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# Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality

Constantinos C. Mylonas [a,*], Neil J. Duncan [b], Juan F. Asturiano [c]

[a] Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, P.O. Box 2214, Iraklion, Crete 71003, Greece
[b] IRTA, Sant Carles de la Rapita, 43540 Tarragona, Spain
[c] Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Edificio 7G, 46022 Valencia, Spain

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ABSTRACT

This article reviews the use of hormonal treatments to enhance sperm production in aquaculture fish and the methods available for evaluating sperm quality. The different types of testis development are examined and a brief review is presented of the endocrine regulation of spermatogenesis in fishes, including the increasing evidence of the existence of spermatozoa subpopulations. Hormonal manipulations are employed to induce spermatogenesis in species such as the freshwater eels, to synchronize maximal sperm volume to ovulation for *in vitro* fertilization and to enhance sperm production in species with poor spermiation. The hormones that are employed include gonadotropins (GtHs) of piscine or mammalian origin, and gonadotropin-releasing hormone agonists (GnRHa) administered by injections or controlled-release delivery systems, with or without dopaminergic inhibitors. Pheromones in the culture water and hormones added to the sperm *in vitro* have also been employed to enhance spermiation and sperm quality, respectively, in some fishes. Hormonal therapies usually do not affect sperm quality parameters, except in cases where fish fail to spermiate naturally or produce very small volumes of high-density sperm. Different parameters have been used to evaluate fish sperm quality, including sperm volume and density, spermatozoa motility and morphometry, and seminal plasma composition. The development of Computer-Assisted Sperm Analysis (CASA) systems made possible the estimation of a higher number of sperm motion parameters using an objective, sensitive and accurate technique. The development of Assisted Sperm Morphology Analysis (ASMA) software has introduced a new approach for sperm evaluation studies, demonstrating changes in the spermatozoa related to reproductive season, hormonal treatments or the cryopreservation processes, and how these may be related to changes in sperm motility and fertilization capacity. The article concludes with a few practical protocols for the enhancement of sperm production in aquaculture species.



## 1. Introduction

Production of high quality eggs and sperm is a prerequisite for the sustainable expansion of aquaculture. In captivity, control of reproductive function begins with the manipulation of the environment, in order to provide the necessary conditions and information - such as photoperiod and thermal cyclicity where they exist, and at times spawning substrate - in order to condition the fish and stimulate them to undergo gametogenesis (oogenesis and spermatogenesis), maturation and spawning. However, in many commercially produced species, there are important reproductive dysfunctions that hinder the efficient and reliable production of fertilized eggs (Mylonas et al., 2010). Reproductive dysfunctions are most often seen in females, with the failure of oocyte maturation, ovulation and/or spawning being the most common. As an exception to the above rule of females being the problematic sex in aquaculture, various flatfishes produce very small amounts of sperm (also referred to as semen or milt) during the spawning period (Agulleiro et al., 2007; Guzmán et al., 2011b; Vermeirssen et al., 1998, 2004). Furthermore, hatchery-produced males (F1 generation) of some fishes do not exhibit any breeding behavior and fail to spawn with the females, even if females complete ovulation and spawning. Such an example is the Senegalese sole (*Solea senegalensis*) (Norambuena et al., 2012). In many other fishes where males usually complete spermatogenesis and spermiation in captivity, it is often observed that the amount of good quality sperm produced may be diminished. Hormonal manipulations using a variety of exogenous hormones have been used in many fishes, in order to address the problems exhibited by male breeders. The objective of this article is to summarize the current knowledge on the reproductive function of male fish in aquaculture, broodstock management and methods to enhance spermatogenesis and sperm production. In addition, the article provides an extensive review of the available sperm quality evaluation methods.

* Corresponding author.
*E-mail address:* mylonas@hcmr.gr (C.C. Mylonas).

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