Science and Technology, 4 (1999) 1-7

Spermatogenesis and Spermiogenesis of the *Aphanius Dispar* (Rüppell 1828) (Pisces: Cyprinodontidae)

Taher A. Ba-Omar

Department of Biology, College of Science, Sultan Qaboos University, P.O.Box 36, Al-Khod 123, Muscat, Sultanate of Oman.

تكون المني والحيوانات المنوية لسمك الصد

طاهر بن عبدالرحمن باعمر

خلاصة : تم استخدام المجهر الضوئي والمجهر الألي لدراسة مراحل نمو الخلايا الجرثومية الذكرية لسمك الصد ، وتظهر هذه الدراسة أن خصية هذا السمك تتكون من كيسات تحتوي كل منها على مرحلة واحدة من مراحل النمو للخلايا الجرثومية المذكرة ، وكذلك وصف الشكل والتركيب لنوايا وعضيات الخلايا الجرثومية المذكرة.

ABSTRACT: Light and transmission electron microscopy were used to study the structure of various stages of germ cells of the *Aphanius dispar* (Rüppell 1828) in Oman. *A. dispar* testes were of cystic type. Different stages of spermatogonia, spermatocytes and spermatids were observed within the cysts. Each cyst is occupied by a group of germ cells almost all in the same stage. The morphology and ultrastructure of the nuclei and cytoplasmic organelles of the germ cells are described. Some flagella possessing lateral side fins and some biflagellated spermatozoa were seen.

Spermatogenesis and spermiogenesis of teleost have been the subject of several studies (Afzelius, 1977; El-Hawawi *et al*, 1981; Guha *et al*, 1986; Saez *et al*, 1990 and Iliadou & Fishelson, 1995). However very little information is available on *Aphanius dispar* (Rüppell 1828), a cyprinodont fish in Oman. *A.dispar* is common and widely distributed in fresh and brackish waters. The biology of *A. dispar* in the Sultanate of Oman is at present receiving considerable attention (Ba-Omar & Prentis, 1992; Ba-Omar *et al* 1998). This paper attempts to provide some information on the variation of spermatogenic and spermiogenic differentiation of *A. dispar*, at the ultrastructural level.

Material and methods

Male specimens of *Aphanius dispar* with a total length range of 31-48 mm were collected from Wadi Al-Khoud (Muscat) near the Sultan Qaboos University in Northern Oman (Lat.23° 30' N; Long.58° 40' E) and from springs near Salalah in the Dhofar Region, Southern Oman (Lat.17° 01' N; Long.54° 01' E). Fish collected were subsequently scarified and the testes were immediately removed and immersed in Karnovsky fixative buffered with sodium cacodylate to a pH of 7.4 for four hours. The tissues were then post-fixed in 1% aqueous solution of osmium tetroxide for 1 hour and dehydrated in a series of alcohol before embedding in Agar 100 resin. Semi-thin and ultra-thin sections were cut using Leica ultamicrotome R. The semi-thin sections were stained with toluidine blue and the ultra-thin sections were stained with uranyl acetate and post-stained in lead citrate. The sections were examined using a Zeiss EM900 TEM.

Results

LIGHT MICROSCOPY: Semi-thin sections examined by light microscopy indicated the general feature of the testes in *Aphanius dispar* (Fig.1). The testes were composed of several cysts. Each cyst was occupied by several germ cells usually in the same stage of development. The cysts with different stages of germ cells were not arranged in any definite pattern and were randomly distributed (Fig.1). Synchrony at each stage of development was maintained within each cyst by the close apposition of those germ cells.

Figure 1. Light Micrograph (LM) of a transverse section of the testis showing cysts with different stages (arrows). Bar = 25 μm.

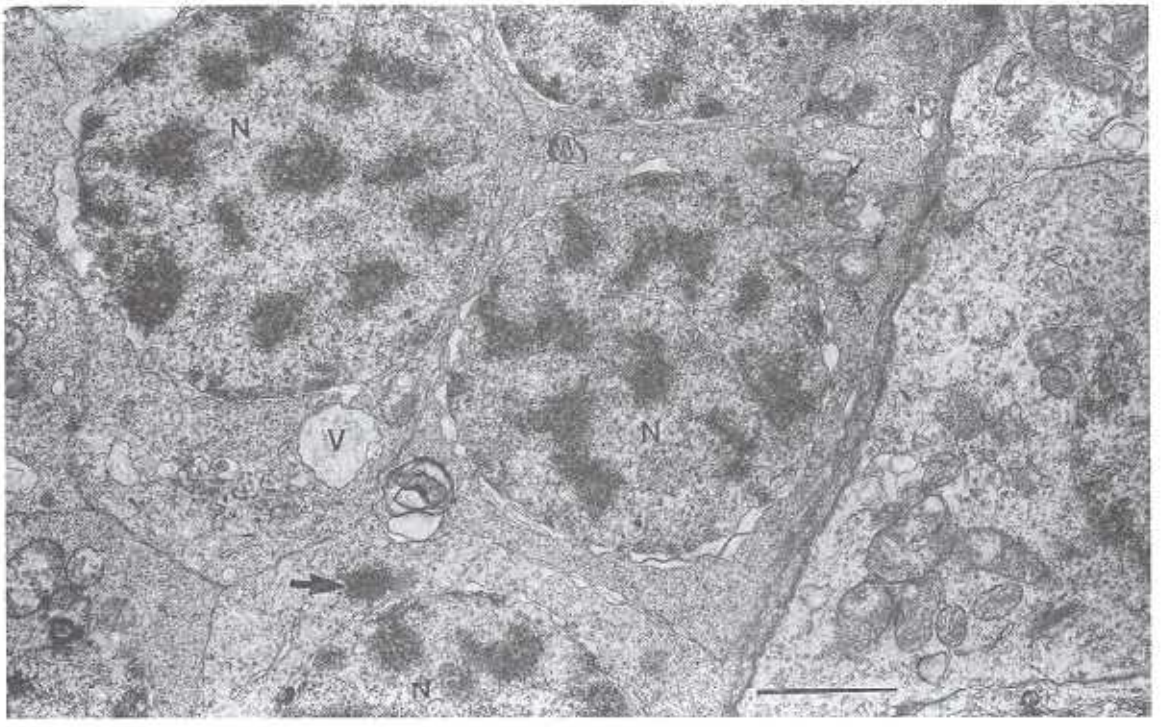

Figure 2. Transmission electron micrograph (TEM) of spermatogonia showing the nucleus (N) with heterochromatin, mitochondria (small arrows), nuage-like materials (large arrow heads) and membrane bound vacuoles (v). Bar = 1 μm.

Transmission Electron Microscopy

SPERMATOGONIA: The spermatogonia were grouped in cysts. Each cyst was composed of primary and secondary spermatogonia. The primary spermatogonia mitotically divide to form secondary spermatogonia. The nuclei of both primary and secondary spermatogonia were spherical in shape with clumps of heterochromatin (Fig. 2). Cytoplasmic organelles such as ribosomes and mitochondria were present (Fig. 2). The mitochondria were quite small with different shapes; some were spherical and others were elongate. Nuage-like materials were generally in close association with the outer leaflet of the nuclear envelope of the spermatogonia (Fig. 2). Membrane bound vacuoles were also present but few (Fig. 2).

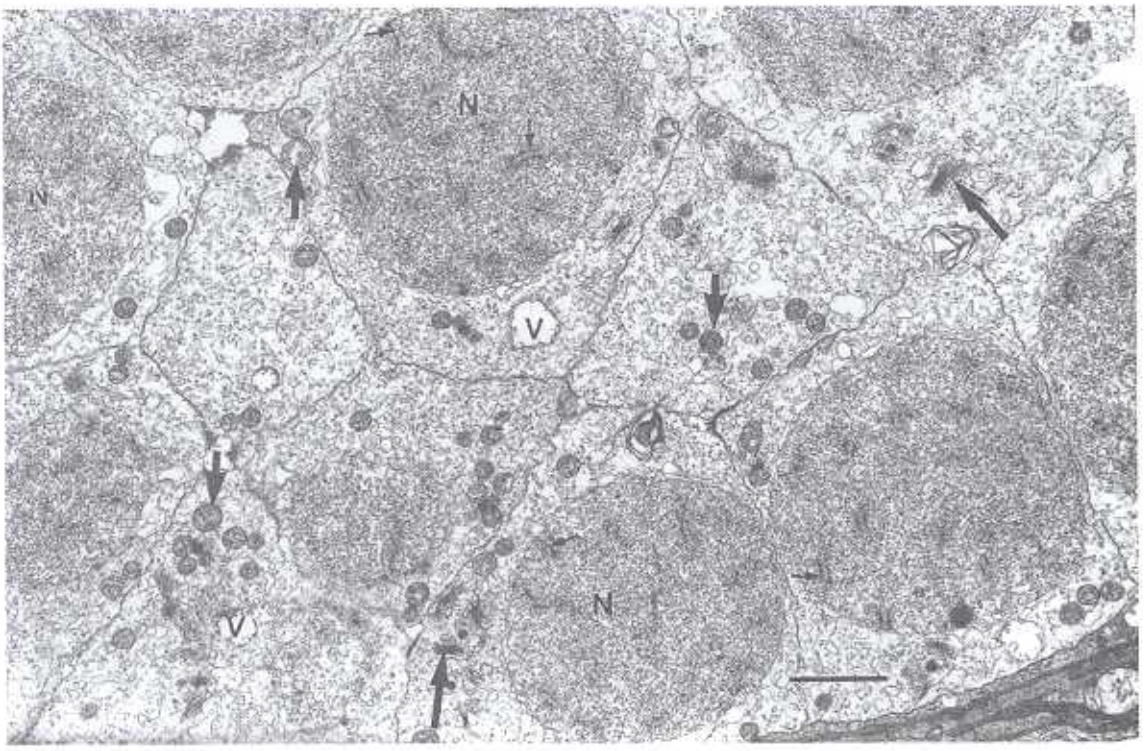

Figure 3. Transmission electron micrograph (TEM) of spermatocytes showing nuclues (N), mitochondria (large arrow heads), synaptonemel complex (small arrows), Golgi complex (large arrows) and membrane bound vacuoles (v). Bar = 2 μm.

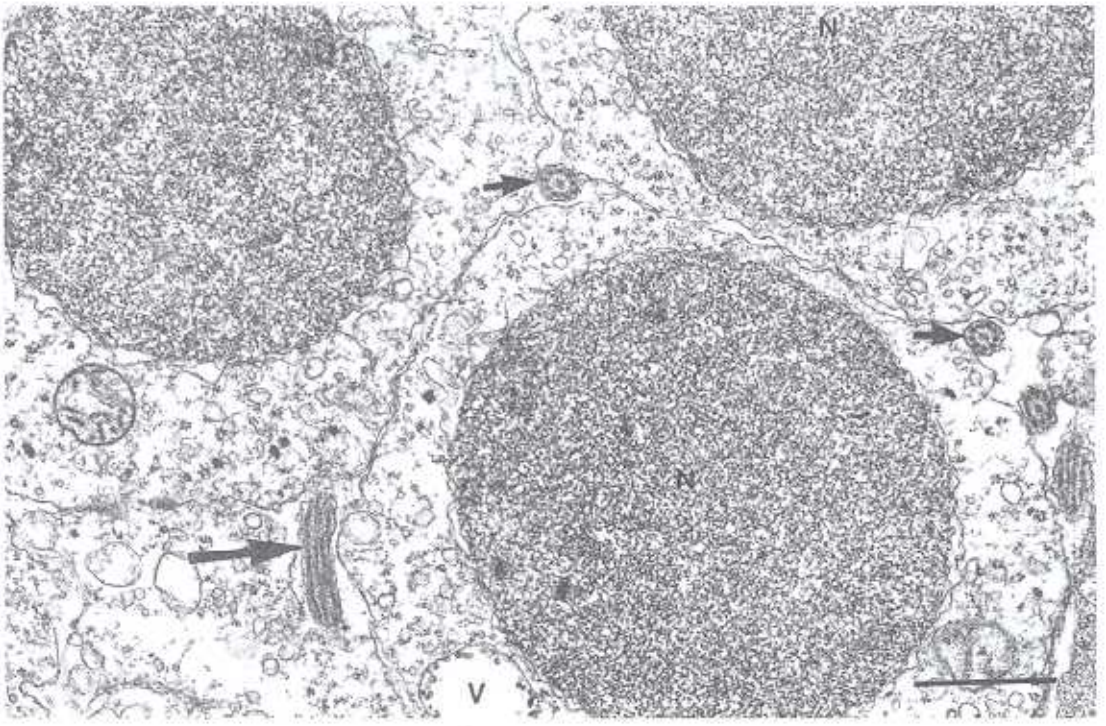

Figure 4. Transmission electron micrograph (TEM) of spermatocytes showing nucleus (N), a centriole (arrow heads), flagella (large arrow heads) among spermatocytes and membrane bound vacuoles(v). Bar = 1 μm.

SPERMATOCYTES: The secondary spermatogonia produce primary spermatocytes through mitosis. The nuclei were spherical in shape with finely granulated chromatin, homogeneously distributed throughout the nucleus (Fig. 3). Some synaptonemal complexes were noted adhering to the nuclear envelope (Fig. 3). Cytoplasmic organelles such as mitochondria, endoplasmic reticulums (ERs), Golgi complex and ribosomes are present (Fig. 3). The mitochondria were small in size where some were spherical whereas the others were elongated. Pairs of centrioles and groups of flagella were frequently seen (Fig. 4). Membrane bound vacuoles were present in variable sizes (Fig. 3, 4).

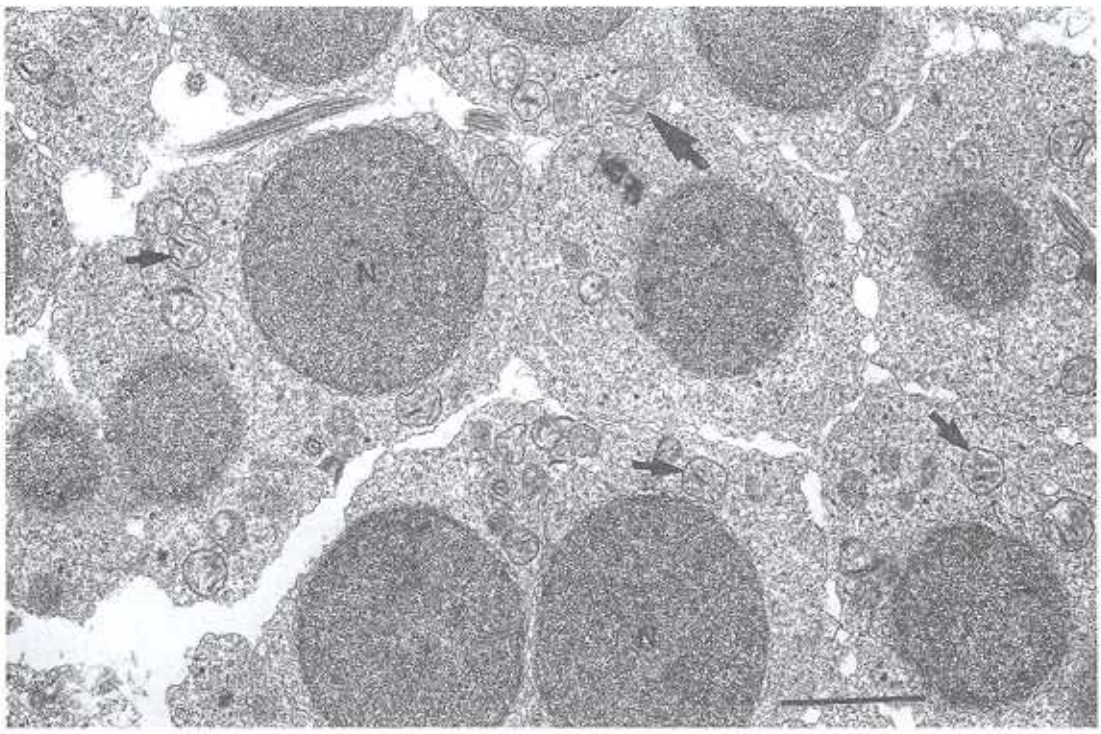

Figure 5. Transmission electron micrograph (TEM) of spermatids showing the lose contact of spermatids with their neighbor. Nucleus (N), Golgi complex (large arrow heads) and mitochondria (small arrow heads). Bar = 1.7 μm.

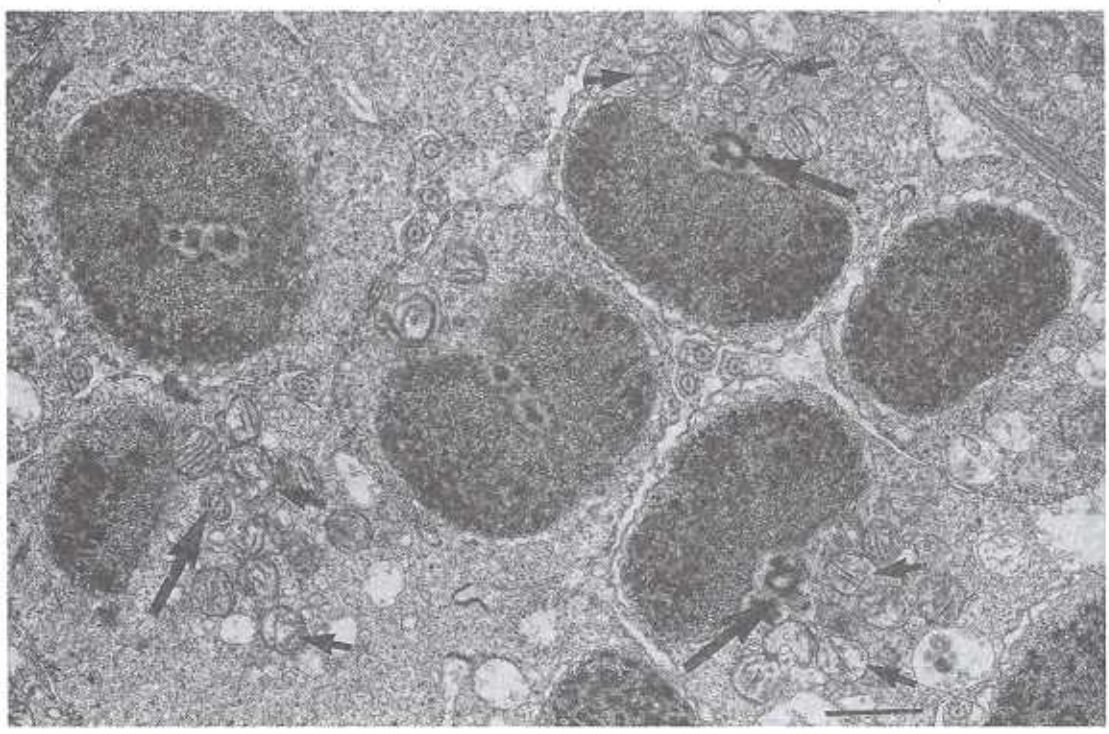

Figure 6. Transmission electron micrograph (TEM) of spermatids showing the concentration of mitochondria (arrow heads) in the area of the centrioles (arrows). Bar = 1.7 μm.

SPERMATIDS: The spermiogenesis took place within each cyst. The spermatids lost contact with each other in preparation for the formation of spermatozoa (Fig.5). The early spermatids possessed spherical nuclei with dense chromatin and few mitochondria (Fig. 5). Cytoplasmic organelles such as mitochondria, ribosomes, small quantity of smooth endoplasmic reticulum (SER) and Golgi complex were present (Fig.5). The centrioles were centrally located in what was becoming the head-piece. During this differentiation, the mitochondrial complex moved to the area distal of chromatin condensation and associates with the centrioles (Fig. 6). The late spermatids possessed nuclei deeply indented on the

posterior surface in the area around the centrioles forming a groove where mitochondria were seen (Fig. 6). The nucleus with its granular chromatin was condensed into a kidney-shaped mass towards one of the cell poles (Fig. 7). This condensation occupied most of the nucleus. The axoneme consisted of the usual 9+2 microtubules (Fig.8). There were two lateral side fins present within the flagella (Fig.9). Some developing spermatozoa were seen to possess two flagella (Fig. 9).

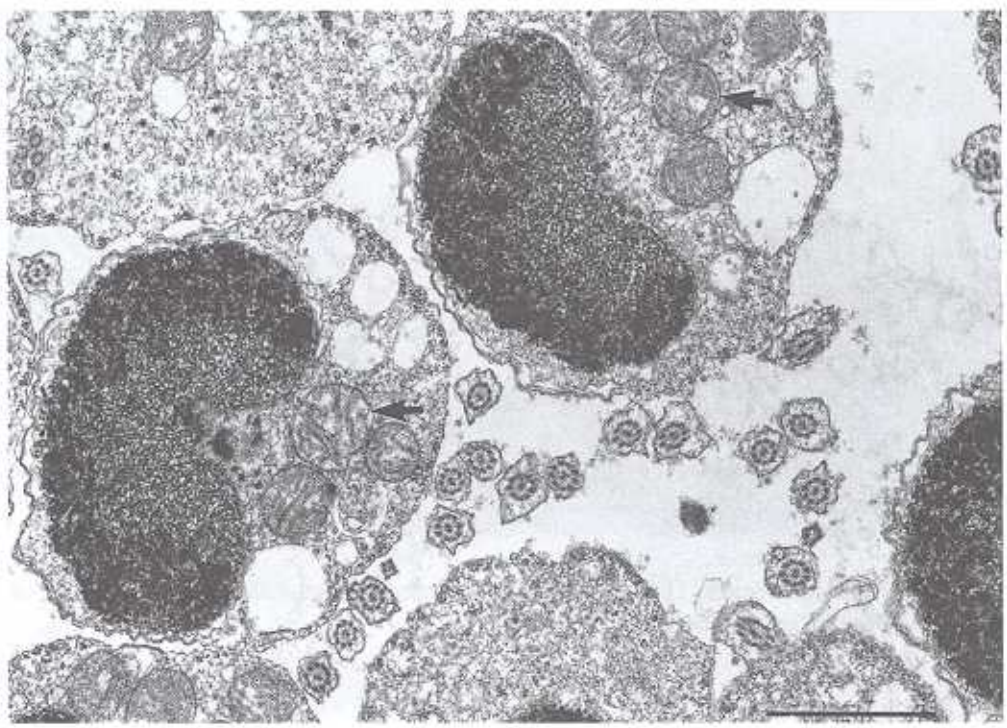

Figure 7. Transmission electron micrograph (TEM) of spermatids showing the condensation of the granular chromatin in the kidney-shaped nucleus (N) and mitochondria (arrow heads). Bar = 1.7 µm.

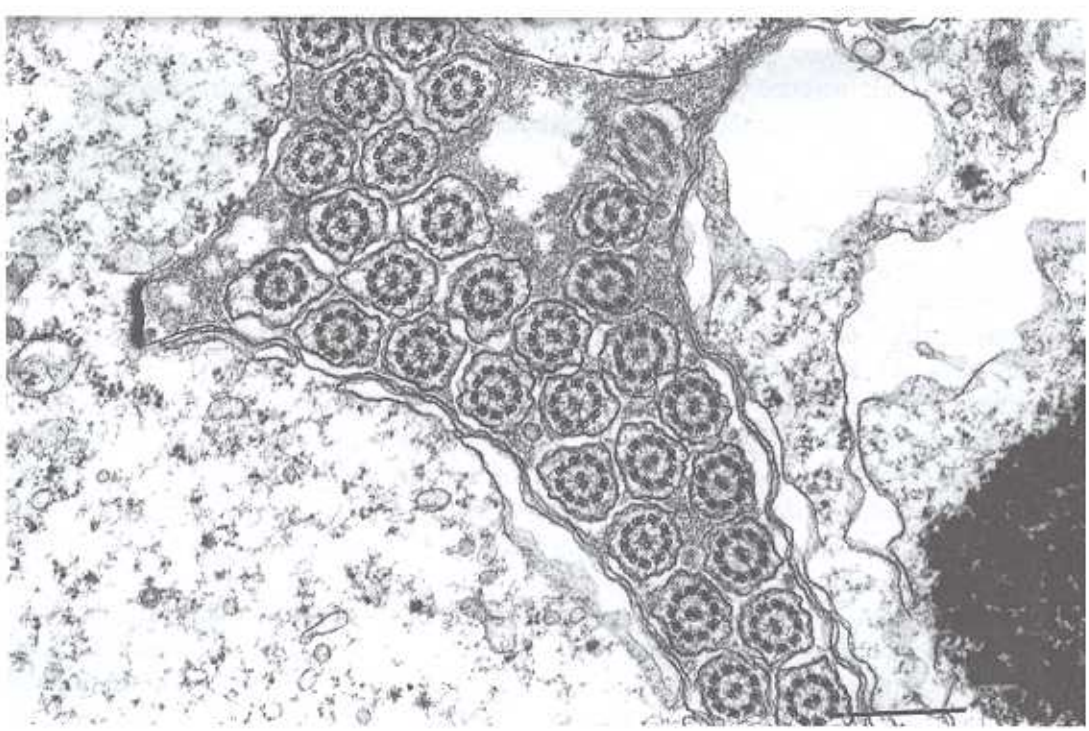

Figure 8. Transmission electron micrograph (TEM) showing a group of axonemes with the usual 9+2 microtubules. Bar = 0.5 µm.

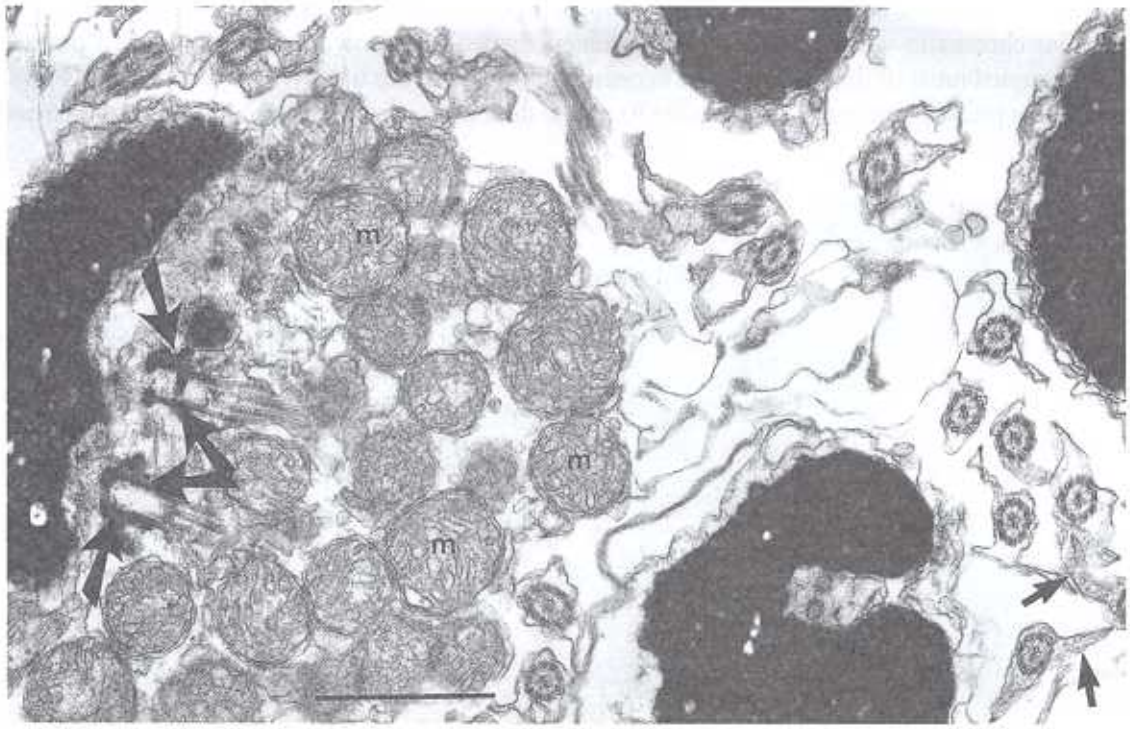

Figure 9. Transmission electron micrograph (TEM) of a developing spermatozoon with two flagella (large arrows), mitochondria (M) and 2 lateral side fins flagella (arrow heads). Bar = 1.1 μm.

Discussion

Testes of *Aphanius dispar* were of cystic type. The different stages of spermatogenesis and spermiogenesis including spermatogonia, spermatocytes and spermatids were observed within the each cyst. These germ cells apparently undergo division and maturation within the same cyst before being released as spermatozoa. Each cyst maintained synchrony to a high degree via the intercellular bridges which exist between spermatogonia and spermatocytse. The study of marbled newt, *Triturus marmoratus* showed that a single cell type was present in each testicular lobule (Fraile *et al*, 1992). Similar observations have been recorded for the mullet, *Liza dumerili* and very close physical contact between adjacent cells maintained a high degree of synchrony within each cyst (Horst, 1978).

These germ cells during their formation and development were subjected to various morphological changes. The cytoplasmic organelles such as rough endoplasmic reticulum, smooth endoplasmic reticulum and mitochondria were well developed during spermatogenesis. Saez *et al* (1990) showed that rough endoplasmic reticulum and smooth endoplasmic reticulum are well developed in the spermatogonia of the marbled newt *T. marmoratus marmoratus*. Other structures such as nuage materials were noticed in spermatogenesis in close association with the outer leaflets of the nuclear membrane. It has been shown that spermatogonia of amphibians and mammals possesses nuage materials which are an RNA - containing cytoplasmic inclusions (Kerr & Dixon, 1974; Paniagna *et al*, 1985 and Saez *et al*, 1990). Annulate lamellae were well defined and found in the secondary spermatogonia of fish collected from Southern Oman. Similar structures were described by Billard (1984), in the spermatogonia of *Poecilia reticulata*.

Some synaptonemal complex of *A. dispar* spermatocytes were shown adhering to the nuclear membrane. A similar synaptonemal complex has been founded in the spermatocyte nucleus of fowl, *Gallus domesticus* (Lin *et al*., 1995).

The membrane bound vacuoles were present among the spermatogonia and spermatocytes are well known to be associated with the removal of lipoidal substances.

In *A. dispar*, it appears that spermiogenesis possesses two stages, the early and the late. The main characteristics of the early stage were the gathering of the mitochondria at one pole, the future posterior pole. Afzelius (1977) showed that mitochondria of the spermatids are found in the posterior pole of crinoid *Antedon petasus*. In the late stage, nucleus showed an indentation, where a groove is going to be formed, forming a kidney shaped structure.

A group of flagella were observed among the secondary spermatocytes of the *A. dispar*. Secondary spermatocyte formation in *Xenopus laevis*, both in *in vitro* and in *in vivo* indicated the formation of flagella (Abe *et al.* 1988). The two lateral side fins on the flagella were obvious. The presence of these side fins may assist in the movement of spermatozoa.

Afzelius (1978) and Guha *et al* (1988) showed the lateral side fins were present in the spermatozoa of the garfish *Lepisosteus osseus* and the tilapia *Oreochromis niloticus*.

Acknowledgements

The author wishes to thank Sultan Qaboos University for the use of facilities and Professor R. Victor for reading the manuscript, Mr. P. Prentis of the Electron Microscopy Unit College of Medicine is thanked for his technical help.

References

ABE, S-I, ASAKURA, S. and UKESHIMA, A. 1988. Formation of flagella During interphase in secondary spermatocytes from *Xenopus laevis in vitro*. *J. Experimental Zoology* **246**: 65-70.

AFZELIUS, B.A. 1977. Spermatozoa and spermatids of the Crinoid *Antedon petasus*, with note on primitive spermatozoa from deuterostome animals. *J Ultrastructure Research* **59**: 272-281.

AFZELIUS, B.A. 1978. Fine structure of the garfish spermatozoon. *J. Ultrastructure Research* **64**: 309-314.

BA-OMAR T.A. and PRENTIS P.F. 1992. An ultratructural study of Sertoli cells in two geographically isolated populations of *Aphanius dispar*, pp548-49. In: *Proceedings of the 50th annual meeting of the Electron Microscopy Society of America*, Part I: 935 pp.

BA-OMAR, T.A.; VICTOR, R. and TOBIAS, D.B. 1998. Histology of the stomach of *Aphanius dispar* (Rüppell 1828), a cyprinodont fish, with emphasis on changes caused by stress from starvation. *Tropical Zoology* **11**:11-17.

BILLARD, R. 1984. Ultrastructural changes in the spermatogonia and spermatocytes of *Poecilia reticulata* during spermatogenesis. *Cell and Tissue Research*. **237**: 219-226.

EL-HAWAWI, A.S.N., AL-YOUSIF, M.S. and AL-ABDUL-LATIF, S. 1981. The ultrastructure of spermiogenesis in the fish *Aphanius dispar* (Rüppell 1828) (Family:Cyprinodontidae). *J. College of Science, University of Riyadh* **12**(1): 115-125.

FRAILE, B., SAEZ, F.J., CODESAL, J. and PANIAGUA, R. 1992. Characteristics of secondary spermatocytes in the marbled newt (*Triturus marmoratus*). *J. Anatomy*. **180**: 81-88.

GUHA, T., SIDDIQUI, A.Q. and PRENTIS, P.F. 1986. Ultrastructure of primary spermatocyte in the fish (Tilapia: *Oreochromis niloticus*). The synaptonemal complex. *Proceedings of the 44th annual meeting of the Electron Microscopy Society of America*. pp288.

GUHA, T., SIDDIQUI, A.Q. and PRENTIS, P.F. 1988. Ultrastructure of testicular spermatozoon of the fish *Oreochromis niloticus*. Proceedings of the 46th annual meeting of the Electron Microscopy Society of America. pp278.

HORST, G.VAN DER. 1978. The structure of the testis of the mullet, *Liza Dumerili* (Teleostei;Mugilidae) with special reference to spermatogenesis. *Zoological Africana* **13**(2): 233-243.

ILIADOU, K. and FISHELSON, L. 1995. Histology and cytology of testes of the catfish *Prarsilurus aristotelis* (Siluridae, Teleostei) from Greece. *Japan J. Ichthyology* **41**(4): 447-454.

KERR, A. and DIXON, K.E. 1974. An ultrastructural study of germ plasm in spermatogenesis of *Xenopus laevis*. *J. Embryology and Experimental Morphology* **32**: 573-592.

LIN, M., THORNE, M.H., MARTIN, I.C.A., SHELDON, B.L. and JONES, R.C. 1995. Electron microscopy of the seminiferous epithelium in the triploid (ZZZ and ZZW) fowl, *Gallus domesticus* . *J. Anatomy* **186**: 563-576.

PANIAGUA, R.; NISTAL, M.; AMAT, P. and RODRIGGUEZ, M.C. 1985. Presence of ribonucleoproteins and basic proteins in the nuage an intermitochondrial bars of human spermatogonia. *J. Anatomy* **143**:201-206.

SAEZ, F.J., FRAILE, B. and PANIAGUA, R. 1990. Histological and quantitative changes in the annual testicular cycle of *Triturus marmoratus marmoratus*. *Canadian J. Zoology* **68**: 63-72.

Received 7 September 1998
Accepted 17 April 1999