

Influential Factors of Quality of Fish Gametes for Use of *In Vitro* Fertilization

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Abstract

Aquaculture is the fastest growing livestock activity in Brazil, producing important sources of protein for human consumption. Most Brazilian fish farms that work with native species perform *in vitro* reproduction, given that these migrating species cannot release gametes without hormonal induction. To succeed with *in vitro* reproduction, the evaluation of reproductive parameters of both genders is necessary. The objective of this review is to describe the main reproductive parameters of fish, which must be evaluated for *in vitro* reproduction.

Introduction

It is estimated that, in Brazil, there are 2,835 species of freshwater fish and 1,201 marine species [1]. This is significant data, and directly related to the geographical location in the tropics, with the territorial dimensions and the quantity and size of the watersheds. According to the Food and Agriculture Organization of the United Nations - FAO [2], the aquaculture is advanced in several countries within Latin America, and Brazil is considered as one of the leading producers in developing and producing increasing amounts of various fish species. Some groups of fish reproduce naturally in lentic waters, others, including most of the native species, must migrate toward the headwaters of the rivers for reproduction. The last have great importance to national fish farming [3].

Native migratory fish don't spawn in captivity, requiring the manipulation of the reproductive cycle. In captivity, the induction of fish breeding, by application of hormones that induce the final maturation and release of gametes, constitutes an efficient technique, allowing rheophilic fish to complete their reproductive cycle at the desired time and under controlled conditions [4].

Knowledge of species biology, especially in regard to reproductive aspects, is essential when aiming to conduct intensive fish farming [5]. *In vitro* reproductive technologies allied to the study of species biology can improve production and survival of the offspring.

The main cause for low reproductive efficiency in captive fish is related to the non-development and appropriate gonad maturation, which are determined by the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) [6]. Thus, this article aims to describe the different techniques for evaluating reproductive parameters in females and males of native fish.

Reproductive Parameters in Females

Various techniques have been employed to solve problems that occur with *in vitro* reproduction of fish. Among these problems, we can highlight the difficulty of identifying sexual dimorphism in most native fish and the ability to determine the quality of oocytes and the characteristics that influence their ability to succeed in fertilization [6].

Several methods have been described to determine the gonadal development stage of maturation in fish. In some species, the gonads can be viewed with help to endoscope [7-9]. However this method, although efficient in the evaluation of advanced stages of vitellogenesis, it can present problems when the animals are at early stages, where only fat can be collected, it is necessary to perform various collections, which can be stressful to the reproducer.

Methods such as ultrasound and the use of cannulas have also been tested in several species [10,11]. The use of analysis of the distribution frequency percentage of the diameters of intra-ovarian oocytes and gonadal development indicator of the degree of females are also used [12]. In freshwater species of fish the diameter of the oocyte may vary from 0.60 to 4.2 mm. For some species, the indicative diameter of the spawning quality has been defined.

Another methodology used as indicator of the level gonadal development for selection of the females capable of reproduction and spawning quality is the analysis of position of the nucleus in the oocytes. Oocytes in the final maturation present the nucleus located at the periphery of the cell, contrary to occurring in immature or in maturing oocytes in which the nucleus is in central position [6].

In evaluating the spawning other parameters can also be evaluated, among them we can mention the spawning index (SI=weight of spawning \times 100/body weight of the female) which indicates the efficiency of spawning compared to the body weight of the individual and the gonadosomatic index (IG=weight of spawning+weight of the ovaries depleted \times 100/body weight) which expresses the percentage that the gonads represent the total weight of individuals, ranging mainly due to the species, spawning type, the time of year and environmental conditions and management [13-15].

Felizardo et al. [15], also mentioning the evaluations of absolute frequency, absolute frequency on the weight and absolute frequency relative to the standard length of the fish. The absolute fecundity is the total number of oocytes produced by fish, the absolute fecundity relative for weight is the number of oocytes per gram of specimen and absolute fecundity relative to length is the number of oocytes per centimeter of the animal.

The parameters of evaluation of spawning mentioned are important

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to verify its quality, but this certification will only take place upon estimated fertilization rate [6]. Paulino et al. [16], emphasizes the importance of evaluating the fertilization rate when performing *in vitro* reproduction of fish.

Reproductive Parameters in Males

Semen analysis

The knowledge of semen features is fundamental to *in vitro* reproduction routine, due to it allows the right use of gametes and the number of reproductives as well [17].

The sperms of most teleosts fish show immobility on the gonads and their (its) motility in the moment that there is water contact [18], and the osmolarity difference between the semen and the activating solution is responsible for this activation [6]. Quality sperm can be influenced by many reasons such as individual size, reproductive age, the action of successive semen collecting, season of year, hormonal induction and the ionic composition variety of seminal plasma.

Sperm motility is one of main parameters to be considered in the analysis of fish semen quality. This variable is influenced by numerous factors such as temperature, nutritional status, health status, analysis conditions employed activating solutions and studied species [6].

The importance of sperms' motility duration is the time needed for the sperm to penetrate in the micropyle oocyte fertilization to occur. In the most teleosts, the micropyle opening time is approximately 60 seconds [5].

Although there is none minimal value for the sperm motility parameters in fish that able to ensure semen effectiveness, the values obtained in most studies are used as a parameter and considered such as a guarantee for the semen quality. According to Murgas et al. [19], the reduction in the duration of the motility of spermatozoa in part occurs by a drop in supply of energy, which occurs during the period of mobility.

However, the sperm motility should not be the only indicator of semen quality, and other features need to be considered. Among them, it is possible highlight the features such as volume, motility, vigor and concentration and morphological sperm characteristics [20]. It is important the knowledge of these values to optimize the artificial fertilization process.

Semen volume can be influenced by season, mood, sexual rest period and collection method, which makes it very variable between different species of fish and even for the same specie [13].

Hormone treatments can also affect semen volume and sperm concentration. In most species of fish that receive this treatment, it is observed a significant increase in this parameter [21]. However, the volume does not have a biological intrinsic value, but it has an amount of fertilizing cells which are enough.

The sperm concentration assessment is traditionally used for *in vitro* fertilization. The sperm concentration presents the amount of sperm per ml of semen and can be determined by counting in volumetric chambers, by diluting semen in formolcitrato [22]. Concentration values may vary according to weight and age of the fish, season [13], collection frequency and ejaculation volume [6].

According to Silva et al. [13], sperm concentration is positively correlated with the period that it is occurring hormone induction and becoming higher at the end of the reproductive period. In general, a low sperm concentration is followed by an increasing in semen volume in fish hormonally induced.

Sperm morphology is an important feature which can influence the fertilization rate *in vitro* reproduction, due to abnormal sperm may be unable to fertilize the oocytes. The fish sperm are morphological divided into head, mid-piece and tail [23]. In most groups of fish, the acrosome is a missing structure which is compensated by the presence of the micropyle in the oocyte, which is the entrance of spermatozoa [24].

Anomalies in sperm can be divided into primary and secondary. The primary changes are related to failures during spermatogenesis and the secondary changes, such as broken and isolated tail or head isolated, are related to smear preparation [25]. In fish, the morphological changes in spermatozoa may occur after the osmolarity increasing or decreasing of the habitat.

The morphological assessment of fish sperm can assist in the semen samples formation, making inference about its fertilizing potential or frozen semen samples and explaining reproductive failures taken as fit conventional sperm motility analysis [26].

Miliorini et al. [27] report that a percentage above 50% of normal sperm is necessary for a good fertilization in artificial reproduction.

Final Considerations

Reproduction of fish in fish farms, mainly migratory species, the success of *in vitro* reproduction is dependent on the quality of reproductive parameters of the reproductives in both sexes. In females, the quality assessing of oocytes is the main way to verify spawning yield. In males, the seminal assessing, including sperm motility rate, concentration and morphology of spermatozoa generate information about fertilizing capacity of the sperm produced. Through this, the evaluation of fish reproduction parameters becomes an indispensable tool for successful reproduction *in vitro*.

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