The Cycle of the Seminiferous Epithelium in the Greater Japanese Shrew Mole, *Urotrichus talpoides*

Takuo Mizukami1,2, Sachi Kuwahara1, Masako Ohmura1, Yasuko Inuma1, June Izumikubo1, Mio Hagiwara1, Masamichi Kurohmaru2, Yoshihiro Hayashi3 and Takao Nishida1

1) Laboratory of Anatomy and Physiology, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 252–8510 and 2) Department of Veterinary Anatomy, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113–8657, Japan

(Received 24 May 2000/Accepted 12 September 2000)

**ABSTRACT.** Spermatogenesis and acrosomal formation in the greater Japanese shrew mole, *Urotrichus talpoides*, were studied by light microscopy. On the basis of acrosomal changes, morphology of spermatid head, nuclear shape, appearance of meiotic figures, location of spermatid and period of spermiation, the cycle of the seminiferous epithelium was classified into 12 stages, and developing spermatids could be divided into 15 steps. The mean relative frequencies of stages from I to XII were 10.9, 8.7, 9.8, 7.3, 8.5, 10.3, 12.5, 8.7, 5.8, 5.4, 5.1 and 7.1%, respectively. Similar to the case in the musk shrew, the spermatid nucleus of the greater Japanese shrew mole remained in the middle region of the seminiferous epithelium and only the acrosome extended towards the basement membrane. The elongation of the acrosome, however, was not prominent. The proacrosomal vesicle first appeared in stage II and then one large and round granule was seen in stage III. The acrosomal vesicle became flattened on the surface of the nucleus in stage IV. Spreading of the acrosomic system has been recognized from stage VII. In stage VII, spermiation occurred. In stage IX, the spermatid nucleus began to elongate. Elongation and condensation of the nucleus were clearly observed in stage X. In stage XII, pachytene spermatocytes divided into diplonate spermatocytes. In stage XII, meiotic figures and secondary spermatocytes were observed.

**KEY WORDS:** cycle of the seminiferous epithelium, greater Japanese shrew mole, insectivore, spermatogenesis, spermiation.

---

Spermatogenesis is one of the most fascinating processes in the male reproduction. In the seminiferous epithelium, the cells are organized in a series of well-defined cellular associations, called “stages” [16, 17, 24]. The cycle of the seminiferous epithelium has been defined in a number of mammalian species [1–3, 5–16, 19–23, 27]. The stage is very useful to compare the expression and localization of some important genes and proteins in the seminiferous epithelium among mammalian species. While, insectivores have been thought to be a primitive mammal and do not form a scrotum. There is no critical information why spermatogenesis normally occurs in these ascratal mammals. In order to clarify the mechanism of spermatogenesis in ascratal mammals, it must be needed to classify the stages of the seminiferous epithelium in these mammals at the first step. In insectivores, however, there are only a few reports on the cellular association of seminiferous epithelium [1, 8, 27]. For example, in the musk shrew, the cycle of the seminiferous epithelium has been divided into 13 stages and developing spermatids have been subdivided into 13 steps [8]. In the Watase’s shrew (*Crocidura watasei*), the cycle of the seminiferous epithelium has been divided into 12 stages [1]. Additionally, during acrosomal formation, the spermatid nucleus remained in the middle region of the seminiferous epithelium and only the acrosome extended towards the basement membrane in each species. This characteristic acrosomal elongation in these two species is quite different from that in mice [20], rats [18], and other mammalian species. At present, it is still obscure whether this feature is common throughout the Order Insectivora, and it is therefore necessary to investigate the seminiferous epithelium of other species belonging to this order. Greater Japanese shrew moles (*Urotrichus talpoides*), distributed only in Japan and belonging to the Order Insectivora, are relatively easy to obtain in the field and belong to a different family (Family Talpidae) from the musk and Watase’s shrews (Family Soricidae) [4]. In the present study, an attempt was made to classify the cycle of the seminiferous epithelium of the greater Japanese shrew mole. This research aims to establish the criteria to evaluate the spermatogenic activity and male reproductive state of greater Japanese shrew mole, to observe its acrosomal formation and to compare these features with those of the musk shrew, Watase’s shrew and other mammalian species.

**MATERIALS AND METHODS**

Five adult greater Japanese shrew moles, weighing 14–20 g, were collected at the university forest in Chichibu, Saitama Prefecture. Most of greater Japanese shrew moles were captured with a Sherman’s trap baited with flour paste or soybean. The animals were anesthetized with pentobarbital (5 mg/100 g body weight, i.p.). Then, the testes were surgically excised and immersed in Bouin’s fixative. The samples were cut into slabs, dehydrated in a graded series of ethanol, and embedded in paraffin wax. They were sectioned at 4 µm, stained with hematoxylin-eosin or periodic acid Schiff (PAS)-hematoxylin [17] and observed by light microscopy.
Five hundred round seminiferous tubules were selected from each testis. A total of 5000 round seminiferous tubules (500 tubules \times 10 testes) were evaluated and classified into each stage.

### RESULTS

**Gross anatomy of the male reproductive tract in the greater Japanese shrew mole:** In the greater Japanese shrew mole, each testis descends into the cremasteric scrotum. Three kinds of accessory glands—the seminal vesicle, prostate gland, and bulbourethral glands—are clearly visible. In the musk shrew and Watase’s shrew, the testis is greenish in color, whereas in the greater Japanese shrew mole, it shows no peculiar color. The bulk of the spermatic cord is associated with the testicular artery and vein. The artery branches from the abdominal aorta and pursues a fairly direct course towards the testis. Although the testicular veins form a pampiniform plexus, in most mammalian species including the musk shrew [3, 24, 25], the veins do not form such a pampiniform plexus in the greater Japanese shrew mole.

*Cycle of the seminiferous epithelium* (Figs. 2, 3): Based on evaluations in this study, the acrosomal formation in the greater Japanese shrew mole was quite characteristic. The spermatid nucleus remained in the middle region of the seminiferous epithelium and only the acrosome extended towards the basement membrane, though the elongation of the acrosome was not prominent. The cycle of the seminiferous epithelium was divided into 12 stages, and developing spermatids could be subdivided into 15 steps (Figs. 2, 3) based on acrosomal changes, morphology of spermatid head, nuclear shape, appearance of meiotic figures, location of spermatid, and period of spermiation. The mean relative frequencies of the stages from I to XII were 10.9, 8.7, 9.8, 7.3, 8.5, 10.3, 12.5, 8.7, 5.8, 5.4, 5.1, and 7.1%, respectively. The characteristics of each stage are described below.

**Stage I**

Two kinds of spermatids were present in stages I to VII. No acrosomal system was recognized at step 1 spermatids. While step 13 spermatids were located at the luminal surface of the seminiferous epithelium, pachytene spermatocytes were located near the basement membrane. They were observed in stages from I to X. Type A spermatogonia, attached to the basement membrane, were seen throughout all stages.

**Stage II**

Step 2 spermatids are larger than step 1 spermatids. A proacrosomal vesicle with some proacrosomal granules was first observed at step 2 spermatids. Pachytene spermatocytes increased in size in comparison with those in the previous stage.

**Stage III**

One large and round granule was seen within the vesicle at step 3 spermatids. The vesicle had no contact with the nucleus.

**Stage IV**

The acrosomal system spread over the nucleus of step 5 spermatids. The expanding rim of the vesicle circumscribed a maximum angle approaching 40° on the nuclear profile. Pachytene spermatocytes gradually increased in size. Step 13 spermatids were deeply embedded within the crypts of the Sertoli cell. The acrosome continued to protrude forward from the nucleus.

**Stage V**

The acrosomal system spread over the nucleus of step 5 spermatids.

**Stage VI**

The expanding rim of the vesicle circumscribed an angle from 40° to a maximum of 90°. The acrosome and sub-acrosomal space continued to protrude forward from the nucleus and embedded within the crypts of the Sertoli cell at step 14 spermatids.

**Stage VII**

Spreading of the acrosomic system was still recognized at step 6 spermatids. The expanding rim of the vesicle circumscribed an angle from 90° to a maximum of 120°. Preleptotene spermatocytes were clearly seen in this stage.

**Stage VIII**

Early in stage VII, step 15 spermatids were located at the

![Fig. 1. Gross anatomy of the male genital organ of the greater Japanese shrew mole. 1: testis, 2: epididymis, 3: spermatic cord, 4: penis, 5: glans penis, 6: guctus deferens, 7: seminal vesicle, 8: prostate gland, 9: urinary bladder, 10: kidney, 11: testicular artery. The testis is oval in shape and located within a cremasteric scrotum. The ligament of the tail of epididymis, which is attached to the testis, connects to the cremasteric scrotum. Ductus deferens forms a meshed plexus.](image-url)
luminal surface of the seminiferous epithelium. A cytoplasmic lobe of step 15 spermatids was small, but very densely stained. In late stage VII, spermiation occurred. Leptotene spermatocytes were located near the basement membrane. They are observed in stages from I to X. Type A spermatogonia attached to the basement membrane are seen throughout all stages. Stage II: A proacrosomal vesicle with some proacrosomal granules is first observed at step 2 spermatids. Stage III: The vesicles in the spermatid proacrosomal vesicle have no contact with the nucleus. Stage IV: The acrosomal vesicle becomes flattened on the surface of the nucleus at step 4 spermatids. Step 13 spermatids are deeply embedded within the crypts of the Sertoli cell (arrow). The acrosome continues to protrude forward from the nucleus. Stage V: The acrosomic system spreads over the nucleus of step 5 spermatids. Stage VI: Spreading of the acrosomic system is still recognized at step 6 spermatids. Stage VII: In early stage VII, step 15 spermatids are located at the luminal surface of the seminiferous epithelium (arrow). A cytoplasmic lobe of step 15 spermatids is small, but very densely stained. In late stage VII, spermiation occurs. The nucleolus and Golgi complex of pachytene spermatocytes become more prominent (arrowhead). Stage VIII: Matured spermatids completely disappear from the seminiferous epithelium. Zygotene spermatocytes are seen in this stage. Stage IX: The ovoid nucleus of spermatids becomes flattened and more elongated than that in the previous stage. Stage X: Chromatin condensation begins at the apex of the nucleus (arrow). Stage XI: Diplotene spermatocytes differentiate from pachytene spermatocytes in this stage. Stage XII: Meiotic anaphase or telophase of meiosis I and secondary spermatocytes are observed.

Stage VIII
Mature spermatids completely disappeared from the seminiferous epithelium. One kind of spermatids was recognized in stages VIII to XII. The shape of step 8 spermatids was slightly elongated. The nucleus came intact with the cell surface. Zygotene spermatocytes were seen in this stage.

Stage IX
Remodeling of the nuclear shape began at step 9 spermatids. The ovoid nucleus of spermatids became flattened and more elongated than at step 8 spermatids.

Stage X
The nucleus underwent flattening and elongation at step 10 spermatids. Chromatin condensation began at the apex of the nucleus. The nucleus of the pachytene spermatocyte was round and stained lightly in a distinct region.

Stage XI
The nucleus of step 11 spermatids continued its flattening, elongation and condensation of chromatin. Diplotene spermatocytes differentiated from pachytene spermatocytes in this stage.

Stage XII
The nucleus still continued its flattening and condensation of chromatin. The spermatid head decreased in length at step 12 spermatids. Meiotic anaphase or telophase of meiosis I and secondary spermatocytes were observed.
DISCUSSION

As in most of other mammals, one cross section of the seminiferous epithelium in the greater Japanese shrew mole showed a single stage of the cycle. Although spermatogenesis in the greater Japanese shrew mole fundamentally resembles that in the mouse, musk shrew, Watase’s shrew and other mammalian species, some slight differences were detected. While the cycle of the seminiferous epithelium of the greater Japanese shrew mole was divided into 12 stages and spermatids were subdivided into 15 steps, the cycle of the seminiferous epithelium of the musk shrew was divided into 13 stages and developing spermatids were subdivided into 13 steps [16]. That of the Watase’s shrew was divided into 12 stages and spermatids were subdivided into 13 steps [1]. That of the Spanish mole was divided into 10 stages and spermatids were subdivided into 13 steps [27]. In the greater Japanese shrew mole, the extension of the acrosome towards the basement membrane took a shorter period (2 steps) than in the musk shrew (3 steps) and Spanish mole (3 steps) and almost the same as that in the Watase’s shrew, which showed differences in number of stages among these four kinds of insectivores. In most mammals, both the acrosome and spermatid nucleus migrate towards the basement membrane during acrosomal formation, whereas in the musk shrew, the spermatid nucleus remains in the middle region of the seminiferous epithelium and only the acrosome extends towards the basement membrane. The spermatid nucleus of the greater Japanese shrew mole also remained in the middle region of the seminiferous epithelium and only the acrosome extended towards the basement membrane, though the elongation of the acrosome was not prominent. Similar to the greater Japanese shrew mole, in the Spanish mole [27] and European common shrew [22], the spermatids’ nucleus remained in the middle region of seminiferous epithelium and only the acrosome extended towards the base to a lesser degree. At this point, the acrosomal formation in the greater Japanese shrew mole was more similar to that in the Spanish mole (Talpa occidentalis) [27] and European common shrew (Sorex araneus) [22] than in the musk shrew and Watase’s shrew. It is well known that insectivores have a large acrosome in comparison with other mammalian species [22]. The development of the large acrosome should have a strong relation with the peculiar acrosomal formation in insectivores. It seems likely that the prominent extension of acrosome induces enlargement of acrosome.

![Fig. 3. Schematic drawing of the cycle of the seminiferous epithelium in the greater Japanese shrew mole. Roman numerals represent each stage. Arabic numerals beneath the Roman numerals show the relative frequency of each stage as a percentage. Arabic numerals beneath each spermatid show each step. A, In, B, differentiated spermatogonia (A, B, Intermediate); PL, L, Z, P, D, primary spermatocytes; PL, preleptotene phase; L, leptotene phase; Z, zygotene phase; P, pachytene phase; D, diplotene phase; II, secondary spermatocyte; M, meiotic figure; RB, residual body.](image-url)
ACKNOWLEDGMENT. We wish to thank Dr. Ken Ishida (Department of Forestry Science, The University of Tokyo) for guiding us to the sampling point of the university forest in Chichibu.

REFERENCES


