The fine structure of the sperm and spermatid differentiation in the brown mussel *Perna perna*

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The mature sperm of *Perna perna* is 50–55 μm long and comprises three regions: a head, a mid-piece and a tail. The head incorporates a round electron-dense nucleus and an elongated cone-shaped acrosome. The mid-piece consists of a ring of five mitochondria, in the centre of which is the distal centriole from which the tail arises. The early spermatid is characterized by a large nucleus and perinuclear cytoplasm containing numerous mitochondria and proacrosomal vesicles. By mid-spermiogenesis the nuclear chromatin begins to condense, the proacrosomal vesicles coalesce to form the acrosome and the mitochondria are reduced to five in number. At this stage the tail first appears. During late spermiogenesis the acrosome elongates and invaginates on its adnuclear surface. The structure of the sperm of *P. perna* is therefore similar to that of other mytilaceans, thus supporting the contention that sperm ultrastructure could be used in studies on bivalve phylogeny.

S. Afr. J. Zool. 1985, 20: 5–9

Die volwasse sperma van *Perna perna* is 50–55 μm in lengte en bestaan uit drie onderdele: 'n kop, 'n middelstuk en 'n stert. Die kop omsluit 'n ronde elektrondigte kern en 'n verlengde keëlvormige akrosoom. Die middelstuk bestaan uit 'n ring van vyf mitochondria, in die middel waarvan die distale sentriool geleë is. Die stert het sy ontstaan in hierdie sentriool. Die vroeë spermatied word gekenmerk deur 'n groot kern en omliggende sitoplasma wat heelwat mitochondria en pro-akrosomale vesikels bevat. Teen middelspermiogenese begin die kernchromatien verdig, die pro-akrosomale vesikels vloei saam en vorm die akrosoom en die getal mitochondria word na vyf verminder. Die stert verskyn gedurende hierdie stadium. Die akrosoom verleng en vou op sy kemgerigte oppervlak na binne toe. Gevolglik is die struktuur van die sperma van *P. perna* soortgelyk aan die van ander Mytilacea en dit onderskraag die bewering dat die ultrastruktuur van sperma in die studie van die filogenese van tweekleppiges gebruik kan word.

S.-Afr. Tydskr. Dierk. 1985, 20: 5–9

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Received 20 July 1984; accepted 22 August 1984

Studies on the reproductive biology of South African bivalves are limited, information being restricted to the reproductive cycles and gametogenesis (using light microscopy) of *Donax serra* (De Villiers 1975), *Choromytilus meridionalis* and *Aulacomya ater* (Griffiths 1977). To date there have been no ultrastructural studies on the morphology of sperm or the process of spermatogenesis for any of the South African bivalves.

Franzen (1956) catagorized invertebrate sperm into two basic types: 'primitive sperm', produced by species exhibiting external fertilization and 'modified sperm' produced by those species with internal fertilization. Bivalve sperm is considered to be 'primitive' (Popham 1979). Despite the fact that information on the ultrastructure of bivalve sperm is limited, the studies which have been done have revealed that the sperm exhibit marked morphological variation, the acrosome being particularly variable (for review see Popham 1979). Such variation has been attributed to a difference in reproductive habits and has been used to correlate sperm structure with bivalve phylogeny (Popham, Dickson & Goddard 1974; Popham 1979). Of the superfamily Mytilacea only two species have received attention. *Mytilus edulis* (Niijima & Dan 1965; Longo & Dornfeld 1967) and *Mytilus perna* (Bourcart, Lavellard & Lubet 1965).

Perna perna (Linn.), a member of the Mytilacea, is found on rocky shores of South Africa from the mid-tide level down. It has a wide distribution, but is more abundant from False Bay to Durban (Day 1974). There are no published reports on the reproductive biology of this species. In this paper we describe the fine structure of the mature sperm and examine the process of spermatid differentiation.

Materials and Methods

Specimens (6 cm long) were collected during January and February 1984 from rocky shores of the eastern Cape and transported back to the laboratory where they were kept in an aquarium with circulating sea water (35‰; 20°C).

The external structure of the sperm was observed using both scanning and light microscopy. Whilst in the aquarium several individuals released sperm which was immediately collected and fixed overnight in 5% glutaraldehyde in sea water at 4°C. Once fixed, the sperm was washed in phosphate buffer (pH 7,2) and the suspension filtered onto 0,45 μm filter paper. The filtrate was then dehydrated through a graded ethanol series, critical point dried and coated with gold. The sperm was then examined with a JEOL U3 SEM and JEOL 100 CXII Temscan.

For TEM, the testes, found predominantly in the mantle

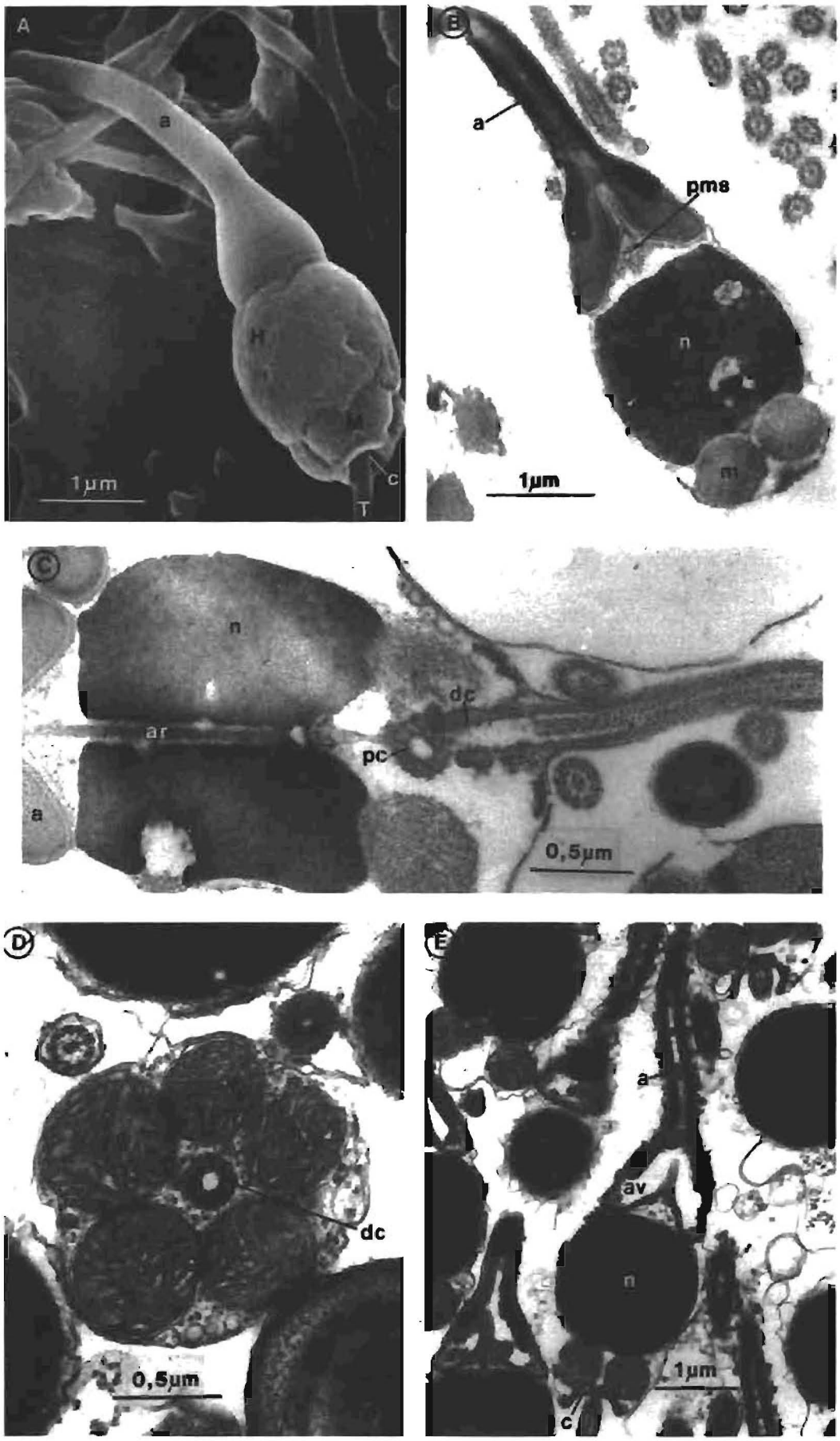

Figure 1 Ultrastructure of the mature sperm of *P. perna*. (A) Scanning electron micrograph showing external features. (B) Longitudinal section through the head and mid-piece showing an acrosome consisting of two materials of differing electron densities. (C) L.S. sperm showing axial rod passing through the nucleus, connecting posteriorly with the proximal centriole. The tail arises from the distal centriole. (D)T.S. mid-piece illustrating ring of five mitochondria and distal centriole. (E)L.S. showing collar surrounding the base of the tail. a, acrosome; ar, axial rod; av, acrosomal vesicle; c, collar; dc, distal centriole; H, head; M, mid-piece; m, mitochondrion; n, nucleus; pc, proximal centriole; pms, premembranoid sleeve material; T, tail.

lobes, were excised from animals, placed in 5% glutaraldehyde in sea water (4°C) and left in the fixative overnight. After buffer washing (phosphate buffer pH 7,2) small pieces of tissue were post-fixed in 1% osmium tetroxide for 90 min, dehydrated and embedded in TAAB 812 resin through propylene oxide. Thin sections (65 nm) cut with a diamond knife were stained in uranyl acetate and lead citrate and examined with a JEOL 100 CX II microscope.

Results

Structure of the mature sperm

The mature sperm of *Perna perna* (50–55 µm long) comprises three regions: a head, a mid-piece and a tail (Figures 1a, b). The head incorporates a round electron-dense nucleus (1,7 µm diameter) and an acrosome. The acrosome has the form of an elongated (5,3 µm) conical vesicle which is invaginated at the posterior end to form a lumen. The acrosomal vesicle is differentiated into two zones suggesting that it is composed of two types of material. There is an outer electron-dense zone and an inner more electron-lucent zone (Figure 1b).

Extending posteriorly into the nucleus and anteriorly into the hollow of the acrosomal vesicle is an axial rod (53 nm diameter) (Figure 1c). The mid-piece consists of a ring of five mitochondria (0,6 µm diameter) (Figure 1a, d) in the centre of which are located the proximal and distal centrioles (Figure 1c). The tail (45 µm long; 0,25 µm diameter) originates from the distal centriole. The base of the tail is surrounded by a collar of plasma membrane characteristically thickened and electron dense (Figures 1a, e).

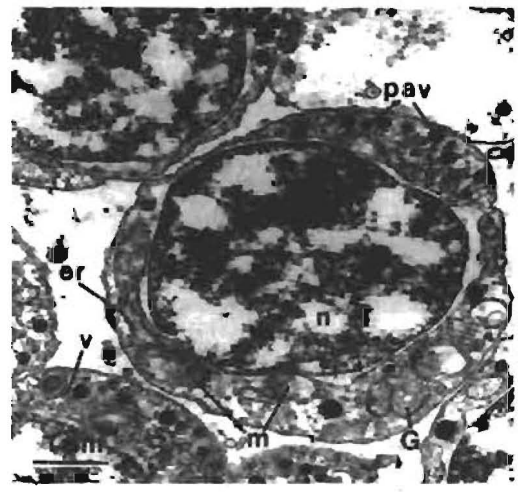

Figure 2 Early spermatids showing large central nucleus (n) and cytoplasm with several mitochondria (m), endoplasmic reticulum (er), Golgi bodies (G), vesicles (v) and osmiophilic proacrosomal vesicles (pav).

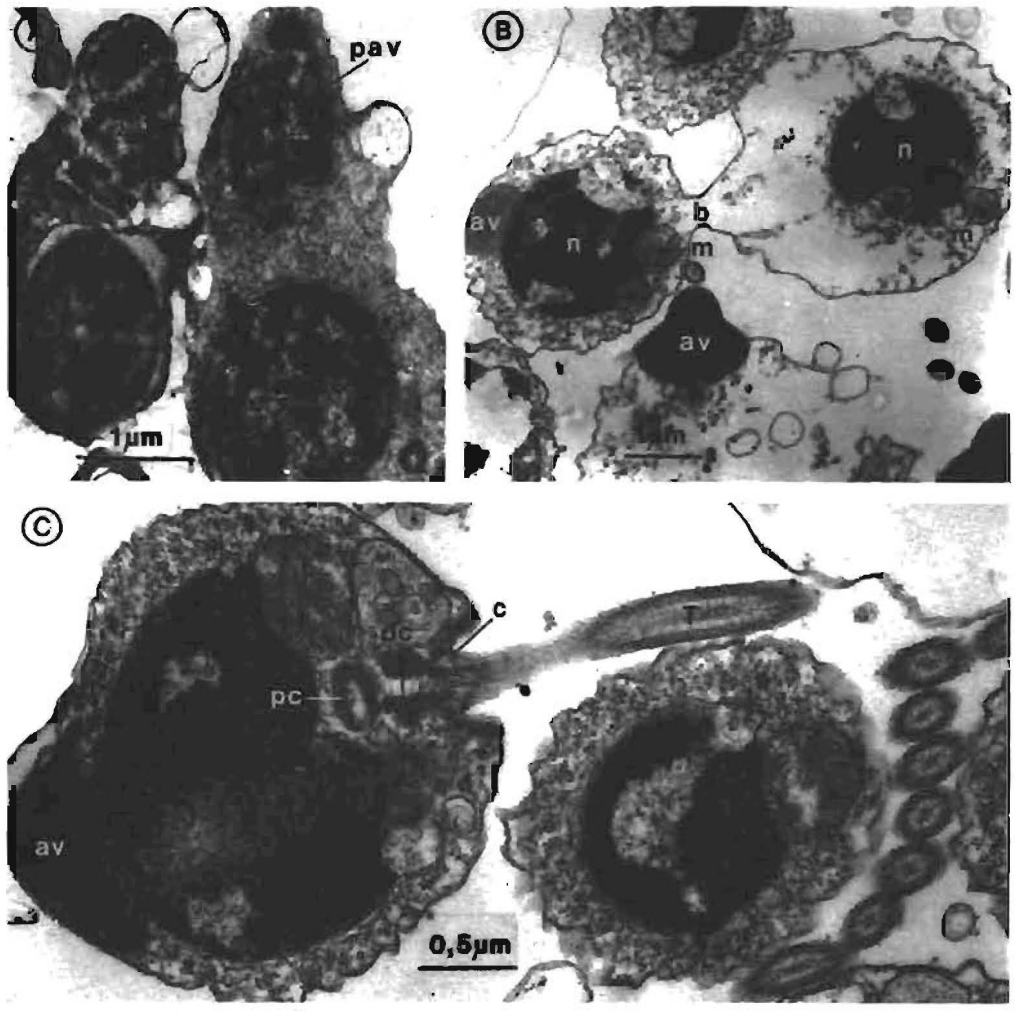

Figure 3 Stages in spermiogenesis. (A) Mid-spermatid stage illustrating aggregation of proacrosomal vesicles (pav) at the presumptive anterior end. The nuclear chromatin is now more condensed. (B) A pair of spermatids connected by a cytoplasmic bridge (b). Note the juxtaposition of mitochondria (m) and nucleus (n), and the single acrosomal vesicle (av). (C) Late spermatid. The tail (T) is now fully formed. dc, distal centriole; c, collar; pc, proximal centriole.

Spermatogenesis

Most stages of spermatogenesis can be observed in the testis throughout the year (Hodgson, unpublished results). The early spermatid is characterized by the large spherical nucleus which occupies the centre of the cell. The perinuclear cytoplasm contains a few mitochondria, diffuse endoplasmic reticulum and dense osmiophilic granules (0,2 µm diameter) which are sometimes closely associated with the lamellae of Golgi bodies (Figure 2). These osmiophilic granules are thought to represent proacrosomal vesicles. Intracellular cytoplasmic bridges connect some of the early (and mid-) spermatids (Figure 3b), these bridges being similar in structure to those found in *M. edulis* (Longo & Dornfeld 1967).

As development progresses to a mid-spermatid stage, cytoplasm is lost from the cell by sloughing. The contents of the nucleus condense and the proacrosomal vesicles migrate to the presumptive anterior end of the cell where they probably coalesce forming one large electron-dense acrosomal vesicle (Figures 3a, b). Very little endoplasmic reticulum is now present within the cell and the mitochondria, which are reduced in number, increase in size, having an internal structure which is more electron dense. The mitochondria form a close association with the nucleus and in many cases are tightly apposed to the nuclear envelope (Figures 3b, c). As further development occurs the mitochondria occupy the opposite end of the cell to the acrosomal vesicle so forming the sperm mid-piece. The tail first appears at the mid-spermatid stage, developing from the distal centriole; the proximal centriole occupying a position between the distal centriole and the nucleus (Figure 3c).

During the late spermatid stage, the single cone-shaped acrosomal vesicle begins to assume the mature form. The morphogenesis of the acrosome is shown in Figures 3 and 4. As the cone elongates it becomes invaginated on its adnuclear surface and the lumen which is formed is filled with granular premembranoid sleeve substance and fibrous material. The granular substance adheres to the periphery of the acrosomal vesicle as it develops, whereas the fibrous material extends posteriorly into the nucleus and anteriorly into the lumen to form the axial rod. Posteriorly, when seen in longitudinal section, the axial rod bisects the nucleus (Figure 1c & 4b).

Discussion

The morphology of the mature sperm of *Perna perna* is almost identical to previous descriptions given for *Mytilus edulis* Niijima & Dan 1965; Longo & Dornfeld 1967). The sperm of both species have a mid-piece consisting of a ring of five mitochondria and a head capped by an elongated acrosome, the axial rod of which penetrates through the nucleus. However the nucleus of *P. perna* differs in shape to that of *M. edulis*, being spherical in the former and ovoid in the latter. Popham (1979) suggests that the large acrosome of the sperm of *Mytilus* may be associated with penetration of the thick jelly coat of the egg of this species. Studies on oogenesis of *P. perna* (Hodgson & Bernard, in prep.) have revealed that this species also has an egg with a thick jelly coat, thus adding further support to Popham's theory.

The observations presented here on spermatogenesis of *P. perna* agree with the findings of Longo & Dornfeld (1967) for *Mytilus*. In particular the acrosome would appear to originate by the coalescence of a number of small osmiophilic vesicles secreted by Golgi bodies, and not from a single large cup-shaped dictyosome as described by Franzen (1955). In addition, as the sperm matures, there is an increase in the size but a decrease in the number of mitochondria until only five remain. Unfortunately we were unable to ascertain how increase in size and reduction in number were achieved.

Popham *et al.* (1974) suggested that sperm ultrastructure could be used for taxonomic purposes and that it should be possible to predict sperm characteristics from the study of related species. They therefore predicted that genera having a common ancestry with *Mytilus* would have an axial rod deeply inserted into the nucleus. Our findings from *P. perna* support this hypothesis. We therefore predict that an examination of *Choromytilus* and *Aulacomya* (two other South African mytilid bivalves) would reveal that both these molluscs will have sperm with an elongated acrosome, an axial rod passing through the nucleus, and a mid-piece with five mitochondria.

Acknowledgements

We would like to thank Mr R. Cross and Mr A. Hartley of the Electron Microscope Unit, Rhodes University, for technical and photographic services. This work was supported

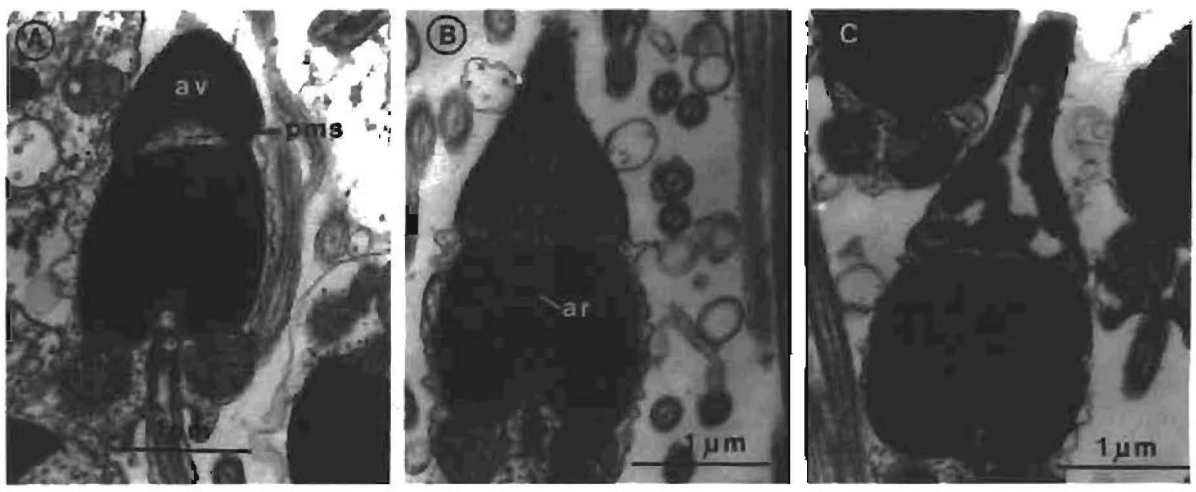

Figure 4 Morphogenesis of the acrosome during late spermatid development. (A) The acrosomal vesicle (av) is beginning to invaginate on its adnuclear surface and the space thus formed is filled with premembranoid sleeve material (pms). The chromatin is now fully condensed. (B) Elongation of the acrosome; the axial rod (ar) has now penetrated the nucleus (n). (C) The acrosome has almost assumed its mature form, that of a hollow vesicle.

from a grant from Rhodes University.

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