

## FECAL STEROID PROFILE AND GENITAL SWELLING OF FEMALE JAVAN GIBBONS (*Hylobates moloch* Audebert 1797) MAINTAINED IN INDIVIDUAL CAGE

(Profil Steroid di Feses dan Pembengkakan Organ Kelamin Luar Owa Jawa (*Hylobates moloch* Audebert 1797) yang Dipelihara di Kandang Individu)

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### ABSTRAK

Pengukuran estron terkonjugasi (E<sub>1</sub>C) dan pregnanediol glucuronat (PdG) di feses dilakukan pada tiga ekor Owa Jawa (*Hylobates moloch* AUDEBERT 1797) betina untuk mengevaluasi fungsi ovarium ketiga owa tersebut yang masing-masing dipelihara di kandang individu di Pusat Studi Satwa Primata IPB dan di Taman Margasatwa Ragunan, Jakarta. Contoh feses dikoleksi secara regular pada jam 07.00-09.00, 5-7 kali seminggu selama 4-9 bulan. Setelah melalui proses pengeringbekuan, contoh feses diekstraksi menggunakan metanol dan asai E<sub>1</sub>C dan PdG dilakukan dengan enzyme immunoassay (EIA). Profil hormon yang diperoleh menggambarkan adanya satu betina yang bersiklus dengan panjang siklus 21-25 hari. Lama fase folikular yang diperoleh pada betina tersebut bervariasi antara 11-18 hari, dan fase luteal yang relative konstan dengan lama 8-12 hari. Akan halnya dua betina yang lain, tidak diperoleh adanya gambaran pola yang menunjukkan siklus ovarium yang regular. Pembengkakan organ kelamin luar yang berfluktuasi juga hanya teramati pada betina yang bersiklus dengan lama pembengkakan 3-5 hari, dan perdarahan menstruasi terjadi selama 2-3 hari. Hasil yang diperoleh menunjukkan bahwa pengukuran steroid di feses merupakan suatu metoda nonivasif yang dapat diterapkan untuk mengevaluasi fungsi ovarium Owa Jawa. Keadaan fisiologis dari individual betina mungkin menjadi bahan pertimbangan lain dibandingkan tipe perkandangan sebagai faktor yang mempengaruhi profil hormonal.

Kata kunci: Owa Jawa, steroid di feses, pembengkakan organ kelamin luar

### INTRODUCTION

The Javan Gibbon (*Hylobates moloch* AUDEBERT 1797) is one of the primate species endemic to the tropical rainforests of Java, Indonesia and has attained the unenviable ranking of 'critically endangered' (CR) by the IUCN and is listed on Appendix I in CITES (Hilton-Taylor, 2000). The majority of individuals lives in the western part of Java and only a small population remains in Central Java (Nijman & van Ballen, 1998). Because of extensive destruction of the native habitat, combined with illegal hunting and live capturing have reduced the number of wild silvery gibbons to about 2,000 individuals and the distribution of this species is fragmented (Gates, 1998; Rinaldi, 2003).

Javan Gibbons are invariably monogamous and territorial, and defend their territories through regular loud morning songs and occasional encounters with neighbors and intruders (Leighton, 1987). The population of this species can become extinct faster than other primates

because of its inter-group spacing (Chivers, 1977), small group size (Chivers, 1989), long inter-birth interval, as wild silvery gibbon pairs give birth to single offspring every three to four years (Supriatna & Wahyono, 2000) and late sexual maturity (Geissmann, 1991).

In order to promote population growth as well as to preserve genetic diversity in captivity, a lot of efforts have been placed in improving its captive breeding management. However, little available information on reproductive biology of the Javan Gibbon to date, makes the reproductive success of this species disrupted. The knowledge in this area would not only be important for assessing the fertility status, but would also provide the basis for enhancing reproduction by assisted reproductive technology. Reports on reproductive hormone profiles and genital swelling in female white-handed gibbon (Dahl & Nadler, 1992; Nadler *et al.*, 1993; Collins *et al.*, 1994) have given such valuable supports in conducting research in Javan Gibbon. This study was thus designed to evaluate the ovarian function of the

female Javan Gibbon kept in individual cage using the measurement of steroid metabolites in fecal as a noninvasive approach combining with the observation of genital swelling and menstruation blood.

## METHODS

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 was used in this study. One of the female is maintained at Primate Research Center, IPB (Mimis) and the other two females (Owa 1 and Owa 2) are kept at Ragunan Zoo, Jakarta, all in a single-typed cage. The size of the cage at Primate Research Center, IPB is (15 m x 2.8 m) x 4.0 m in height, whereas the cage size at Ragunan Zoo is (4.5 m x 1.5 m) x 2 m in height. A mixture of chopped fruits, vegetables, supplemented with monkey chow (for Mimis) was given twice a day, and access to water was *ad libitum*.

Fecal sample was collected between 0700h and 0900h, 5-7 days per week over 4-9 months period, and following collection, samples were immediately stored at  $-20^{\circ}\text{C}$  without preservative until assayed. Daily records of menstruation were monitored and visual inspections of the perineal swelling were also carried out everyday at the same time as samples collection. The degree of genital swelling was scored as 0) no swelling, 1) partial swelling, no colour change and no discharge, 2) relative increase in swelling, reddish but no discharge, 3) maximum swelling with discharge and red in colour (Dahl and Nadler, 1992; Czekala & Sicotte, 2000).

Prior to analyze, the samples were extracted as described by Heistermann *et al.* (1993) for  $\text{E}_1\text{C}$  and PdG measurements. A total amount of fecal 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  $2200 \times g$  for 10 minutes. The supernatant was decanted into a clean glass tube and after appropriate dilution in assay buffer (5.96 g  $\text{Na}_2\text{HPO}_4$ , 8.50 g NaCl, and 1 g BSA Fr. V in 1 L  $\text{H}_2\text{O}$ , pH 7.2) was taken directly to assay. Individual extraction efficiencies were monitored by the recovery of [ $^3\text{H}$ ]-progesterone (35,000 cpm; NEN Du Pont, Bad Homburg, Germany), which was added to the fecal powder before extraction.

Microtitreplate EIAs previously characterized by Heistermann and Hodges (1995) were used to determine immunoreactive  $\text{E}_1\text{C}$  and PdG in feces. The samples were diluted in assay buffer with a certain dilution depending on

the reproductive status. Parallelism test was also performed to the samples in replicate dilutions using  $\text{E}_1\text{C}$  and PdG assay to validate the assay used.

The follicular phase was defined as the interval between the first day of menstruation until the day of the  $\text{E}_1\text{C}$  peak, whereas the luteal phase comprised the interval from the day after the  $\text{E}_1\text{C}$  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 of 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 AND DISCUSSION

The extraction efficiencies of the individual sample that were assessed by the recovery of [ $^3\text{H}$ ]-progesterone gave the mean  $\pm$  SD values ranged from  $78.2 \pm 6.6\%$ .  $\text{E}_1\text{C}$  and PdG EIAs of serial dilutions of the fecal extracts from different phases (follicular and luteal) exhibited curves parallel to those  $\text{E}_1\text{C}$  and PdG standards (data not shown). Intraassay 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 concentration, whereas interassay coefficients of variations were 14.2% for low concentration and 9.4% for high concentration.

The profile of fecal  $\text{E}_1\text{C}$  and PdG in one of the female observed (Mimis) showed regular ovarian cycle, and the pattern of excretion of those steroid metabolites throughout the period of observation together with the profile of genital swelling is illustrated in Figure 1.

Compare to the profile of  $\text{E}_1\text{C}$  which seemed to be more vary in fluctuations, the profile of PdG showed clearer patterns with discrete follicular and luteal phases. The PdG measurements showed the consistent low levels of 0.20-1.06  $\mu\text{g/g}$  during the follicular phase and increase in the concentration during the luteal phase to the level of 2.14-9.78  $\mu\text{g/g}$ , whereas the concentration of  $\text{E}_1\text{C}$  during the follicular phase was 0.16-0.42  $\mu\text{g/g}$  and decline to the level of 0.05-0.12  $\mu\text{g/g}$  at the luteal phase. The length of the ovarian cycle obtained from this study was 21-25 days with 11-18 days of follicular phase length and 8-12 days of luteal phase length. Menstruation blood flow was also seen during observation which lasted for 2-3 days and was consistent with the hormonal pattern during the ovarian cycle.

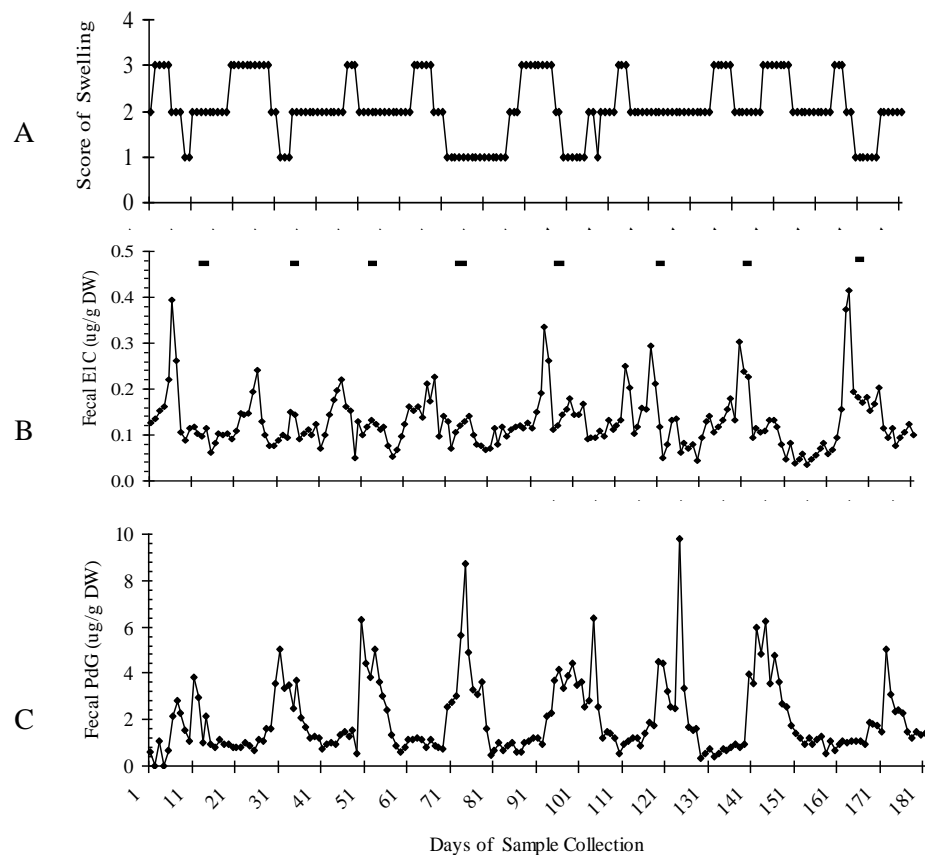


Figure 1. Profiles of E<sub>1</sub>C (B) and PdG (C) in feces of Mimis in relation to the stage of genital swelling (A) during the 9-months period of observation. Black bars indicate periods at which menstrual blood flow was seen.

Of the cycling female, the degree of swelling, changes in genital swelling and the appearance of the discharge showed clear fluctuation during the cycle which increase in consistent with the high level of E<sub>1</sub>C. The data obtained from the cycling female was different from those obtained from the other two females as presented in Figure 2. (Owa 1) and Figure 3. (Owa 2).

The hormonal profiles of those females did not reflect a regular ovarian cycle. As seen in Figure 2., Owa 1 showed a non regular swelling and long follicular phase with non conclusive pattern in luteal phase, whereas Owa 2 (Figure 3.) did not show any change in genital swelling consistent with the erratic profile of E<sub>1</sub>C, although in its PdG profile showed 1 to 2 profiles that were similar to the luteal phase pattern of cycling female.

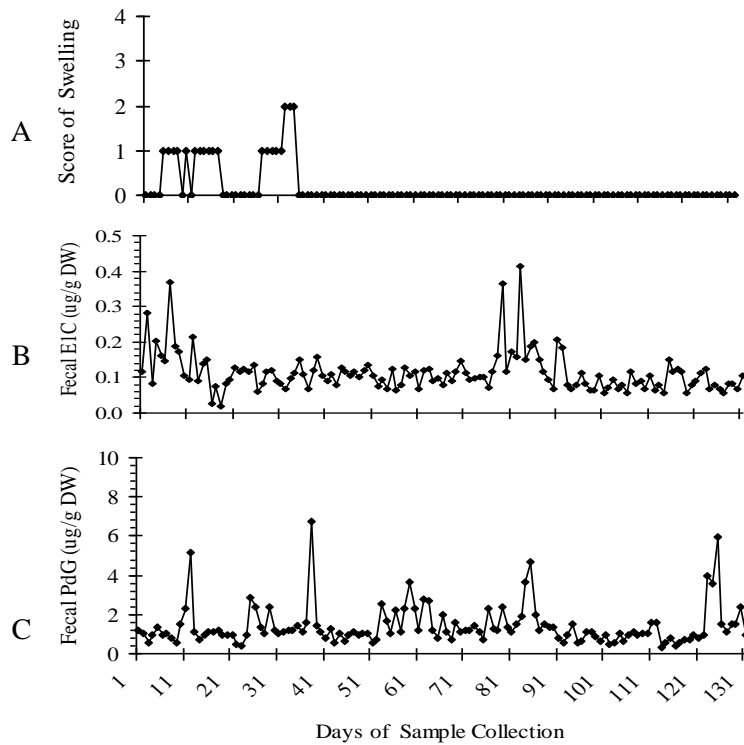


Figure 2. Profiles of E<sub>1</sub>C (B) and PdG (C) in feces of Owa1 in relation to the stage of genital swelling (A) during the 4-months period of observation. Black bars indicate periods at which menstrual blood flow was seen.

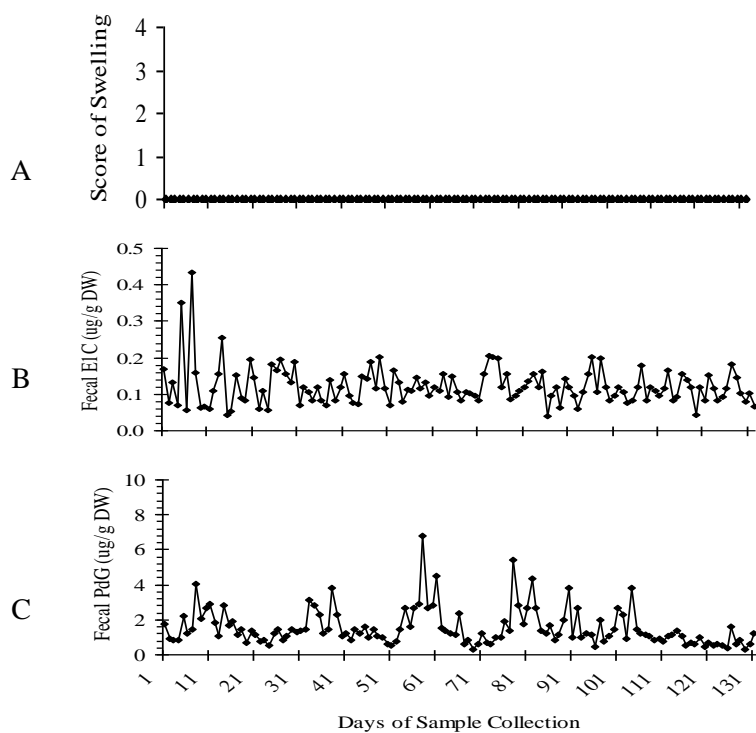


Figure 3. Profiles of E<sub>1</sub>C (B) and PdG (C) in feces of Owa2 in relation to the stage of genital swelling (A) during the 4-months period of observation. Black bars indicate periods at which menstrual blood flow was seen.

This study has demonstrated the feasibility and the potential use of steroid metabolite analysis from fecal samples to evaluate the ovarian function of Javan Gibbon in captivity. Since for many non-domestic species, regular blood collection can be difficult in captive situations and nearly impossible in the wild so that the fecal analysis will be more practical. The same work has been reported by Nadler *et al.* (1993) in *Hylobates lar*. and other primates species (Ziegler *et al.*, 2000; Heistermann *et al.*, 2001; O'Neill, 2004). Regarding the lack of published information on the reproductive endocrinology of captive gibbons, this present study also provides the first information on this area.

Despite the small sample and variability among animals, the length of the ovarian cycle or intermenstrual intervals reported here are similar to the length of the ovarian cycle in *Hylobates lar* reported by Nadler *et al.* (1993) that was found to be 19 to 22 days, but shorter than that of in *Hylobates syndactylus* (37-42 days) (Yundiarto *et al.*, 2004). With the exception of the follicular phase that ranged from 7 to 11 days, the length of luteal phase of *Hylobates lar*. seemed to be similar with our finding that ranged from 8-15 days (Nadler *et al.*, 1993).

This finding about the occurrence of genital swelling in one of the cycling female with the score of three (maximum swelling), similar to that of found in *Hylobates lar*. (Dahl & Nadler, 1992). However, given the monogamous mating system of this species, one should be more careful to confirm that this sexual swelling does indeed occur in this species. The incidence of the swelling in the female observed might be because of variation in individual animal, considering genitalia externa is one of the target organ for the estradiol (Ferin *et al.*, 1993). Of the Javan Gibbon in the wild, habitat or stratum use may place constraints on visual communication is logical, but this may have been over-emphasized when considering sexual swelling and arboreality (Dahl & Nadler, 1992).

The condition of the two non-cycling females might be related to the environment that influences their reproduction. The environment could be the external and the internal factors. The external factors such as cage condition, mating pair, social interaction, and may be visitors. On the other hand, internal factors depend on external factors that may influence Central Nervous System (CNS) in which the reproductive steroids are induced to be secreted and the pathways to the target organs (Ferin *et al.*, 1993; Leighton, 1987).

Although the three females observed were caged individually, the profile of their hormones described different states of their physiological conditions. There are some possibilities to elucidate the differences between conditions of gibbons from Primate Research Center (PRC) IPB and from the Zoo in Jakarta. The females at Ragunan Zoo faced some visitors, keepers and vets or sometimes

researchers, even though they can still have visual and auditory contact with other primates surrounding, especially with *Presbytis comata*, which lives sympatric with them in the wild. As for the female at PRC, apart from visited by keeper and the vet, this female could still have visual contact with the male Javan Gibbon which is housed in individual cage as well in a close distance. Contact with a lot of people could make them frightened, worried or disturbed, resulting in a very stressful conditions.

Another possible reason why the females at Ragunan Zoo did not display irregular cycle is those females might not have contact with the males. Reproduction is only one of a host of activities in which an individual may engage (Johnson & Everitt, 2000). Since it is known that mating system of this species is monogamous, partners would influence the time of estrus. Unwilling partners would make the female does not come into estrus, so that would affect their menstrual patterns (Leighton, 1987).

There are numerous conditions under which the menstrual cycle may become abnormal, that is, irregular, extended, shortened, anovulatory, or simply absent. Since the gonadotropin appears to be programmed to respond only to pulsatile GnRH stimulation, it is clear that an intact GnRH pulse generator is required for a normal menstrual cycle. 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. As the level of Luteinizing Hormone (LH) was not be measured to accurately place the ovulation day, 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. By using the profile of E<sub>1</sub>C and PdG as independent markers of the female cycle stage and characteristic changes in genital swelling pattern, it was likely to determine the time of presumed ovulation (Ferin *et al.* 1993).

## CONCLUSION

In conclusion, the methods and data presented in this study provide the basis for a practical approach to evaluation and monitoring of ovarian events in the female Javan Gibbon, particularly the use of metabolite steroids profile for any effort to evaluate the ovarian function and may predict the time of ovulation.

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