Spermatogenesis in the Watase's Shrew, *Crocidura watasei* —A Light and Electron Microscopic Study—

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Spermatogenesis in the Watase's shrew, *Crocidura watasei*, was investigated by light and transmission electron microscopy. The cycle of the seminiferous epithelium was divided into 12 stages using the development of spermatids as a main criterion. The steps of spermatids were characterized by morphological changes of the nucleus and acrosomal structure. The relative frequencies of the stages 1 to $\times 11$ were 11.0, 10.3, 6.8, 10.6, 24.0, 6.4, 4.4, 7.9, 6.4, 4.9, 3.7 and 3.6%, respectively. Four types of spermatogonia (A_1 , A_2 , In and B) could be discerned by the observation of whole mount samples. The development of spermatids was divided into four phases (Golgi, cap, acrosome and maturation phases), as in other mammals. In Golgi phase of the spermatid, several acrosomal granules were encountered. In cap phase, the acrosome gradually spread over the nuclear surface. In early acrosome phase, the acrosome began to elongate and reached the maximal length in step 8 spermatids. The acrosome of step 8 spermatids was twice as long as that of spermatozoa. In late acrosome phase, the acrosome was on the way of shrinkage. Finally, the fan-shaped acrosome was formed in maturation phase. These findings suggested that the process of acrosomal formation was quite characteristic in the Watase's shrew in that the spermatid acrosome elongated most prominently in the mammals hitherto examined. — KEY WORDS : acrosome, spermatid, spermatogenesis, spermiogenesis, Watase's shrew

The Watase's shrew distributed in Nansei Islands is 5–7 cm in body length (excluding the tail) and 5–8 g in body weight in the adult [14]. Recently, it has been suggested that the Watase's shrew is not a subspecies of the long-tailed shrew, *Crocidura horsfieldi*, but an independent species from chromosomal and osteometrical standpoints [11, 14, 17, 36, 37]. Therefore, in the present study, the scientific name of the Watase's shrew is designated as *Crocidura watasei*. For the last decade, the Watase's shrew has been attempted to be domesticated as a new laboratory animal belonging to the Order Insectivora at Amami

Branch, Institute of Medical Science, the University of Tokyo [13, 14]. Some peculiar anatomical characteristics have been reported in this small insectivore [12, 18].

Although the spermiogenesis have been investigated in many mammalian species from rodents [2, 6, 7, 10, 15, 19–26, 28, 31] to livestock [4, 8, 21, 33, 34], the information on spermatogenesis of insectivores is very few at present [29]. Therefore, the present study was designed to classify the stages of the seminiferous epithelium and to elucidate spermatogonial renewal in the testis of Watase's shrew. As to the definition of stages, because develop-

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mental changes of the spermatid acrosome were frequently used to divide the cycle of the seminiferous epithelium into stages [1, 3, 19, 28], these morphological characteristics were employed as criteria in this study. In addition, ultrastructural changes of spermatids during spermiogenesis were examined by transmission electron microscopy.

Materials and Methods

Seven adult Watase's shrews, weighing 5-7 g, were captured at Amami and Tokunoshima Islands. Under pentobarbital anesthesia, the testes of 4 animals were perfused with 0.9% saline followed by Bouin's solution. The samples were immersed in the same fixative for 2-3 hr, dehydrated in a graded series of ethanol and embedded in paraffin. Then, they were sectioned at 5 μ m, stained with periodic acid Schiff (PAS)-hematoxylin and examined by light microscopy. The cross-sections were used to determine the relative duration of the stages of the cycle of the seminiferous epithelium. In both testes of each animal, the stage of the cycle was determined in 200 cross-sectioned seminiferous epithelium. The relative duration of each stage was decided by the observation of 1,000 cross-sections.

Two right testes from 2 Watase's shrews were surgically excised and used for the examination of spermatogonial renewal with whole mount samples. The other testes of the same animals were provided for transmission electron microscopy. The whole mount samples were prepared according to the method of Clermont and Bustos-Obregon (1968) as follows. Firstly, the tunica albuginea was cut open to remove the seminiferous tubules. The detached tubules were then immersed in Bouin's fixative for 2-3 hr and separated from each other with fine needles. The tubules were placed in 3 consecutive baths containing 80% ethanol, 70% ethanol and distilled water, respectively. They were stained with hematoxylin, washed in distilled water and dehydrated in a graded series of ethanol. Finally, the tubules were mounted on a glass slide and examined as whole mount samples by light microscopy.

Four testes from 3 Watase's shrews were used for transmission electron microscopy. The testes were perfused through the heart with 0.9% saline followed by 2.5% glutaraldehyde

in 0.05 M cacodylate buffer, pH 7.4. The testes were cut into smaller pieces and immersed in the same fixative for an additional hour. After washing with 0.05 M cacodylate buffer, they were postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol and embedded in Quetol 812. Semithin sections were cut at 1 μ m, stained with toluidine blue and examined with a light microscope to classify their seminiferous epithelia into different stages. Each stage of the epithelium was selected for the observation by transmission electron microscopy. Thin sections were cut on an LKB ultratome, stained with uranyl acetate and lead citrate and examined with a JEM-1200 EX transmission electron microscope at 80 kV.

Results

The spermiogenesis of the Watase's shrew could be divided into 4 phases (Golgi, cap, acrosome and maturation phases). In the Golgi phase, several acrosomal granules were found in the Golgi zone. In the cap phase, the granules changed into the acrosome. The head cap appeared to cover a part of the nucleus. In the acrosome phase, the acrosome extended to be slender. The nucleus flattened to become half-discoidal in shape. In the maturation phase, the spermatid completed its morphological change to become a spermatozoon. These 4 phases were subdivided into 13 steps (Table 1). Steps 1-3, 4-5, 6-10 and 11-13 of spermatids corresponded to the Golgi, cap, acrosome and maturation phase, respectively.

Cycle of the seminiferous epithelium: Judging from acrosomal changes of spermatids and presence and/or absence of the residual body, meiotic figure and spermiation, the cycle of the seminiferous epithelium was divided into 12 stages (Table 1). The stages of the cycle are numbered with Roman numerals to avoid a confusion with the steps of spermatogenesis numbered with Arabic numerals.

Stage I was identified by the presence of newly-formed step 1 spermatids. Only a few acrosomal granules were seen in the Golgi zone of these cells. In addition to step 1 spermatids, maturing spermatids at step 11 were also observed in this stage. The nucleus of step 11 spermatid was flat and discoidal in shape. The acrosome was blade-like in shape. Along the

Table 1. Diagram of 12 stages of the cycle of the seminiferous epithelium in the Watase's shrew

relative % frequency	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
	11.0	10.3	6.8	10.6	24.0	6.4	4.4	7.9	6.4	4.9	3.7	3.6
	11	12	12	12	13							
	1	2	3	4	5	5	5	6	7	8	9	10
	P	P	P	P	P	P	P	P	P	P	D	M
	A ₂	A ₂	In	In	B	B	PL	L	Z	P	P	SS
	A ₁	A ₁	A ₁	A ₁	A ₁	A ₁	A ₁	A ₁	A ₁	A ₁	A ₂	A ₂

A₁, A₂, In, B: differentiated spermatogonia/PL, L, Z, P and D: primary spermatocytes (PL, preleptotene step: L, leptotene step: Z, zygotene step: P, pachytene step: D, diplotene step) SS: secondary spermatocyte M: mitotic figure Roman numerals: each stage of the cycle Arabic numerals at the upper part: relative frequency of each stage in percentage Arabic numerals at the lower part: each step of spermatids

basement membrane, 2 types of spermatogonia (type A₁, A₂) were recognizable at this stage. The type A₁ spermatogonia had an oval nucleus containing pale-stained granular chromatin. While, the type A₂ had a round nucleus with dark-stained granular chromatin. The type A₂ was smaller in size than the type A₁. The type A₁ and A₂ spermatogonia were found at all stages of the cycle. On the other hand, all spermatocytes present at this stage belonged to early pachytene stage of the meiotic prophase. The dark-stained chromosome of their nucleus was thick, but still long and tortuous (Fig. 1).

In stage II, one large acrosomal granule appeared in the Golgi zone of the spermatid. The maturing spermatids (step 12) were recognizable in this stage. These spermatids were morphologically similar to spermatozoa, although they were located in the middle portion of the seminiferous epithelium and rich in cytoplasm. The A₁, A₂ spermatogonia and early pachytene spermatocytes were also present at this stage.

In stage III, the acrosomal granule began to flatten on the surface of the spermatid nucleus. The acrosomal granule became hemispherical and could be designated as the acrosome. In addition to the type A₁ spermatogonia, a new type of spermatogonia (intermediate type; type In) appeared at the base of the tubule. Although morphological characteristics of the type In spermatogonia were similar to those of the type A₂, the type In contained an increasing amount of chromatin

flakes attached to the nuclear membrane. The maturing spermatids (step 12) and early pachytene spermatocytes were also present at this stage.

Stage IV was discerned by the formation of the head cap. The head cap was a delicate membranous structure. The maturing spermatids (step 12), type A₁ and type In spermatogonia, and early pachytene spermatocytes were still present at this stage (Fig. 2).

In stage V, the acrosome covered a half of the nuclear surface. The matured spermatids (step 13) without the residual cytoplasm were lining the luminal surface of the seminiferous epithelium. Type A₁ spermatogonia and newly-emerged type B spermatogonia were recognizable along the basement membrane. The nucleus of type B spermatogonia had large flakes of dark-stained chromatin. The early pachytene spermatocytes were also present.

In stage VI, the sperm release occurred. The residual body remained within the seminiferous epithelium. Only one generation of spermatids (step 5) was recognized in the epithelium. Type A₁ and type B spermatogonia, and early pachytene spermatocytes were present in this stage.

In stage VII, the residual body disappeared. The round spermatids (step 5) were still present. Type A₁ spermatogonia and a new generation of primary spermatocytes formed by division of type B spermatogonia were found in the vicinity of the basement membrane. The newly-emerged preleptotene spermatocytes had a small round nucleus containing large chro-

matin flakes. The older generation of spermatocytes reached the late pachytene step (Fig. 3).

In stage VIII, the acrosome of the spermatids came in contact with the plasma membrane and oriented towards the basement membrane with rotation of the nucleus. The nucleoplasm of the spermatid (step 6) showed a tendency to condense. The young generation of primary spermatocytes was differentiated into the leptotene step. No change was noticed in late pachytene spermatocytes except for a gradual increase of their nuclear volume.

In stage IX, the acrosome gradually extended towards the base of the epithelium. The leptotene spermatocytes were differentiated into the zygotene step. Type A₁ spermatogonia and late pachytene spermatocytes were also present (Fig. 4).

In stage X, the acrosome further extended to reach the maximal length. Although the acrosome of the step 8 spermatid extended into the basal region of the seminiferous epithelium, the flattened nucleus remained in the middle region. The younger generation of primary spermatocytes was differentiated into the early pachytene step of the meiotic prophase. Type A₁ spermatogonia and the older generation of primary spermatocytes at late pachytene step were recognizable in this stage (Fig. 5).

In stage XI, the acrosome of step 9 spermatids expanded to a lesser degree, while the nucleoplasm condensed to a greater degree. Type A₂ spermatogonia appeared by division of type A₁ spermatogonia. The younger spermatocytes at early pachytene step and the older spermatocytes at diplotene step were recognizable at this stage.

In stage XII, meiotic figures and secondary spermatocytes were present. The acrosome of step 10 spermatids shrank to be flat sword-like in shape. Their nuclei were half discoidal in shape. The younger spermatocytes at pachytene step and type A₂ spermatogonia were also detected (Fig. 6).

Ultrastructural changes of spermatids during spermiogenesis: Morphological changes of spermatids during spermiogenesis were investigated by transmission electron microscopy. The morphological features of spermatids could be divided into 4 phases (Golgi, cap, acrosome and maturation phases).

In the Golgi phase, the spermatid and its nucleus were round in shape. The precursor of acrosome was composed of acrosomal granules and a capsule and situated between the nucleus and the Golgi complex. Some oval mitochondria and a quantity of smooth ER were observed in the spermatid cytoplasm. The spermatids were connected with each other by the intercellular bridges (Fig. 7).

In the cap phase, the spermatid and its nucleus were still round. The homogeneous acrosome gradually expanded along the nuclear membrane and finally covered a half of the whole nucleus in the later cap phase, as observed by light microscopy. The acrosomal cap was concavo-convex in outline (Fig. 8). The well-developed Golgi complex was present in the vicinity of the acrosome.

In the early acrosome phase, the acrosome began to elongate towards the base of the tubule. The elongation of the acrosome reached the peak in the late acrosome phase. The manchette consisting of bundles of microtubules appeared in elongating spermatids in the early phase (Fig. 9).

In the maturation phase, the nucleus of the maturing spermatid condensed conspicuously and was surrounded by the homogeneous dense acrosome (Fig. 10). The acrosome decreased in length and increased in width than in the previous phase.

Discussion

There are 2 methods to subdivide the cycle of the seminiferous epithelium in mammals. One method is based on the nuclear shape and the location of spermatids, meiotic figures and spermiation. The cycle of the seminiferous epithelium is usually divided into 8 stages by this method [9, 10, 16, 27, 31-35]. The other method is based on the morphological changes of the spermatid acrosome. In this case, the cycle is divided into 12-14 stages according to authors [1, 3, 5, 19, 28]. Because of the greater number of divided stages, the latter method seems to be useful to classify the morphology of the seminiferous epithelium in detail. In the present study, the cycle of the seminiferous epithelium in the Watake's shrew could be divided into 12 stages with the latter method.

The developing spermatids were subdivided into 19 steps in the rat [2, 30] and 16 steps in

the mouse [23, 30]. In the case of the Watase's shrew, however, only 13 steps were discernable. The acrosomal formation (namely, the duration of spermiogenesis) of the Watase's shrew takes less than 1.5 cycles, while it takes more than 1.5 cycles in the rat [2, 30], mouse [23, 30], prairie vole [31] and cotton rat [19]. Although the acrosomal formation of the Watase's shrew seems to be complicated owing to the prominent elongation and subsequent shrinkage of the acrosome, the acrosomal formation of the Watase's shrew takes a shorter period as compared with that in other small mammals. From the observation of whole mount samples, differentiated spermatogonia of the Watase's shrew were classified into 4 types (A₁, A₂, In and B). The types of differentiated spermatogonia seem small in number in the Watase's shrew, since their number was 5 in the golden hamster [5] and 6 in the rat [2], mouse [22, 26] and Chinese hamster [28].

Among some characteristics as described above, the most conspicuous feature in the spermatogenesis of the Watase's shrew is the prominent elongation of the spermatid acrosome. To our knowledge, no other mammals show such prominent elongation of the acrosome. For example, the acrosome of the European common shrew, *Sorex araneus*, is small in size and different from the well-developed acrosome of the Watase's shrew [29]. This may suggest that the Watase's shrew is specialized separately from *Sorex*. Further studies concerning the spermatogenesis and/or testicular morphology of other kinds of shrews will be required to confirm whether this peculiar feature is specific to the Watase's shrew.

In most mammals studied [9, 10, 16, 19], the nucleus of the maturing spermatid moves to the basal region of the seminiferous tubule and then moves towards the luminal side before spermiation. On the other hand, the nucleus of the spermatid remains in the middle region of the seminiferous epithelium at that time in the Watase's shrew. The prominent elongation of the acrosome to the basal region of the tubule in the Watase's shrew may be the compensation of the basal migration of the nucleus of the maturing spermatid in other mammals.

In conclusion, the present study provided

the fundamental information on the spermatogenesis of the Watase's shrew. Because of the prominent elongation of the acrosome, the Watase's shrew may be one of useful mammals to clarify the complicated mechanism of the acrosomal formation.

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References

- [1] Clermont, Y. and Harvey, S. C. (1965). Duration of the cycle of seminiferous epithelium of normal, hypophysectomized and hypophysectomized-hormone treated albino rats. *Endocrinology*, **76**, 80-89.
- [2] Clermont, Y. and Bustos-Obregon, E. (1968). Re-examination of spermatogonial renewal in the rat by means of seminiferous tubules mounted "in toto". *Am. J. Anat.*, **122**, 237-248.
- [3] Clermont, Y. and Antar, M. (1973). Duration of the cycle of the seminiferous epithelium and the spermatogonial renewal in the monkey, *Macaca arctoides*. *Am. J. Anat.*, **136**, 153-166.
- [4] Courtens, J., Amir, D., and Durand, J. (1980). Abnormal spermiogenesis in bulls treated with ethylene dibromide: An ultrastructural and ultracytochemical study. *J. Ultrastruct. Res.*, **71**, 103-115.
- [5] De Rooij, D. G. (1968). Stem cell renewal and duration of spermatogonial cycle in the goldhamster. *Z. Zellforsch.*, **89**, 113-136.
- [6] De Rooij, D. G. and Janssen, J. M. (1987). Regulation of the density of spermatogonia in the seminiferous epithelium of the Chinese hamster: I. Undifferentiated spermatogonia. *Anat. Rec.*, **217**, 124-130.
- [7] De Rooij, D. G. and Lok, D. (1987). Regulation of the density of spermatogonia in the seminiferous epithelium of the Chinese hamster: II Differentiating spermatogonia. *Anat. Rec.*, **217**, 131-136.
- [8] Ekstedt, E., Söderquist, L., and Plöen, L. (1986). Fine structure of spermatogenesis and Sertoli cells (*Epitheliocytus sustentans*) in the bull. *Anat. Histol. Embryol.*, **15**, 23-48.
- [9] Foote, R. H., Swierstra, E. E., and Hunt, W. L. (1972). Spermatogenesis in the dog. *Anat. Rec.*, **173**, 341-352.
- [10] Grocock, C. A. and Clarke, J. R. (1975). Spermatogenesis in mature and regressed testes of the vole (*Microtus agrestis*). *J. Reprod. Fertil.*, **43**, 461-470.
- [11] Harada, M., Yoshida, T. H., Hattori, S., and Takada, S. (1985). Cytogenetical studies on Insectivora. III. Karyotype comparison of two *Crocicidura* species in Japan. *Proc. Jpn. Acad.*, **61**, 371-374.
- [12] Hattori, S. and Yamanouchi, K. (1984). Gross anatomy of the Watase's shrew, *Crocicidura horsfieldi watasei*. *Exp. Anim.*, **33**, 519-524.
- [13] Hattori, S., Noboru, Y., and Yamanouchi, K. (1986). Domestication of the Watase's shrew, *Crocicidura horsfieldi watasei*, for a laboratory animal. *Jpn. J. Exp. Med.*, **56**, 75-79.
- [14] Hattori, S. (1991). Study on *Crocicidura watasei*. The Doctoral Thesis, The University of Tokyo. (In

- Japanese).
- [15] Huckins, C. (1971). The spermatogonial stem cell population in adult rats. I. Their morphology, proliferation and maturation. *Anat. Rec.*, **169**, 533-558.
 - [16] Kennelly, J. J. (1972). Coyote reproduction : I. The duration of the spermatogenic cycle and epididymal sperm transport. *J. Reprod. Fertil.*, **31**, 163-170.
 - [17] Krishna, R. S. and Aswathanarayana, N. V. (1978). Karyological analysis of Horsfield's shrew from peninsula India. *J. Heredity*, **69**, 202-240.
 - [18] Kurohmaru, M., Nishida, T., Mochizuki, K., Hayashi, Y., and Hattori, S. (1982). Morphology of the intestine of the Watase's shrew, *Crocidura horsfieldi watasei* *Jpn. J. Vet. Sci.*, **44**, 795-799.
 - [19] Kurohmaru, M., Tiba, T., Nishida, T., and Hayashi, Y. (1988). Spermatogenesis and ultrastructural changes of spermatids during spermiogenesis in the cotton rat, *Sigmodon hispidus*. *Okajimas Folia Anat. Jpn.*, **65**, 203-220.
 - [20] Leblond, C. P. and Clermont, Y. (1952). Definitions of stages of the cycle of the seminiferous epithelium in the rat. *Ann. N.Y. Acad. Sci.*, **55**, 548-573.
 - [21] Lok, D., Weenk, D., and De Rooij, D. G. (1982). Morphology, proliferation, and differentiation of undifferentiated spermatogonia in the Chinese hamster and the ram. *Anat. Rec.*, **203**, 83-99.
 - [22] Monesi, V. (1962). Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine. *J. Cell Biol.*, **14**, 1-18.
 - [23] Oakberg, E. F. (1956 a) A description of spermatogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium. *Am. J. Anat.*, **99**, 391-414.
 - [24] Oakberg, E. F. (1956 b). Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. *Am. J. Anat.*, **99**, 507-516.
 - [25] Oakberg, E. F. (1957). Duration of spermatogenesis in the mouse. *Nature*, **180**, 1137-1138.
 - [26] Oakberg, E. F. (1971). Spermatogonial stem-cell renewal in the mouse. *Anat. Rec.*, **169**, 515-532.
 - [27] Osman, D. I. and Plöen, L. (1986). Spermatogenesis in the camel (*Camelus dromedarius*). *Anim. Reprod. Sci.*, **10**, 23-26.
 - [28] Oud, J. L. and De Rooij, D. G. (1977). Spermatogenesis in the Chinese hamster. *Anat. Rec.*, **187**, 113-124.
 - [29] Plöen, L., Ekwall, H., and Afzelius, B. A. (1979). Spermiogenesis and the spermatozoa of the European common shrew (*Sorex araneus* L.). *J. Ultrastruct. Res.*, **68**, 149-159.
 - [30] Russell, L. D. (1990). Staging for laboratory species. In *Histological and Histopathological Evaluation of the Testis*, pp 62-193, Russell, L. D., Ettlin, R. A., Sinha Hikim, A. P., and Clegg, E. D. (eds.), Cache River Press.
 - [31] Schuler, H. M. and Gier, H. T. (1976). Duration of the cycle of the seminiferous epithelium in the prairie vole (*Microtus ochrogaster ochrogaster*) *J. Exp. Zool.*, **197**, 1-12.
 - [32] Swierstra, E. E. and Foote, R. H. (1963). Cytology and kinetics of spermatogenesis in the rabbit. *J. Reprod. Fertil.*, **5**, 309-322.
 - [33] Swierstra, E. E. (1968). Cytology and duration of the cycle of the seminiferous epithelium of the boar : Duration of spermatozoan transmit through the epididymis. *Anat. Rec.*, **161**, 171-186.
 - [34] Swierstra, E. E., Gebauer, M. R., and Pickett, B. W. (1974). Reproductive physiology of the stallion. I. Spermatogenesis and testis composition. *J. Reprod. Fertil.*, **40**, 113-123.
 - [35] Tiba, T., Ishikawa, T., and Murakami, A. (1968). Histologische Untersuchung der Kinetik der Spermatogenese beim Mink (*Mustela vison*). I Samenepithelzyklus of der Paarungszeit. *Jpn. J. Vet. Res.*, **16**, 73-87.
 - [36] Tsuchiya, K. (1979). A contribution to the chromosome study in Japanese mammals. *Proc. Jpn. Acad.*, **55**, 191-195.
 - [37] Yoshida, T. H., Moriguchi, Y., and Sonoda, J. (1968). Karyological studies of three species of Insectivora collected in Japan. *Ann. Rep. Nat. Inst. Genet. Jpn.*, **18**, 24-25.

ワタセジネズミ *Crocidura watasei* の精子発生 ——光顕および電顕による観察——

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ワタセジネズミ *Crocidura watasei* の精子発生について光顕および電顕による観察を行った。精上皮周期は精子細胞尖体の形態変化を主な指標として解析した結果、12のステージに区分可能であった。ステージⅠからⅫの出現頻度は各々11.0, 10.3, 6.8, 10.6, 24.0, 6.4, 4.4, 7.9, 6.4, 4.9, 3.7および3.6%である。またホルマウント標本の観察から分化中の精祖細胞はA₁, A₂, Intermediate, Bの4つのタイプに区分された。一方、精子細胞はその成熟程度に応じてゴルジ相(ステップ1—3), 頭帽相(ステップ4, 5), 尖体相(ステップ6

—10) および成熟相(ステップ11—13)の4相に区別された。ゴルジ相では複数の尖体果粒が存在し、頭帽相ではこれらが融合して尖体を形成し、精子細胞核表面に広がった。尖体相では核質の濃縮とともに尖体の伸長が始まり、尖体はステップ8の精子細胞で最長となった。尖体相後期では尖体の収縮が進行し、成熟相で幅広の尖体が完成した。ワタセジネズミに認められた精子細胞分化過程での尖体の著しい伸長とその後の収縮は、他の哺乳類に見られない極めて特徴的な現象である。

Explanation of Figures

Fig. 1. Stage I. ×700 Round spermatid (arrow) and maturing spermatids (step 11, arrowheads) were present at this stage. Early pachytene spermatocyte (P) and some spermatogonia were also recognizable. BM: basement membrane L: lumen Ser: Sertoli cell

Fig. 2. Stage IV. ×700 Similar to stage I, 2 generations of spermatids were found. Younger spermatids (arrows) had an obvious acrosomal cap.

Fig. 3. Stage VII. ×700 Only one generation of spermatids (step 5, arrowheads) was present at this stage. Preleptotene spermatocyte (arrow) newly appeared in the vicinity of the basement membrane. P: pachytene spermatocyte

Fig. 4. Stage X. ×700 The acrosome of the spermatid extended towards the base of the tubule. Newly-emerged zygotene spermatocyte (Z) was recognized. P: pachytene spermatocyte

Fig. 5. Stage X. ×700 Although the acrosome reached the basal region of the seminiferous epithelium, the nucleus remained in the middle

region. Early (Pe) and late (Pl) pachytene spermatocytes were also found.

Fig. 6. Stage XII. ×700 The secondary spermatocyte (arrow) and mitotic figures (arrowheads) appeared at this stage.

Fig. 7. Spermatid in Golgi phase. ×6,000 Some acrosomal granules (arrowheads) were clearly observed near the Golgi complex. A quantity of smooth endoplasmic reticulum and several mitochondria were found within the cytoplasm. N: nucleus

Fig. 8. Spermatid in cap phase. ×7,500 The acrosome (arrow) surrounded more than half of the round nucleus. N: nucleus

Fig. 9. Spermatid in acrosome phase. ×3,800 Both the acrosome (arrowheads) and the nucleus (N) elongated to a greater degree. Although the acrosome condensed to be homogeneous, the nucleoplasm was not yet condensed. Ser: Sertoli cell

Fig. 10. Spermatid in maturation phase. ×5,300 Five matured spermatids with condensed nucleoplasm were harboured in the Sertoli cell (Ser). N: nucleus Arrow: tail of spermatid