Arab Gulf J. Scient. Res., **8** (2), pp. 153-162 (1990)

The Fine Structure of Spermiogenesis and the Mature Spermatozoon of *Brachidontes variabilis* (Bivalvia)

Hameed A. Al-Hajj

Department of Biological Sciences,
University of Jordan, Amman, Jordan

Abstract. The mature sperm of *Brachidontes variabilis* is of the primitive type, with a head, a midpiece, and a tail. The head comprises an inverted Y-shaped acrosome which has an anterior electron-dense region and a posterior electron-lucent one. The subacrosomal space is filled with flocculent material, but with no acrosomal rod. The nucleus is barrel-shaped with a posterior fossa. The midpiece consists of five mitochondria which surround the proximal and distal centrioles. Early spermatids have large central nuclei, and a number of scattered proacrosomal granules. In mid-spermatids, these granules coalesce into a large basal granule which migrates apically where it invaginates at its adnuclear surface and becomes an inverted Y-shaped vesicle. Nuclear condensation is of the granular pattern.

Studies on the reproductive biology of bivalves from the Gulf of Aqaba (Red Sea) are lacking; the only information is that provided by Hulings (1986) in his survey of aspects of reproduction of rocky intertidal molluscs form this Gulf. There have been no ultrastructural studies on sperm morphology or spermiogenesis of any bivalve from this region.

The available reports on the fine structure of bivalve sperm have shown marked variations, especially in regard to the acrosomal region (Popham *et al.* 1974, Popham 1979, Franzen 1983, Hodgson and Bernard 1986a,b, Van der Horst *et al.* 1986, Hodgson *et al.* 1987). Such variations have been attributed to differences in reproductive habits, and have been used to correlate sperm structure with bivalve phylogeny. Of the family Mytilidae, sperm of *Mytilus edulis* (Niijima and Dan 1965, Longo and Dornfeld 1967, Hodgson and Bernard 1986a), *Mytilus perna* (Bourcart *et al.* 1965), *Perna perna* (Bernard and Hodgson 1985), *Aulacomya ater, Choromytilus meridionalis,* and *Mytilus* *galloprovincialis* (Hodgson and Bernard 1986b) and *Brachidontes virgiliae* (Bernard *et al.* 1988) have been described.

Brachidontes variabilis (Krauss 1848), a species within the Mytilidae, is found as dense beds in depressed areas of beach rock or fossil reef in the Jordan Gulf of Aqaba (Hulings 1986). No information on the fine structure of the mature sperm and spermiogenesis of this species is available. The present investigation reports on this aspect of reproductive biology of this species.

Materials and Methods

Specimens of *B. variabilis*, ranging in diameter from 12.8 to 27.3 mm were collected monthly for one year, and were kept in a seawater table with continuously circulating seawater until time of use. The cream-colored testes were separated from the mantle lobe, cut into small pieces, and fixed in 2.5% glutaraldehyde in seawater at 4°C for 2 hr, at pH 7.4. After washing with seawater, the tissues were postfixed in 1% OsO_4 in seawater for 90 min, then dehydrated in a graded series of acetone, infiltrated and embedded in Spurr resin (1969). Ultrathin sections were stained with alcoholic uranyl acetate for 10 minutes, and lead citrated for 5 minutes, and examined with a Zeiss EM10B electron microscope.

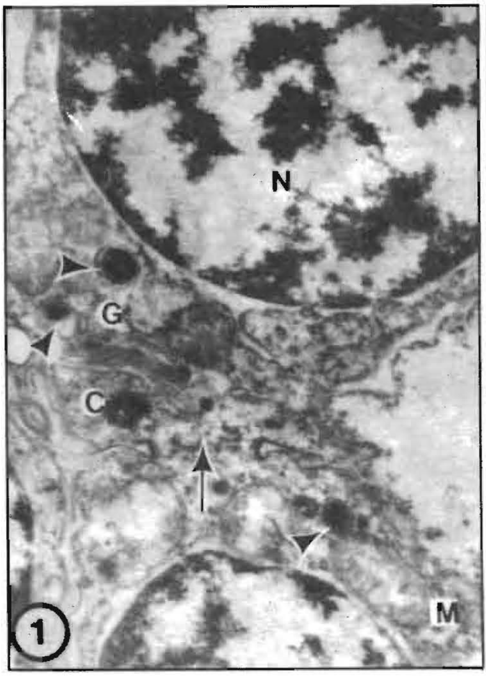
Results

Spermiogenesis

In early spermatids, the nucleus is spherical-oval and is central. The cytoplasm is basically granular with few organelles; it contains a basal Golgi body, scattered proacrosomal granules, a centriole, and a few mitochondria. Intercellular bridges connect some spermatids (Fig. 1).

As spermiogensis proceeds, the proacrosomal granules fuse into a large basal, oval-spherical acrosomal granule which has an electron-dense core and an electron-lucent cortex (Fig. 2). The nucleus shows peripheral clumping of the chromatin material and the nuclear envelope has occasional pores. The mitochondria become reduced in number and occupy indentations in the posterior nuclear region (Fig. 2).

By the mid spermatid stage, the acrosomal granule migrates (Fig. 3) and eventually assumes an apical position opposite to the mitochondrial complex. By now, the middle piece, with its mitochondria closely associated with the nucleus, has become established (Fig. 4).



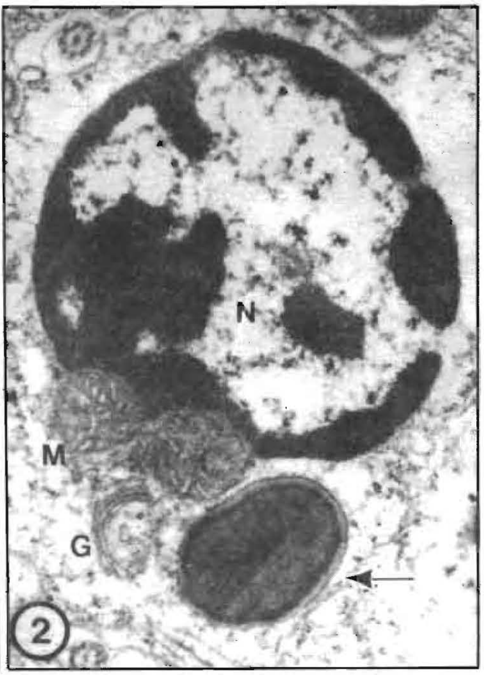


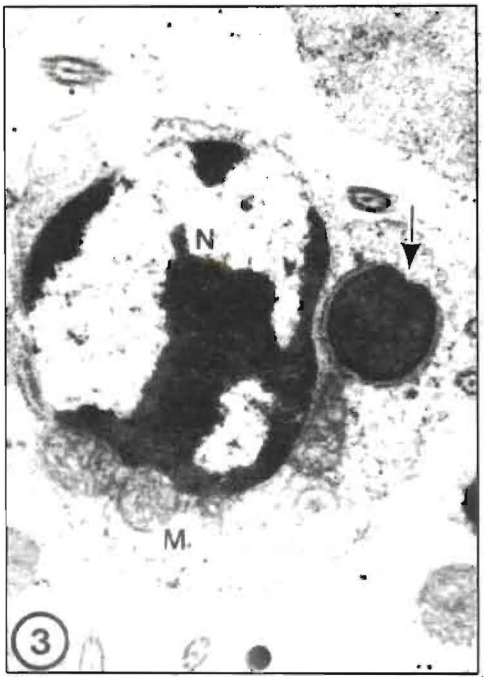
Fig. 1. Two early spermatids connected by a cytoplasmic bridge (arrow). Golgi body (G), centriole (C), mitochondria (M), nuclei (N) and proacrosomal granules (arrow heads) are shown. X 18,400.

Fig. 2. Early spermatid showing a basal Golgi body (G), mitchondria (M), nucleus (N), and a large proacrosomal granule (arrow). X 21,400.

During the late spermatid stage, the chromatin material becomes granular and the nucleus attains a barrel-shape with a posterior fossa into which the proximal centriole fits. The proacrosome invaginates at its adnuclear surface, and an electron-dense material appears in its apex. The cytoplasm is completely sloughed (Fig. 5). Later, the acrosomal vesicle attains an inverted V-shaped form, the apical dense material spreads posteriorly, and the ends of the electron-lucent acrosome become bulbous (Figs. 6,7). As the spermatid matures, the nucleus becomes condensed. The acrosomal vesicle assumes an inverted Y-shaped appearance, and is closely associated with the nucleus. The subacrosomal space becomes bell-shaped, and is filled with a posteriorly aggregated flocculent material (Figs. 8,12).

Sperm Ultrastructure

The mature sperm of *B. variabilis* (58 µm long) comprises three regions: a head, a middle piece, and a tail. The head (2.4 µm long) consists of an inverted



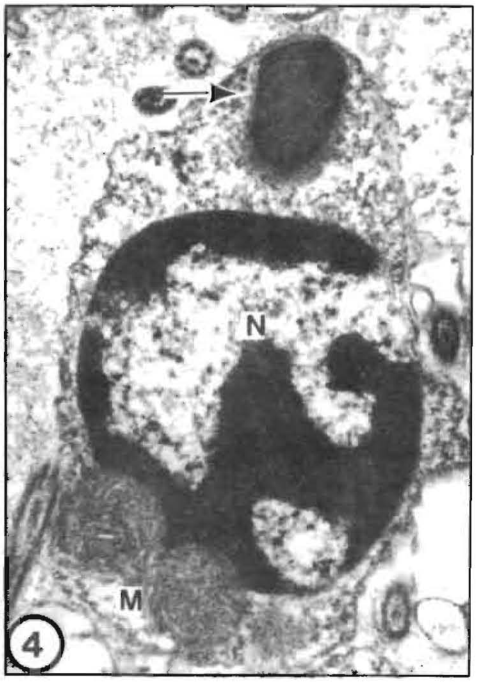
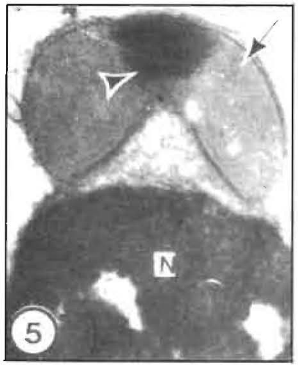


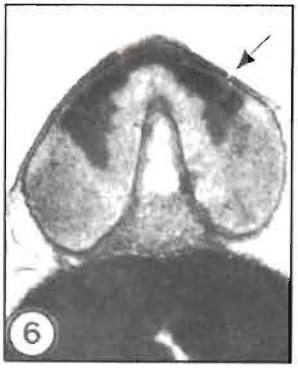
Fig. 3. Mid-spermatid showing a migrating proacrosomal granule (arrow), mitochondria (M), and a nucleus (N). X 18,300.

Fig. 4. Mid-spermatid showing an apical proacrosome (arrow), mitochondria (M), and a nucleus (N). X 21,500.

Y-shaped acrosome, and a nucleus. The acrosome (0.8 µm long) is differentiated into two zones: an anterior electron-dense zone, and a posterior electron-lucent one. The adnuclear acrosomal surface is invaginated to form a subacrosomal space which contains flocculent materials, but no acrosomal rod (Figs. 8,12). The nucleus, about 1.6 µm long, is electron-opaque, barrel-shaped, and with a posterior fossa (Figs. 10,12).

The middle piece consists of five oval-spherical mitochondria (0.6 µm in diameter) surrounding the proximal and distal centrioles. Pericentriolar bodies originated from the distal centriole and project towards the plasmalemma, close to an annulus (Figs. 9,10). Glycogen granules are observed around the centrioles (Fig. 11). The tail, about 55 µm long, originates from the distal centriole (Fig. 10).





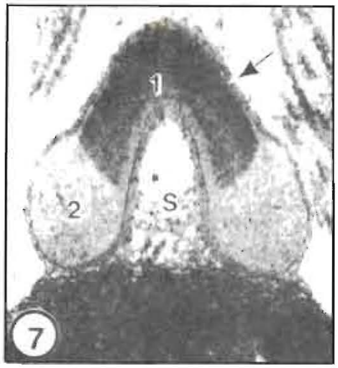


Fig. 5. Anterior region of a late spermatid showing an indented proacrosome (arrow), with an apical electron-dense material (arrow head) and a granular nucleus (N). X 40,000.

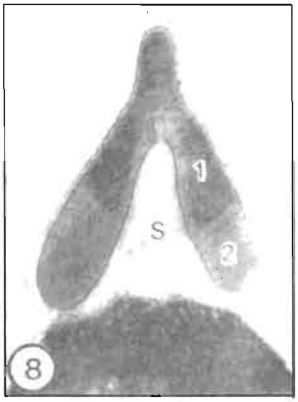
Fig. 6. Anterior region of a late spermatid showing a V-shaped proacrosome (arrow) with more spreading of the electron-dense material. X 45,000.

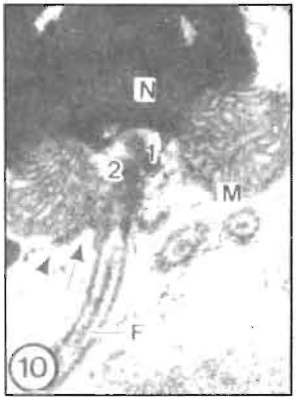
Fig. 7. Anterior region of a late spermatid showing a proacrosome (arrow) with an anterior electron-dense zone (1), a posterior electron-lucent one (2) with bulbous ends, and a flocculent subacrosomal space (S). X 36,000.

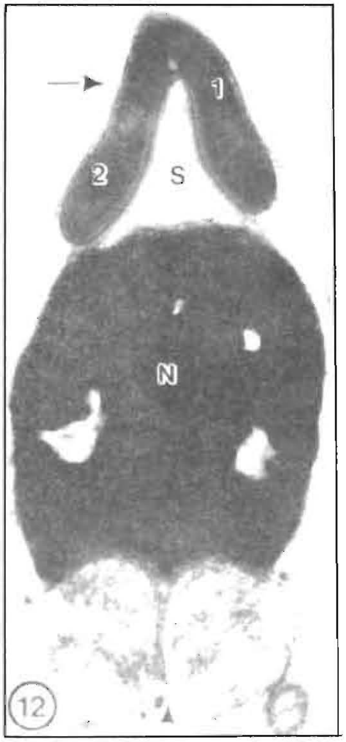
Discussion

This investigation shows that sperm of *B. variabilis* are of the primitive type (Franzen 1955, 1970), and similar to those of other bivalves studied thus far, particularly in the tail and the midpiece regions (Trimble and Gaudin 1975, Popham and Marshall 1977, Lewis *et al.* 1980, Sakai *et al.* 1982, Franzen 1983, Hodgson and Bernard 1986a). This adds support to the contention of Popham (1979) that these two regions represent the least variable structures in bivalve sperm, and consequently could not be of help in using sperm ultrastructrue in determining taxonomic relationships. In contrast, the nuclei of bivalve sperm show more morphological diversity. The nucleus of *B. variabilis* sperm is barrel-shaped, as in some other bivalves (Longo and Anderson 1969, Popham *et al.* 1974, Franzen 1983, Hodgson and Bernard 1986a,b, Bernard *et al.* 1988). Other nuclear shapes of bivalve sperm, like the ovoid-round (Daniels *et al.* 1971, Hodgson and Bernard 1986b, Popham 1979), cylindrical (Sakai *et al.* 1982) conical (Popham 1979, Trimble and Gaudin 1975, Popham and Marshall 1977), or bullet-shaped (Popham 1979) have been reported.

A higher level of structural diversity in bivalve sperm involves the shape and size of the acrosome, its proximity to the nucleus, and the presence or absence of an acrosomal rod. Bell-shaped (present study; Popham *et al. 1974,* Sakai *et al.* 1982), cup-shaped (Popham and Marshall 1977, Popham 1979, Hodgson *et al.* 1987), bullet-shaped (Lewis *et al.* 1980) or cone-shaped acrosomes (Popham 1979, Franzen 1983, Bernard and Hodgson 1985, Hodgson







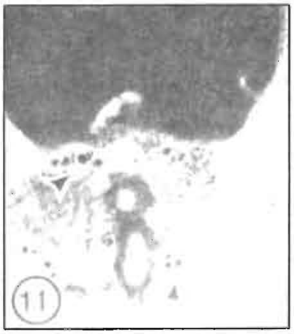


Fig. 8. Acrosome of a sperm with anterior electron-dense zone (1), a posterior electron-lucent one (2), a bell-shaped subacrosomal space (S). X 40,000.

Fig. 9. Cross section through the mid-piece, showing five mitochondria surrounding the distal centriole. X 22,400.

Fig. 10. Posterior portion of a sperm showing a nucleus (N) with a fossa, mitochondria (M) surrounding the proximal (1) and distal centrioles (2). Note the annulus (arrowhead), the pericentriolar process (arrow), and the flagellum (F). X 21,400.

Fig. 11. Mid-piece region of a sperm showing glycogen granules (arrow heads). X 30,600.

Fig. 12. A mature sperm showing the acrosome (arrow) with an anterior electron-dense zone (1), a posterior electron-lucent one (2), subacrosomal space (S), nucleus (N), and mid-piece (arrow-head). X 40,700.

and Bernard 1986a,b, Hodgson *et al.* 1987) have been described. Within the mytilids studied thus far, variations in acrosomal shape and dimensions are very apparent, namely *Choromytilus meridionalis, Aulacomya ater* (Hodgson and Bernard 1986b), and in closely related species like *Mytilus edulis, M. galloprovincialis* (Hodgson and Bernard 1986a), *B. variabilis* (present study) and *B. virgiliae* (Bernard *et al.* 1988), *Bankia australis* and *B. carinata* (Popham *et al.* 1974); *Solen cylindraceus* and *S. capensis* (Hodgson *et al.* 1987).

The acrosomal rod is of particular interest in bivalve spermatology. This structure is lacking in sperm of *B. variabilis* and some twelve other species studied thus far (Higashi 1964, Trimble and Gaudin 1975, Popham and Marshall 1977, Franzen 1983, Hodgson and Bernard 1986a,b, Hodgson *et al.* 1987, Bernard *et al.* 1988). This contradicts with the prediction (Popham *et al.* 1974, Bernard and Hodgson 1985) that genera having common ancestry with *Mytilus* would have an acrosomal rod deeply inserted in the nucleus. The absence of an acrosomal rod has been described as a primitive feature (Baccetti 1979). When present, this rod is either short and limited to the acrosome lumen (Longo and Anderson 1969, Popham *et al.* 1974, Franzen 1983), or penetrates the nucleus either slightly (Pochon-Masson & Gharagozlou 1970, Popham *et al.* 1974, Lewis *et al.* 1980, Sakai *et al.* 1982; Franzen 1983) or deeply (Niijima and Dan 1965, Daniels *et al.* 1971, Bernard and Hodgson 1985, Hodgson and Bernard 1986a). This rod may play a role in fertilization of oocytes of the bivalves concerned (Niijima and Dan 1965, Popham 1974b).

The acrosome of *B. variabilis* sperm is made of an anterior electron-dense region and a posterior electron-lucent one, as in other bivalves (Popham *et al.* 1974, Hylander and Summers 1977, Bernard and Hodgson 1985, Hodgson and Bernard 1986b, Hodgson *et al.* 1987, Bernard *et al.* 1988). This regional difference has been suggested to reflect different functions of the acrosome during fertilization (Hylander and Summers 1977). The acrosome of this species develops from scattered proacrosomal granules associated with a Golgi body. Such granules coalesce into a large spherical, basally located acrosomal granule which migrates apically where it develops into its final form. This contrasts with earlier findings (Bernard and Hodgson 1985, Hodgson and Bernard 1986b, Bernard *et al.* 1988) which refer to "the migration of proacrosomal granules to the presumptive anterior end of the spermatid where they probably coalesce into one large acrosomal vesicle".

Results of this study support the suggestion of other investigators (Popham 1979, Hodgson and Bernard 1986a,b, Hodgson *et al.* 1987) that the acrosomal region is a more useful structure in assessing bivalve phylogentic relations than other sperm regions.

Acknowledgements

I would like to thank Dr. Neil Hulings for his help in collecting the specimens, Mrs. Suha Janakat and Miss Hiyam Shaltaf for technical assistance, Mr. Fattouh Ahmad for his help in photography, and Miss Basema Al-Khiat for typing this manuscript.

References

Baccetti, B. (1979) The evolution of the acrosomal complex. *In:* **Fawcett, D.W., Bedford, J.M.** (eds.) *The spermatozoon.* Urban and Schwarzenberg, Baltimore, Munich, pp. 305-329.

Bernard, R.T.F., Davies, B.R., and **Hodgson, A.N.** (1988) Reproduction in a Brackish-Water Mytilid: Gametogenesis and Embryonic Development. *The Veliger* **30**(3): 278-290.

Bernard, R.T.F., Hodgson, A.N. (1985) The fine structure of the sperm and spermatid differentiation in the brown mussel *Perna perna. S. Afr. J. Zool.* **20:** 5-9.

Bourcart, C., Lavallard, R., Lubet, P. (1965) Ultrastructure du spermatozoide de la moule *Mytilus perna* von Shering. *C.R. Hebd. Seanc. Acad. Sci., Ser D. Sci. Nat.,* **260**D: 5096-5099.

Daniels, E.W., Longwell, C., McNiff, J.M., Wolfgang, R.W. (1971) Ultrastructure of spermatozoa from the American oyster *Crassostrea virginica. Trans. Am. Micros. Sco.* **90**(3): 275-282.

Franzen, A. (1955) Comparative morphological investigations into the spermiogenesis among Mollusca. *Zoo. Bidr.* Uppsala **30:** 399-456.

Franzen, A. (1970) Phylogenetic aspects of morphology of spermatozoa and spermiogenesis. *In:* **Baccetti, B.** (ed.) *Comparative spermatology.* Academic Press, New York, pp. 29-46.

Franzen, A. (1983) Ultrastructural studies of spermatozoa in three bivalve species with notes on evolution of elongated sperm nucleus in primitive spermatozoa. *Gamete Res.* **7:** 199-214.

Higashi, S. (1964) Electron microscope studies on spermatogenesis of the freshwater mussel, *Heriopsis schlegelii. Bull. Jap. Sco. Fish.* **30:** 564-569.

Hodgson, A.N., Bernard, R.T.F. (1986a) Observations on the ultrastructure of the spermatozoon of two mytilids from the south-west coast of England. *J. Mar. Biol. Ass. U.K.* **66:** 385-390.

Hodgson, A.N., Bernard, R.T.F. (1986b) The ultrastructure of the sperm and spermatogenesis in three species of bivalve molluscs. *Gamete Res.* **15:** 123-135.

Hodgson, A.N., de Villiers, C.J., Bernard, R.T.F. (1987) Comparative spermatology of two morphologically similar species of *Solen (Mollusca: Bivalvia). S. Afr. Tydskr. Dierk.* **22**(4): 264-268.

Hulings, N. (1986) Aspects of the reproduction of rocky intertidal mollusks from Jordan Gulf of Aqaba (Red Sea). *The Veliger* **28**(3): 318-327.

Hylander, B.L., Summers, R.G. (1977) An ultrastructural analysis of the gametes and early fertilization in two bivalve molluscs, *Chama macerophylla* and *Spisula solidissima* with special reference to gamete binding. *Cell Tissue Res.* **182:** 469-489.

Lewis, C.A., Leighton, D.L., Vacquier, V.D. (1980) Morphology of Abalone spermatozoa before and after the acrosome reaction. *J. Ultrastruct. Res.* **72:** 39-46.

Longo, F.J., Anderson, E. (1969) Spermiogenesis in the surf clam *Spisula solidissima* with special reference to the formation of the acrosomal vesicle. *J. Ultrastruc. Res.* **27:** 435-443.

Longo, F.J., Dornfeld, E.J. (1967) The fine structure of spermatid differentiation in the mussel *Mytilus edulis. J. Ultrastruct. Res.* **20:** 462-480.

Niijima, L., Dan, J. (1965) The acrosome reaction in *Mytius edulis.* I. Fine structure of the intact acrosome. *J. Cell Biol.* **25:** 243-248.

Pochon-Masson, J., Gharagozlou, I.D. (1970) Particularite morphologique de l'acrosome dans-le spermatozoide de *Tapes decussatus* L. (Mollusque, Lamellibranche). *Ann. Sci. Natur. Zool.* Paris, **12:** 171-180.

Popham, J.D. (1974) The acrosome reaction in the sperm of the shipworm *Bankia australis* Calman (Bivalvia, Mollusca). *Cell Tissue Res.* **151:** 83-101.

Popham, J.D. (1979) Comparative spermatozoon morphology and bivalve phylogeny. *Malacocol. Rev.* **12:** 1-20.

Popham, J.D., Dickson, M.R., Goddard, C.K. (1974) Ultrastructural study of the mature gametes of two species of *Bankia* (Mollusca, Teredinidae). *Aust. J. Zool.* **22:** 1-12.

Popham, J.D., Marshall, B. (1977) The fine structure of the spermatozoon of the Protobranch Bivalve *Nucula hartvigiana* Pfeiffer. *The Veliger* **19**(4): 431-433.

Sakai, Y.T., Shiroya, Y. Haino-Fukushima, K. (1982) Fine structural changes in the acrosome reaction of the Japanese Abalone *Haliotis discus. Develop. Growth Differ.* **24**(6): 531-542.

Spurr, A. (1969) A low viscocity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26:** 31-43.

Trimble, J. Gaudin, D. (1975) Fine structure of the sperm of the fresh-water clam *Ligumia subrostrata* (Say 1831), (Mollusca, Bivalvia). *The Veliger* **18**(1): 34-36.

Van der Horst, G., Hodgson, A.N., Bernard, R., Salie, W., Maasdorp, J.W. (1986) Sperm ultrastructure of two *Donax* species. *Proc. Electron Micros. Soc. South. Afr.* **16:** 69-70.

(Received 03/01/1989;
in revised form 24/02/1990)

التركيب الدقيق لتكوّن المني والحيوانات المنوية الناضجة في الرخوي ثنائي الصدفة براكي دونتس ڤاريابايليس

حميد أحمد الحاج

قسم العلوم الحياتية - الجامعة الأردنية - عمان - الأردن

يعتبر الحيوان المنوي الناضج للرخوي ثنائي الصدفة براكي دونتس ڤاريابايليس من النوع البدائي، ويتركب من رأس وقطعة وسطى وذيل، ويتكون الرأس من جسم قمي على هيئة حرف Y مقلوب، وله منطقة أمامية داكنة الكترونيا وأخرى خلفية فاتحة الكترونيا. ويمتلىء الحيّز الواقع تحت الجسم القمي بمادة حبيبية - خيطية، ولكنه لا يحتوي قضيب. أما النواة فإنها برميلية الشكل، ولها نقرة خلفية. وبالنسبة للقطعة الوسطى فإنها تتكون من خمسة ميتوكوندريا تحيط بالمريكزين الداني والقاصي. وللطلائع المنوية المبكرة أنوية مركزية كبيرة، وعدد من الحبيبات المبعثرة التي ستشكل الجسم القمي فيما بعد. أما في الطلائع المنوية المتوسطة، فإن هذه الحبيبات تندمج في حبيبة قاعدية كبيرة، لا تلبث أن تتحرك في الطلائع المنوية المتقدمة لتحتل موقعاً أمامياً بالنسبة للنواة، حيث تنغمد في الجهة المقابلة للنواة، وتصبح حوصلة على شكل حرف Y مقلوب. وبالنسبة للتكثف النووي، فإنه من النوع الحبيبي.