

Ultrastructure of the Abnormal Head of the Epididymal Spermatozoa in the Big White-Toothed Shrew, *Crocidura lasiura*

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우수리땃쥐, *Crocidura lasiura*의 부정소 미부 정자의 비정상 두부 미세구조

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ABSTRACT

Normal and abnormal morphology of the epididymal spermatozoa in the big white toothed shrew, *Crocidura lasiura* were studied with the light and electron microscopy.

Normal spermatozoa were observed with a few abnormal spermatozoa. This indicates that abnormal morphology is no absolute indicator of infertility. However, the existence of the abnormal spermatozoa is related to infertility. Especially abnormal morphology of the sperm head is definitely associated with infertility.

The following types of abnormal head morphology of the epididymal spermatozoa in the wild healthy adults of the *C. lasiura* were described: 1) Nucleus with lack of condensation of the nucleoplasm 2) Destructed acrosome 3) Folded acrosome and plasma membrane 4) Separation of the acrosome from the nucleus 5) Acrosome with irregular condensation 6) Wrongly located granules of the apical body.

Key words : *Crocidura lasiura*, Crocidurinae, Infertility, Sperm abnormality

INTRODUCTION

Insectivora including Soricidae is known as the most primitive mammal and the ancestor stock of the present

mammals (Tsuchiya, 1985; Churchfield, 1990). Soricidae are generally considered to comprise two subfamilies, Soricinae and Crocidurinae by morphological characters (Jones & Johnson, 1960; Won, 1967; Corbet, 1978) and distinctive reproductive characteris-

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tics (Tsuchiya, 1985; Churchfield, 1990). Crocidurinae inhabiting Korea is composed of three species, *Crocidura suaveolens*, *C. dsinezumi* and *C. lasiura*. Above all, *C. lasiura* are widespread in the Korean peninsula (Jones & Johnson, 1960; Won, 1967). Crocidurinae is not only a useful environmental indicator having the sensitivity about pollutions (Diamond & Sherburne, 1969; Braham & Neal, 1974; Roberts et al., 1978; Andrew et al., 1984), but also is an important mediator controlling the population of the invertebrates and small vertebrates in ecosystem (Holling, 1959; Buckner, 1969; Churchfield,

1990). However the all of them are very rare in the wild and their population also are decreased, they have never been studied with exceptions of the brief comments, taxonomical review and studies of the sperm ultra-structure (Jones & Johnson, 1960; Won, 1967; Han, 1997; Jeong & Yoon, 2001; Yoon & Jeong, 2001).

Sperm morphology, especially sperm head is important to infertility, because morphology of the sperm head is basically conditioned by the nuclear structure (Holstein, 1975; Jager, 1990; Ferrari et al., 1998). However abnormality of the sperm head in *C. lasiura* having extra-

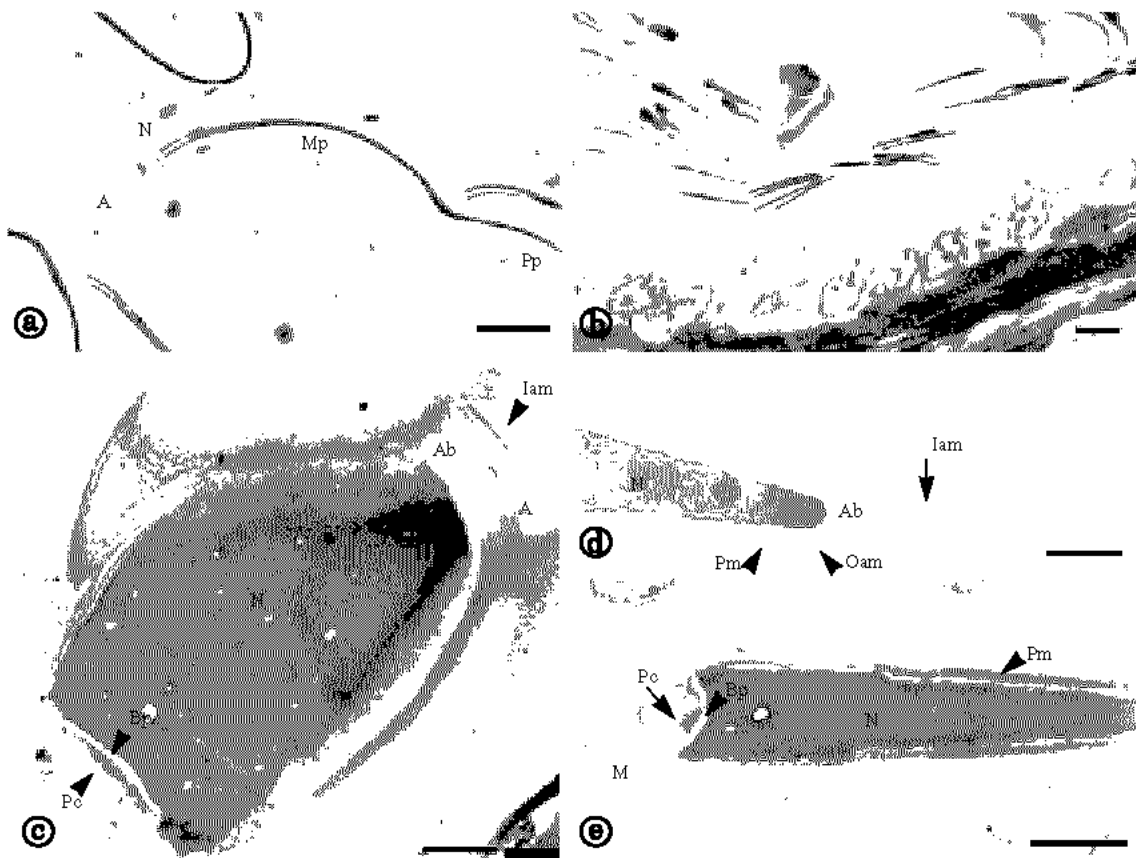


Fig. 1. Light and electron micrographs showing the epididymal spermatozoa with normal head of *Crocidura lasiura*.

a) Planar view of the living epididymal spermatozoa. b) Light micrograph of the caudal epididymis with many spermatozoa. c) Frontal section of the sperm head showing shield-shaped nucleus (N), serrated inner acrosomal membrane (Iam) and apical body (Ab) consists of the fine granules. d) Sagittal section of the flat sperm nucleus and acrosome (A). e) Sagittal section of the flat sperm neck and nucleus. Bp, Basal plate; M, Mitochondrion; Mp, Middle piece; Oam, Outer acrosomal membrane; Pc, Proximal centriole; Pm, Plasma membrane; Sp, Subacrosomal space. Scale bars = 10 μm (a, b) and 1 μm (c-e).



Fig. 2. Electron micrographs showing the epididymal spermatozoa with abnormal head of *Crocidura lasiura*. a) Nucleus (N) with lack of the condensation of the nucleoplasm. b) Destroyed acrosomes (A) independent from the nuclei. c) Spermatozoal head with an extremely folded acrosome and plasma membrane (Pm), wrongly located granules of the apical body (Ab) and partial separation of the acrosome dependent from the nucleus. d) Spermatozoal head with an irregular concentrated and folded acrosome and irregular inner acrosomal membrane (Iam). oam, outer acrosomal membrane; ss, sub-acrosomal space. Scale bars = 1 μ m (a-d).

ordinary big acrosome has not been studied. The aim of the present study is to compare ultrastructure of the

normal and abnormal sperm head and to describe types of the abnormal sperm head morphology.

MATERIALS AND METHODS

Crocidura lasiura of the seven adult males were collected at June and October 1999 and March 2000 from Mt. Jiri.

In other to observe living spermatozoa of the epididymis, one epididymis from the sacrificed specimen was finely cut in the Millonig's phosphate buffer, pH 7.4 and was observed with the light microscope.

To observe ultrastructure of spermatozoa, the other epididymis was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in the Millonig's phosphate buffer. The materials were post-fixed with 1.3% osmium tetroxide in the same buffer for 2 hr, dehydrated with a series of the graded ethyl alcohol 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 of the thin sections were examined with a JEOL 100S transmission electron microscope.

RESULTS

Epididymal spermatozoa in the wild healthy adults of the *Crocidura lasiura* were observed with the light and electron microscopy. As a result, a few abnormal spermatozoa and many normal spermatozoa are observed.

From the observation of the planar views and microscopic specimens using the light microscopy, normal spermatozoon of *C. lasiura* has the flatten head with vary large fan-shaped acrosome (Figs. 1a, 1b). From the observation using the electron microscopy, the nucleus of the sperm head is shield-shaped in the frontal section and a serrated inner acrosomal membrane and a common apical body consists of fine granules were observed on the top of the nucleus (Fig. 1c). A nucleus and acrosome were very flat in the sagittal section (Figs. 1d, 1e). The

subacrosomal space contacting with the top of the nucleus was filled with a apical body consists of fine granules, and empty subacrosomal spaces of the oval type also were seen (Fig. 1d). From the observation of the abnormal spermatozoa using electron microscopy, a nuclei with lack of the condensation of the nucleoplasm (Fig. 2a), destructed acrosomes independent from the nuclei (Fig. 2b), an extremely folded acrosome and plasma membrane, wrongly located granules of the apical body and partial separation of the acrosome dependent from the nucleus (Fig. 2c) and an irregular concentrated and folded acrosome and an irregular inner acrosomal membrane (Fig. 2d) were seen.

DISCUSSION

Soricinae and Crocidurinae belong to the Soricidae can be classified by the following morphological characteristics of the spermatozoa (Fawcett & 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 and electron-dense apical body and the neck has a solid proximal centriole is filled with electron-dense materials (Mori et al., 1991; Mori, 1994). In the 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 (Koehler, 1977; Mori & Uchida, 1985; Mori et al., 1991; Mori, 1994). *Crocidura lasiura* inhabiting the Korea peninsula shows also the characteristics of the Crocidurinae already reported. It is very interesting that existence of the extraordinary large acrosome in crocidurinae. In this study, a few abnormal and many normal spermatozoa were observed in the wild healthy adult *C. lasiura* (data not shown). The existence of a few abnormal spermatozoa is maybe associated with a unique morphology of the acrosome in the sperm head. Also, this indicates that the existence of the abnormal sperm morphology is no

absolute indicator of infertility. However certain distinct types of abnormal morphology are not only definitely associated with infertility (Holstein, 1975; Jager, 1990; Matallioyakis et al., 1996), but also very useful to evaluate toxicity of the chemical products in the experiments using various animals (Creasy et al., 1986; Chowdhury et al., 1989).

The sperm malformation is divided into three types, head malformation, tail malformation and broken spermatozoa (Kuzminsky et al., 1996). Either a single anomaly of each of sperm structural components, acrosome, nucleus, axoneme and accessory structures or a combination of these anomalies are observed in the malformed spermatozoa (Dadoune, 1988). Especially sperm head morphology is very important to fertility. Because sperm head morphology basically conditioned by the nuclear structure (Jager, 1990; Ferrari et al., 1998). In case of men, the percentage of abnormal spermatozoal head, pyriform head was 22.0+/-14.9% in subfertile and 13.0+/-7.8% in fertile men; 16.0% of the subfertile men presented a higher percentage of abnormal spermatozoal head than the normal. Moreover, morphological abnormalities in the neck and the tail, as also a cytoplasmic droplet, are significantly more frequent in sperm with a pyriform head than in sperm with a normal head (Rouso et al., 2002). On the other hand, Jager (1990) reported that human sperm nuclei show an exceptional variability is caused by a variable content of chromatin stabilizing disulphide bridges, which in turn is determined by differences in composition of basic proteins: protamine types and subtypes, histones, and intermediate forms. An abnormal state of the chromatin can be related to an abnormal DNA configuration or an abnormal DNA content: diploid instead of haploid. He explained abnormality of the nuclear chromatin is probably one of the causes of morphological aberrations of the sperm head. Thus sperm head abnormality in the *C. lasiura* also is caused by abnormal chromatin or DNA.

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<국문초록>

야생의 우수리땃쥐, *Crocidura lasiura*의 부정소 미부의 정상과 비정상 형태의 정자를 광학 및 전자현미경으로 비교 관찰하였다.

정상 정자와 함께 다수의 형태 비정상 정자가 관찰되었다. 이것은 비정상 정자의 존재가 개체의 생식력 없음을 나타내는 절대적 지표가 아님을 의미한다. 하지만 비정상 정자의 존재는 개체의 생식력 없음과 연관되며 특히 두부의 형태의 이상은 생식력 없음과 밀접한 관계가 있다.

건강한 야생의 우수리땃쥐, *C. lasiura*의 부정소 미부에서 관찰된 정자의 비정상 두부의 형태는 다음과 같은 유형으로 나타났다. 1) 핵질의 응축이 이루어지지 않거나 부분적으로 이루어진 형태 2) 첨체의 분열 혹은 파손이 나타나는 형태 3) 첨체 혹은 원형질막이 부분적 혹은 완전히 접혀진 형태 4) 첨체가 핵과 분리되는 형태 5) 첨체가 불규칙적으로 응축하는 형태 6) 잘못된 위치에서 관찰되는 첨단체