Testicular volume and concentration of spermatozoa in the monogarnus male Javan Gibbons (*Hylobates moloch)*

P. Astuti[1], T. L.Yusuf[2], E. Hayes[3], H. Maheshwari[2], L. Sjahfirdi[4], A, Junaidi[1], D Sajuthi[5]

[1] Faculty of .Veterinary Medicine of Gadjah Mada University, Yogyakana, Indonesia
[2] Faculty of Veterinary Medicine of the Bogor Agricultural University, Indonesia
[3] Washington National Primate Research Center, Seattle, Washington, USA
[4] Faculty of Mathematics. and Science University of Indonesia, Jakarta, Indonesia
[5]Primate Research Center, IPB Bogor, Indonesia

In non-human primates testicular size related to patterning of mating behavior and sperm production. Among non-human primates. Javan Gibbons (*Hylobates moloch*) have a very strict and rigid social system consisting of monogamous family groups. The objectives of this study were I) to observe basal reproductive traits in male Javan Gibbons (*Hylobates* moloch) including concentration of spermatozoa, testicular volume and plasma concentration of testosterone and 2) to compare the ratio of body weight with testicular volume in both Javan Gibbons and *Macaca fascicularis* (as a polyginandrous comparator).

Six adult males (7-15 years old) with mean body weights of 6.56±0.73 kg were used in the study and were maintained in zoo and primate center captive breeding programs. All animals were anesthetizied (ketamine 10 mg/kg) prior to semen and blood collection. Semen was collected several times from each male over a ten month by rectal probe electro-stimulation. Testosterone was measured in plasma sampels using Enzyme Link lmmunosorbent Assay Average sperm concentration was 87.52±7.54 mil/ml (n=10): testicular volume was 10.35±2.23 cm3 (n-20); the ratio of testicular volume to body weight was 1.61±0.40 cm3/kg (n=20); and plasma testosterone concentration was 6.41±2.08 ng/ml (n=20). For *Macaca fascicularis*, average sperm concentration were 91.10±12.93 mil/ml (n=10), testicular volume was 46.95±5.97 cm3(n=10); the ratio of testicular volume to body weight was 10.52±1.45 cm3/kg (n=10) and plasma testosterone concentration was 4.50±0.59 ng/ml(n=10).

It was concluded that the testicular volume and ratio testicular volume to body weight for Javan gibbons as a monogamous primates was very small compared to a polyginandrious comparator species of old world monkey, even concentration of sperm and plasma testosterone are almost the same. Given the monogamus breeding habits of the Javan Gibbons testicular size and function may be adapted to the needs and timing of a single mating relationship.

Introduction

Javan Gibbons (*Hylobates* moloch) is an endemic primate which is categorized critically endangered by the CI (Llanos et al. 2000). The captive population is getting decrease because of external and internal factors, such as their mating system namely monogamous. even they live in small groups.

In non-human primates, testicular size is very related to mating system, resulting in sexual selection when the gametes of two or more males compete for access to a given set of ova (Jolly *et al.* 2003; Anderson *et* al. 2004), and sperm production for Holstein bulls (Bailey *et al.* 1996). As comparing data, it was used *Macaca fascicularis*, because they have a difference mating system (polygyniandrous). The objectives of study were 1) to observe basal reproductive traits in male Javan Gibbons (*Hylobates* moloch) including concentration of spermatozoa, testicular volume

and plasma concentration of testosterone and 2) to compare the ratio of body weight with testicular volume in both Javan Gibbons and *Macaca fascicularis* (as polyginandrous). We hypothetizied, as monogamus, Javan Gibbons only has a small testicular volume because they have no sperm competition where sperm from several males occupy the female reproductive tract.

Materials and Methods

Six male adult Javan Gibbons (7-15 years old) with the body weight of 5-8 kg were used in this study. They were maintained in Primate Research Center , IPB, Bogor, Taman Sari zoo, Bandung and Ragunan zoo, Jakarta. As comparing data, it was used six male adult Macaca *fascicularis* (5-10 years old) with the body weight of 3-5 kg.

Semen was collected by rectal probe electro-stimulation, after all animals were anesthetizied using Ketamine 10 mg/kg bw. Immediately after collection, sperm concentration was determined by haemocytometer count and microscopic examination of a drop of semen dilution.

Testicular volume was estimated using caliper as describe by Stanger *et al.* (1995). The length and width of testis were measured. Each measurement was taken 3 times and the values averaged to give the recorded measurement. The volume of an ellipsoid $V=0.1667$ ($3.14\ W^2\ L$; L=length/2, W=total width) was to estimate testicular volume.

Besides semen collection and testicular volume, we collected blood samples for measuring the concentration of plasma testosterone using Testosterone kit (DRG production, Germany) for ELISA. The blood samples were placed into 10 ml tubes and immediately centrifuged at 500 g for 10 minutes (Monfort *et al.* 1994).

Results and discussion

The testicular volume, ratio testicular volume-body weight and concentration of spermatozoa of *Hylobates moloch* are presented in table 1. In this research, the ratio testicular volume and body weight is 1.61±0.42 cm^3/kg). This ratio is almost the same as langurs (*Presbytis spp*), *Hylobates spp* and man, which are not multi-male from 0.9 to 1.3 Smith (1984).

Comparing with *Macaca fascicularis*, the testicular volume of *Hylobates moloch* is much smaller: 1.61±0.42 versus 10.52±1.45 cm^3/kg) (Table 2). *Macaca fascicularis* is one of non-human primates which characterized by multi-male multi-female. Males guard receptive females, but female copulate with different mates. As expected sperm competition is intense and they have much larger testicular volume (46.95±5.97 cm^3) than *Hylobates moloch* (10.36±2.23 cm^3) (Table 1). Hamadryas society is structured around permanent one-male harem, while in anubis baboons males compete for access to estrous female. The inherent differences in overt male competition is indicated biologically by the larger testes of anubis (Magruder. 2003). In the grey mouse Lemur (*Microcebus murinus*) mating system, an intense sexual precopulatory competitions develops among males, whereas females also played an important role in sexual competitions among males, since their presence enhances the aggressive interaction between males. Concequensly. they also have high relative testes to suppon the presence of sperm competition (Andres and Solignac. 2003).

Table 1. Mean±SD body weight; testicular volume; ratio testicular volumelbw; and concentration of spermatozoaof male Javan Gibbons

Variable	Ari	Riko	Hml	Kiki	Jack	Emed	Average
Body weight (kg)	7.5±0.4	6 2 (n=1)	6.1 (n=1)	5.9 (n=1)	5.7±0.2 (n=6)	6.7±0.6 (n=5)	6.6±0.7 (n=20)
Testicular vol. (cm^3)	9.8±1.4 (n=6)	6.0 (n=1)	10.4 (n=1)	6.9 (n=1)	9 2±1.2 (n=6)	13 9±1.9 (n=5)	10.4±2.2 (n=20)
Ratio Tes.vol/bw	1.3±0.2 (n=6)	I.0 (n=1)	1.7 (n=1)	1.2 (n=1)	1.6±0.2 (n=6)	2.1±0.4 (n=5)	1 6±0 4 (n=20)
Sperm concent (jt/ml)	76.2±26.3 (n=6)	- (n=0)	- (n=0)	- (n=0)	89.7±2.1 (n=2)	93.7±8.0 (n=2)	87.5±7.5 (n=10)

The other mating system where the male dominates a harem of females (e.g gorillas) would have testes half the size of human while the highly promiscous chimpanzees have testes much larger than human (Short and Balaban, 1994). Preston *et al.* (2002) reported that testicular size was positively associated with copulatory activity and subsequent paternity success. Based on the starting growth of testes, it was reported that in the *Macaca radiata* was occurred at the males development namely 5-6 years (Moudgal *et al.* 1993), whereas anubis baboons reach a larger Anal testis size much earlier in their devetopment, reflecting on the divergent social system (Magruder 2003)

Tabel 2. Comparison data of body weight; testicular volume; ratio testicular volume/bw; and concentration of spermatozoa between male Javan Gibbons and *Macaca fascicularis*

Variable	*Hylobates moloch*	*Macaca fascicularis*
Body weight(kg	6.55±0.72 (n=20)	4.50±0.59 (n=10)
Testicular vol. (cm^3)	10.35±2.22 (n=20)	46.95±5.97 (n=10)
Ratio Tes.vol/bw (cm^3/kg)	1.60±0.41 (n=20)	10.52±1.45 (n=10)
Sperm concent. (mil/ml)	87.52±7.54 (n=10)	91 10±12 93 (n=10)
plasma Testosterone ng/ml	6.4±2.08 (n=20)	4.50±0.59 (n=10)

Multi-male species not only have larger testes but also have a higher sperm production capacity per unit weight of testes tissue, because the sperm of each male would have to compete with those of other male, the most sperm was most likely to generate offspring (Harvey and Clutton-Bmk, 1983). This opinion is not likely as present study. Here, the concentration of sperm both of Javan Gibbons or Macaca *fascicularis* is similar. In primates, sperm midpiece volume (not sperm head or flagellum volume) is significantly greater where females mate with multiple partner so that sperm competition is likely to occur. The midpiece is concerned because this contains the

mitochondria required to power sperm motility (Anderson and Dixson, 2003). Mammals that have multiple partner mating system, and large testes in relation to body weight, have shorter and more muscular vasa deferentia than mammals where single partner mating system, and smaller relative testes sizes, are norm (Anderson *et al.* 2004). Social structure reinforcing the benefits that come with a hierarchical orientation, for example a harem, would evidence males with relatively low sperm counts (smaller testes) and increased aggressive tendencies associated with higher testosterone levels.

It can be concluded that the testicular volume of Javan gibbons as a monogamous primates was very small. They would have no need of plentiful concentration sperm and so small testes would suffice.

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