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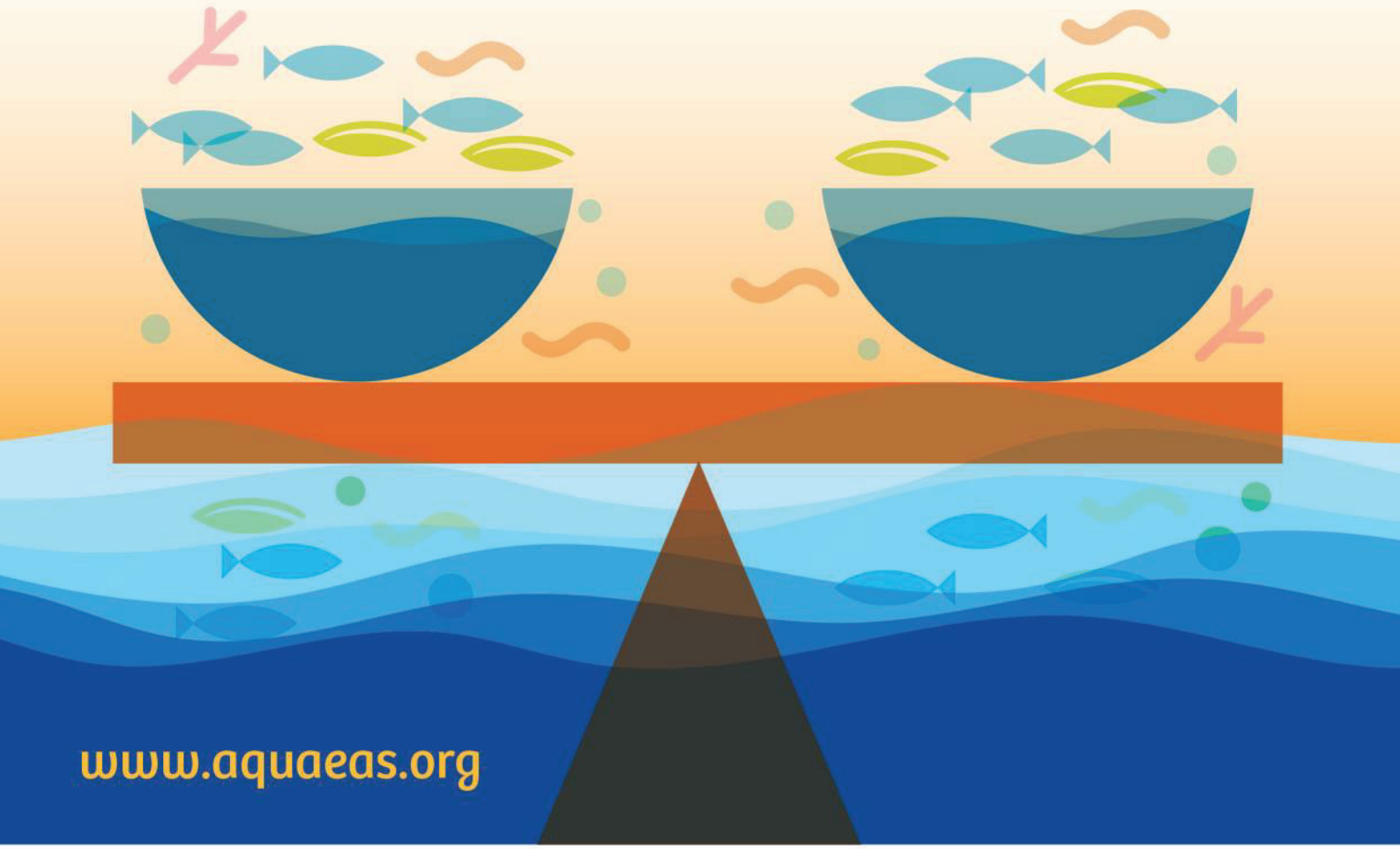

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SPERMATOGONIAL PROLIFERATION AND APOPTOSIS IN PREPUBERTAL MEAGRE *Argryrosomus regius* TREATED WITH RECOMBINANT FOLLICLE STIMULATING HORMONE, AND COMPARISON WITH ADULTS

R. Zupa[1*], N. Duncan[2], I. Giménez[3], C. C. Mylonas[4], C. Pousis[1], G. Ventriglia[1], L. Valentini[5], C. Varvara[1], A. Corriero[1]

[1]Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano 70010, Bari (Italy)
[2]Institute of Agrifood Research and Technology, 43540 La Ràpita, Tarragona, Spain
[3]Rara Avis Biotec, S. L., Calle Moratín 17, 46002 Valencia, Spain
[4]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research Heraklion 71003, Crete, Greece
[5]DiMePRe-J, University of Bari Aldo Moro, Valenzano 70010, Bari (Italy)
E-mail: rosa.zupa@uniba.it

Introduction

The meagre *Argyrosomun regius* (Asso, 1801) is a promising aquaculture species and advancing puberty using recombinant gonadotropins could shorten the generation time for selective breeding programs (Zupa et al., 2023). The aim of this study was to assess the effects of recombinant follicle stimulating hormone (rFsh) administration on spermatogonial proliferation and apoptosis in prepubertal meagre reared in indoor tanks through a comparison with adult fish reared in sea cages.

Material and Methods

Prepubertal meagre males (18-months old) reared in indoor tanks at IRTA (La Ràpita, Spain) underwent a six-weeks treatment with increasing doses of rFsh (week 0: 6 μg/kg; week 1: 9 μg/kg; week 2 to week 6: 12 μg/kg); control prepubertal males were injected weekly with 1 mL of saline solution. Prepubertal fish samplings took place before the treatment (week 0; control fish, N = 6) and after 6 weeks of treatment (week 6; control fish, N = 9 and rFsh-treated fish, N = 4). Adult males (6-years old) belonging to a commercial stock reared in sea cages in the Gulf of Taranto (Ionian Sea, Italy) were sacrificed during early (March-April 2021; N = 7) and advanced (June 2021; N = 4) phases of spermatogenesis. Testis samples were fixed in Bouin's solution and embedded in paraffin wax. Deparaffinized sections were stained with hematoxylin-eosin; proliferating spermatogonia were identified through the immunohistochemical detection of the proliferating cell nuclear antigen (PCNA); apoptotic germ cells were identified through the TUNEL method.

Results and Discussion

The rFsh-treated fish had larger testes compared to both control groups, had larger seminiferous tubules that contained all stages of spermatogenesis and had more abundant luminal spermatozoa (Fig. 1a, b). The testes of adult fish sampled in March-April were in active spermatogenesis with all germ cell types in the germinal epithelium and luminal spermatozoa (Fig. 1c); while in June, all adults were fully mature, showing residual spermatogenetic activity in a thin germinal epithelium and plenty of luminal spermatozoa (Fig. 1d). Anti-PCNA immunostaining was observed in the nuclei of single spermatogonia, spermatogonia in cysts and primary spermatocytes (Fig. 1e), but only single spermatogonia were considered for quantitative analysis. The TUNEL reaction labelled spermatogonia and spermatocytes (Fig. 1f). Fish treated with rFsh showed a significant decrease of proliferating and apoptotic single spermatogonia. In adults, spermatogonial proliferation was significantly higher during the early phase of spermatogenesis compared with the advanced phase in June, and apoptosis significantly increased from the early to the advanced phase of spermatogenesis (Table 1).

The treatment with rFsh stimulated spermatogenesis advancement in prepubertal meagre and induced a significant reduction in spermatogonial proliferation and apoptosis. In adult fish, germ cell apoptosis was low during the early spermatogenesis phase and increased during the advanced spermatogenesis phase. This observation confirms that apoptosis plays a major role in regulating germ cells/Sertoli cells ratio and in preventing aberrant germ cell development during spermatogenesis in adult fish (Prisco et al., 2003; Zupa et al., 2013). Moreover, the present data support our previous hypothesis that in prepubertal meagre apoptosis is involved in the inhibition of spermatogonial survival and progress towards meiosis (Zupa et al., 2023).

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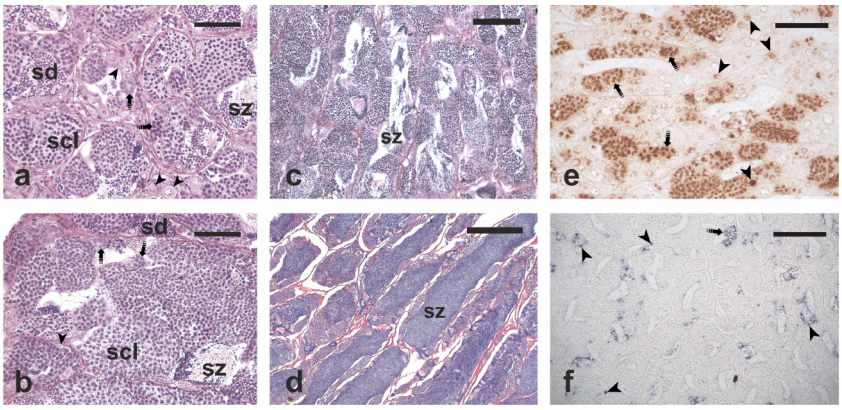

Fig. 1. Micrographs of meagre testis sections. a) control prepubertal fish; b) rFsh-treated prepubertal fish; c) adult fish in early phase of gametogenesis; d) adult fish in advanced gametogenesis phase; (e) anti-PCNA positive germ cells with nuclei stained in brown; (f) TUNEL-positive germ cells appearing as dark blue dots. Magnification bars = 50 μm in (a, b, e), 100 μm in (c, f), 300 μm in (d). Arrowhead = single spermatogonium; dashed arrow = spermatogonial cyst; scI = primary spermatocyte cyst; sd = spermatid cyst; sz = spermatozoa.

Table 1. Density of proliferating single spermatogonia and apoptotic germ cells in prepubertal and adult meagre.

	Mean (± sd) density of anti-PCNA proliferating single spermatogonia (n/mm^2 germinal epithelium)	Mean (± sd) surface occupied by apoptotic germ cells (μm^2/mm^2 germinal epithelium)
Prepubertal, control week 0	98.2 ± 47.2^a	58799.8 ± 12903.7^a
Prepubertal, control week 6	121.3±52.9^a	23451.4±1554.8^b
Prepubertal, rFsh-treated week 6	12.4±4.8^b	1700.8±297.3^c
Adult, early spermatogenesis (March-April)	487.7±291.0^a	11531.7±7536.6^a
Adult, advanced spermatogenesis (June)	22.7±12.8^b	32147.3±18796.2^b

Different letters indicate significant differences among prepubertal fish groups or between adult fish sampled in early and advanced phases of gametogenesis (ANOVA; $P < 0.05$).

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