



Title	A Fine Structural Study of Spermatogenesis in the Brittle-Star <i>Ophiura sarsii</i> (Echinodermata: Ophiuroidea), with a Demonstration of the Precocious Formation of the Acrosome (With 5 Text-figures)
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**A Fine Structural Study of Spermatogenesis in the  
Brittle-Star *Ophiura sarsii* (Echinodermata:  
Ophiuroidea), with a Demonstration  
of the *Precocious* Formation  
of the Acrosome<sup>1)</sup>**

By

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*(With 5 Text-figures)*

It is well known that in many animals acrosome formation and differentiation occurs during the spermatid stage (spermiogenesis). On the other hand, a recent study concerned with the spermatogenesis of the brittle-star *Amphipholis kochii* has noted that the ophiuroid spermatogenesis has remarkable characteristic, viz. the acrosome formation is initiated in the spermatogonia (Yamashita and Iwata, 1983a). It is, however, problematical that a finding obtained from only one example of the ophiuroids may be regarded as characteristic of the ophiuroids in general. It is therefore necessary to investigate if the same characteristic is detectable in other ophiuroid species.

The present paper, therefore, sets out to describe the fine structural changes during the spermatogenesis of another brittle-star, *Ophiura sarsii* Lütken, in order to examine whether the previous finding is applicable to the ophiuroids generally.

**Materials and Methods**

The brittle-stars, *Ophiura sarsii*, were collected at the depth of about 300 m in Uchiura Bay, south-western Hokkaido, Japan, during the season from April to September. During this season, the maturity of the testes in *O. sarsii*, as well as the ovaries, varied extensively according to individuals and showed no distinct seasonal fluctuations, suggesting an absence of a distinct annual reproductive cycle in this species. We are therefore able to observe various maturation states of the testis during this season.

The testes were fixed successively with 5% glutaraldehyde in 75% sea water

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and 1% OsO<sub>4</sub> in 75% sea water, dehydrated in acetone and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a JEOL JEM-100S electron microscope.

### Observations

The spermatogenic cells are roughly arranged in zones from the outside to the inside of the testis according to their maturity, viz. the spermatogonia are located near the testicular wall, the spermatozoa gather to form a large mass in the central portion of the testis, and the spermatocytes and the spermatids occupy the intermediary portion of the germinal epithelium (Fig. 1).

#### *Spermatogonia*

The spermatogonia are distinguishable from other kinds of spermatogenic cell by their nuclear morphology: the nucleus is large, comprising patches of condensed chromatins scattered throughout the nucleoplasm and one or two prominent nucleoli (Figs. 1, 2A and B). The cytoplasm of the spermatogonia contains small, randomly distributed mitochondria (Figs. 2A and B), two centrioles situated perpendicular to each other (Fig. 2C), Golgi apparatus usually found near the centrioles (Figs. 2A-C), a striated rootlet associated with the distal centriole (Fig. 2D), and abundant ribosomes. It should be noted that proacrosomal vesicles produced by the Golgi apparatus are already observable in the spermatogonia (Fig. 2A). The proacrosomal vesicles are membrane-bound coarse accumulations of fine granules (Fig. 2E). The membrane of the proacrosomal vesicles appears more electron-dense than that of other ordinary vacuoles. The spermatogonia also contain electron-dense materials with or without surrounding membrane (Fig. 2B). Perhaps these materials are specific to the germ-line cells as reported in the echinoids (Longo and Anderson, 1969; Houk and Hinegardner, 1981), holothuroids (Atwood, 1974) and ophiuroids (Yamashita and Iwata, 1983a). Unlike the spermatogonia of the echinoids (Longo and Anderson, 1969), the spermatogonia of the present species (like other echinoderms) have no flagellum (Bruslé, 1968; Atwood, 1974; Krishnan and Dale, 1975; Bickell *et al.*, 1980; Yamashita and Iwata, 1983a). The adjacent spermatogonia are jointed by desmosome-like structures (Fig. 2A).

#### Abbreviations

AR, acrosomal vesicle	P, periacrosomal material
DC, distal centriole	PC, proximal centriole
DJ, desmosome-like junction	S, spermatozoon
F, flagellum	SD, diplotene primary spermatocyte
G, Golgi apparatus	SL, pre-leptotene/leptotene primary spermatocyte
M, mitochondrion	SG, spermatogonium
N, nucleus	ST, spermatid
NO, nucleolus	SZ, zygotene/pachytene primary spermatocyte
NV, nuclear vacuole	

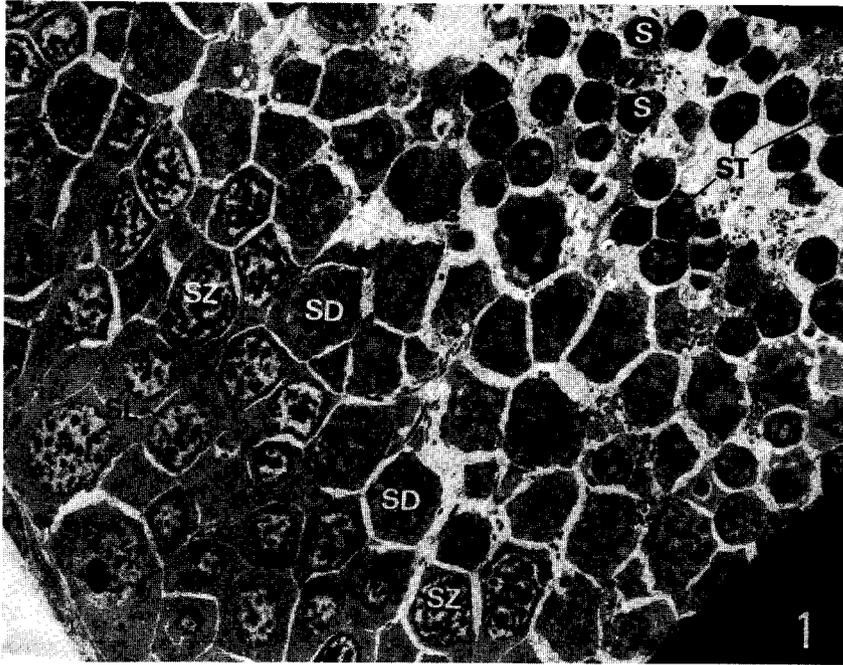


Fig. 1. Low magnification of the testis of *Ophiura sarsii*, showing the arrangement of the spermatogenic cells.  $\times 1,860$ .

### *Spermatocytes*

The primary spermatocytes have a more condensed nucleus than that of the spermatogonia. We can find at least three types of the primary spermatocytes according to their nuclear morphology (Fig. 1): the nucleus of the first type contains chromatins condensed in irregular clumps and one or two nucleoli; the second type of nucleus is composed of more condensed chromatins than that of the first and bears no nucleoli; the third type is relatively homogeneous in density of chromatins. Although a synaptonemal complex, the most prominent marker for classification of the primary spermatocytes, may not be detected unambiguously in this species, these three types of primary spermatocyte seem to be classifiable as pre-leptotene /leptotene primary spermatocyte, zygotene/pachytene primary spermatocyte, and diplotene primary spermatocyte respectively, as has been defined by Bickell *et al.* (1980) for the crinoid primary spermatocytes. The secondary spermatocytes may not be observed either presumably because they have no cellular characteristics that differ from the primary spermatocyte and because they pass through this stage in a short time.

The cytoplasm of the spermatocytes contains the same organelle found in the spermatogonia (Fig. 3). The formation of the proacrosomal vesicles are still

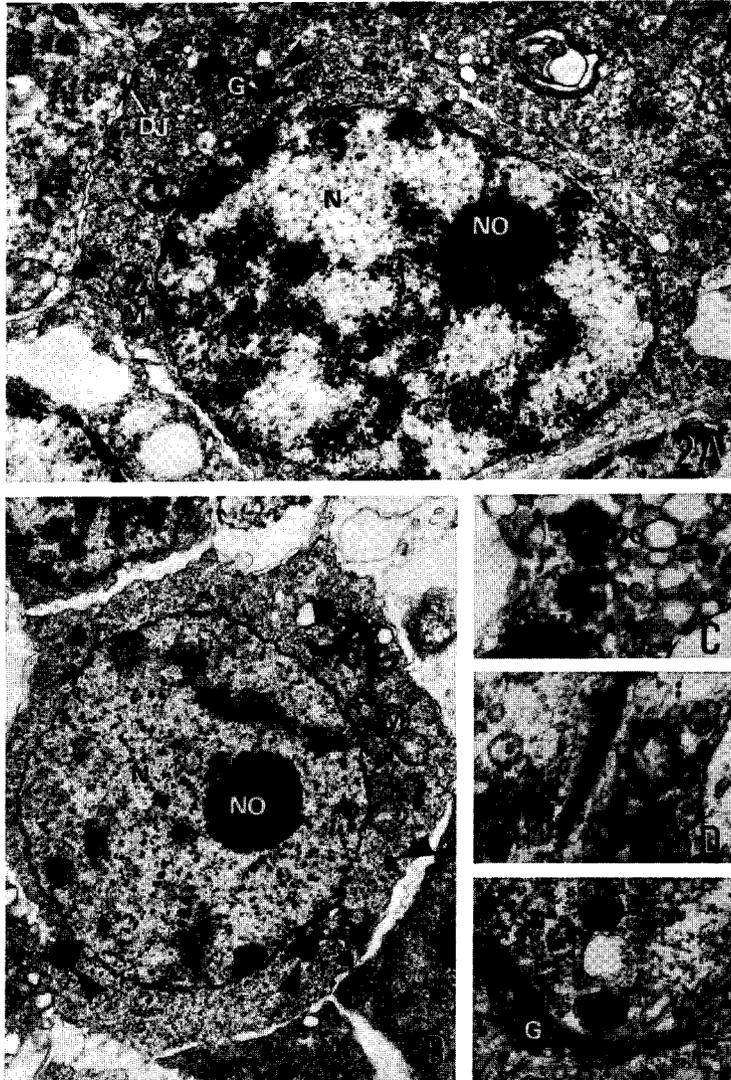


Fig. 2. Spermatogonia in *Ophiura sarsii*. A: The proacrosomal vesicles (arrowheads) are formed by Golgi apparatus.  $\times 9,600$ . B: Arrowheads indicate the dense materials specific to the germ line cells.  $\times 8,000$ . C: The proximal and distal centrioles.  $\times 16,000$ . D: The rootlet associated with the distal centriole.  $\times 19,200$ . E: High magnification of the Golgi region of the spermatogonium in Fig. 2A, showing the proacrosomal vesicles (arrowheads).  $\times 23,600$ .

detectable in the primary spermatocytes (Figs. 3A and B). The flagellum makes its appearance in the primary spermatocytic stage (Fig. 3C). The desmosome-like junctions found between the spermatogonia can still be observed during this stage (Fig. 3A).

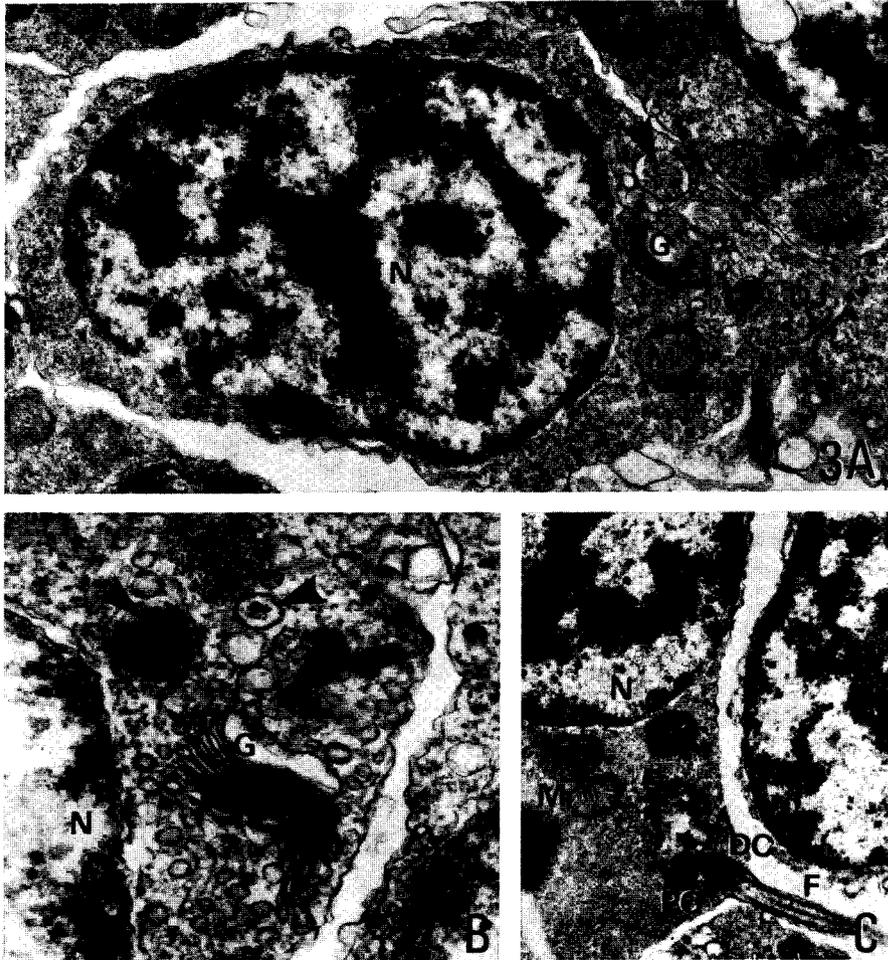


Fig. 3. Spermatocytes in *Ophiura sarsii*. A: The acrosome formation is seen in the primary spermatocyte (arrowhead).  $\times 15,000$ . B: The Golgi region of the primary spermatocyte, showing the proacrosomal vesicles (arrowheads).  $\times 37,500$ . C: The primary spermatocyte with flagellum. Arrowhead shows the rootlet associated with the distal centriole.  $\times 12,000$ .

### *Spermatids*

The nucleus of the early spermatids consists of condensed chromatins which are aggregated heterogeneously (Fig. 4A). During spermiogenesis, the condensation of the chromatins is accelerated, leaving several decondensed regions known as nuclear vacuoles (Figs. 4B and C). In concord with the condensation of the chromatins, the nucleus alters its shape, from circular to ellipsoidal (Figs. 4A-C). The cytoplasm is confined to the posterior region of the spermatids where the scattered small mitochondria gather and fuse to each other and form a single doughnut-shaped mitochondrion (Figs. 4A and B). The proacrosomal vesicles fuse to form an acrosomal vesicle (Fig. 4D). The acrosomal vesicle of the early spermatids is found in the caudal portion of the cell (Fig. 4A); during spermiogenesis it is transported to the anterior portion (Figs. 4B and C). During this movement, the homogeneous contents of the acrosomal vesicle differentiate into an electron-dense and an electron-lucent region (Fig. 4E). Close to the acrosomal vesicle on the side of the electron-lucent region, we are able to find a dense plate-like structure (Fig. 4E). The electron-lucent region becomes the basal region of the acrosome in the subsequent stage (Figs. 4F and G). The axis of the acrosome has therefore been determined before sitting on the nucleus. The anteriorly transported acrosomal vesicle is situated in a small indentation of the nucleus (Fig. 4B). Between the indentation and the acrosomal vesicle, fibrous materials are present (Fig. 4F), and they surround the acrosomal vesicle, becoming periacrosomal materials (Fig. 4G). During spermiogenesis the indentation of the nucleus becomes deeper, especially at its center, as well as wider (Figs. 4B and C); this is now called an acrosomal fossa. The spermatids are jointed to each other by intercellular bridges, suggesting that cytokinesis is incomplete during meiosis (Fig. 4H).

### *Spermatozoa*

The spermatozoa comprise an ellipsoidal head, an ellipsoidal middle piece and a long flagellated tail (Figs. 5A and B), thus conforming to a typical primitive spermatozoa as defined by Franzen (1970).

The head consists of the acrosome and the nucleus. The acrosome is semi-circular and surrounded by a limiting membrane (Fig. 5A). The basal half of the acrosomal vesicle membrane is more electron-dense than that of the upper half (Fig. 5C), as has been reported in other ophiuroids (Summers *et al.*, 1975; Hylander and Summers, 1975; Yamashita and Iwata, 1983a). The contents of the acrosome are composed of electron-dense fine granular materials, which occupy the main part of the acrosome and are denser at the upper side, and an electron-lucent irregular region situated at the relatively basal part of the acrosome (Fig. 5C). Just beneath the acrosome, the dense plate-like structure is detectable (Fig. 5C), as has already been seen during the later spermiogenesis (cf. Figs. 4E-G). The periacrosomal materials are made up of fine fibers (Fig. 5C). The nucleus is highly condensed with several nuclear vacuoles (Fig. 5A). The acrosomal fossa is deeper at its center

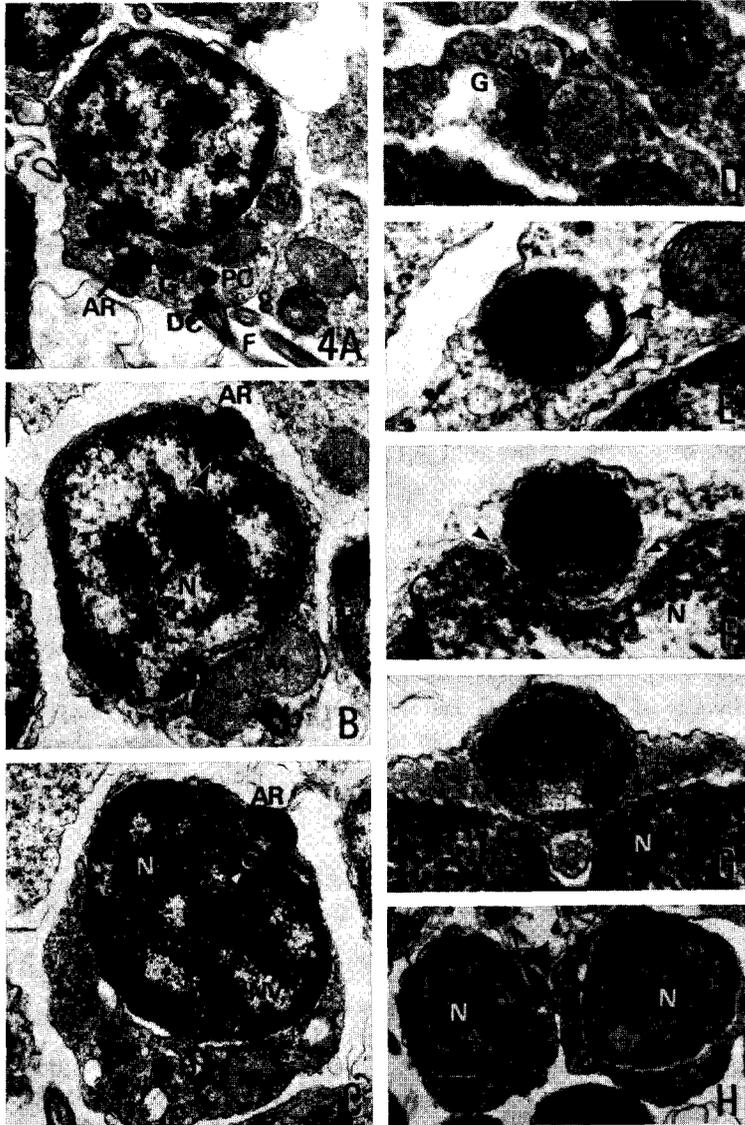


Fig. 4. Spermatids in *Ophiura sarsii*. A: The early spermatid.  $\times 9,720$ . B: More developed spermatid than that of Fig. 4A. Arrowhead shows the indentation of the nucleus.  $\times 10,730$ . C: The late spermatid. Arrowhead indicates that the acrosomal fossa is deeper at its center.  $\times 13,970$ . D: The proacrosomal vesicles in the early spermatid, showing their fusion (arrowhead).  $\times 24,300$ . E: The acrosomal vesicle in the early spermatid. Arrowhead shows the plate-like structure closely associated with the vesicle membrane. Note that the contents of the acrosomal vesicle differentiate into two regions.  $\times 32,400$ . F: High magnification of the acrosomal region in Fig. 4B. Arrowheads indicate fibrous materials which are the precursors of the periacrosomal materials.  $\times 42,500$ . G: High magnification of the acrosomal region in Fig. 4C.  $\times 42,500$ . H: The spermatids jointed by the intercellular bridge (arrowhead).  $\times 7,450$ .

(Fig. 5A). The posterior portion of the nucleus is also indented slightly (Fig. 5B): this indentation is known as a centriolar fossa.

The middle piece consists of a doughnut-shaped mitochondrion and some

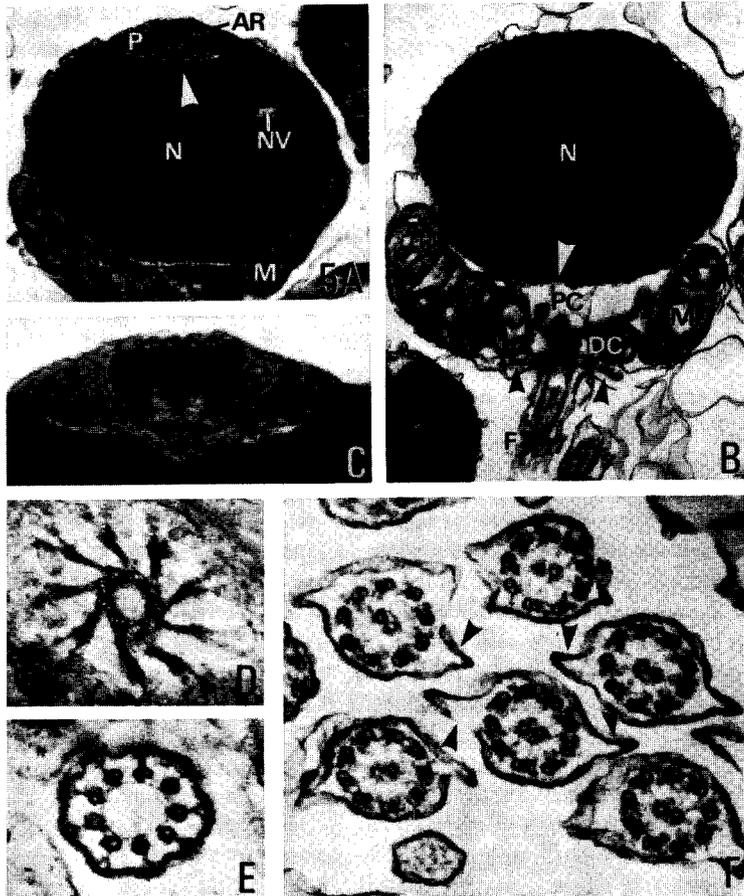


Fig. 5. Spermatozoa in *Ophiura sarsii*. A: The longitudinal section of the spermatozoon. Arrowhead shows the acrosomal fossa which is deeper at its center.  $\times 16,000$ . B: The longitudinal section of the spermatozoon. The nucleus is slightly indented at the posterior end (white arrowhead). Black arrowheads show the centriolar satellite complex associated with the distal centriole.  $\times 16,800$ . C: High magnification of the acrosomal region of the spermatozoon in Fig. 5A. The plate-like structure is found beneath the acrosome (arrowhead).  $\times 40,000$ . D: The transverse section through the distal centriole, showing the nine spoke-like satellites.  $\times 40,000$ . E: The transverse section through the proximal tip of the central microtubules of the flagellum, showing the nine Y-shaped connectives.  $\times 60,000$ . F: The transverse section of the flagellum, showing the lateral expansions of the flagellar membrane (arrowheads).  $\times 60,000$ .

residual cytoplasm, including two centrioles (Fig. 5B). In contrast with the perpendicular orientation of the two centrioles in the previous stages, the proximal centriole now lies at an angle of about  $30^\circ$  from the axis of the distal centriole (Fig. 5B). This reorientation of the proximal centriole has been detected during spermiogenesis. We are now able to observe a centriolar satellite complex associated with the distal centriole (Fig. 5B) instead of the rootlet present during the previous stages. It is made of nine spoke-like satellites and nine Y-shaped connectives: the former radiates from the dense matrix of the distal centriole and bifurcates into secondary spokes (Fig. 5D); the latter is detectable in the transverse section through the proximal tip of the central microtubules in the flagellum, and connects the peripheral microtubules and the flagellar membrane (Fig. 5E).

The flagellum is composed of an ordinary 9+2 structure (Fig. 5F). The flagellar membrane expands laterally and these lateral expansions are roughly aligned with the central microtubules of the flagellum when observed in a transverse section (Fig. 5F).

### Discussion

The present observation shows that, irrespective of the difference in their taxonomic families, the entire processes of the spermatogenesis of *A. kochii* and *O. sarsii* are very similar (cf. Yamashita and Iwata, 1983a), confirming that the findings obtained from the previous and present studies may be regarded as indicating the general characteristics of ophiuroid spermatogenesis.

The basic features of ophiuroid spermatogenesis resemble those of other echinoderm spermatogenesis, as we pointed out in our previous paper (Yamashita and Iwata, 1983a). The remarkable fact about ophiuroid spermatogenesis is that the acrosome formation, i.e. the production of the proacrosomal vesicles, is initiated in the spermatogonia, unlike that of many other animals, where it begins in the spermatids. The beginnings of the acrosome formation in other echinoderms hitherto reported is in the spermatogonia in the holothuroids (Atwood, 1974; Pladerollens and Subirana, 1975), in the primary spermatocytes in the crinoids (Bickell *et al.*, 1980) and in the spermatids in the echinoids (Longo and Anderson, 1969). Although the beginning of the acrosome formation in the asteroids is presumed to occur in the spermatids, the exact beginning is still uncertain, because only spermiogenesis has been observed for the asteroids (Dan and Shirakami, 1971). My unpublished observation reveals that in the asteroid *Asterina pectinifera* the proacrosomal vesicles are already found in the primary spermatocytes. This therefore allows us to say that acrosome formation in the echinoderms is initiated at the latest in the primary spermatocytes, with the exception of the echinoids. The significance of the *precocious* production of the acrosome in the ophiuroids has been discussed in our previous papers, in relation to the fact that spermatogenesis in the ophiuroids is of short duration (Yamashita and Iwata, 1983a and b).

It should also be noted in the spermatogenesis of *O. sarsii* that the axis of the acrosome is already determined before the acrosome sits in the indentation of the

nucleus. This finding was not observed in *A. kochii* (Yamashita and Iwata, 1983a). Generally speaking, the axis of the acrosome develops after contact with the nucleus, suggesting that the determination of the axis depends on the nucleus. The present observation, however, suggests that the acrosome of *O. sarsii* develops its axis independently of the nucleus. A similar situation has been reported in the asteroids. In this case, however, the axis of the acrosome is in concord with the axis of the Golgi apparatus (Dan and Shirakami, 1971). In the present species, however, the axes of the acrosome and the Golgi apparatus can be seen to be independent of each other. It may therefore be presumed that the determination of the acrosomal axis in *O. sarsii* depends directly neither on the nucleus nor Golgi apparatus.

Such structures as the plate-like formation found beneath the base of the acrosome in the present species have also been reported for other echinoderms (cf. Yamashita and Iwata, 1983a), and it has been generally thought that these structures are the center for the polymerization of G-actin into F-actin in order to elongate the acrosomal process during the acrosome reaction (Mabuchi and Mabuchi, 1973; Tilney, 1976 and 1978; Tilney *et al.*, 1973 and 1978). Many observations hitherto reported for echinoderm spermatogenesis agree that these structures are organized from a part of the periacrosomal materials (Summers *et al.*, 1975; Hylander and Summers, 1975; Yamashita and Iwata, 1983a, etc.). However, the present observations clearly demonstrate that the plate-like structure is already found before an appearance of the precursors of the periacrosomal materials. This finding suggests that the plate-like structure, probably the organization center of the actin filaments which support the acrosomal process, is derived not from the periacrosomal materials but from other materials. The most likely origin of this structure seems to be substances associated closely with the acrosomal vesicle membrane, but much more detailed observation as to the origin of this structure well necessary before we can be certain of this.

### Summary

This paper deals with the fine structural changes of the spermatogenic cells during spermatogenesis in the brittle-star *Ophiura sarsii* Lütken (Echinodermata: Ophiuroidea). The remarkable point in the spermatogenesis of *O. sarsii* is that the acrosome formation, i.e. the formation of the proacrosomal vesicles, is initiated in the spermatogonia, unlike that in many other animals, where it occurs in the spermatids. This finding has also been described in another ophiuroid, *Amphipholis kochii*, suggesting that the *precocious* formation of the acrosome is a general characteristic of ophiuroid spermatogenesis. Besides the *precocious* formation of the acrosome, the spermatogenesis of *O. sarsii* is notable in that the axis of the acrosome is determined before contact with the nucleus and that the plate-like structure found beneath the acrosome is detectable before an appearance of the

precursors of the periacrosomal materials. These findings are discussed in comparison with other echinoderm spermatogenesis.

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