Cycle of the Seminiferous Epithelium in the Java Fruit Bat (*Pteropus vampyrus*) and the Japanese Lesser Horseshoe Bat (*Rhinolophus cornutus*)

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**ABSTRACT.** The cycle of the seminiferous epithelium in the Java fruit bat, *Pteropus vampyrus*, and the Japanese lesser horseshoe bat, *Rhinolophus cornutus*, was investigated by light microscopy and the characteristics of spermiogenesis were compared between these two species. In the Java fruit bat, the cycle of the seminiferous epithelium was divided into 11 stages and developing spermatids were subdivided into 13 steps. While in the Japanese lesser horseshoe bat, the cycle of the seminiferous epithelium was divided into 10 stages and developing spermatids were subdivided into 13 steps. Excepting slight morphological differences, the characteristics of acrosomal formation in both species were almost similar with each other. In the Java fruit bat after stage VII, the acrosome gradually elongated, flattened and finally became scoop-like in shape. In the Japanese lesser horseshoe bat after stage VIII, the acrosome elongated, flattened and then slightly shortened. Before spermiation, the acrosome became long spatula-like in shape. The elongation and flattening of spermatids in these two species were similar to those in insectivores. The finding may reflect the fact that the order Chiroptera is phylogenetically close to the order Insectivora.

**KEY WORDS:** acrosome, cycle of seminiferous epithelium, Japanese lesser horseshoe bat, Java fruit bat, spermiogenesis.

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Several studies have been reported on the testes of bats [6, 10, 14–17, 23]. Some of them were carried out from a morphological viewpoint. For example, Mori [14] compared the sperm structure of bats among each species. Oh et al. [16] investigated the spermiogenesis in the Japanese greater horseshoe bat by transmission electron microscopy. However, only one report is available on the cycle of the seminiferous epithelium in bats [23].

The cycle of the seminiferous epithelium has been studied in various mammals [1, 2, 4, 5, 7–9, 11, 12, 16, 19, 24] and it has become obvious that the morphological characteristics of acrosomal formation are different among each species. Especially, the acrosome formation is quite peculiar in the musk shrew [8] and Watase’s shrew [1]. The acrosome displays the prominent extension, subsequent shortening and flattening during its formation, and finally becomes the enormous fan-shaped acrosome. In the present study, we picked up 2 kinds of bats, one (Java fruit bat) belongs to the suborder Microchiroptera and the other (Japanese lesser horseshoe bat) belongs to the suborder Megachiroptera, and compared the characteristics of acrosomal formation between the two species. Then, we attempted to compare the acrosome formation of these two kinds of bats with those of rodents and insectivores.

**MATERIALS AND METHODS**

**Animals:** Two adult Java fruit bats (average weight 567 g, average forearm length 175 mm) obtained in East Java, Indonesia and 3 adult Japanese lesser horseshoe bats (average weight 7.0 g, average forearm length 41.3 mm) captured in Aomori Prefecture, Japan were used in the present study. All of these bats showed active spermatogenesis according to histological analysis described below. These materials have already been used in our previous lectin-histochemical study [15].

**Light microscopy:** Under pentobarbital anesthesia, the animals were perfused with 0.9% physiological saline followed by Bouin’s fixative through the left ventricle. The testes were excised, sliced into slabs and immersed in the same fixative overnight. They were dehydrated in a graded series of ethanol, infiltrated in xylene and embedded in paraffin wax. Sections were cut at 4 µm, deparaffinized, stained with periodic acid Schiff (PAS)—hematoxylin and examined by light microscopy. In both species, more than 500 round seminiferous tubules were evaluated and classified into each stage.

**RESULTS**

**Cycle of the seminiferous epithelium in the Java fruit bat:** Spermiogenesis of the Java fruit bat could be divided into 4 phases (Golgi, cap, acrosome and maturation phases). Developing spermatids were subdivided into 13 steps. Steps 1–2, 3–5, 6–10 and 11–13 of spermatids corresponded to the Golgi, cap, acrosome and maturation phases, respectively. In the Golgi phase, a proacrosomal vesicle was found near the nucleus of step 2 spermatids. In the cap phase, the vesicle developed to an acrosome. Then, the acrosome gradually expanded and covered the half of the
nuclear surface. In the acrosome phase, the nucleus of spermatids gradually elongated and condensed. The acrosome became flattened. In the maturation phase, the spermatids completed their morphological change, and the spermiation occurred. On the basis of acrosomal changes, nuclear shape, location of spermatids, period of spermiation and appearance of meiotic figures, the cycle of the seminiferous epithelium was divided into 11 stages in the Java fruit bat. The characteristics of each stage were shown as follows (Figs. 1, 3 and 5).

**Stage I:** Type B spermatogonia, pachytene spermatocytes, round spermatids (step 1) and elongate spermatids (step 11) were observed in the seminiferous epithelium. In step 1 spermatids, no acrosomal granules were recognized. Step 11 spermatids were located in the middle region of the epithelium. Pachytene spermatocytes, located in the basal region, were seen throughout all stages except stages XI and XII.

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Fig. 1. Light micrographs of the seminiferous epithelium of the Java fruit bat at each stage. (I) Stage I. Early and elongate spermatids are observed. The acrosome becomes scoop-like in shape in step 11 spermatids. (II) Stage II. The proacrosomal vesicle is recognized in step 2 spermatids. (III) Stage III. The acrosome first appears and flattens on the nuclear surface of step 3 spermatids. (IV) Stage IV. The acrosome expands on the nuclear surface of step 4 spermatids. The cytoplasm of elongate spermatids decreases to a great degree. (V) Stage V. Matured spermatids (step 12 spermatids) are arranged on the luminal surface. (VI) Stage VI. Spermiation occurs. Preleptotene spermatocytes are found in the basal region of the epithelium. (VII) Stage VII. The acrosome of step 7 spermatid comes in contact with the plasma membrane and orients towards the base. Leptotene spermatocytes are observed in the basal region. (VIII) Stage VIII. The elongation of spermatids proceeds. The nucleus becomes rhomboid in shape. Leptotene spermatocytes change into zygotene ones. (IX) Stage IX. The elongation of spermatids proceeds to a great degree. Step 9 spermatids become flattened. (X) Stage X. The elongate spermatids come most close to the basement membrane. Pachytene spermatocytes give rise to diplotene ones. (XI) Stage XI. The elongation and condensation of the spermatid nucleus progress further. Meiotic figures and secondary spermatocytes are clearly seen. L: leptotene spermatocyte. Z: zygote spermatocyte. P: pachytene spermatocyte. D: diplotene spermatocyte. SS: secondary spermatocyte. M: meiotic figure. 1–12: step 1–12 spermatid. × 330.
**Stage II**: The proacrosomal vesicle first appeared in step 2 spermatids. Step 11 spermatids began to move to the apical region of the epithelium.

**Stage III**: The acrosome first appeared and flattened on the nuclear surface of step 3 spermatids. The cytoplasm of elongate spermatids decreases. Preleptotene spermatocytes are found in the basal region of the epithelium. The nucleus of pachytene spermatocytes became larger in size.

**Stage IV**: The acrosome expanded to cover one-third of the nuclear surface of step 4 spermatids. The cytoplasm of step 12 spermatids decreased to a great degree.

**Stage V**: The acrosome expanded further to cover the half of the nuclear surface of step 5 spermatids. Thus, the acrosomal formation almost completed. Step 13 spermatids, just prior to spermiation, were arranged on the luminal surface. Residual bodies were found near the nucleus of step 13 spermatids.

**Stage VI**: Spermiation occurred. Therefore, matured spermatids disappeared from the epithelium, and only one generation of spermatids (step 6) were present in the epithelium. The nucleus of step 6 spermatids changed to be oval in shape. Preleptotene spermatocytes derived from type B spermatogonia appeared in the basal region of the epithelium.

Stage VIII: The elongation of the acrosome and nucleus in step 8 spermatids proceeded to a degree. The nucleus became rhomboid in shape and began to condense. Step 8 spermatids moved to the basal region. Leptotene spermatocytes changed into zygotene ones. The nucleus of zygotene spermatocytes became condensed.

Stage IX: The elongation of spermatids proceeded to a great degree. Step 9 spermatids became flattened and were located in the basal region.

Stage X: The elongate spermatids (step 10) came most close to the basement membrane. Pachytene spermatocytes changed into diplotene ones.

Stage XI: The elongation and condensation of the spermatid nucleus progressed further. The acrosome of step 11 spermatids became scoop-like in shape. Meiotic figures and secondary spermatocytes were clearly seen.

Cycle of the seminiferous epithelium in the Japanese lesser horseshoe bat: Spermiogenesis of the Japanese lesser horseshoe bat could also be divided into 4 phases (Golgi, cap, acrosome and maturation phases). Developing spermatids were subdivided into 13 steps. Steps 1–2, 3–5, 6–10, 11–13 of spermatids corresponded to the Golgi, cap, acrosome and maturation phases, respectively. The cycle of the seminiferous epithelium in the Japanese lesser horseshoe bat was divided into 10 stages, on the basis of the same criteria described above. The characteristics of each stage were shown as follows (Figs. 2, 4 and 6).

Stage I: Intermediate spermatogonia, pachytene spermatocytes, round spermatids (step 1) and elongate spermatids (step 11) were recognized in the seminiferous epithelium. In step 1 spermatids, no acrosomal granules were observed. The acrosome of step 11 spermatids became spatula-like in shape. Intermediate spermatogonia and pachytene spermatocytes were located in the basal region of the epithelium. Pachytene spermatocytes were seen throughout all stages except stage IX.

Stage II: The proacrosomal vesicle first appeared in step 2 spermatids. Step 11 spermatids began to move to the luminal surface of the epithelium. Intermediate spermatogonia changed into type B ones.

Stage III: The acrosomal vesicle was recognized on the nuclear surface of step 3 spermatids.

Stage IV: The acrosome first appeared and expanded to cover one-third or one-fourth of the nuclear surface of step 4 spermatids. The cytoplasm of step 11 spermatids decreased to a great degree.

Stage V: The acrosome expanded to cover the half of the nuclear surface of step 5 spermatids. The flagellum began to form. The acrosome showed a slight projection at its center. Step 12 spermatids were arranged on the luminal surface. Residual bodies were found near their nucleus.

Stage VI: Spermiation occurred. Only one generation of spermatids (step 6) and preleptotene spermatocytes were
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... seen in the epithelium. Preleptotene spermatocytes were smaller than type B spermatogonia.

Stage VII: Step 7 spermatids began to elongate. The acrosome of these spermatids came in contact with the plasma membrane and oriented towards the base. Preleptotene spermatocytes changed into leptotene ones.

Stage VIII: The acrosome of step 8 spermatids became bullet-like in shape and continued to elongate. Step 8 spermatids began to move to the basal region and some of them reached the middle region of the epithelium. Leptotene spermatocytes gave rise to zygotene ones.

Stage IX: The nucleus of step 9 spermatids became flattened and condensed. The spermatids still proceeded to elongate. Spermatids came most close to the basal region. Pachytene spermatocytes changed into diplotene ones.

Stage X: The elongation and condensation of the spermatid nucleus completed. In this stage, meiotic figures, secondary spermatocytes and pachytene spermatocytes were recognized in the epithelium.

DISCUSSION

The cell association of the seminiferous epithelium in the Java fruit bat was almost similar to that in the Japanese lesser horseshoe bat. The cycle of the seminiferous epithelium was divided into 11 stages in the Java fruit bat, and into 10 stages in the Japanese lesser horseshoe bat. The number of steps in these bat species was also similar with each other. In these bats, steps 1–2, 3–5, 6–10 and 11–13 of spermatids...
corresponded to the Golgi, cap, acrosome and maturation phases, respectively. However, there were slight differences in the acrosomal formation. In the Java fruit bat, the shape of acrosome became rhomboid in the acrosome phase and changed into scoop-like in the maturation phase. On the contrary, the acrosome of the Japanese lesser horseshoe bat became bullet-like in shape in the acrosome phase, and then slightly shortened. In the maturation phase, it became long spatula-like in shape. Thus, in the Java fruit bat, developing spermatids could be subdivided into 10 steps (step 1–10 spermatids) during one cycle, while in the Japanese lesser horseshoe bat, developing spermatids could be subdivided into 11 steps (step 1–11 spermatids) during one cycle, but in the Japanese lesser horseshoe bat, developing spermatids could be subdivided into 10 steps (step 1–10 spermatids) during one cycle. This finding leads to the difference in the number of stages between the two species.

It is well known that the morphological differences are observed in the acrosomal formation among species [7]. In the rat, mouse [22] and hamster [12], the spermatid nucleus elongated and then began to curve at its tip. Thus, it changed into sickle-like in shape after the cap phase. In the rat, it took 12 steps during acrosome and maturation phases. On the other hand, in the musk shrew [8] and Watase’s shrew [1], the acrosome elongated prominently and flattened. In the late acrosome phase, it slightly shortened and finally became fan-shaped in the maturation phase. In 2 kinds of insectivores, it took only 8 steps during these two phases. In 2 kinds of bats examined here, the elongation of acrosome was also recognized and took 8 steps during these two phases. In addition, the shortening of acrosome was observed in the Japanese lesser horseshoe bat as in insectivores above mentioned.

Conclusively, the characteristics of spermatogenesis were similar between these two bat species. These characteristics were relatively similar to those of insectivores than those of rodents. Rouse and Robson [21] reported that the morphological features of the outer dense fiber of spermatozoa are in common between the order Insectivora and the order Chiroptera. Additionally, some molecular studies [3,18] pointed out the phylogenetic similarity between these two orders. The present finding may also reflect the fact that the order Chiroptera is phylogenetically close to the order Insectivora. Since only a few informations are available on the spermatogenesis in bats, a further study on other kinds of bats should be needed in the future.

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REFERENCES

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