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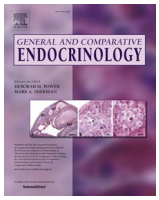

Effects of different hormonal treatments on spermatogenesis advancement in hatchery-produced greater amberjack *Seriola dumerili* (Risso 1810)

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ABSTRACT

In earlier studies, wild-caught greater amberjack *Seriola dumerili* (Risso, 1810) males reared in sea cages showed gametogenesis impairment and low sperm production and quality. Here, we (a) examined if F1 hatchery-produced males reared in sea cages also exhibit reproductive dysfunctions and (b) evaluated the effects of gonadotropin releasing hormone agonist (GnRHa) administration through injections ($GnRHa_{inj}$) or sustained-release implants ($GnRHa_{impl}$), and human chorionic gonadotropin (hGC) injections on spermatogenesis/spermiation enhancement. Fish were given a hormone treatment just prior to the spawning season, and were transferred to land-based tanks, according to an established spawning induction protocol. Blood samples (n = 6) were obtained on Days 0, 7 and 13 after treatment. Testis samples were obtained on Days 0 (n = 4) and 13 (n = 2 per treatment). The fish prior to their transfer from the sea cages to the land-based tanks, exhibited a low gonadosomatic index, altered sex steroid hormone profile and high density of testicular apoptotic cells. After transfer to tanks, there was a general depression of sex steroid plasma levels parallel to an increase in cortisol concentrations. Despite the negative effect on steroidogenesis by the transfer from the sea, the hormonal treatments increased the number of fish from where sperm could be obtained, as well as testis growth, and reduced testicular apoptosis. Treatment with hCG resulted in the most significant changes in spermatogenesis, while $GnRHa_{impl}$ appeared to induce less intense, but likely longer-lasting effects. The study indicated that F1 hatchery-produced males also exhibited reproductive dysfunctions as wild-caught captive-reared greater amberjack, and that the observed positive effects of the hormone treatments on spermiation/spermatogenesis were likely mediated by factors other than sex steroid hormones.

1. Introduction

The greater amberjack *Seriola dumerili* (Risso, 1810) is a species of worldwide reputation that has become an excellent candidate for aquaculture production (Nakada, 2000, 2008). Despite the research efforts aimed at its domestication in the Mediterranean region (García-Hernández et al., 1997; Jover et al., 1999; Micale et al., 1999 Fakriadis et al., 2019, 2020a, b; Corriero et al., 2021; Fakriadis and Mylonas, 2021), its aquaculture production remains very low due, to a large extent, to difficulties associated with the reproductive maturation in captivity.

Fishes reared in captivity are often affected by reproductive dysfunctions that must be first identified and then alleviated, in order to obtain large quantities of fertilised eggs for larval rearing (Mylonas et al., 2010). Wild greater amberjack females caught as juveniles in the Mediterranean and reared in sea cages showed a limited capacity to finalise vitellogenesis and underwent extensive atresia of late vitellogenic follicles (Zupa et al., 2017a; Pousis et al., 2018, 2019), and males showed small gonad mass, a precocious cessation of spermatogenesis and a high level of germ cell apoptosis (Zupa et al., 2017b). Moreover, males showed a marked deterioration of sperm quality during the reproductive season (Zupa et al., 2017b; Fakriadis and Mylonas, 2021).

The objective of the present study was to (a) examine if F1 hatchery-produced males reared in sea cages also exhibit reproductive dysfunctions and (b) evaluate the effects on testicular growth and spermatogenesis of three different hormonal treatments. We used gonadotropin releasing hormone agonist (GnRHa) administration through injections and sustained-release implants, and human chorionic gonadotropin

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(hGC) injections, administered during the advanced spermatogenesis phase, and just prior to the expected spawning season, in view of a potential incorporation in a commercial spawning induction protocol for the species. Due to the late puberty of greater amberjack, the large size of breeders and the scarcity of captive and cultured stocks, the availability of individuals in the present study was limited. Therefore, it was not possible to carry out the full experimentation that would require sacrificing an adequate number of fish to examine fully and deeply the effect of the hormonal treatments on the gonad in histological detail. Although the results may be considered as preliminary, nevertheless they provide useful information on the ameliorating effects of the treatments on the reproductive dysfunctions observed in hatchery-produced greater amberjack males.

2. Material and methods

2.1. Ethical statement

The experimental protocol was approved by the Greek National Veterinary Services (AP 31337). All procedures involving animals were conducted in accordance to the "Guidelines for the treatment of animals in behavioral research and teaching" (Anonymous, 1998), the Ethical justification for the use and treatment of fishes in research: an update (Metcalfe and Craig, 2011) and the "Directive 2010/63/EU of the European parliament and the council of 22 September 2010 on the protection of animals used for scientific purposes" (EU, 2010).

2.2. Fish husbandry, treatments and samplings

Fish used in the present study were produced from eggs obtained in Argosaronikos Fish Farm S.A. (Salamina Island, Greece) in 2017, after spawning induction of wild-caught breeders with GnRHa (H-4070, Bachem, Switzerland) implants (Fakriadis et al., 2020a, b). The hatchery-produced F1 juveniles were stocked at the same farm in sea cages and were maintained following common aquaculture practices and fed with a commercial broodstock diet after 3 years of age (Skretting, Vitalis Cal). On 18th of May 2021 (day 0), 28 four-year-old sexed and PIT tagged adult males (Lancerotto et al., 2023) (Supplementary File S1) were tranquilized in a 10–20 m^3 anesthesia sack placed inside the cage using clove oil (0.01 ml/L), and from there the fish were transferred one-by-one to a 1 m^3 tank located on an adjacent service platform, in order to be anesthetized completely (clove oil, 0.03 ml/L) (Mylonas et al., 2005). A sperm sampling was attempted, by using a catheter (Pipelle de Cornier, Laboratorie CCD, France) inserted at the opening of the genital pore, as hand stripping is not always possible in greater amberjack, due to the thick musculature of the abdominal wall (Fakriadis and Mylonas, 2021). Blood samples from the caudal vasculature were obtained from all fish using 5-mL heparinized syringes. Four fish were sacrificed on day 0 (Control-day 0) in order to obtain testicular data (size and stage of reproductive development), after they were euthanized by remaining in the anesthetic bath until cessation of opercular ventilation, and severing one of the gill arches. The remaining 24 fish were divided randomly in four treatment groups (n = 6). Fish were treated with either a GnRHa injection ($GnRHa_{inj}$) at an effective dose of 24 ± 7 μg GnRHa kg^{-1} (mean ± SD), a GnRHa implant ($GnRHa_{impl}$) at an effective dose of 139 ± 17 μg GnRHa kg^{-1} (mean ± SD), or an hCG injection (hCG_{inj}) at an effective dose of 972 ± 120 IU hCG kg^{-1} (mean ± SD); a fourth group of fish was left untreated (Control). Then, the fish were transferred one-by-one by boat (in a 0.5-m^3 tank) to four round 23-m^3 tanks according to their hormonal therapy. Each tank was supplied with a mixture of surface (90 %) and well (10 %) seawater and was exposed to ambient photo-thermal conditions. Seven days and 13 days after treatment (25 and 31 May), a blood and sperm sample was collected from all fish (n = 6 per treatment). On 31 May, two of the sampled fish from each treatment group were sacrificed as described above, in order to evaluate the effect of the treatment on testis histology and apoptosis (Control-day 13, $GnRHa_{inj}$-day 13, $GnRHa_{impl}$-day 13, hCG_{inj}-day 13). For each sacrificed fish, biometric data (fork length, FL, nearest cm; body mass, BM, nearest kg; gonad mass, GM, nearest g) were registered and the gonadosomatic index was calculated as GSI = 100 GM BM^{-1}. The remaining fish (n = 4 per treatment) were not sacrificed, as they formed the productive broodstock needed by the farm to produce fertilized eggs for the following reproductive seasons.

2.3. Sperm quality evaluation

Sperm samples were collected on board the service boat, immediately upon anaesthesia and before treating the fish with the hormones or taking a blood sample. Sperm samples (50 μl) were stored in microvials and were kept on ice until the completion of the work on board. The samples were then moved to shore and processed for sperm quality evaluation. Sperm samples were assessed using computer assisted sperm analysis (CASA, ISAS, Spain). Spermatozoa were activated in seawater containing 2 % bovine serum albumin (BSA) (1:668), in an appropriate dilution to obtain 200–300 cells in the field. Spermatozoa movement was recorded 15 sec after activation on a reusable counting chamber with a fixed depth (SpermTrack), using a digital camera at 100 fps attached to a light microscope (Zeiss Primo Star) under 100x magnification (10x objective, negative phase contrast). The software settings were adjusted to 1 to 90 μm for head area; Curvilinear Velocity (VCL) < 10 μm sec^{-1} to classify a spermatozoon as immotile; and spermatozoa were considered rapid when VCL was higher than 100 μm sec^{-1}. The analysed sperm quality parameters included: (a) sperm density (number of spermatozoa ml^{-1} milt, szoa ml^{-1}), (b) survival of spermatozoa under cold storage at 4 °C (>5% motile cells in each sample, days), (c) duration of ≥ 5 % of the motile spermatozoa in the field of view (motility duration, min), d) motile cells (%), e) VCL (μm sec^{-1}), f) Straight Line Velocity (VSL) (μm sec^{-1}), g) Average Path Velocity (VAP) (μm sec^{-1}), and h) Straightness (STR) (%).

2.4. Sex steroid hormone and cortisol plasma concentrations

Quantification of testosterone (T), 11-Ketotestosterone (11-KT) (the main androgen in many fish species), 17,20β-dihydroxypren-4-en-3-one (17,20β-P) (a putative maturation-inducing steroid; MIS) and cortisol was performed according to the methods developed and validated in Papadaki et al. (2021) and Lancerotto et al. (2023), using an Agilent 1260 Infinity binary pump HPLC system coupled to an Agilent 6460C triple quadrupole mass spectrometer equipped with an Agilent Jet Stream Electrospray source (Agilent Technologies). The chromatographic separation of analytes was achieved on a Poroshell 120 column fitted with a guard column (EC-C18, 150 mm x 3 mm, 2.7 μm particles; Agilent Technologies).

2.5. Testis histology and identification of apoptotic cells

The effects of the treatments on testis histology and apoptosis were evaluated on the four pre-treatment Control fish sacrificed on day 0 (Control-day 0) and two fish per hormone treatment group that were sacrificed on day 13 (Control-day 13, $GnRHa_{inj}$-day 13; $GnRHa_{impl}$-day 13, hCG_{inj}-day 13). One-cm thick testis slices were cut and fixed in Bouin's solution, dehydrated in ethanol, clarified in xylene and embedded in paraffin wax. Five-μm thick sections were then stained with haematoxylin-eosin. The identification of apoptotic germ cells was performed using the terminal deoxynucleotidyl transferase-mediated d'UTP nick end labeling (TUNEL) method with an in-situ Cell Death Detection Kit, AP (Roche Diagnostics, Mannheim, Germany) that was used in accordance with the manufacturer's instructions.

2.6. Statistics

Since sperm could be analysed only from some of the treated fish and

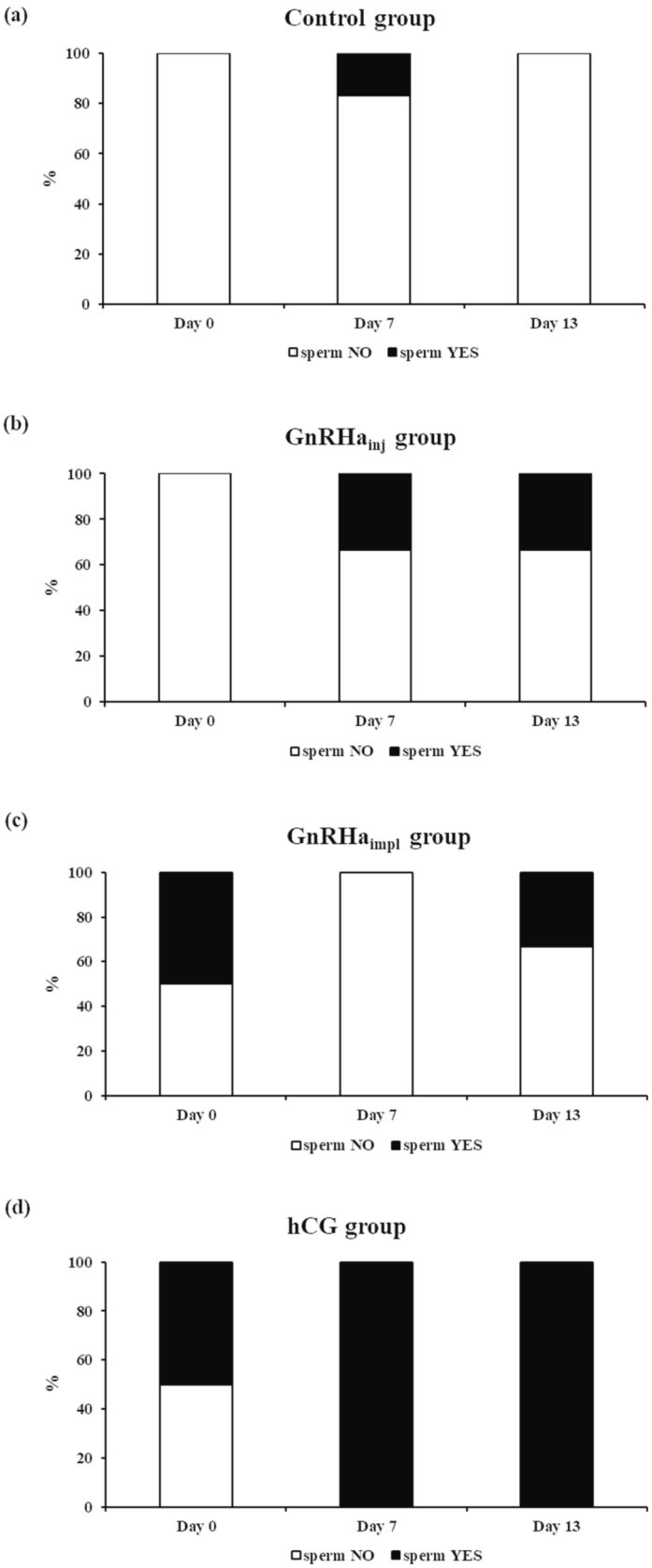

Fig. 1. Percentage (%) of hatchery-produced F1 generation greater amberjack that provided sperm samples for CASA analysis.

Table 1
Sperm quality parameters (mean ± SD) of hatchery-produced F1 generation greater amberjack.

	Day 0 (n = 6)	Treated-day 7 (n = 8)	Treated-day 13 (n = 10)
Density (10^9 szoa/ml)	72.8 ± 18.8	65.0 ± 28.3	71.8 ± 17.0
Survival (days)	2.2 ± 1.0[a]	1.8 ± 1.0[a]	3.8 ± 0.6[b]
Duration (min)	5.1 ± 1.9	3.4 ± 1.6	4.2 ± 1.6
VCL (μm/sec)	142.3 ± 17.0	145.7 ± 20.5	146.3 ± 15.4
VSL (μm/sec)	112.1 ± 18.4	123.1 ± 20.7	119.1 ± 19.7
VAP (μm/sec)	121.1 ± 19.7	131.0 ± 21.8	126.9 ± 18.9
Motile cells (%)	34.3 ± 14.1	48.0 ± 19.8	36.7 ± 11.7
STR (%)	92.5 ± 1.8	93.9 ± 2.0	93.6 ± 1.9

VCL, curvilinear velocity; VSL, straight line velocity; VAP, average path velocity; STR, straightness. Lowercase letters indicate statistically significant differences between Day 0 group and Treated-day 7 group or Treated-day 13 group (Student's *t* test; $P < 0.05$).

from none of the fish from the Control-day 0 group (see Results section), differences in sperm quality parameters were assessed by comparing the pooled fish sampled before the treatment on day 0 (Day 0 group), independently of the treatment group they were later assigned to, with treated fish sampled on day 7 (Treated-day 7 group) and with treated fish sampled on day 13 (Treated-day 13 group) through the Student's *t* test. Differences in sex steroid and cortisol plasma concentrations were tested using a two-way repeated measures Analysis of Variance (ANOVA) followed by Tukey's HSD post hoc test. Differences in GSI between Control-day 0 and Control-day 13 groups were assessed by Student's *t* test. Differences in GSI among Control-day 13, $GnRHa_{inj}$-day 13, $GnRHa_{impl}$-day 13, and hCG-day 13 were assessed through one-way ANOVA followed by Duncan's New Multiple Range post hoc test. Prior to the application of Student's *t* test and ANOVA, GSI data were arcsine transformed, as appropriate with proportions (Sokal and Rohlf, 1981). All the results are presented as means ± SD, and the statistical probability significance was established at the $P < 0.05$ level.

3. Results

Twenty-five sperm samples from the fish used in the study were analyzed through CASA, because the other samples obtained in the three sampling times (Day 0, 7 and 13) were contaminated by blood and could not be analysed. The percentage of fish in each experimental group (n = 6) from which sperm could be collected is reported (Fig. 1) and the sperm quality parameters of each analysed fish are reported in Supplementary File S1. No statistically significant changes were observed between fish that did not receive any treatment and were sampled on day 0 (Day 0) and fish that received a hormonal treatment and were sampled on day 7 (Treated-day 7) or on day 13 (Treated-day 13) for any of the sperm parameters analysed, except for spermatozoa survival of Treated-day 13 that was significantly higher ($P < 0.05$) than Day 0 and Treated-day 7 groups (Table 1). All treatments, including Control, exhibited a significant reduction ($P < 0.05$) of the examined sex steroids, except for T, which had a small, but significant ($P < 0.05$) elevation on day 7 (Fig. 2). On the contrary, cortisol plasma levels increased significantly ($P < 0.05$) after the onset of the experiment and remained high on day 13 compared to day 0, regardless of hormonal treatment.

To reduce the number of sacrificed fish to the absolute minimum, the analysis of testis histology and apoptosis was limited to four fish at the start of the experiment (Control-day 0) and only two of the six fish used per hormonal treatment, on day 13. At the time of the onset of the experiment (Control-day 0), the GSI was 1.4 ± 0.4 (Table 2) and testes showed seminiferous tubules containing a moderate number of luminal spermatozoa and all stages of spermatogenesis in the germinal epithelium (Fig. 3a). Control-day 13 fish did not show significant changes in GSI compared with the Control-day 0 group ($P = 0.30$) (Table 2). The GSI of treated groups at day 13 was at least 2 times higher than Control-day 13 group (Table 2), although the limited number of samples did not allow to obtain a statistically significant difference ($P = 0.46$). The histological evaluation showed a germinal epithelium in active spermatogenesis in Control-day 13, $GnRHa_{impl}$-day 13 and $GnRHa_{inj}$-day 13 groups (Fig. 3b, c), whereas spermatogenesis clearly declined in hCG_{inj}-day 13 fish (Fig. 3d). Seminiferous tubules of fish given any of the hormonal therapies appeared to be larger than control groups and showed wider lumina with more abundant spermatozoa. This was especially evident in fish of the hCG_{inj}-day 13 group which showed large seminiferous tubules filled with spermatozoa (Fig. 3d). All the analysed testes showed TUNEL-positive testicular cells. A lower density of apoptotic cells was observed in the hormone-treated than in Control fish. This was particularly evident in the hCG_{inj}-day 13 group (Fig. 4).

4. Discussion

The present study indicates that F1 male greater amberjack are even more severely affected by reproductive dysfunctions than their wild-caught parents reared in captivity, and that rearing in captivity from egg did not result in better adaptation to aquaculture conditions. In fact, the GSI was about half that of wild captive-reared fish and about 1/8 of adults sampled from the wild during the same phase of the reproductive cycle (Zupa et al., 2017a). An altered release of gonadotropins from the pituitary is one of the main causes of the reproductive dysfunctions occurring in fish reared in captivity, hence the available technologies used commonly to alleviate reproductive dysfunctions include administration of hormonal treatments (Mylonas and Zohar, 2001; Zohar and Mylonas, 2001; Mylonas et al., 2010). Several trials with greater amberjack of wild origin showed that GnRHa administration is effective in inducing maturation in both sexes and resulted in spawning of fertilised eggs (Fakriadis et al., 2019, 2020a, b; Fakriadis and Mylonas, 2021). However, lower fertilization success was obtained compared to other fish species that reproduce in captivity, and the need to study sperm production and quality after spermiation enhancement was suggested previously (Fakriadis and Mylonas, 2021). All the applied hormonal treatments induced testicular development in the present study, both at the macroscopic and microscopic level. In particular, the treatments were associated with an increased GSI trend, a higher germinal epithelium and more abundant luminal spermatozoa. As a result of the stimulation of the spermatogenic process, the fish injected with hCG showed the most dramatic testicular changes, characterized by the presence, in the internal testicular region, of seminiferous tubules containing large sperm masses. Moreover, the hCG injection increased spermiating fish from 50 to 100 % and prolonged significantly – as the other treatments did- spermatozoa survival under cold storage. Sperm motility, density and duration values from the present study fall within the range of those reported for F1 greater amberjack in the Canary Islands (Jerez et al., 2018) and wild-caught captive-reared males in the Mediterranean (Fakriadis and Mylonas, 2021). However, VCL, VSL, VAP and STR were higher compared to the latter study. On the other hand, survival under cold storage was lower than previous studies (Jerez, et al., 2018; Fakriadis and Mylonas, 2021). Lower short-term storage survival of sperm was also found in turbot *Scophthalmus maximus* 6 days before the onset of the spawning period for females, compared to 18 days after the onset of spawning (Suquet et al., 1998). In the present study, the initial sampling was held in mid-May at 18–18.5 °C, some days earlier than the onset of the spawning period for the females and outside the verified temperature range of 19–24 °C for spawning of greater amberjack in the Mediterranean (Fakriadis et al., 2020a, b; Fakriadis and Mylonas, 2021). Therefore, the extent that both temperature and timing of hormonal therapy affected sperm production is unknown and should be considered, especially when it is well-known that both at the beginning and at the end of the reproductive period sperm production is low (Contreras et al., 2019).

The hormonal treatments performed in the present study had no effect on sex steroid secretion, as treated groups had similar sex steroid

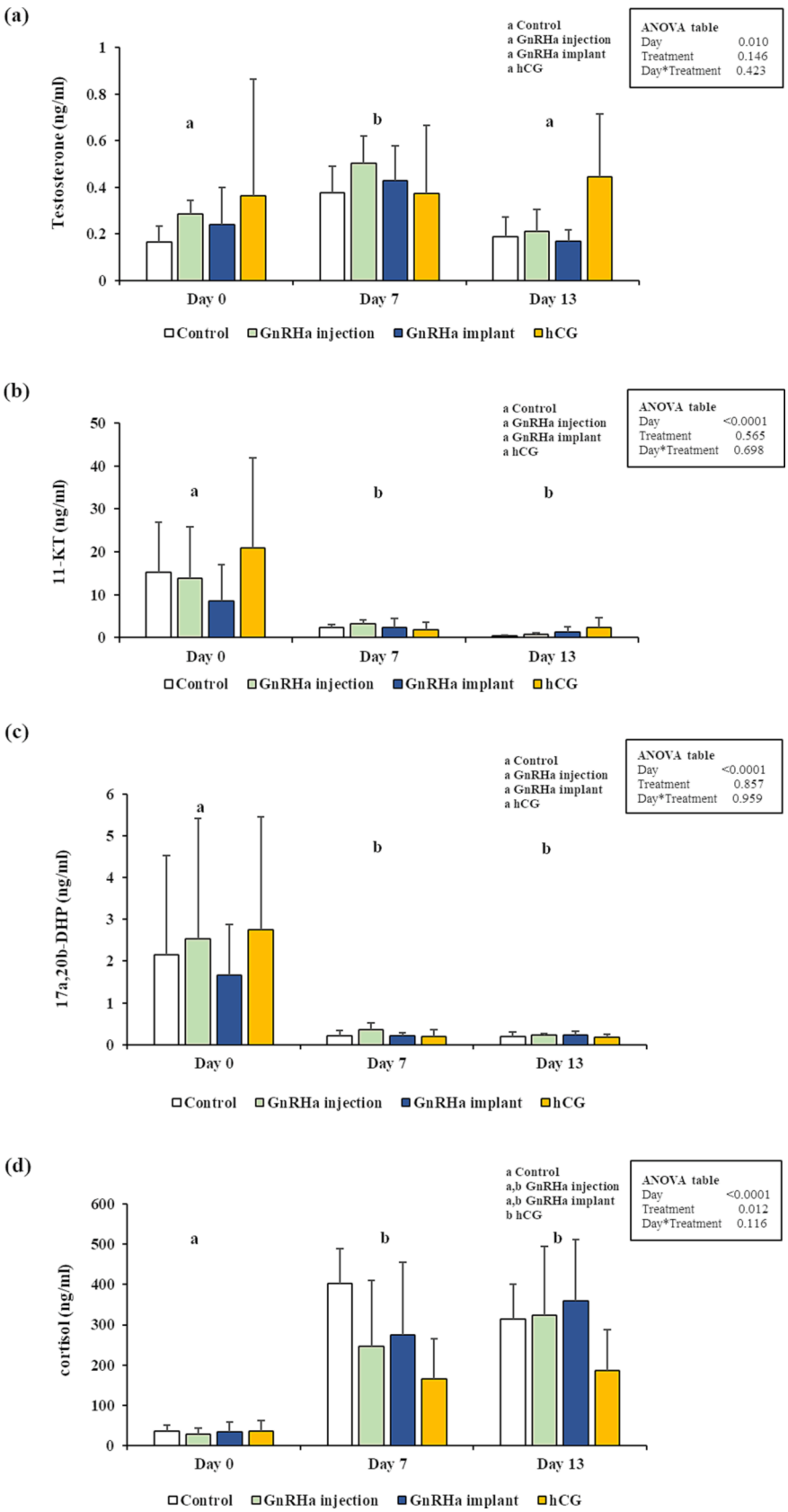

Fig. 2. Plasma sex steroid and cortisol concentrations (ng/ml) of hatchery-produced greater amberjack males (n = 6) before (Day 0) and after (Day 7 and Day 13) treatments with single GnRHa injection, sustained release GnRHa implant or hCG injection. Lowercase letters in legends and above bars indicate statistically significant differences among the different treatment groups and sampling times, respectively (two-way ANOVA, Tukey's HSD; $P < 0.05$).

Table 2
Mean ± SD gonadosomatic index (GSI) in hatchery-produced greater amberjack treated with GnRHa injection ($GnRHa_{inj}$), GnRHa implant ($GnRHa_{impl}$) or hCG injection (hCG_{inj}) on Day 0 and sampled after thirteen days (Day 13).

Treatment date	Treatment type	GSI
Day 0	Control (N = 4)	1.4 ± 0.4
Day 13	Control (N = 2)	1.0 ± 0.2
	GnRHa injection (N = 2)	2.1 ± 0.8
	GnRHa implant (N = 2)	2.0 ± 1.4
	hCG (N = 2)	2.6 ± 0.7

plasma levels as the control group sampled on day 13, and significantly lower than the values of the control group on day 0. This reduction of sex steroids might be due to the effect of stress imposed on the fish by the transfer from the sea cages to the land-based tanks, as indicated by the high levels of cortisol in all the groups on Days 7 and 13. For instance, in an earlier experiment with Atlantic bluefin tuna *Thunnus thynnus*, GnRHa implants were administered through a supergun or jabstick to fish allowed to spawn in their rearing cage, with limited exposure to stress (Mylonas et al., 2007), whereas treatments in the present study involved fish exposure to a handling procedure (*i.e.* crowding, anesthetizing and transferring to a tank) that might have caused the observed elevation of cortisol plasma levels on Day 13. Unfortunately, collecting fertilized eggs from sea cages is not possible in greater amberjack, as it has been done successfully in Atlantic tuna (Mylonas et al., 2007; De Metrio et al., 2010), because eggs are not very positively buoyant and they are lost through the bottom of the spawning cage (Fakriadis et al., 2020b). Although the role of cortisol in fish reproduction has received significant attention (Milla et al., 2009, Murugananthkumar and Sudhakumari, 2022), its complex role on fish spermatogenesis is not yet fully elucidated. For example, it has been shown that cortisol inhibits testicular development in common carp *Cyprinus carpio* (Consten et al., 2002a) by directly decreasing androgen levels in pubertal, but not in adolescent males (Consten et al., 2002b). In a recent study on zebrafish *Danio rerio*, it was shown to have a direct positive effect on spermatogenesis, without affecting 11-KT levels, and when found at very high levels it affected the expression levels of the androgen receptor gene (Tovo-Neto et al., 2020). Hence, the eventual effects of the present hormonal treatments on spermatogenesis and testicular growth derived, likely, from the combined -and opposite- effects of gonadotropins and sex steroid withdrawal or cortisol. In fact, in some fish species it has been shown that normal spermatogenesis occurs even if androgen plasma levels are very low (Mylonas et al., 2013a, b; Zupa et al., 2023) and this is explained by the fact that Fsh stimulates spermatogenesis through two different mechanisms, the classical one mediated by sex steroids and a non-classical, more recently discovered, steroid-independent mechanism mediated by insulin-like growth factor 3 (Nóbrega et al., 2015). Moreover, treatment with exogenous hormones has been shown not always to affect sex steroid levels in several relevant experiments (Canario and Scott, 1991; Podhorec et al., 2017; Woo et al., 2021).

In the present study, based on the subjective evaluation of TUNEL-stained testis sections, a reduction of testicular apoptosis was observed in treated fish, and particularly in hCG-injected fish. In captive-reared Atlantic bluefin tuna (Corriero et al., 2009; Zupa et al., 2013, 2014) and greater amberjack (Zupa et al., 2017b), a higher density of apoptotic testicular cells was observed compared to wild breeders in the same reproductive phase and GnRHa administration through sustained-release implants reduced significantly testicular apoptosis in Atlantic bluefin tuna (Corriero et al., 2009) and administration of rFsh reduced significantly testicular apoptosis in prepubertal meagre (Zupa et al., 2023).

The different intensity of the effects of the applied GnRHa or hCG

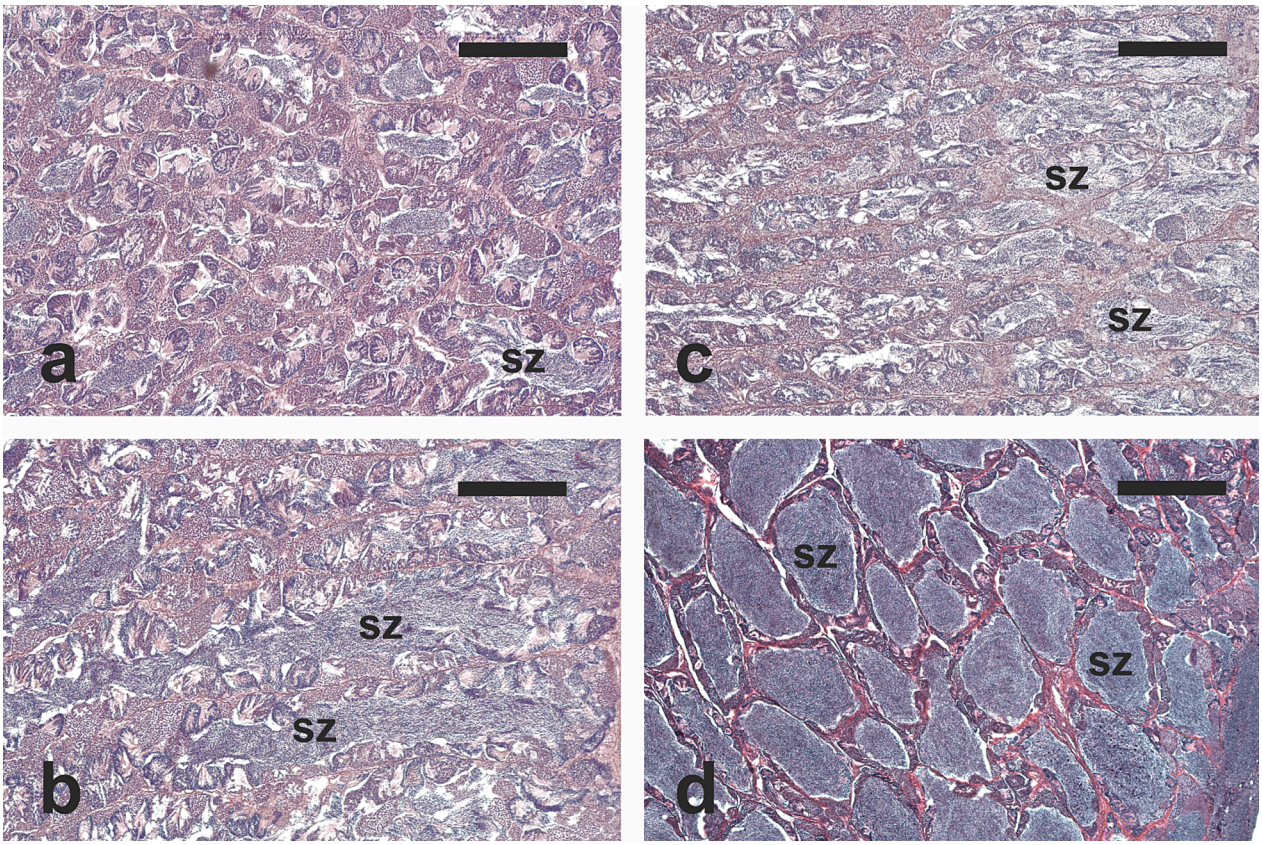

Fig. 3. Microphotographs of histological sections from hatchery-produced F1 generation greater amberjack testes. a) Control-day 0; b) $GnRHa_{inj}$-day 13; c) $GnRHa_{impl}$-day 13; d) hCG-day 13. Compared with fish of the control group, testes of treated fish show larger seminiferous tubules with wider lumina and more abundant spermatozoa (sz). Hematoxylin-eosin staining. Magnification bars = 200 μm.

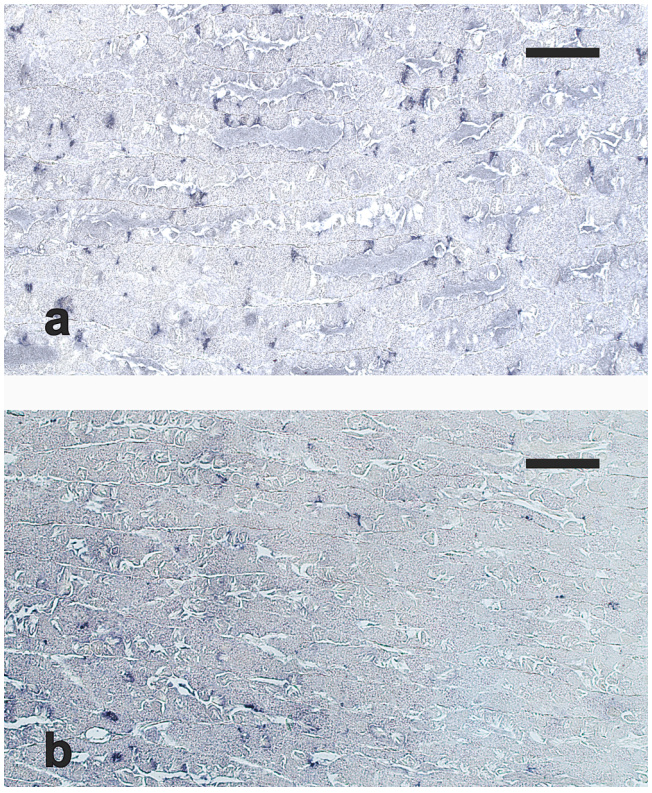

Fig. 4. Microphotographs of testes sections from F1 generation hatchery-produced greater amberjack sampled on 31/05/2021 and stained with the terminal deoxynucleotidyl transferase-mediated 2′-deoxyuridine 5′-triphosphate nick end labelling (TUNEL) method, identifying apoptotic cells as dark blue dots. a) Control-day 13; b) hCG_{inj}-day 13. Magnification bars = 250 μm.

treatments on hatchery-produced greater amberjack spermatogenesis can be explained by the specific mechanisms and kinetics of the treatments: hCG surrogates deficient endogenous gonadotropins and modulates directly spermatogenesis, whereas the effect of GnRHa is indirect as this hormone stimulates pituitary release of gonadotropins that in turn stimulate spermatogenesis. Moreover, a less marked effect of treatment with GnRHa implants compared to the administration of the same hormone through injection is explained by the slow delivery GnRHa and the consequent smoother and longer-lasting action. On the contrary, the intense effect of hCG on spermatogenesis and spermiation suggests a time-limited, breaking out efficacy of the therapy on spermatogenesis advancement. Since greater amberjack females have an asynchronous oocyte development and have the potential to spawn many times during each reproductive season (Fakriadis et al., 2019, 2020a, b), the choice of GnRHa administration via implants should be preferred if a long-lasting production of fertilised eggs is required. Moreover, the choice of the treatment should take into consideration that treatments with exogenous non-native gonadotropins may be less effective in the following years, due to their immunogenicity (Mylonas and Zohar, 2001).

In conclusion, F1 generation hatchery-produced greater amberjack did not prove to be better adapted to farming conditions than fish caught from the wild and reared in sea cages. In addition, sampling and transferring of the fish from sea cages to land-based tanks resulted in a stress-induced reduction of sex steroid hormone production, which could not be overcome by the exogenous hormonal therapies. Among the three applied treatments, hCG injection showed the most marked effects on spermatogenesis and reduction of germ cell apoptosis. The observed effects of hCG eventually resulted in an increased percentage of spermiating fish, but had limited effects on sperm quality enhancement. The observed effects on spermatogenesis were likely mediated by factors other than sex steroids. Further studies on a larger number of individuals are needed before the present data can be applied at the industrial level.

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CRediT authorship contribution statement

G. Ventriglia: Investigation, Visualization, Writing – original draft. **I. Fakriadis:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **M. Papadaki:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **R. Zupa:** Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **C. Pousis:** Investigation, Writing – review & editing. **M. Mandalakis:** Investigation, Methodology. **A. Corriero:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. **C.C. Mylonas:** Conceptualization, Investigation, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data are included in the main text and in the supplementary file

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2024.114447.

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