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Review

Sperm cryopreservation in oysters: A review of its current status and potentials for future application in aquaculture

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ABSTRACT

Cryopreservation has been expected to improve the efficiency of hatchery operation by supplying gametes on demand without live broodstocks and genetic improvement programs by achieving desired mating and establishing across-generation controls. The preservation of genetic materials of improved stocks and the original population is immensely important for oyster aquaculture industry to prepare the potential impacts from epidemic diseases and natural disasters. This review summarizes the research progress of sperm cryopreservation in oysters and discusses the scope of application of preserved sperm in aquaculture. A bulk of about 50 reports have been published on oyster sperm cryopreservation since 1971, nevertheless the application of this technique in aquaculture is limited. These studies primarily focused on the development of protocol for individual species by optimizing a set of interacting variables at different steps from sperm collection to post-thawing and fertilization. A number of approaches such as sperm motility, morphological integrity and fertility are used to evaluate the cryopreserved sperm quality but there are considerable variations in practice. We synthesized the outcomes in the existing literature in an attempt to suggest the standardization of sperm cryopreservation technique and provide directions for future research.

Contents

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1. Introduction

Cryopreservation is the preservation of living cells and tissues in liquid nitrogen (LN) at −196 °C for a longer period without compromising biological functionality. This technique offers an opportunity to transport living cells to multiple locations with less disease or biosecurity concerns because frozen cell is less likely to carry diseases than a whole organism. As cryopreservation has wider applications in aquaculture, biotechnology, ecotoxicology and basic research (Chen et al., 1998; Paredes and Bellas, 2009), there is a need to develop a reliable method for sperm cryobanking to meet the demand of different applications (Lubzens et al., 1997). The earliest successful cryopreservation is reported by Luyet and Hodapp (1938) in frog sperm and Jahnel (1938) in human sperm. A major breakthrough came after the use of glycerol as a cryoprotective agent in human sperm cryopreservation (Polge et al., 1949). In livestock, sperm cryopreservation is a proven technique for developing, maintaining and distributing genetic materials. Since the first report of Pacific herring sperm cryopreservation in 1953 (Blaxter, 1953), the protocols have been developed for more than 200 aquatic species around the world (Gwo, 2000; Tiersch, 2000), and many more in the last decade.

Along with terrestrial vertebrates and fishes, molluscs have received attention in cryopreservation studies including oysters (Dong et al., 2005b; Adams et al., 2008; Yang et al., 2012), mussels (Di Matteo et al., 2009; Smith et al., 2012), scallops (Yang et al., 2007; Espinoza et al., 2010), pearl oysters (Acosta-Salmón et al., 2007; Kawamoto et al., 2007), clams (Dupré and Guerrero, 2011) and abalones (Zhu et al., 2014). While sperm is noted for a wider range of studies, the eggs, embryos and larvae are also considered for cryopreservation in these species (Chao et al., 1997; Paniagua-chavez et al., 2000; Smith et al., 2001). Among the molluscan species in sperm cryopreservation, oysters are most widely studied with more than 50 published papers (including research articles, thesis, book chapters, short papers, technical reports and abstracts) since the first report on Pacific oyster sperm cryopreservation (Lannan, 1971). The Pacific oyster is most extensively investigated with 70% of the reports having been published on its sperm cryopreservation.

Sperm cryopreservation has become a billion dollar global business for artificial insemination in dairy cattle whereas the technology has been limited to research exploration in oysters (Tiersch et al., 2007). The dairy cattle industry almost entirely depends on cryopreserved sperm for breeding programs thus offering a model for commercial application (Caffey and Tiersch, 2000). The strategies for application of cryopreserved fish sperm for aquaculture are already in place (Tiersch, 2008). Gene bank of cryopreserved sperm for Atlantic salmon, *Salmo salar* is established in Norway to preserve the genetic diversity of the natural stocks (Walso, 1998). Moreover, high throughput application of cryopreserved sperm for processing at commercial scale has already been established in fish and oyster (Hu et al., 2011; Yang et al., 2012).

Sperm cryopreservation in oysters has progressed over the last a couple of decades by developing protocols for a number of species, but many issues are still unresolved for the application of research results. Application of cryopreserved sperm for aquaculture is constrained by technical requirements from research findings to commercial operations. The commercial scale sperm cryopreservation has been evaluated (Dong et al., 2005a; Adams et al., 2009), and the use of existing livestock cryopreservation facility has been proven practical for oyster sperm (Dong et al., 2007b). In addition, the application of cryopreserved sperm for selective breeding has been assessed in Pacific oysters (Adams et al., 2008). Considering the biological potentials, we would like to draw attention towards the feasibility of application of cryopreserved oyster sperm in aquaculture.

There are a number of reviews that focused on the sperm cryopreservation in aquatic shellfishes and invertebrates (Gwo, 2000; Chao and Liao, 2001; Tiersch et al., 2007). However, the information on oyster sperm cryopreservation remains scattered in literature. There has been a general lack of standardization among studies, rendering it difficult for the future researcher to reproduce the result (Dong et al., 2011), therefore, standardization of cryopreservation procedure is required to ensure repeatability. Moreover, the feasibility of application of cryopreserved oyster sperm should be discussed to represent the potential of cryopreserved sperm in breeding programs, such as selective breeding, hybridizations through the cross of inbred lines established by self-fertilization and production of triploids. There has been substantial progress in Pacific oyster and eastern oyster sperm cryopreservation and can be used as a model for other oysters. Furthermore, inclusion of new approaches and improvement of the existing procedure is also desired in future research.

In this review, we attempt to (1) summarize the procedures and the factors affecting sperm quality in cryopreservation (2) discuss the standardization of cryopreservation procedures, (3) explore the feasibility of applications of cryopreserved oyster sperm to selective breeding program in aquaculture, and (4) discuss the directions for future research.

2. Procedures in cryopreservation

Cryopreservation procedures involve a series of steps which are optimized by experimental trials. The steps include: 1) sperm collection, 2) selection of extenders for sperm dilution, 3) Choice of cryoprotectants for sperm equilibration and evaluation of toxicity, 4) packaging of sperm, 5) cooling and freezing and 6) thawing. Optimization of a set of interacting variables at each of the steps is crucial in any cryopreservation development. However, all the mentioned steps vary considerably within and among species (Tiersch, 2000). Cryopreservation includes the entire cycle from sperm collection to thawing without major change in functional capability of sperm. Some of the steps (e.g., sperm collection and dilution) in cryopreservation are relatively mild, while the other steps (e.g., cooling, freezing and thawing) are extremely stressful for sperm. The steps in sperm cryopreservation of oysters are discussed as follows.

2.1. Sperm collection

The suitability of sperm collection methods for cryopreservation needs critical appraisal based on the merits and purposes of the cryopreservation. Sperm is collected from individual males or pooled from several males during cryopreservation. The pooled sperm would minimize individual variations in sperm quality but it cannot be used in selective breeding programs for a single pair mating. Oyster sperm can be collected by natural spawning (Hughes, 1973; Yang et al., 2013), biopsy with notching or anesthesia to access gonad (Li, 2009; Yang et al., 2013) and stripping (Dong et al., 2007b; Adams et al., 2008; Yang et al., 2012). The male oysters can be induced to spawn without sacrificing the animal by keeping at 4 °C overnight in air and subsequently increasing water temperature and adding algae after immersion (Wang and Wang, 2008). Injection of serotonin in the gonadal tissue also induces a mature oyster to release sperm within 30 min (Gibbons and Castagna, 1984). Cryopreservation involves maintenance of sperm at a suitable concentration for sample handling at different stages, but natural spawning fails to yield desired sperm concentration. The method for obtaining sperm should be determined by the purpose of cryopreservation and the case of study.

Notching or applying anesthesia is another non-lethal sperm collection technique for cryopreservation. Notching is the process of making hole on the shell by a grinding wheel that ensures collection of a desired volume of sperm for cryopreservation but this technique damages mantle tissue and reduce the chances of animal survival. Anesthetic agents such as dead sea salt (33.3% $MgCl_2$, 24.3% KCl, 5.5% NaCl, 0.2% $CaCl_2$, 0.5% Br^-, 0.15% sulfates and 36.4% crystallization water) and Epson salt ($MgSO_4 \cdot 7H_2O$) have been used to collect sperm. These salts do not induce spawning activity but help opening of shells which are otherwise tightly closed by the adductor muscles. Dead Sea salt is used as an anesthetic in Pacific oysters (Namba et al., 1995; Suquet et al., 2009), American oysters (Yang et al., 2013), Sydney rock oysters