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Morphological characterization of spermatozoa of the night monkey

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Abstract: The morphology, sizes and abnormality rates of spermatozoa in the night monkey were revealed in the present paper. Motile spermatozoa of three males, 7, 8 and 12–13 years old, were squeezed from the ducts of the cauda epididymis after cutting the ducts in cryopreservation media. The morphology of the spermatozoa and abnormalities in them were observed, and the sizes of the spermatozoa were measured in smear samples. The spermatozoa of the night monkey had heads with rounded and thick shapes. Measurement of the spermatozoa revealed that the average widths and lengths of the heads, average lengths of the middle pieces, and average total lengths from the head to tail tip were 4.7 ± 0.8 *μ*m and 2.8 ± 0.4 *μ*m, 6.6 ± 2.2 *μ*m and 55.1 ± 6.2 *μ*m, respectively (average ± SD). The total rates of abnormal spermatozoa were different among the 7-, 8- and 12–13-year- old night monkeys, 41.8%, 24.0% and 36.5%, respectively. Freezing of semen was also attempted using the procedure contained in a commercial kit for the mouse. Although the motility of the spermatozoa from the night monkeys was poor in fresh samples, the motility of their spermatozoa frozen-thawed with the commercial kit was similar to that before freezing.

Key words: Night monkey, Cauda epididymis, Sperm, Morphological abnormality, Cryopreservation

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Introduction

Primates are the most closely related species to humans, and they are used as experimental animals in the field of medicine. The night monkey (*Aotus lemurinus*) is a species of new world monkeys living in South America and the only nocturnal monkey in simiiformes. There are some subspecies of night monkey [1]. The night monkey, which is an annual breeding animal having an estrus cycle of 16 days, reaches puberty at about 3 years old in both sexes and forms socially monogamous pairs [2]. Their gestation period is 133 days [3], and they give birth to 1 or 2 infants at a time.

Squirrel monkeys (*Saimiri boliviensis*), used in the present study as a control, have a mating season from September to March and socially form a harem. Puberty in these monkeys is from 2 to 3 years old in females and 4 to 5 years old in males, and their gestation period, 155.2 ± 13.8 days [4], is longer than that of the night monkey.

In the field of developmental biology, many investigators have researched about cryopreservation, *in vitro* fertilization and production of offspring from embryos produced *in vitro* in primates [5, 6]. In new world monkeys, marmosets and squirrel monkeys have been mainly researched, but no details have been reported regarding gametes, embryo production *in vitro* and cryopreservation of gametes and embryos in the night monkeys up to now. In the present study, we revealed the morphology, sizes and abnormality rates of the spermatozoa of the night monkey.

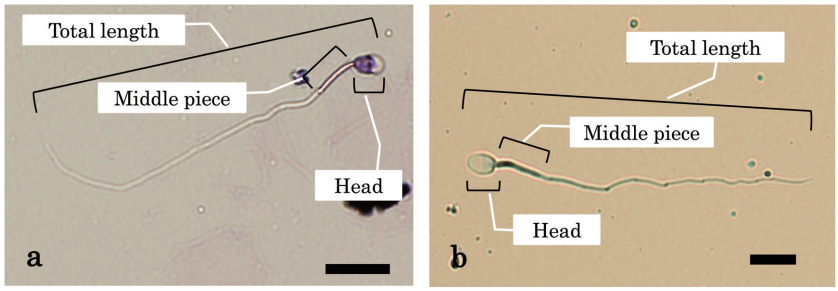

Fig. 1. Morphology of spermatozoa in the night monkeys and squirrel monkey. a: A spermatozoon from a night monkey. The head has a rounded and thick shape. b: A spermatozoon from the squirrel monkey. The head has a rounded and flatted shape (Bar=10 μm).

Materials and Methods

Animals

The night monkeys and the squirrel monkey used in the experiments were kept in the Amami Laboratory of the University of Tokyo, which is located on Amami Island, Kagoshima, Japan. Three male night monkeys, which were 7, 8 and 12–13 years old, and a male squirrel monkey, which was 10 years old, were euthanized, and their testes and epididymides were removed as samples.

Media

Preparation of sperm suspensions was performed with different methods appropriate to the individuals. Motile spermatozoa of the night monkeys were squeezed from and pushed out of 5–7 parts of the ducts of the cauda epididymis after cutting the ducts in a medium for cryopreservation (FERTIUP, Kyudo Co., LTD., Saga, Japan). Motile spermatozoa of the squirrel monkey were squeezed from 2 or 3 parts of the ducts of the cauda epididymis held between fingers, and the obtained spermatozoa were suspended in the medium for cryopreservation (FERTIUP).

Sperm Characterization

Smear samples of spermatozoa for morphological observation and measurement of sizes were made from the sperm-suspended media described above. After smear samples were stained with 2% Giemsa solution for 10 mins, the morphology of the spermatozoa was observed, and several sizes of the spermatozoa were measured, including the width and length of heads, length of middle pieces, and total lengths of spermatozoa.

Sperm Freezing and Thawing

Freezing of semen was carried out according to the procedure of a commercially available kit (FERTIUP, Kyudo Co., LTD.). Frozen spermatozoa of the night monkeys were treated according to the thawing protocol for FERTIUP (Kyudo Co., LTD.), and then the motility was observed in the preincubation medium from the FERTIUP kit.

All procedures for the animal experiments were performed in accordance with the guidelines for animal care and use of the University of Tokyo.

Results

All cauda epididymides of the three night monkeys were found to be withered and flavescent, while both cauda epididymides of the squirrel monkey were found to be completely white seminiferous tubules filled with spermatozoa. The size of the cauda epididymis in the three night monkeys, about 1.5 × 2.0 mm, was smaller than that of the squirrel monkey, about 3 × 5 mm.

The spermatozoa of the night monkeys showed heads with rounded and thick shapes (Fig. 1-a), while those of the squirrel monkey showed heads with rounded and flatted shapes (Fig. 1-b).

Measurement of the spermatozoa in the three night monkeys revealed that the average widths and lengths of heads, average lengths of middle pieces, and average total lengths from head to tail tip were 4.7 ± 0.8 μm and 2.8 ± 0.4 μm, 6.6 ± 2.2 μm and 55.1 ± 6.2 μm, respectively (average ± SD in Table 1). In the squirrel monkey, the average widths and lengths of heads, average lengths of middle pieces, and average total lengths from the head to tail tip of spermatozoa were 6.1 ± 0.4 μm, 4.3 ± 0.3 μm, 10.5 ± 1.1 μm and 76.8 ± 5.1 μm, respectively (average ± SD).

The rates of abnormal spermatozoa relative to the total number of spermatozoa observed were different among the three 7-, 8- and 12–13-year-old night monkeys, 41.8%

Table 1. Results of measurement of spermatozoa in the night monkeys and squirrel monkey

	Total no. of sperm	Ave. width of heads	Ave. length of heads	Ave. length of middle pieces	Ave. total lengths
Night monkey (n=3)	631	4.7 ± 0.8	2.8 ± 0.4	6.6 ± 2.2	55.1 ± 6.2
Squirrel monkey (n=1)	205	6.1 ± 0.4	4.3 ± 0.3	10.5 ± 1.1	76.8 ± 5.1

Average ± SD μm.

Table 2. Rates of abnormal spermatozoa in the night monkeys and squirrel monkey

Individuals (age in years)	Total no. of spermatozoa	No. of normal spermatozoa	No. of abnormal spermatozoa				
			Total (%)	Head	Neck	Middle piece	Tail
Night monkey (7)	505	294	211 (41.8)	143	14	13	41
Night monkey (8)	405	308	97 (24.0)	61	16	6	14
Night monkey (12–13)	430	273	157 (36.5)	107	11	18	21
Squirrel monkey (10)	411	404	7 (1.7)	1	2	0	4

Table 3. Motility of spermatozoa after the freeze-thawing treatment with FERTIUP in the night monkeys and squirrel monkey

Individuals (age in years)	Motility of spermatozoa	
	Before freezing (fresh)	After thawing
Night monkey (7)	10+	10+
Night monkey (8)	10+	10+
Night monkey (12–13)	10+	10+
Squirrel monkey (10)	90+++	10++

Values with +, percentage of live spermatozoa showing slow forward motion; Values with ++, percentage of live spermatozoa showing active forward motion; Values with +++, percentage of live spermatozoa showing very active forward motion.

(211/505), 24.0% (97/405) and 36.5% (157/430), respectively (Table 2). In each of the night monkeys, abnormalities were more frequently observed on the head than on the neck and tail. On the other hand, the rate of abnormal spermatozoa in the 10 year-old squirrel monkey was lower (1.7%, 7/411) than those of night monkeys.

After thawing spermatozoa of the night monkeys according to the protocol of Kyudo Co. Ltd., the motility was observed in the preincubation medium from the FERTIUP kit. Although the motility of the spermatozoa of the night monkeys was poor in each fresh sample, the motility of the live spermatozoa after the freeze-thawing treatment according to the FERTIUP procedure was similar to that before freezing, which showed slow forward motion (Table 3). However, the motility of the squirrel monkey's spermatozoa after the freeze-thawing treatment was significantly decreased compared with that of fresh spermatozoa, which showed very active forward motion.

Discussion

The morphology of the heads of the spermatozoa of the night monkeys, which showed a thick, rounded shape, looks similar to that of human spermatozoa. The morphology of the spermatozoa of the squirrel monkey corresponded to that in the report by Bennet (1967) [7]. The average total length of the spermatozoa in the night monkeys was rather smaller than that of the squirrel monkey in the present study, which was similar to the result for the squirrel monkey reported by Bennett (1967) [7].

It is known that mammalian spermatozoa, excluding those of humans and gorillas [8], show high rates of normality; for example, 80% of ejaculated sperm in both Cynomolgus monkey and Captive bonnet monkey were found to be normal [9, 10]. In the present study, although the squirrel monkey's spermatozoa also showed a high rate of normal spermatozoa, all of the samples of sper-

matozoa from the night monkeys showed high rates of abnormality.

In the present study, it was suggested that the rates of abnormal sperm did not correlate with night monkey age because the rate of abnormal spermatozoa in the youngest night monkey (7 years old) was the highest, 41.8% (211/505). The night monkeys used in the present study had not mated with females for a long time. This fact may be related to the poor conditions of their cauda epididymides and high rates of abnormal spermatozoa. However, to reveal the relationship between poor condition of the cauda epididymis and a high rate of abnormality of spermatozoa in night monkeys, additional samples are needed.

The motility of the squirrel monkey's spermatozoa was very good compared with those of the night monkeys. Because sampling was only performed during the breeding season of the squirrel monkey, the spermatozoa might have shown good motility and morphological normality.

After freezing and thawing, many of the spermatozoa in the squirrel monkey lost their motility, but the movement of only a few motile spermatozoa was equivalent to that of fresh spermatozoa. On the other hand, in the night monkeys, the rates of moving spermatozoa after freezing and thawing were similar to those of fresh spermatozoa. Sankai *et al*. (2003) reported that in cryopreservation of spermatozoa from different primates by several methods, the spermatozoa of the Cynomolgus monkey did not show good activity [11]. They described that the survival of cryopreserved spermatozoa was different among species. Successful cryopreservation has been reported in some species, but a common method has not been established in primates. Therefore, we tried to cryopreserve spermatozoa of the night monkey and squirrel monkey with FERTIUP [12], which shows good results in cryopreservation of mouse spermatozoa in our experience. Because frozen-thawed spermatozoa of the night monkey showed good results, FERTIUP may be able to be used for cryopreservation of sperm in the night monkey.

The results of the present study may be useful for production of embryos or offspring of the night monkey *in vitro* in the future.

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