female). Subsequent to reaching these peak values, the P_4 concentrations decreased to 40–60 ng/g in all bears. Only one obvious peak was observed in each of the three sun bears during the study period.

Lactation: Lactation was observed by nipple squeezing in all three of the sun bears (Fig. 3).

DISCUSSION

We hypothesized that the sun bear may have a pseudopregnancy period in its reproductive cycle. In the present study, the three females showed increasing fecal P_4 concentrations and lactation without mating stimulus. An increased serum P_4 level has been reported in pregnant polar bears (*Ursus maritimus*), pregnant American black bears (*Ursus americanus*), pregnant Hokkaido brown bears (*Ursus arctos yesoensis*) and non-pregnant Hokkaido brown bears that were segregated from males during breeding season [7, 15, 25, 26]. Non-pregnant and pregnant Hokkaido brown bears exhibited the similar annual change of serum P_4 levels, and a similar phenomenon was reported in unmated female American black bears [24, 25]. Although it has been assumed that ovulation in bears occurs in response to a mating stimulus [29], the event of spontaneous ovulation and pseudopregnancy in Hokkaido brown bears and American black bears were suggested [24, 26]. The results of the present study also suggest the occurrence of spontaneous ovulation and pseudopregnancy in the sun bear. This suggestion was supported by the observation of lactation in all three bears between February and March 1999 (Fig. 3).

Although the sun bear has been considered to be a non-seasonal breeder [20], a peak of P_4 concentrations was

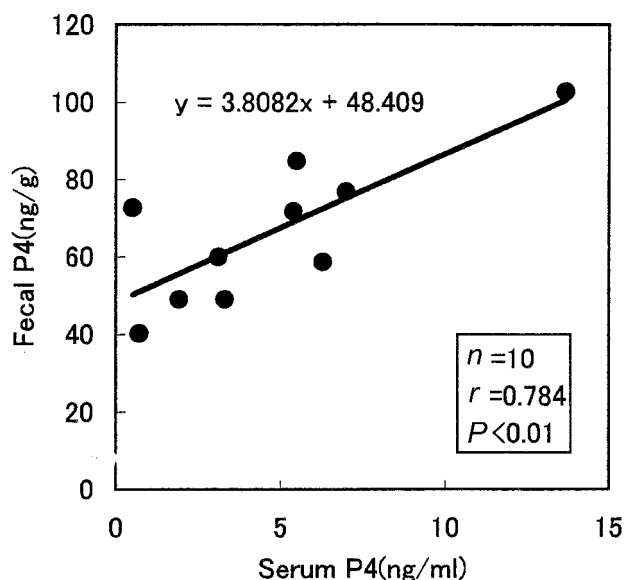


Fig. 1. Correlation between fecal and serum progesterone concentrations in sun bears measured by EIA using the Boehringer Mannheim ES300 system.

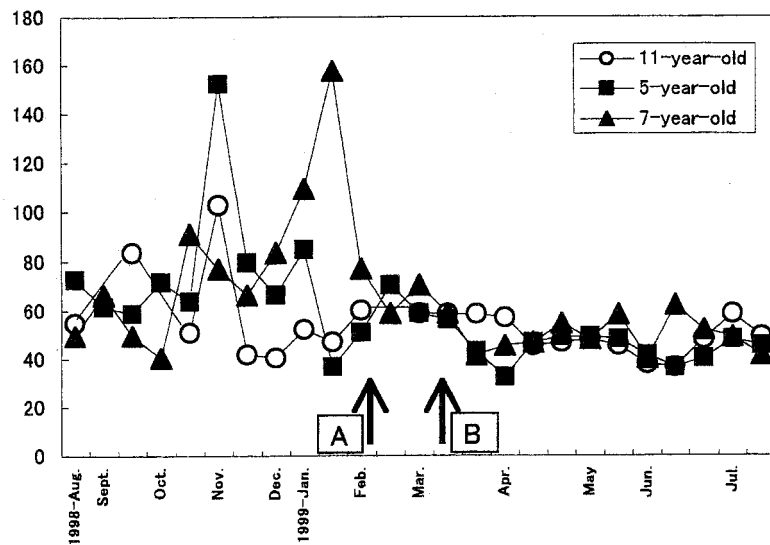


Fig. 2. Annual changes in fecal progesterone concentrations in three female sun bears. Two arrows indicate the time when the bears were immobilized to check lactation (A: 11-year-old female, B: 5- and 7-year-old females).



Fig. 3. Lactation was observed in all of three sun bears without mating stimulus.

observed once during a 12-month period, which suggests that the sun bear may have a breeding season in Sarawak. Breeding season was observed in some species in tropical areas during the rainy season [1]. Notably, the rainy season in the study area lasts from November to February. Flowering and fruition around this region occur during the rainy season [28]. Food resources are closely linked to reproduc-

tive success [19]. Thus, sun bear gestation might be associated with the rainy season, at which time high food availability would contribute to increased nutritional needs. On the other hand, the period from June to August in the study area is dry. The high temperature results in low sperm quality and testosterone concentration [1]. In female animals, increased incidences of following, in response to elevated temperature, were reported: decrease in gonadotropin levels and follicular growth; higher occurrence of abnormal ova; lower conception rates; and high embryonic mortality and teratology [1]. Thus, it would be beneficial for the sun bear to gestate during the rainy season not only from a nutritional aspect but also relative to gonadal organ function. However, confirmation of this hypothesis requires large-scale observation not only of captive sun bears in the original distribution area but also of the wild population.

Delayed implantation, which phenomenon occurs in other species of bears, has been suspected to occur in the sun bear [20]. The results of the present study cannot confirm this event, though our findings suggest the possibility of seasonal breeding in the sun bear, in which case delayed implantation might be an useful characteristic by which to cope with deficiency in food resources, which might result from unexpected climate changes (e.g., prolonged dry season). Delayed implantation in bears was directly confirmed by embryo recovery in the Hokkaido brown bear [27]. Unfortunately, the facilities at SWRC veterinary clinic do not allow us to conduct this procedures in the sun bear. Alternatively, the measurement of estrogen concentration will help detecting delayed implantation indirectly. During delayed implantation, no increase or tendency to decrease of estrogen levels was reported in several species [13, 15, 17, 18, 21, 24]. Observation of estrogen values in the serum,

urine, or feces of mated female sun bears for at least one year is essential to find possibility of delayed implantation.

The combination of methanol extraction and Enzymum-Test® Systems was applied to measure fecal P₄ levels in this study. Although the result showed a significant positive correlation between the fecal P₄ concentrations and serum P₄ value, this method tended to indicate a higher fecal P₄ value when the serum P₄ level was low. This might be understood from the difference of recovery rate of steroids in feces due to variation of fecal contents or conversion from conjugated P₄ to an unconjugated form by intestinal micro-organisms [9]. We recommend, therefore, that the same food items must be fed and fecal samples must be frozen at a fixed time after defecation during a study period. However, observing longitudinal fecal P₄ profile is more important than measuring an exact fecal P₄ value to monitor annual ovarian cycle. Thus our method was suitable to achieve the objective of the present study.

In conclusion, two things are suggested by the present study, 1) the sun bears may have a breeding season that is associated with the rainy season in its area of distribution, and 2) spontaneous ovulation and pseudopregnancy occur in the sun bear.

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