Fecal Steroid Profile of Female Javan Gibbons (Hylobates moloch) Maintained in Pairing-Typed Cage

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Estrone conjugate (E1C) and pregnanediol glucuronide (PdG) were predominant steroid metabolites of estrogen and progesterone in feces of most primates and could be used to evaluate ovarian function. These metabolites were determined along with records of genital swelling throughout 3-4 months period from three female Javan Gibbons (Hylobates moloch) maintained in pairing-typed cage at Schmutzer Primate Center, Jakarta (Ullah) and at Taman Margasatwa Taman Sari, Bandung (Donna and Citah). Following methanolic extraction of lyophilized fecal powder, samples were analyzed using enzyme linked immunosorbent assay (ELISA) for E1C and PdG. In all of the three females observed, both hormone profiles did not indicate any regular cycle of ovarian function even though genital swellings were sometimes observed. In one female (Donna) the hormone patterns showed clear signs of cycle irregularities with extended luteal phase of 40 days and erratic pattern of follicular phase. Of the other two females, no ovarian cycle was found.

The data indicate that the fecal steroids analysis is a practical and valuable diagnostic tool for providing reliable information on ovarian function in Javan Gibbon. Factors affected reproductive hormonal profile should be taken in consideration in trying to achieve success in captive breeding program for this species.

Key words: Javan Gibbon, fecal steroid, genital swelling

INTRODUCTION

The Javan Gibbon or Owa Jawa (Hylobates moloch) is an endemic species in Java and critically threatened. This species is listed on the Red list of Threatened Animals, IUCN as ‘endangered’ and also listed on the Appendix I in CITES (Hilton-Taylor 2000). IUCN/CBSG Primate Working Group registered this species as a critically endangered species. The number of wild Javan Gibbons (Hylobates moloch) maintained in pairing-typed cage at Schmutzer Primate Center, Jakarta (Ullah) and at Taman Margasatwa Taman Sari, Bandung (Donna and Citah). Following methanolic extraction of lyophilized fecal powder, samples were analyzed using enzyme linked immunosorbent assay (ELISA) for E1C and PdG. In all of the three females observed, both hormone profiles did not indicate any regular cycle of ovarian function even though genital swellings were sometimes observed. In one female (Donna) the hormone patterns showed clear signs of cycle irregularities with extended luteal phase of 40 days and erratic pattern of follicular phase. Of the other two females, no ovarian cycle was found. The data indicate that the fecal steroids analysis is a practical and valuable diagnostic tool for providing reliable information on ovarian function in Javan Gibbon. Factors affected reproductive hormonal profile should be taken in consideration in trying to achieve success in captive breeding program for this species.

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Javan Gibbons are arboreal animal and live in primary and secondary tropical rainforest, from sea level up to 1500m above sea level and are found only on the island of Java, and specifically only in West Java and the western parts of Central Java. They live in a group with monogamy style consisting of a prime couple of adult male and female together with one to four juveniles and young (Chivers 1989).

Considering with the status of the Javan Gibbon, it is essential to develop and implement a conservation program, particularly for increasing their population growth as well as to manage its habitats. To date, the captive population which are mostly maintained in zoos are being managed to achieve success in promoting population growth. However, there are still numerous problems associated with breeding of this species in captivity, including high proportion of non-reproducing adult female. The causes for the difficult to breed this species are not clear, but are likely to be both behaviour and reproductive physiology basis (Leighton 1987).

Lack of information on reproductive biology of this species may contribute to the unsuccessful breeding program at present. Studies dealing with endocrinology of reproduction in gibbons (Hylobatidae) referred to Hylobates lar (Nadler et al. 1993; Barelli et al. 2007) or other species of Hylobates. Our previous study of fecal steroid analysis for female Javan Gibbon maintained in individual cage, we found that the individual variation was high (Maheshwari et al. 2007). Reports on reproductive hormone profiles and genital swelling in
female White-handed Gibbon (Dahl & Nadler 1992; Nadler et al. 1993; Collins et al. 1994) were also valuable for Javan Gibbon. This study was designed to evaluate the ovarian function of the female Javan Gibbon kept in pairing-typed cage by measuring their fecal steroid estrone conjugate (E1C) and pregnanediol glucuronide (PdG), and combining with the observation of genital swelling and menstruation blood. The information obtained will be valuable in improving management system of these species to achieve their captive breeding success.

MATERIALS AND METHODS

Animals and Housing. Prior to this study, survey was conducted to find the location where the female maintained. Three sexually adult female Javan Gibbons, non pregnant, 7-8 years of age and 6-8 kg of body weight with no history of breeding were used in this study. One of the female is maintained at Schmutzer Primate Center, Jakarta (Ullah, Figure 1) and the other two females (Donna & Citah, Figure 2) are kept at Taman Sari Zoo, Bandung, all with in pairing-typed cage. The size of the cage at Schmutzer Primate Center is (17 x 9.0 m) x 14.5 m in height, whereas the cage size at Taman Sari Zoo is (4.5 x 4.0 m) x 6.0 m in height. A mixture of chopped fruits and vegetables was given twice a day, and access to water was ad libitum.

Sample Collection and Genital Observation. Fecal sample was collected between 07.00 and 08.00 am, 5-7 days per week over 3-4 months period, and following collection, samples were immediately stored at -20 °C without preservative until assayed. Daily records of menstruation were monitored and visual inspections of the genital swelling were carried out everyday at the same time. The degree of genital swelling was scored as (0) no swelling, (1) partial swelling, no color change and no discharge, (2) relative increase in swelling, reddish but no discharge, (3) maximum swelling with discharge and red in color (Dahl & Nadler 1992; Czekala & Sicotte 2000).

Sample Preparation. Prior to analyze, the samples were extracted as described by Heistermann et al. (1993) for E1C and PdG measurements. A total amount of fecal

Figure 1. Schmutzer Primate Center, cage size: (17 x 9.0 m) x 14.5 m in height.

Figure 2. Taman Sari Zoo, cage size: (4.5 x 4.0 m) x 6.0 m in height.
samples collected were first lyophilized, and the resulting dried pellets were pulverized and extracted with 3 ml of 80% methanol in water by vortex for 10 minutes followed by centrifugation at 2200 x g for 10 minutes. The supernatant was decanted into a clean glass tube and after appropriate dilution in assay buffer (5.96 g Na2HPO4, 8.50 g NaCl, and 1 g BSA Fr. V in 1 L H2O, pH 7.2) was taken directly to assay.

**Assay Validation.** Validations of assay were evaluated by monitoring individual extraction efficiencies, parallelism test, intra- and interassay coefficient of variation of quality control at low and high concentration, and the concentration at which 90% of the antibody binds to the conjugate (sensitivity). Individual extraction efficiencies were monitored using the recovery of [3H]-progesterone (35,000 cpm; NEN Du Pont, Bad Homburg, Germany), which was added to the fecal powder before extraction. Parallelism test was performed to the fecal extracts in serial dilutions using E1C and PdG assays.

**E1C and PdG Analysis.** Microtitreplate EIAs previously characterized by Heistermann and Hodges (1995) were used to determine immunoreactive E1C and PdG in feces. The samples were diluted in assay buffer with a certain dilution depending on the reproductive status.

**Data Analysis.** The follicular phase was defined as the interval between the first day of menstruation until the day of the E1C peak, whereas the luteal phase comprised the interval from the day after the E1C peak until the day before the menstruation. A threshold value of two standard deviations (SD) above the mean of the preceding follicular phase values was taken in order to determine the first increase in fecal PdG concentration. An increase in concentrations above this threshold value indicates a statistically significant rise with P < 0.05 (Jeffcoate 1983).

**RESULTS**

**Validation of Assays.** Recovery of [3H]-progesterone gave the mean ± SD values ranged from 78.2 ± 6.6%. Serial dilutions of fecal extracts gave displacement curves parallel to those of E1C and PdG standards (Figure 3). Intra assay coefficients of variation calculated from replicate determinations of fecal quality control pools gave values of 8.6% for low concentration and 6.9% for high

![Figure 3. Parallelism Test of E1C (A) and PdG (B) Assays.](image)

**Figure 4.** Profiles of genital swelling (a), E1C (b), and PdG (c) in feces of female Javan Gibbon (Ullah) during the 4-months period of observation.
concentration, whereas inter assay coefficients of variations were 14.2% for low concentration and 9.4% for high concentration. Sensitivity of assay was 1.56 pg for E$_1$C and 25 pg for PdG per well.

**Fecal E$_1$C and PdG Profiles.** The profile of fecal E$_1$C and PdG in individual female was depicted in Figure 4 for Ullah, showed the patterns of both hormones that did not reflect any regularity of ovarian function. The genital swelling profile also showed no correlation with the concentration of E$_1$C throughout the period of observation. The level of E$_1$C was fluctuated erratically closed to the basal concentration (0.07-0.13 mg/g), although several high concentration was reached at the level of 0.27-0.33 mg/g, however did not seemed to be periovulatory increase. Compare to the profile of E$_1$C which seemed to be more vary in fluctuations, the profile of PdG showed patterns with some fluctuation.

Similar to Ullah’s, the profile of E$_1$C and PdG of Donna (Figure 5) also indicated irregularities in the cycling, with the concentration of E$_1$C ranged from 0.03-0.15 mg/g without any significant increase. During the observation, there was two pattern of PdG indicated luteal phase with extended follicular phase. The degree of swelling and changes in genital swelling showed no fluctuation during the cycles observed which may consistent with the low level of E$_1$C. No menstruation blood flow was seen for this female.

The profile of E$_1$C and PdG of Citah (Figure 6) also indicated that this female was not cycling, and its E$_1$C’s level ranged from 0.03-0.13 mg/g without any significant increase.

![Figure 5. Profiles of genital swelling (a), E$_1$C (b), and PdG (c) in feces of female Javan Gibbon (Donna) during the 3-months period of observation.](image)

![Figure 6. Profiles of genital swelling (a), E$_1$C (b), and PdG (c) in feces of female Javan Gibbon Citah during the 3-months period of observation.](image)
increase. Changes in genital swelling and menstruation blood flow was not seen for this female as well. Without menstruation blood flow that could be seen, the overall conclusion was difficult to draw for this female.

**DISCUSSION**

This study focused on E\(_1\)C and PdG because these hormones are known to be primary or major metabolites in other primates (Ziegler et al. 1989; Čezkala et al. 1991) and the enzyme immunoassays for this metabolite were immediately available. The present study can also be applied to examine the relationship between the pattern of female genital swelling and underlying hormonal changes during the ovarian cycle. Compare to the menstrual cycle (Barelli et al. 1994), genital swellings were programmed to respond only to pulsatile Gonadotropin-gonadotropin is required since the gonadotropin is a cyclic pattern that coincides with the fertile periods (Hodgkiss 2007), and contrast, the sexual swellings of four females did not coincide with the fertile periods (Hodgkiss 2007), and Anzenberger (2009) found that the average of ovarian cycle in *Hylobates* spp.: 20.0-25.4 days, and longer menstrual cycles in *Hylobates moloch* reported for an average of 25.4 ± 8.4 days, including some unusually long cycles of up to 38 days (Hodgkiss 2007). Cycle durations in gibbons could be determined using different markers such as endocrinological methods, genital swelling, menstrual bleeding, or copulation observation. Very long cycles might be resulted from determination using copulation marker as gibbons copulate in very short time and easily escape detection, and may even occur during the night (van Schaik et al. 1999; Hodgkiss 2007). Cycles may also remain undetected if menstrual bleedings are used as marker.

In captive breeding management of primates, monitoring ovarian cycles is of considerable importance. These non-cycling females might be resulted from many factors that influence their reproduction system, including external and internal factors. The external factor that had most effect to the disruption of the ovarian cycle was assumed to be the size of cage, followed by feeding condition, mating pair, social interaction, and visitors (van Schaik et al. 1999). These external factors furthermore can affect the Central Nervous System (CNS) and its axis as internal factors in which regulate reproductive steroids secretion and transportation (Leighton 1987; Ferin et al. 1993).

Even though these three females were maintained in pairing system as their natural life, there were some possibilities to bring about the unpleasant conditions of these gibbons. Recall that gibbons are arboreal animals and in its natural habitat, it rarely descends to the forest floor. It spends most of its time in the forest canopy, with locomotion in the form of brachiation, where it swings by its long arms from branch to branch (Leighton 1987), the size of the cage such in these two locations will affect very much for inducing distress condition. The existence of unwanted mating would also be a potential factor to induce stress. At some captive breeding locations other than zoo, the animals mostly encounter with keepers. In zoo, however those animals faced visitors almost everyday with different behavior. Contact with a lot of different people could also generate very stressful conditions.

For a normal menstrual cycle, an intact GnRH pulse generator is required since the gonadotropin is programmed to respond only to pulsatile Gonadotropin-Releasing Hormone (GnRH) stimulation. In the absence of a functional pulse generator, known as abnormalities of the GnRH pulse generator, gonadotropin secretion is lacking and the ovaries remain unstimulated (Richards et al. 2002). In such cases, follicle stimulation by Follicle Stimulating Hormone (FSH) is insufficient to produce adequate growth and maturation of ovarian follicles, with resulting decrease in ovarian estradiol secretion. Even though follicles have developed and produced estrogen, the failure of the preovulatory Luteinizing Hormone (LH) surge to occur will also disrupt normal ovarian and menstrual function (Taylor et al. 2004). Abnormal state of the menstrual cycle may reflect as irregular, extended, shortened, anovulatory, or simply absent. Although the
advisory significance of this finding has been poorly understood, the duration of the follicular phase has been reported to be the main source of variability and diversity of ovarian cycle length (Rosen & Cedars 2004). The hypothalamic-pituitary-ovarian axis is capable of maintaining ovulatory cyclicity on its own, multiple endogenous or environment influences may impinge on the normal activity of the pulse generator usually to decrease GnRH pulse frequency and thereby induce cyclic dysfunction (Richards et al. 2002).

The present study also examined the relationship between the pattern of genital swelling and underlying hormonal changes during the ovarian cycle. As the level of LH was not measured to accurately place the ovulation time, it is assumed that the approach of ovulation is signaled by an estradiol peak, followed by the fall of estradiol and the rise of progesterone (Barelli C, Heistermann M, Boesch C, Reichard UH. 2007. Sexual swellings of female gibbons (Hylobates (H.) lar). Am J Primatol 53:193-255).


Hodgkiss S. 2007. Characterising the physical and hormonal correlates of the ovarian cycle in female Javan gibbons (Hylobates moloch) [Thesis]. Oxford Brookes University, UK.


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REFERENCES


