INITIAL PHASE OF THE INTRAOVARIAN SPERM STORAGE OF *HELICOLENUS DACTYLOPTERUS*

Sílvia Vila *, Maria Sàbat, Marta Muñoz and Margarida Casadevall

Àrea de Zoologia, Departament de Ciències Ambientals, Universitat de Girona, Campus Montilivi, Girona, Spain

* silvia.vila@udg.es, maria.sabat@udg.es, marta.munyoz@udg.es, margarida.casadevall@udg.es

Abstract

Sperm storage crypts located within the ovaries of *Helicolenus dactylopterus* were analysed during the first phase of this storage. Electron and optical microscope observations showed some degree of organization of the stored spermatozoa. Besides, desmosomic unions among cryptal cells denoted the isolation of spermatozoa inside the crypts and were probably related to the protection of these cells from the immune system of the female.The retainment of an important portion of cytoplasm by the spermatozoa at the beginning of the storage could be explained as an initial source of nutrients while there is not nutritional supply from the female.

Key words: Helicolenus dactylopterus, sperm storage, histology, ultrastructure

The bluemouth rockfish, *Helicolenus dactylopterus*, is a zygoparous oviparous species with internal fertilization. Females present specialized structures known as crypts where the spermatozoa are stored and mantained viable for periods up to 10 months, from April and May onwards. When spawning starts, between January and February, the spermatozoa stored in the crypts must be reactivated, with subsequent fertilization of mature eggs (1).

We found that *Helicolenus dacctylopterus* shows the most complex structures for sperm storage than those previously described in viviparous species within the same family (2-3). Thus, in this species spermatozoa remain grouped within differentiated structures instead of floating freely like in *Alcichthys alcicornis* (3) or singly adhered to the ovarian epithelium as found in viviparous species like *Sebastes taczanowskii* that retain spermatozoa adhered to the epithelium of the ovigerous lamella or wrapped in its microvilli. The prolonged time period during which spermatozoa must reside in the ovary may offer an explanation about the existence of these specialised storage crypts.

The aim of this paper is to analyze the structure and ultrastructure of these intraovarian crypts during the initial period of storage. From June onwards there is a marked increment of testicular activity (4), thus our work has been focused on two fresh samples caught in July (Palamós, Costa Brava, northwest Mediterranean), when the crypts are full of "new" spermatozoa.

In both cases several portions of the central ovarian area were analysed. Small pieces of ovary were fixed in glutaraldehyde (2.5%)-paraformaldehyde (2%) mixture in a 0.1 M cacodylate buffer.

Spermatozoa storage structures are located very near the muscular-connective rachis of the gonad, at the base of the interlamellar gaps (5). The cryptal epithelial cells present a nucleus with some strongly condensed heterochromatin, a lot of free ribosomes, vesicles and RER, and this is a clear evidence of important protein synthesis.

We also observed an enormous quantity of desmosomal unions among the cryptal cells (Fig. 1), forming thus a continuous cellular layer that surrounds the crypt cavity, probably related to the protection of the spermatozoa from the immune system of the female which stores them (3). Lining the epithelial cells there are some cells of different morphology, with a nucleus slightly extended and much more electrodense.

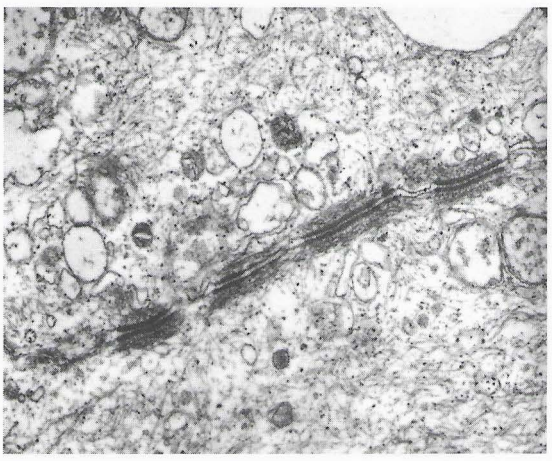

Fig. 1. Transmission electron micrograph showing desmosomal unions among cryptal epithelial cells.

Our observations through the optical microscope suggested some organization of the male gametes located in the crypts, a fact that later was corroborated by the transmission electronic microscope. We can clearly see that these organizations are not due to the existence of a separate membrane like the ones which surround spermatozeugmata.(6), but the spermatozoa are arranged in some kind of bundles seemingly independent one from one another.

An outstanding fact is that the spermatozoa remaining inside the crypts keep an important portion of cytoplasm (Fig. 2), in which we can observe many mitochondria, vacuola and tiny granules. A possible explanation could be that this cytoplasm represents an initial reserve of nutrients for spermatozoa at the beginning of the storage period, while later there would be evidence of a supply of nutritional substances from the female towards the male sexual cells, a fact that we aim to confirm in further studies.

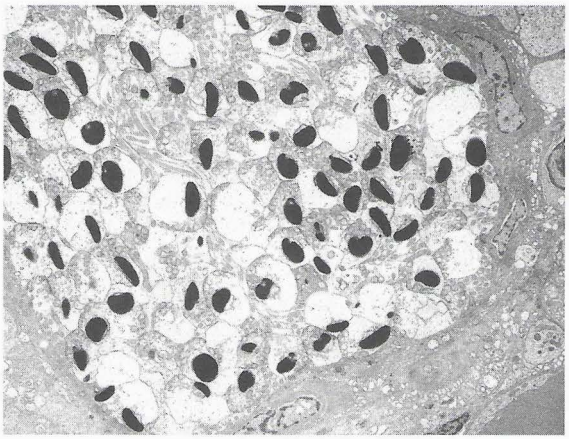

Fig. 2. Intraovarian sperm storage crypts. Electron microscopy view showing a sperm bundle surrounded by cryptal epithelial cells.

References

1 - Muñoz M., Casadevall M., Bonet S.,1999. Annual reproductive cycle of *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaeniformes) with special reference to the ovaries sperm storage. *J. Mar. Biol. Ass. UK.* 79:521-529.

2 - Moser, H.G. 1967. Seasonal histological changes in the gonads of *Sebastodes paucispinis* Ayres, an ovoviviparous teleost (family Scorpaenidae). *J. Morphol.* 123: 329-354.

3 - Koya Y., Munehara H.,and Takano K., 1997. Sperm storage and degradation in the ovary of a marine copulating sculpin, *Alcichthys alcicornis* (Teleostei: Scorpaeniformes): role of intercellular junctions between inner ovarian epithelial cells. *J Morphol.* 233: 153-163.

4 - Muñoz, M. and M. Casadevall 2002. Reproductive indices and fecundity of *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaenidae) in the Catalan Sea (western Mediterranean). *J. Mar. Biol. Ass. U.K.* 82: 995-1000.

5 - Muñoz M., Casadevall M., Bonet S.,and Quagio-Grassiotto I., 2000. Sperm storage structures in the ovary of *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaeniformes): an ultrastructural study. *Environmental Biology of Fishes.* Kluwer Academic Publ., Netherlands. 58: 53-59.

6 - Downing, A. L. and J. R. Burns (1995). Testis morphology and spermatozeugma formation in three genera of viviparous halfbeaks: *Nomorhamphus, Dermogenys,* and *Hemirhamphodon* (Teleostei: Hemiramphidae). *Journal of Morphology.* 225: 329-343.