Aquaculture xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

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

ARTICLE INFO

Article history:
Received 20 May 2015
Received in revised form 25 March 2016
Accepted 18 April 2016
Available online xxxx

Keywords:
Spermiation
Broodstock management
Sperm enhancement
Sperm quality

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).

http://dx.doi.org/10.1016/j.aquaculture.2016.04.021
0044-8486/

2. Spermatogenesis and spermiation

The process of gametogenesis in male fishes has been separated into three phases (Schulz et al., 2010). In phase one, type A spermatogonia proliferate and differentiate into early B spermatogonia, which then undergo multiple mitotic divisions – their total number being species-specific and genetically determined – that result in late B spermatogonia. In phase two, after their last mitotic multiplication, late B spermatogonia undergo meiotic division into spermatocytes I and then spermatocytes II, eventually becoming haploid spermatids. In phase three – referred to as spermiogenesis – the spermatids differentiate into flagellated spermatozoa and are released in the testicular lumen during spermiation (reviewed by Billard, 1986; Miura and Miura, 2001; Miura et al., 2002; Schulz and Miura, 2002; Schulz et al., 2010; Vizziano et al., 2008).

The spermatozoa are released from the spermatocysts into the sperm ducts where maturation - the process that renders them capable of vigorous motility and fertilization (Schulz et al., 2010) - takes place prior to sperm release, during the spawning season. Fish ejaculate sperm spontaneously during spawning, and with the exception of catfishes (Kazeto et al., 2008; Mansour et al., 2004; Viveiros et al., 2002), sperm can also be expressed easily from the sperm ducts after application of gentle abdominal pressure (referred to as "stripping"). Sperm release can be synchronized with female spawning *via* tactile or pheromonal communications (Stacey, 2003).

2.1. Different types of testis development

Testicular development, *i.e.* the changes in the testis structural morphology during the reproductive cycle, has been reviewed extensively (Billard, 1986; Mañanos et al., 2009; Schulz and Miura, 2002; Schulz et al., 2010). Testis development has many common features among fishes, as well as vertebrates in general, such as for example the progress through the different developmental stages of germ cells. The main differences in testis development among different species relate to: (a) the timing of the progress through the different developmental stages of the germ cells in relation to the seasonality of spawning of the fish species, (b) the testis structure and migration of the spermatocysts, and (c) the stage of development of the germ cells that leave the spermatocysts.

Different species of fish present different seasonality in spermatogenesis and spermiation, ranging from the production of sperm all-year-round, to the production of sperm for a short or long reproductive period each year or to sperm production once in a life-time. Spermatogenesis can be considered an asynchronous type of maturation, as most species present periods of development with all stages of germ cells. The different seasonality in spermatogenesis among species results in differences in the presence and abundance of the different developmental stages of germ cells observed among species. Guppies (Poecilidae) that produce sperm all-year-round were described to have an asynchronous testis all-year-round, which was also termed continuous, as sperm was produced continually (Billard, 1986). Rainbow trout (*Oncorhynchus mykiss*), on the other hand, has a seasonal production of spermatozoa in separate annual cycles of spermatogenesis to produce releasable sperm for a short reproductive period. In general, much of the annual spermatogenesis cycle in fish is asynchronous, with all stages of germ cell development present at the same time. Nevertheless, there is a clear progression through the different germ cell stages and at a given time of the reproductive cycle, the testis is dominated by a certain stage of germ cells. Towards the end of the spawning period the testis is almost entirely full of spermatozoa, with a limited presence of earlier stage germ cells. Therefore, germ cells appear to be synchronized to achieve sperm availability for a short fixed period (Billard, 1986, 1992).

The different structural types of testis (*e.g.* anastomosing tubular and lobular) have been ordered by phylogeny (Parenti and Grier, 2004) with clearly defined structural criteria, based on the tubule or lobule network and the position of spermatogonia and spermatocysts (Grier et al., 1980; Grier, 1993; Parenti and Grier, 2004). A tubule is defined as a tube with both ends open and connecting to other tubular structures, while a lobule was defined as essentially a tube with one end being blind, forming a lobe (Grier, 1993). When observed in a two-dimensional histological section, these tubules and lobules can appear similar, resulting in considerable confusion in the literature. The lobular structure of the testis has been further divided into restricted and unrestricted (Grier et al., 1980). Restricted lobular testes contain the different stages of spermatogonia and spermatocysts in different restricted areas. The spermatogonia are situated at the distal section (blind end) of the lobule, and away from the lumen (open end), while spermatocysts with spermatids are principally observed close to the lumen. As the germ cells undergo the different stages of spermatogenesis, the spermatocysts appear to move towards the lumen, so that the spermatozoa are released into the lumen when the spermatocysts rupture. Unrestricted lobular testes have spermatogonia and spermatocysts at different stages of development throughout the lobules, and spermatocysts may move only slightly towards the lumen prior to the release of the spermatozoa.

Important aquaculture species have both tubular and unrestricted lobular testis type. Tubular type testes are found in various fishes (Parenti and Grier, 2004), ranging from the primitive non-teleost order Acipenseriformes (which includes paddlefish and sturgeons) to early teleost orders such as Cypriniformes (carps), Siluriformes (catfishes), Salmoniformes (salmons and trouts) and Esociformes (pikes). Lobular testes are found in Neoteleostei, with unrestricted lobular testes present in the orders Perciformes and Mugiliformes. The different testis structures (tubular or unrestricted lobular testes) do not appear to be related to the amount of sperm that can be produced, as very different volumes of sperm can be collected from species from orders classified with the same testis structure. For example, sperm collection from rainbow trout (a Salmoniformes species with tubular type testes) (Billard, 1992) and European seabass (*Dicentrarchus labrax*, a Perciformes species with unrestricted lobular type testes) (Asturiano et al., 2001; Sorbera et al., 1996) is easy and large volumes can be collected. On the contrary, sperm collection from African catfish (*Clarias gariepinus*, a Siluriformes species with tubular type testes) (Viveiros et al., 2002) and spotted rose snapper (*Lutjanus guttatus*, a Perciformes species with unrestricted lobular type testes) (Ibarra-Castro and Duncan, 2007) is difficult and the amount of sperm collected is usually not sufficient for aquaculture purposes. In the case of catfishes, the problem with low volumes of sperm collected with stripping is related to the presence of seminal vesicles in the efferent duct (Viveiros et al., 2002).

Testicular development can also be classified as cystic or semicystic. In cystic development, when the spermatocysts rupture they release spermatozoa, while in semicystic development they release spermatids, which then differentiate into spermatozoa and complete development in the lumen. Cystic development is the most prevalent among fishes, but a growing number of species from various taxonomic orders and families have been identified to have semicystic development, including Perciformes–Bleniidae (Lahnsteiner and Patzner, 1990), Ophiliiformes-Ophiliidae (Mattei et al., 1993), Scorpaeniformes–Scorpaenidae (Muñoz et al., 2002) Pleuronectiformes–Soleidae (Garcia-Lopez et al., 2005), Siluriformes–Callichthyidae (Spadella et al., 2007), Syngnathiformes–Syngnathidae (Biagi et al., 2010) and Gymnotiformes–Gymnotidea (Vergílio et al., 2013). The most notable aquaculture species with semicystic development is Senegalese sole (Garcia-Lopez et al., 2005), which has a small testis (gonadosomatic index of <0.15%) and very low-volume sperm production all-year-round (<80 μl per fish) (Garcia-Lopez et al., 2005), which have frustrated aquaculture practices (Morais et al., 2014). However, it is unclear if low sperm production is an aspect associated to semicystic development, as sperm production in other species with semicystic development was either not quantified or the species were of a small size (at reproductive maturity) and small sperm volumes would be expected. Senegalese sole appear to have low sperm volume requirements for successful reproduction due to the paired spawning behavior, where the male and

Please cite this article as: Mylonas, C.C., et al., Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality, Aquaculture (2016), http://dx.doi.org/10.1016/j.aquaculture.2016.04.021