Comparative fine structure of the epididymal spermatozoa from three Korean shrews with considerations on their phylogenetic relationships

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Key words: crocidurinae, shrew, spermatozoa, phylogeny, fine structure

ABSTRACT: This study examined the fine structures of epididymal spermatozoa on the lesser white-toothed shrew (Crocidura suaveolens), the Japanese white-toothed shrew (C. dsinezumi) and the big white-toothed shrew (C. lasiura) belonging to the subfamily Crocidurinae living in Korea.

In the spermatozoa of C. suaveolens, the head has a large acrosome, a smooth inner acrosomal membrane and a wavy, finger-like, electron-dense apical body. The neck has a solid proximal centriole that is filled with electron-dense material. These results showed the spermatozoa of C. suaveolens possess the characteristics of both Crocidurinae and Soricinae. In C. dsinezumi and C. lasiura, the head has a large acrosome, a serrated inner acrosomal membrane and a common apical body. The neck has a fistulous proximal centriole with slightly dense electron granules. These results showed the typical characteristics of Crocidurinae.

Although C. suaveolens belongs to the subfamily Crocidurinae, the spermatozoan morphology is different from C. dsinezumi and C. lasiura because it has conserved characteristics of the subfamily Soricinae.

Abbreviations: a, acrosome; ab, apical body; af, axial filament complex; an, annulus; bp, basal plate; dg, dense granules; fs, fibrous sheath; iam, inner acrosomal membrane; lc, longitudinal column; m, mitochondrion; mp, middle piece; n, nucleus; oam, outer acrosomal membrane; od, outer dense fibers; pc, proximal centriole; pn, post nuclear cap; pm, plasma membrane; pp, principal piece; sc, segmented column; sf, satellite fibers; ss, subacrosomal space; te, terminal end of the middle piece.

Introduction

Soricidae belonging to the Insectivora is generally believed to comprise of two subfamilies, Soricinae and Crocidurinae, as a result of their morphometric characteristics including the color of the tooth tip, the length of the head and body, the length of the tail, the thickness of the tail base and the composition of the tail fur (Jones and Johnson, 1960; Won, 1967; Corbet, 1978; Tsuchiya, 1985; Yoon, 1992) as well as the distinctive reproductive characteristics (Churchfield, 1990). Crocidurinae living in Korea consists of three species:
C. suaveolens, C. dsinezumi and C. lasiura. C. suaveolens and C. lasiura are widespread on the Korean peninsula but C. dsinezumi is found only on Cheju island (Jones and Johnson, 1960; Won, 1967; Yoon, 1992). Crocidurinae is not only a useful environmental indicator on account of its sensitivity to pollution (Diamond and Sherburne, 1969; Braham and Neal, 1974; Robert et al., 1978; Andrew et al., 1984); it also is an important mediator controlling the population of various invertebrates and small vertebrates in the ecosystem (Holling, 1959; Buckner, 1969; Churchfield, 1990). However, all three species are rare in the wild and their population is also decreasing. Moreover, they have never been studied with the exception of the brief comments and taxonomical reviews in Korea (Jones and Johnson, 1960; Won, 1967; Yoon, 1992; Han, 1997).

The morphology of the spermatozoa has been reported to be a distinct characteristic of the family, subfamily, genus and species (Friend, 1936; Hughes, 1965; Fawcett, 1970; Fawcett and Phillips, 1970; Uchida and Mori, 1972; Bedford, 1974; Mori, 1994). In addition, the sperm morphology and morphometry is considered one of the phenotypic traits for the study of the phylogenetic distances among species (Cetica et al., 1997). The sperm morphology in the family Dasypodidae can contribute to the understanding of evolution and phylogeny of the reproductive system in these mammals and similar sperm shapes and sizes support the data on their phylogenetic proximity (Cetica et al., 1997). Soricinae and Crocidurinae belong to the family Soricidae, and are classified by the following morphological characteristics of the spermatozoa (Fawcett and Phillips, 1970; Mori et al., 1991). In the Soricinae spermatozoa, the head has a small acrosome, a smooth inner acrosomal membrane and a wavy, finger-like, electron-dense apical body and the neck has a solid proximal centriole filled with electron-dense material (Ploen et al., 1979; Mori et al., 1991; Mori, 1994). In Crocidurinae, the head has a large acrosome, a serrated inner acrosomal membrane and a common apical body, and the neck has a fistulous proximal centriole with slightly dense electron granules (Cooper and Bedford, 1976; Green and Dryden, 1976; Koehler, 1977; Mori and Uchida, 1985; Mori et al., 1991; Mori, 1994). Therefore, it appears that the fine structure of the spermmatozoa is a useful taxonomical characteristic for classifying the species belonging to the two subfamilies, Soricinae and Crocidurinae. In case of the Japanese water shrew (chimarrogale himalayica) it was indeed classified as Crocidurinae by only the morphometric characteristics, but was revised to Soricinae by an examination of the ultrastructure of the spermatozoa (Mori et al., 1991).

The aim of this study was to compare fine structure and morphometry of the epididymal spermatozoa and to consider the phylogenetic relationships of the lesser white-toothed shrew (Crocidura suaveolens), the Japanese white-toothed shrew (C. dsinezumi) and the big white-toothed shrew (C. lasiura) belonging to the subfamily Crocidurinae in Korea.

Materials and Methods

Three adult males of Crocidura suaveolens were collected between October 1999 and March 2000, and seven adult males of C. lasiura were collected between June and October 1999 and March 2000 from Mt. Jiri. Three adult males of C. dsinezumi were collected from Cheju island in Korea in July 1999.

Observation of the living spermatozoa

In order to observe living spermatozoa of epididymis, one epididymis from a specimen was finely cut in Millonig’s phosphate buffer, pH 7.4, and observed using light microscopy. The length of the head, width of the head, length of the nucleus, length of the middle piece and total length of the sperm tail of C. suaveolens and C. lasiura were measured from photographs of the living spermatozoa.

Transmission electron microscopy

To observe the fine structures of the spermatozoa, the other epididymis was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in Millonig’s phosphate buffer. The materials were post-fixed with 1.3% osmium tetroxide in the same buffer for 2 h, dehydrated in a graded series of ethanol and acetone, and embedded in epoxy resin. Thick sections (0.5-1 μm) were stained with 5% toluidine blue for light microscopy. Thin sections (60-90 nm) were double-stained with uranyl acetate and lead citrate. All thin sections were examined using a JEOL 100S transmission electron microscope.

Statistical analysis

The sperm sizes were analyzed by a t-test. A P-value <0.05 was considered significant.
FIGURES 1-6. Light and electron micrographs showing the sperm head and tail of C. suaveolens.
Scale bars: Fig. 1 = 10 μm. Figs. 2-6 = 0.5 μm.
Figure 1. Planar view of an epididymal spermatozoon.
Figure 2. Frontal section of a large, flattened and shield-shaped head.
Figure 3. Sagittal section of a head with an unusually long, wavy, finger-like and electron-dense apical body in the smooth subacrosomal space.
Figure 4. Longitudinal section of a sperm neck bearing the proximal centriole whose lumen is filled with electron-dense granules.
Figure 5. Longitudinal section of the middle and principal pieces.
Figure 6. Cross section of the sperm tails from middle to end pieces. Note outer dense fibers arranged in a horseshoe fashion, well-developed satellite fibers associated with the inner sides of fibers 5 and 6, and bi-lobed fiber 1 fused in the outer side.
**Results**

*Crocidura suaveolens*

The living epididymal spermatozoa of *C. suaveolens* has a flattened and shield-shaped head with a large acrosome and a comparatively long middle piece in the sperm tail (Fig. 1). The length of each part is as follows: head, 12.61 ± 0.49 μm (n=16); head width, 10.70 ± 0.23 μm (n=10); nucleus, 4.72 ± 0.31 μm (n=11); middle piece, 52.85 ± 2.35 μm (n=12); total length of the tail, 87.58 ± 1.71 μm (n=12) (Table 1).

Transmission electron microscopy revealed the frontal section of the nucleus to be a fan shape (Fig. 2) and the sagittal section to be flat (Fig. 3). The smooth subacrosomal space between the inner acrosomal membrane and the nuclear membrane is filled with a wavy, finger-like, electron-dense apical body and fine granules. The length of the post-nuclear cap is approximately half of the nuclear length (Figs. 2, 3). In the longitudinal section of the spermatozoan tail, the proximal centriole of the sperm neck is fully filled with electron-dense material (Fig. 4), and the axial filament complex and outer dense fibers are surrounded by mitochondria at the middle piece and by fibrous sheaths at the principal piece (Fig. 5). In the cross section of the spermatozoan tail, the nine outer dense fibers are arranged in a horseshoe fashion, well-developed satellite fibers are associated with the inner side of the outer dense fibers 5 and 6, and a small bi-lobed outer dense fiber 1 is fused in the outer side (Fig. 6). The outer dense fibers 3 and 8, are smaller in the principal piece, and disappear according to the fusion with the longitudinal columns. In the end piece, the fibrous sheaths and the outer dense fibers have disappeared and only axial filament complex can be observed (Fig. 6).

*C. dsinezumi*

Because the living epididymal spermatozoa of *C. dsinezumi* was not observed, the measurements of the spermatozoa are not shown in this result. However, the epididymal sperm of the light microscopy section has a flattened and shield-shaped head with a large acrosome and a comparatively long middle piece (Fig. 7). Therefore, the morphology of the *C. dsinezumi* spermatozoa is similar to those of *C. suaveolens*.

From the electron microscopy results, the epididymal spermatozoa of *C. dsinezumi* can be distinguished from *C. suaveolens* by the following fine structural characteristics. The subacrosomal space of *C. dsinezumi* is not surrounded by a smooth acrosomal membrane but is surrounded by a serrated inner acrosomal membrane (Figs. 8, 9). The apical body in the subacrosomal space is not a wavy, finger-like, electron-dense apical body but a common apical body, which is composed only of fine granules (Fig. 10). The post-nuclear cap is not approximately 1/2 of the nuclear length but shorter than that of *C. suaveolens*, approximately 2/5 of the nuclear length (Fig. 9). In the neck, the proximal centriole is

**TABLE 1.**

**Measurements (mean±S.D) of the epididymal spermatozoa of shrews belong to Crocidurinae**

<table>
<thead>
<tr>
<th>Species</th>
<th>Head (μm)</th>
<th>Tail (μm)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total length</td>
<td>Width</td>
<td>Nuclear length</td>
</tr>
<tr>
<td><em>Crocidura suaveolens</em></td>
<td>12.61±0.49 (n=16)</td>
<td>10.77±0.23 (n=10)</td>
<td>4.72±0.31 (n=11)</td>
</tr>
<tr>
<td><em>C. dsinezumi</em></td>
<td>14.0</td>
<td>11.5</td>
<td>3.4</td>
</tr>
<tr>
<td><em>C. laziura</em></td>
<td>14.30±0.69* (n=50)</td>
<td>11.48±0.68* (n=50)</td>
<td>5.71±0.35* (n=50)</td>
</tr>
</tbody>
</table>

*p<0.05 compared with *C. suaveolens*
FIGURES 7-13. Light and electron micrographs showing the sperm head and tail of *C. dsinezumi*.

Scale bars: Fig. 7 = 10 μm. Figs. 8-13 = 0.5 μm.

**Figure 7.** Longitudinal section of the epididymal spermatozoon.

**Figure 8.** Frontal section of a part of a large and flatten shield shaped head.

**Figure 9.** Sagittal section of sperm heads with a common apical body in the subacrosomal space surrounded by the serrated inner acrosomal membrane.

**Figure 10.** Sagittal section of a head showing micro-granules in the apical body.

**Figure 11.** Longitudinal section of the sperm neck bearing the proximal centriole which has a fistulous lumen with a little electron-dense granular material.

**Figure 12.** Longitudinal section of the junction of middle piece and principal piece.

**Figure 13.** Cross section of the middle pieces of sperm tails. Note outer dense fibers arranged in a horseshoe fashion, well-developed satellite fibers associated with the inner side of fibers 5 and 6, and bi-lobed fiber 1 fused in the inner side.
not a solid type but a fistulous type with slightly dense electron granules (Fig. 11), and the outer dense fiber 1 is not fused to the outer side but is fused to the inner side and is almost open on the outer side (Fig. 13).

C. lasiura

According to the light microscopic observations, the living epididymal spermatozoa of C. lasiura have a flattened and shield-shaped head with a large acrosome and a comparatively long middle piece in the sperm tail (Fig. 14). Therefore, the morphology of C. lasiura spermatozoa is similar to those of C. suaveolens and C. disinezumi. The length of each part is as follows: head, 14.30 ± 0.69 μm (n=50); head width, 11.48 ± 0.68 μm (n=50); nucleus, 5.71 ± 0.35 μm (n=50); middle piece, 54.73 ± 2.37 μm (n=48); tail, 88.99 ± 2.65 μm (n=49) (Table 1). It shows that the epididymal spermatozoa of C. lasiura are larger than C. suaveolens (p<0.05, except for the total length of the tail). According to the electron microscopy results, the head, neck and tail of the epididymal sperm of C. lasiura is similar to those of C. dsinezumi (Figs. 15-22).

Discussion

Soricidae belonging to Insectivora consists of the two subfamilies, Soricinae and Crocidurinae (Tsushiya, 1985; Churchill, 1990). Soricinae has a reddish brown tooth tip, small ears, a uniform thickness of the tail and long tail hairs with a uniform length. Crocidurinae has an all white tooth, large external ears, a non-uniform thickness of the tail, in which the closer tail is down with a smaller tail thickness and short and long hairs that are mixed up at the tail (Jones and Johnson, 1960; Won, 1967; Corbet, 1978; Yoon, 1992). The phylogenetic relationships in the subfamilies have been studied through an analysis of the chromosomes (Tsushiya, 1985) and allozyme (George, 1986). These results suggest that species belonging to Soricinae are comparatively conservative, but species belonging to Crocidurinae are derived.

The external morphology and fine structure of the epididymal spermatozoa of C. dsinezumi and C. lasiura are quite similar, and show the typical characteristics of Crocidurinae having a flattened and shield-shaped head with a large acrosome, a serrated inner acrosomal membrane and a common apical body composed only of fine granules, and a neck with a fistulous proximal centriole with slightly dense electron granules (Cooper and Bedford, 1976; Green and Dryden, 1976; Koehler, 1977; Mori and Uchida, 1985; Mori et al., 1991; Mori, 1994). In addition, the dimensions of the spermatozoa are also similar to C. disinezumi (Mori et al., 1991) and C. lasiura. On the other hand, the external spermatozoan morphology of C. suaveolens shows the typical characteristics of Crocidurinae having a flattened and shield-shaped head with a large acrosome and a comparatively long middle piece in tail of the spermatozoa. However, the fine structure of the spermatozoa shows the characteristics of Soricinae having a smooth inner acrosomal membrane, a wavy, finger-like, electron-dense apical body and a solid proximal centriole filled with electron-dense material (Ploen et al., 1979; Mori et al., 1991). These results suggest that the spermatozoa of the C. suaveolens possess the characteristics of both Crocidurinae and Soricinae.

Although it has not been reported that the length of the post-nuclear cap in spermatozoa is a morphological key in classifying Soricinae and Crocidurinae, the length of the post-nuclear cap of C. suaveolens was different from C. disinezumi and C. lasiura in this study. In particular, the length of the post-nuclear cap of C. suaveolens is approximately half of the nuclear length, but those of C. disinezumi and C. lasiura are shorter than that of C. suaveolens, approximately 2/5 of the nuclear length. Therefore, the length of the post-nuclear cap might also be a key to classifying Soricinae and Crocidurinae.

Similar to C. suaveolens, the African shrew, Myosorex varius also has two characteristics of Soricinae and Crocidurinae (Bedford et al., 1998). Even though M. varius is classified as a Crocidurinae, its reproductive system has a hybrid characteristic of Soricinae and Crocidurinae. It shows not only the characteristics of Soricinae i.e. an accessory sperm storage site of midway along the vas deferens, fine structure of spermatozoa, an ampulla with many ciliated crypts and a persistence of the first polar body in ovum, but also those of Crocidurinae, i.e. a gland penis with spines, an unduly large acrosome of spermatozoa, an isthmus of the Fallopian tube with many deep crypts, an ampulla with many ciliated crypts, persistence of the intercellular junctions of the cumulus oophorus, long term stability of the cumulus oophorus and the absence of matrix of the cumulus oophorus, and a lack of unique perizonal space. From these differences it was suggested that M. varius be classified as a primitive subfamily, Crocidosoricinae. It is interesting that C. suaveolens has the combined characteristics of Soricinae with Crocidurinae in terms of the external morphology and fine structure of spermatozoa. It appears that some Crocidurinae species still have the characteristics of Soricinae.
FIGURES 14-22. Light and electron micrographs showing the sperm head and tail of C. lasiura.

Scale bars: Fig. 14 = 10 μm. Figs. 15-22 = 0.5 μm.

Figure 14. Planar view of an epididymal spermatozoon.

Figure 15. Frontal section of a head.

Figure 16. Frontal section of a head with a common apical body in the subacrosomal space surrounded by the serrated inner acrosomal membrane.

Figure 17. Sagittal section of a head showing micro-granules in the apical body.

Figure 18. Sagittal section of the sperm neck.

Figure 19. Sagittal section of the proximal centriole bearing a fistulous lumen with a little electron-dense granular materials.

Figure 20. Longitudinal section of the middle piece.

Figure 21. Longitudinal section of the principal piece.

Figure 22. Cross section of the sperm tails from middle to end pieces.
On the other hand, a recent study (Han, 1997) reported the phylogenetic relationships of three Korean shrews using an analysis of the morphometric characteristics and skull measurements. He reported that the relationships between C. disinezumi and C. lasiura are closer than those of C. suaveolens, and C. suaveolens can be distinguished from the other two species using molecular phylogenetic studies with Cyt B of the mtDNA. This molecular phylogenetic result agrees with the spermatozoan external morphology and fine structure observed in this study.

As mentioned above, the epididymal spermatozoa of C. disinezumi and C. lasiura show the typical external morphology and fine structural characteristics of Crocidurinae but those of C. suaveolens can be distinguished from the above two species by having the characteristics of Soricinae in the sperm fine structure. In particular, C. suaveolens belongs to Crocidurinae but also has the fine structure of the epididymal spermatozoa characteristic of Soricinae. Therefore, there is a closer phylogenetic relationship between C. disinezumi and C. lasiura than between C. suaveolens, and C. suaveolens can be distinguished from the other two species.

References


