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SPERMATOGENESIS AND SPERM STRUCTURE IN THE BLACK-STRIPED PIPEFISH *SYNGNATHUS ABASTER* (TELEOSTEI, SYNGNATHIDAE)

SPERMATOGENESI E STRUTTURA DEGLI SPERMATOZOI IN SYNGNATHUS ABASTER *(TELEOSTEI, SYNGHATHIDAE)*

Abstract *– The gonads of adult males of the black-striped pipefish* Syngnathus abaster *were observed during the reproductive season to describe mature sperm both quantitatively and qualitatively. The results show that: a) the sperms number is always very low and, does not vary significantly during the reproductive season; b) all mature sperms observed may be assigned to the introsperm-like type. These data suggest that the unique and functional sperm of Syngnathids pertain to the introsperm type typical of internally fertilizing teleostean bony fishes.*

Key-words: *introsperm, Syngnathids, testis.*

Introduction – Syngnathids species, provided with a closed and highly specialized brood pouch, (such as *Syngnathus* and *Hippocampus*) are considered internal brooders, whereas those species with an open pouch or without this structure (such as *Nerophis*) are external brooders. Hence, in literature, it is commonly accepted that eggs fertilization of external brooders occurs in the external environment for external brooders and inside the male pouch (or marsupium) in internal brooders, but both theories have gaps. Furthemore, data on sperm morphology are limited and, in some cases, contradictory (Kvarnemo and Simmons, 2004). Therefore, histological investigation on testis morphology, spermatogenesis and sperm structure and function could significantly contribute to better understand the fertilization strategies of Syngnathids.

Materials and methods – Adult males of *Syngnathus abaster* were collected in the Pond of Cabras (Sardinia, Italy) during the reproductive season (2007). Testes were dissected from 10 freshly killed fishes. Gonads of 2 males were fixed in Bouin's fixative, dehydrated in a graded ethanol series, cleared in toluene and embedded in paraffin. Sections (5 μm) were stained with Mallory's trichrome for observed general morphology. To examine contents of the male gonads and the number and morphology of the mature sperm fresh testes, in toto, of 9 males were gently squashed and observed with a Zeiss Axiophot light microscope.

Results - Each testis appears as hollow tube with a large central lumen surrounded by an external tunica albuginea and a thin internal germinal epithelium (Fig. 1a) organized in small and poorly developed cysts of few germ cells enveloped by Sertoli cells (Fig. 1b). The central lumen is occupied by floccular material among which three cells types are distinguished droplets-containing cells, large flagellate cells and mature sperm (Fig. 1c, d, e).

cells are spherical or irregular in shape (30.015±8.497 μm large). Many of the droplets-containing cells have small (1.392±0.304 μm) and numerous droplets and one, two or several large nuclei (9.044±0.643 μm) (Fig. 1b, c). However, like the droplets-containing cells, the flagellate cells are giant cells (25.969±9.188 μm large) of spherical, lobed or irregular shape. One, two or several nuclei are visible in

their cytoplasm. Each nucleus is characterized by the basal fossa and the axoneme (Fig. 1d, e). These cellular type, are recognizable as developing spermatids. Young spermatids have large round nuclei (7.333±1.096 μm diameter) with uncondensed chromatin, except around the nuclear fossa (Fig. 1d). Spermatids in an intermediate developmental stage still have rounded nuclei but they are smaller (3.191±0.574 μm) and with condensed chromatin (Fig. 1e). Mature sperm are always elongated mononuclear cells of 79.565±7.965 μm in total length with a conical head (4.719± 0.415 μm length and 0.976±0.079 μm width) (Fig. 1c, insert) and a long tail (74.853±7.995 μm). For each male we counted 500 to 3000 sperm (1694.444±1068.423).

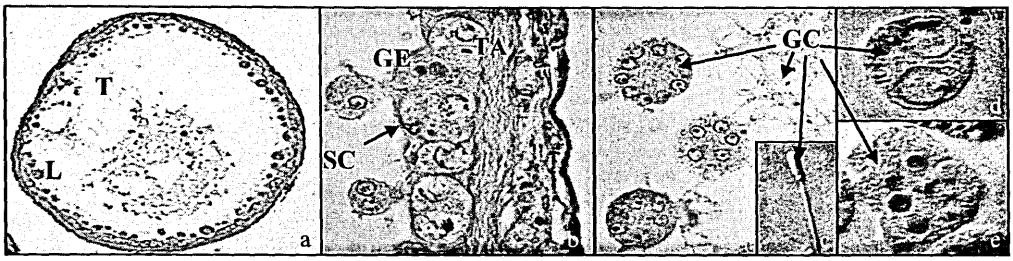

Fig. 1 - a-b) Transversal section of the testis; ×110, ×400. c-d-e) Germinal cells inside the lumen obtained by squashing testes; ×420, insert ×1000, ×800, ×800.
GC: Germinal Cells; GE: Germinal Epithelium; L: lumen; SC: Sertoli Cells; T: Testis; TA: Tunica Albuginea.

a-b) Sezione trasversale del testicolo; ×110, ×400. c-d-e) Cellule germinali all'interno del lume ottenute attraverso lo schiacciamento del testicolo; ×420, inserto ×1000, ×800, ×800.

Conclusions – The present data show that in *S. abaster*, the round cells mixed with mature sperm inside the lumen can be considered germ cells at different developmental stages. Moreover, except for mature sperm, all these cells, can have one or several nuclei, confirming that in *S. abaster* spermatogenesis is of semi-cystic and symplastic type (Carcupino *et al.*, 1999). The estimated number of sperm in *S. abaster* varies from 500 to 3000. The sperm concentration in this small family could be the lowest among fish. These data, together with the simultaneous presence of mature sperm and developing spermatids inside the testes of each male examined, suggest that sperm are formed continuously throughout reproductive season in *S. abaster*. Moreover, the semi-cystic and symplastic spermatogenesis of Syngnathids results in the presence of numerous giant round cells in the lumen, which may hide the few mature sperm produced and hamper a correct morphological interpretation of mature sperm. In fact, the other sperm types, reported for Syngnathids, could merely be developing germ cell.

References

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