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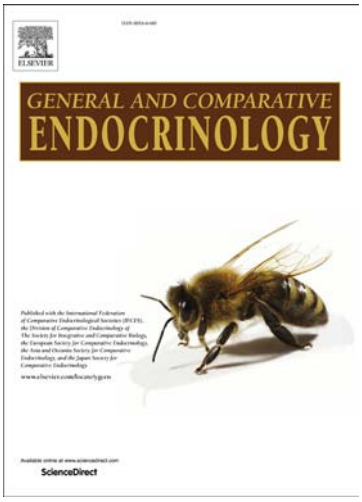

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Progress, challenges and perspectives on fish gamete cryopreservation: A mini-review

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Abstract

Protocols for the cryopreservation of fish gametes have been developed for many different fish species, in special, freshwater salmonids and cyprinids. Methods for sperm freezing have progressed during the last decades due to the increasing number of potential applications: aquaculture (genetic improvement programs, broodstock management, helping with species having reproductive problems), biotechnology studies using model fish species (preservation of transgenic or mutant lines), cryobanking of genetic resources from endangered species, etc.

This mini-review tries to give an overview of the present situation of this area of research, identifying the main challenges and perspectives, redirecting the reader to more in-depth reviews and papers.

Keywords

Sperm; oocyte; aquaculture; cryobanking; endangered species; biotechnology

Highlights

-Freezing protocols have been developed for many fish species

-Gamete cryopreservation has applications in aquaculture, biotechnology or cryobanking

-The lack of standardization limits the industrial use of fish gamete cryopreservation

-PGCs, spermatogonia or somatic cells are alternatives for fish genome preservation

-The improvement of techniques for sperm quality evaluation is required

1. Progress

1.1. Applications of fish gamete cryopreservation

Cryopreservation of fish gametes has evolved during the last decades due to the increasing number of potential applications. The most evident is its use for aquaculture purposes, allowing the improvement of broodstock management at hatcheries (for example, modifying the offspring production season), preserving the genetically selected strains resulting from genetic improvement programs, or helping with species having reproductive problems as lack of synchronization in the gamete production of male and females (as in the case of the European eel, *Anguilla anguilla*; Asturiano et al., 2004) or with those having a low sperm production (as in the case of F1 Senegalese sole, *Solea senegalensis*; Cabrita et al., 2006).

Another potential application is the preservation of genetic material from individuals of natural populations of fish species in the initial phases of the domestication process and genetic modifications. This can assist in maintaining the original wild genotypes for the recovery of genes in the future (becoming a phenotypic backup), contrarily with happened for example in the case of the domestication process of bovine cattle (Vandeputte, 2011). Other possible conservation-related uses include the storage of genetic resources of the increasing number of fish in the lists of endangered species, allowing cryobanking for biodiversity (Van Der Walt et al., 1993; Martínez-Páramo et al., 2009, 2016), or in the case of fish species recently attracting the interest of cryobiologists and aquaculturists, mainly in South America and Asia (Viveiros and Godinho, 2009).

Moreover, the increasing use of aquatic models such as zebrafish in studies of biotechnology, toxicology or pharmacology, requires the use of transgenic lines, knockout and mutant strains that need adequate storage (Kollár et al., 2015; Tiersch et

al., 2011).

1.2. Cryopreservation of fish sperm

Fish genome cryobanking has been attempted using different cell types (see section 2.3.; Labbé et al., 2013). However, spermatozoa have been the objective of most of the studies, making sperm cryopreservation the most established and commercialized technique. The choice of this type of cell is because it is easy to collect in most of the fish species, has a simple cellular structure and a small size and high chilling resistance, making these cells easy to preserve in many fish species. Moreover, reconstruction of individuals can be done by normal fertilization (or androgenesis), but it allows the preservation of only male germplasm.

Some previous publications have reviewed fish sperm cryopreservation subject (Suquet et al., 2000; Cabrita et al., 2009a; Kopeika and Kopeika, 2008; Tiersch and Green, 2011; Figueroa et al., 2014). The Table 1 summarizes studies on cryopreservation of sperm from fish species published during the last 15 years, including the cryoprotectants used (and their concentrations) and the best results obtained in each case in terms of post-thaw motility, cell viability and fertilization rates.

2. Challenges

2.1. When biodiversity means problems

With 25,000 to 30,000 species, fish are the largest group of vertebrates, displaying an extreme biodiversity (Near et al., 2013). This biodiversity is evident in the significant differences found in gamete (spermatozoa) morphology and biology (Mattei, 1991). During the cryopreservation steps of cooling, freezing and thawing, some biophysical and chemical processes such as osmotic changes, dehydration and

rehydration, cell volume changes, ice crystals formation, cryoprotectants toxicity, etc., occur and cells (gametes or others) are more or less sensitive to these changes being species-specific (Cabrita et al., 2014). Thus, cryopreservation protocols must be adapted to find species-specific compromises, and the increasing numbers of studies describing methods to cryopreserve sperm in many species, evidences this diversity (Cabrita et al., 2009a).

2.2. Lack of standardization: a problem to compare results and to arrive to the industry

The main objective has always been maintaining a high sperm fertilizing ability after thawing. However, the difficulties in obtaining reproducible results using sperm cryopreserved using the published methods have limited the use of cryopreserved sperm in production.

Recent scientific discussion have evidenced the need of standardization in different aspects of this area of research, as definition of basic concepts (extenders, cryoprotectant concentrations, dilution ratios), work protocols (sperm concentration determination, sperm cryopreservation methods, equilibration time, handling of straws, polystyrene box or controlled-rate freezer, type of vials, thawing systems, calculation of fertilization and hatching rates, osmolality measurements, sperm quality evaluation, etc) or even reporting of results (Rosenthal et al., 2010; Horváth et al., 2012a).

Although some recent efforts have been made in this regard (Benson et al., 2013; Gallego et al., 2012, 2013; Kása et al., 2014, 2015; Vílchez et al., 2014), new efforts must be made for a complete description and standardization of protocols for sperm cryopreservation, including a very wide area of topics: determination or estimation of sperm motility, substances used for activation of sperm, details of dilution of sperm with extender and cryoprotectants (new ones as the antifreeze proteins, AFPs, or better

combinations of classic ones), use of straws (sealed or unsealed), cooling of samples (dry ice *vs*. liquid nitrogen, styrofoam box *vs*. programmable freezer), methods of calculating fertilization and hatching results.

Regardless of the very high number of publications on this topic, few of the published methods have been adapted to aquaculture practice. There can be several reasons for this failure of application; however, one of them is beyond doubt the lack of standardization not only in methodologies but also in reporting them correctly. The difficulties in interpretation and replication of methods lead to a disappointment and ultimately rejection by the aquaculture industry. We also need to understand that in most fish species sperm is not a limiting factor during induced spawning and, moreover, individual selection is not as advanced in fish as it is in terrestrial livestock.

2.3. Alternative cells

Fish genome cryobanking has been attempted using different cell types: spermatozoa, oocytes, spermatogonia and primordial germ cells (PGCs), as well as somatic cells, blastomeres and embryos (Labbé et al., 2013).

The cryopreservation of fish oocytes has severe limitations because of their large cell volume, the presence of a chorion, the low permeability to cryoprotectants, and a high chilling sensitivity. Different studies have been carried out in zebrafish, as well as other marine and freshwater species, including cryoprotectant toxicity, chilling sensitivity, membrane permeability and cryopreservation (cooling rates, vitrification) of oocytes at different stages of development or ovarian fragments (Zhang et al., 2007; Godoy et al., 2013; Streit Jr. et al., 2014; Marques et al., 2015; reviewed by Martínez-Páramo et al., 2016). However, development of protocols for *in vitro* maturation of ovarian follicles after cryopreservation is required for the use of cryopreserved oocytes (Seki et al., 2008,

2011; Tsai et al., 2010). Thus, oocyte cryopreservation is still in its experimental phase and far from aquaculture applications.

The preservation of spermatogonia and primordial germ cell guarantees the full individual genome. These cells have been cryopreserved successfully in several fish species (Yoshizaki et al., 2011; Robles et al., in press). However, its use requires the development of specific biotechnological tools, such as transplantation.

Fish embryo cryopreservation could be perfect for the establishment and management of genetic selection programs in fish farms. However, they have low membrane permeability, low surface-to-volume ratio, large size, high yolk content and high chilling sensitivity (Hagedorn and Kleinhans, 2000), which is the primary reason for the very limited number of preliminary positive results (Chen and Tian, 2005; Martínez-Páramo et al., 2008; Robles et al., 2005).

However, this procedure requires Thus, these techniques are promising, however, their application in aquaculture practice is still to be developed.

The cryopreservation of somatic (diploid) or embryonic cells (including PGCs) is an alternative to the cryobanking of gametes. They can be a good source of diploid genome to reconstruct fish (reviewed by Labbé et al., 2013). Moreover, they can easily be collected (e.g.: fins clips that regenerate easily). However, the use of these cells means to develop a series of complex and specific techniques as cell culture, nuclear transfer or the transplantation of the thawed cells into recipient fish (of the same or related species) for individual restoration (Siripattarapravat et al., 2011; Chenais et al., 2014), that must be explored in different fish species (reviewed by Martínez-Páramo et al., in press).

2.4. Vitrification

This technique tries to prevent the negative effects of crystallization happening

in the conventional cryopreservation methods mixing cryoprotectants at very high concentration and using very high freezing rates, getting the solidification of external and internal media into an amorphous/glassy state without formation of harmful ice crystals (Fahy et al., 1984).

First applications of vitrification to the cryopreservation of sperm of different species have been published (channel catfish, *Ictalurus punctatus;* Cuevas-Uribe et al., 2011a; green swordtail, *Xiphophorus hellerii*; Cuevas-Uribe et al., 2011b; rainbow trout, *Oncorhynchus mykiss*; Figueroa et al., 2013; Atlantic salmon, *Salmo salar*; Figueroa et al., 2015; Tambaqui, *Colossoma macropomum*; Varela Jr. et al., 2015; Eurasian perch, *Perca fluviatilis* and European eel, *Anguilla anguilla*; Kása et al., in press). However, concentrated cryoprotective solutions are toxic for cells and very high freezing rates can be difficult to achieve with large samples, limiting the applicability of the vitrification to low sperm volumes (2-4 µl; using cryoloops or cryotops). Thus, it does not seem to be able to replace conventional freezing used in most fish species. A real practical application is just feasible in small model species as zebrafish due to the low volumes of sperm that they produce.

Vitrification presents a viable alternative to freezing for the cryopreservation of various teleost tissue types. Vitrification has been tested on somatic cells such as caudal fin explants (Cardona-Costa et al., 2006) as well as testicular cells (Bono-Mestre et al., 2009), embryo blastomeres (Cardona-Costa et al., 2007), oocytes (Guan et al., 2010) and ovarian tissue (Streit et al., 2015). In case of testicular tissue, vitrification was found to be more effective than conventional freezing in terms of cell survival (Bono-Mestre et al., 2009). The efficiency of testicular tissue vitrification and its potential for transplantation and production of germ-line chimeras has been recognized in avian and

mammalian species (Liu et al., 2013; Gouk et al., 2011), thus opening the possibilities of its application in teleost species, as well.

2.5. Evaluation of gamete quality

Fast and accurate techniques for the evaluation of the quality of fish gametes are needed both for the selection of sperm samples and for the establishment of sperm cryopreservation programs by companies. Many different techniques have been developed for gamete quality evaluation (Cosson et al., 2008; Figueroa et al., 2016; Pérez et al., 2009; Sørensen et al., 2013; Valdebenito et al., 2015) including sperm volume, color and density, spermatozoa motility and morphometry parameters (CASA and ASMA software; reviewed by Mylonas et al., in press), and seminal plasma composition (Pérez et al., 2004; Lahnsteiner, 2009).

However, new techniques require new approaches and parameters to evaluate the freezing-thawing processes, and to provide in-depth information on the effects of these processes undergoing during freezing-thawing that reduce sperm quality (Bobe and Labbé, 2010; Cabrita et al., 2009b; Martínez-Páramo et al., in press; Mylonas et al., in press). For example, the cryopreservation process can induce different types of damage to the spermatozoa, such as DNA fragmentation (Bungum et al., 2011; Cabrita et al., 2005b, 2014; Chohan et al., 2006; Pérez-Cerezales et al., 2010; Riesco et al., 2011), and changes in the protein profile of spermatozoa (Zilli and Vilella, 2012) as well as increases on the production of reactive oxygen species (ROS) inducing alterations at DNA level (Aitken and Baker, 2006; Martínez-Páramo et al., 2012; Thomson et al., 2009). Other techniques provide information about specific damage to certain genes and mRNA (Cartón-García et al., 2013; Guerra et al., 2013) and potential epigenetic damages (Labbé et al., submitted). Specifically, in cryopreserved PGCs, different

methylation patterns were found in several genes (e.g *vasa*) (Riesco and Robles, 2013). The improvement of these techniques is allowing the development of better cryopreservation methods, although an evident lack of standardization can compromise the comparison of results between different laboratories and further applications (see section 2.2.).

3. Perspectives

3.1. Cryopreservation and industry

In mammals (both cattle and humans) cryopreservation means an important commercial business, while this is yet to happen in aquatic species. Trying to solve the lack of commercial-scale know-how for scaling-up to industry and practical aquaculture, national or supranational specialized centers should be created to improve the standardization (definitions, methodologies, reporting), and offer quality assessment and cryobanking services (linked to genetic programs, endangered species management, or preservation of special samples). Unfortunately, this activity is currently carried out primarily by research centers that concentrate on conservation-related issues (O'Reilly and Doyle, 2007; Streit et al., 2013) and not by commercial companies that use cryopreserved sperm in their genetic improvement programs.

Commercial application of fish sperm cryopreservation is hindered by several factors. Sperm cryopreservation came to the aid of dairy cattle farming exactly when the industry needed it in the 1950-ies. Due to a continuously increasing selection pressure and the development of artificial insemination (AI) techniques, the individual value of dairy bulls increased significantly (Chandler and Godke, 2011). Cryopreservation solved a very stressing problem: how to use the sperm of a few valuable bulls to simultaneously fertilize the oocytes of several thousand cows on various continents.

However, all this was a result of a very long domestication process. Aquaculture on the other hand is still in its infancy: higher yields can still be achieved using technologies that do not require genetic improvement (e.g. formulation of feeds, stocking rates, vaccination, etc.). Thus, sperm cryopreservation will be achieved commercially in some species where the protocols are better developed, the aquaculture industry has demanded such tools and there are economic interests. For other species, sperm cryopreservation will be used occasionally or in niche areas for solving acute problems. Nevertheless, aquaculture is also a very rapidly developing industry. During the last years the development of genetic improvement programs has happened and there is a growing offer of cryobanking services for several salmonids and other species (reviewed by Martínez-Páramo et al., in press). Several companies that supply the cryopreservation industry have a line of products specifically for fish, such as extenders and activating solutions and there is at least one company based in Norway that offers commercial cryopreservation services to the aquaculture industry, exclusively. All this means that cryopreservation in aquatic species is slowly gaining momentum along with the development of aquaculture, especially in the salmon industry where marker assisted selection has already been introduced and QTL (Quantitative Trait Loci) mapping is becoming increasingly important (Yue, 2014; Tsai et al., 2015).

3.2. Development of alternative techniques

The techniques dedicated to preserving oocytes, embryos or larvae could be a key area of research, although much effort has been made with little success. An alternative or complementary way is to improve emerging biotechnological techniques, as the use of PGCs, spermatogonia or alternative diploid cell sources for genome preservation and transplantation (Labbé et al., 2013; Chenais et al., 2014). However, these techniques

will need improvement in several technical aspects (reviewed by Martínez-Páramo et al., in press) such as cell isolation, identification, labelling, transplantation, nuclear transference or genome inactivation of the recipient. Moreover, better and simpler methods are required for DNA integrity evaluation, types of cryodamage (induced by freezing, thawing or cryoprotectants) on the chromatin and cellular structures and their epigenetics consequences, or regarding the production and effects of reactive oxygen species, to guarantee the sperm viability after every step of these processes and the embryo survival (Chenais et al., 2014). And basic research is required on aspects such as cell reprogramming, to regenerate fish producing gametes from somatic cells after transplantation, or germ cell pluripotency (Martínez-Páramo et al., in press; Robles et al., in press).

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References

Aitken R.J., Baker M.A., 2006. Oxidative stress, sperm survival and fertility control. Mol. Cell. Endocrinol. 250, 66–69.

Asturiano, J.F., Pérez, L., Garzón, D.L., Marco-Jiménez, F., Peñaranda, D.S., Vicente, J.S., Jover, M., 2004. Physio-chemical characteristics of seminal plasma and

development of media and methods for the cryopreservation of European eel sperm. Fish Physiol. Biochem. 30, 283–293.

Asturiano, J.F., Marco-Jiménez F., Peñaranda, D.S., Garzón, D.L., Pérez, L., Vicente. J.S., Jover, M., 2007. Effect of sperm cryopreservation on the European eel sperm viability and spermatozoa morphology. Reprod. Domest. Anim. 42, 162-166.

Asturiano, J.F., Riesco, M.F., Martins, G., Vílchez, M.C., Pérez, L., Gavaia, P., Cabrita, E., 2015. Cryopreservation of zebrafish sperm, first trials and results. 5th International Worshop on the Biology of Fish Gametes. Ancona (Italy). Book of abstracts pp. 157-158.

Asturiano, J.F., Sørensen, S.R., Pérez, L., Lauesen, P., Tomkiewicz, J. First production of larvae using cryopreserved sperm. Effect of preservation temperature and cryopreservation on European eel sperm fertilization capacity. Reprod. Domest. Anim., in press. Doi: 10.1111/rda.12706

Babiak, I., Glogowski, J., Dobosz, S., Kuzminki, H., Goryczko, K., 2002. Semen from rainbow trout produced using cryopreserved spermatozoa is more suitable for cryopreservation. J. Fish Biol. 60, 561-570.

Babiak, I., Bolla, S., Ottesen, O., 2008. Suitable methods for cryopreservation of semen from Atlantic halibut, *Hippoglossus hippoglossus* L. Aquacult. Int. 16, 561–572.

Benson, E.E., Betsou, F., Fuller, B.J., Harding, K., Kofanova, O., 2013. Translating cryobiology principles into trans-disciplinary storage guidelines for biorepositories and biobanks: a concept paper. Cryo-Letters 34, 277–312.

Bernáth, G., Bokor, Z., Kása, E., Várkonyi, L., Hegyi, Á., Kollár, T., Urbányi, B., Żarski, D., Radóczi Ifj, J., Horváth, Á., 2015. Comparison of two different methods in the cryopreservation of Eurasian perch (*Perca fluviatilis*) sperm. Cryobiology 70, 76-78.

Bobe, J., Labbé, C., 2010. Egg and sperm quality in fish. Gen. Comp. Endocrinol. 165, 535–548.

Bono-Mestre, C., Cardona-Costa, J., García-Ximénez, F., 2009. Effects on cell viability of three zebrafish testicular cell or tissue cryopreservation methods. Cryo-Letters 30, 148–52.

Bungum, M., Bungum, L., Giwercman, A., 2011. Sperm chromatin structure assay (SCSA): A tool in diagnosis and treatment of infertility. Asian J. Androl. 13(1), 69–75.

Butts, I.A.E., Litvak, M.K., Kaspar, V., Trippel, E.A., 2010. Cryopreservation of Atlantic cod *Gadus morhua* L. spermatozoa: Effects of extender composition and freezing rate on sperm motility, velocity, and morphology. Cryobiology 61, 174-181.

Butts, I.A.E., Babiak, I., Ciereszko, A., Litvak, M.K., Słowińska, M., Soler, C., Trippel, E.A., 2011. Semen characteristics and their ability to predict sperm cryopreservation potential of Atlantic cod, *Gadus morhua* L. Theriogenology 75, 1290-1300.

Cabrita, E., Robles, V., Alvarez, R., Herráez, M.P., 2001. Cryopreservation of rainbow trout sperm in large volume straws: application to large scale fertilization, Aquaculture 201, 301–314.

Cabrita, E., Robles, V., Cuñado, S., Wallace, J.C., Sarasquete, C., Herráez, M.P., 2005a. Evaluation of gilthead sea bream, *Sparus aurata*, sperm quality after cryopreservation in 5ml macrotubes. Cryobiology 50, 273–284.

Cabrita, E., Robles, V., Rebordinos, L., Sarasquete, C., Herráez, M.P., 2005b. Evaluation of DNA damage in rainbow trout (*Oncorhynchus mykiss*) and gilthead sea bream (*Sparus aurata*) cryopreserved sperm. Cryobiology 50, 144–153.

Cabrita, E., Soares, F., Dinis, M.T., 2006. Characterization of Senegalese sole, *Solea senegalensis*, male broodstock in terms of sperm production and quality. Aquaculture 261, 967-975.

Cabrita, E., Robles, V., Herráez, M.P., 2009a. Methods in Reproductive Aquaculture: Marine and Freshwater Species. CRC Press, Boca Raton, FL, USA, 549pp.

Cabrita, E., Robles, V., Herraez, P., 2009b. Sperm quality assessment, in: Cabrita, E., Robles, V., Herraez, P. (Eds.), Methods in Reproductive Aquaculture: Marine and Freshwater Species. CRC Press Taylor and Francis Group, Boca Raton, pp. 93–148.

Cabrita, E., Engrola, S., Conceição, L.E.C., Pousão-Ferreira, P., Dinis, M.T., 2009c. Successful cryopreservation of sex-reversed sperm from *Epinephelus marginatus*. Aquaculture 287, 152-157.

Cabrita, E., Ma, S., Diogo, P., Martínez-Páramo, S., Sarasquete, C., Dinis, M.T., 2011. The influence of certain aminoacids and vitamins on post-thaw fish sperm motility, viability and DNA fragmentation. Anim. Reprod. Sci. 125(1–4), 189-195.

Cabrita, E., Martínez-Páramo, S., Gavaia, P.J., Riesco, M.F., Valcarce, D.G., Sarasquete, C., Herráez, M.P., Robles, V., 2014. Factors enhancing fish sperm quality and emerging tools for sperm analysis. Aquaculture 432, 389-401.

Cardona-Costa, J., Roig, J., Perez-Camps, M., García-Ximénez, F., 2006. Vitrification of caudal fin explants from zebrafish adult specimens. Cryo-Letters 27, 329-332.

Cardona-Costa, J., García-Ximénez, F., 2007. Vitrification of zebrafish embryo blastomeres in microvolumes. Cryo-Letters 28, 303-309.

Cartón-García, F., Riesco, M.F., Cabrita, E., Herráez, M.P., Robles, V., 2013. Quantification of lesions in nuclear and mitochondrial genes of *Sparus aurata* cryopreserved sperm. Aquaculture 402–403, 106-112.

Chandler, J.E., Godke, R.A., 2011. Cryopreservation of Bull Sperm, in: Tiersch, T.R.,

Green, C.C. (Eds.), Cryopreservation in Aquatic Species, 2nd Edition. World Aquaculture Society, Baton Rouge, Louisiana, USA, pp. 291-298.

Chen, S.L., Tian, Y.S., 2005. Cryopreservation of flounder (*Paralichthys olivaceus*) embryos by vitrification. Theriogenology 63, 1207–1219.

Chenais, N., Depince, A., Le Bail, P.-Y., Labbe, C., 2014. Fin cell cryopreservation and fish reconstruction by nuclear transfer stand as promising technologies for preservation of finfish genetic resources. Aquacult. Int. 22, 63–76.

Chew, P.C., Abd-Rashid, Z., Hassan, R., Asmuni, M., Chuah, H.P., 2010. Semen cryo-bank of the Malaysian Mahseer (*Tor tambroides* and *T. douronensis*). J. Appl. Ichthyol. 26, 726-731.

Chohan, K.R., Griffin, J.T., Lafromboise, M., De Jonge, C.J., Carrell, D.T., 2006. Comparison of chromatin assays for DNA fragmentation evaluation in human sperm. J. Androl. 27(1), 53–59.

Ciereszko, A., Dietrich, G.J., Nynca, J., Liszewska, E., Karol, H., Dobosz, S., 2013. The use of concentrated extenders to improve the efficacy of cryopreservation in whitefish spermatozoa. Aquaculture 408-409, 30-33.

Ciereszko, A., Dietrich, G.J., Nynca, J., Dobosz, S., Zalewski, T., 2014. Cryopreservation of rainbow trout semen using a glucose-methanol extender. Aquaculture 420-421, 275-281.

Cosson, J., Groison, A.L., Suquet, M., Fauvel, C., Dreanno, C., Billard, R., 2008. Studying sperm motility in marine fish: an overview on the state of the art. J. Appl. Ichthyol. 24, 460–486.

Cuevas-Uribe, R., Leibo, S.P., Daly, J., Tiersch, T.R., 2011a. Production of channel catfish with sperm cryopreserved by rapid non-equilibrium cooling. Cryobiology 63(3), 186–197.

Cuevas-Uribe, R., Yang, H., Daly, J., Savage, M.G., Ronald, B.W., Tiersch, T.R., 2011b. Production of F1 offspring with vitrified sperm from a live-bearing fish, the green swordtail *Xiphophorus hellerii*. Zebrafish 8(4), 167–179.

Daly, J., Galloway, D., Bravington, W., Holland, M., Ingram, B., 2008. Cryopreservation of sperm from Murray cod, *Maccullochella peelii peelii*. Aquaculture 285 (1-4), 117–122.

DeGraaf, J.D., Berlinsky, D.L., 2004. Cryogenic and refrigerated storage of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) spermatozoa. Aquaculture 234, 527–540.

Doğu, Z., 2012. Cryopreservation of semen in shabout (*Barbus grypus* Heckel, 1843): sperm motility and fertilization rates. J. Appl. Ichthyol. 28, 952-955.

Dziewulska, K., Rzemieniecki, A., Czerniawski, R., Domagała, J., 2011. Post-thawed motility and fertility from Atlantic salmon (*Salmo salar* L.) sperm frozen with four cryodiluents in straws or pellets. Theriogenology 76, 300–311.

Dzuba, B.B., Kopeika, E.F., 2002. Relationship between the changes in cellular volume of fish spermatozoa and their cryoresistance. CryoLetters 23, 353–360.

Fabbrocini, A., Lavadera, L., Rispoli, S., Sansone, G., 2000. Cryopreservation of seabream (*Sparus aurata*) spermatozoa. Cryobiology 40, 46 –53.

Fahy, G.M., MacFarlane, D.R., Angell, C.A., Meryman, H.T., 1984. Vitrification as an approach to cryopreservation. Cryobiology 21, 407–426.

Figueroa, E., Risopatrón, J., Sánchez, R., Isachenko, E., Merino, O., Valdebenito, I., Isachenko, V., 2013. Sperm vitrification of sex-reversed rainbow trout (*Oncorhynchus mykiss*): effect of seminal plasma on physiological parameters. Aquaculture 372–375, 119–126.

Figueroa, E., Valdebenito, I, Farias, J.G. Technologies used in the study of sperm

function in cryopreserved fish spermatozoa, 2014. Aquacult. Res. 2016, 47(6), 1691-1705.

Figueroa, E., Merino, O., Risopatrón, J., Isachenko, V., Sánchez, R., Effer, B., Isachenko, E., Farias, J.G., Valdebenito, I., 2015. Effect of seminal plasma on Atlantic salmon (*Salmo salar*) sperm vitrification. Theriogenology 83(2), 238–245.

Figueroa, E., Valdebenito, I., Farias, J.G., 2016. Technologies used in the study of sperm function in cryopreserved fish spermatozoa. Aquacult. Res. 47(6), 1691–1705.

Frankel, T.E, Theisen, D.D., Guthrie, H.D., Welch, G.R, Woods III, L.C., 2013.The effect of freezing rate on the quality of striped bass sperm. Theriogenology 79, 940–945.

Gallego, V., Peñaranda, D.S., Marco-Jiménez, F., Mazzeo, I., Pérez, L., Asturiano, J.F., 2012. Comparison of two techniques for the morphometry study on gilthead seabream (*Sparus aurata*) spermatozoa and evaluation of changes induced by cryopreservation. Theriogenology 77, 1078–1087.

Gallego, V., Carneiro, P.C.F., Mazzeo, I., Vílchez, M.C., Peñaranda, D.S., Soler, C., Pérez, L., Asturiano, J.F., 2013. Standardization of European eel (*Anguilla anguilla*) sperm motility evaluation by CASA software. Theriogenology 79, 1034–1040.

Garzón, D.L., Peñaranda, D.S., Pérez, L., Marco-Jiménez, F., Espert, X., Müller, T., Jover, M., Asturiano, J.F., 2008. Effects of pH, sodium bicarbonate, cryoprotectants and foetal bovine serum on the cryopreservation of European eel sperm. Reprod. Domest. Anim. 43, 99-105.

Glogowski, J., Kolman, R., Szczepkowski, M., Horváth, Á., Urbányi, B., Sieczyński, P., Rzemieniecki, A., Domagala, J., Demianowicz, W., Kowalski, R., Ciereszko, A., 2002. Fertilization rate of Siberian sturgeon (*Acipenser baeri*, Brandt) milt cryopreserved with methanol. Aquaculture 211, 367–373.

Godoy, L.C., Streit Jr, D.P., Zampolla, T., Bos-Mikich, A., Zhang, T., 2013. A study on the vitrification of stage III zebrafish (*Danio rerio*) ovarian follicles. Cryobiology 67, 347–354.

Gouk, S.S., Jason Loh, Y.F., Kumar, S.D., Watson, P.F., Kuleshova, L.L., 2011. Cryopreservation of mouse testicular tissue: Prospect for harvesting spermatogonial stem cells for fertility preservation. Fertil. Steril. 95, 2399–2403.

Guan, M., Rawson, D.M., Zhang, T., 2010. Cryopreservation of zebrafish (*Danio rerio*) oocytes by vitrification. Cryo-Letters 31, 230–238.

Guerra, S.M., Valcarce, D.G., Cabrita, E., Robles, V., 2013. Analysis of transcripts in gilthead seabream sperm and zebrafish testicular cells: mRNA profile as a predictor of gamete quality. Aquaculture 406–407, 28–33.

Gwo, J.-C., 2010. Fine structure, motility and cryopreservation of spotted sea bass, *Lateolabrax maculatus* (Moronidae, Teleostei), spermatozoa. J. Appl. Ichthyol. 26, 732-736.

Hagedorn M., Kleinhans F.W. (2000) Problems and prospects in cryopreservation of fish embryos, in: Tiersch, T.R., Mazik, P.M. (Eds.), Cryopreservation in Aquatic Species. World Aquaculture Society, Baton Rouge, Louisiana, pp. 161-178.

Horváth, Á., Urbányi, B., 2000. The effect of cryoprotectants on the motility and fertilizing capacity of cryopreserved African catfish *Clarias gariepinus* (Burchell 1822) sperm. Aquacult. Res. 31(3), 317–324.

Horváth, Á., Wayman, W.R., Urbányi, B., Ware, K.M., Dean, J.C., Tiersch, T.R., 2005. The relationship of the cryoprotectants methanol and dimethyl sulfoxide and hyperosmotic extenders on sperm cryopreservation of two North-American sturgeon species. Aquaculture 247, 243– 251.

Horváth, Á., Urbányi, B., Mims, S.D., Bean, W.B., Gomelsky B., Tiesch, T.R., 2006.

Improved cryopreservation of sperm of paddlefish (*Polyodon spathula*). J. World Aquacult. Soc. 37(4), 356-362.

Horváth, Á., Wayman, W.R., Dean, J.C., Urbányi, B., Tiersch, T.R., Mims, S.D., Johnson, D., Jenkins, J.A., 2008. Viability and fertilizing capacity of cryopreserved sperm from three North American acipenseriform species: a retrospective study. J. Appl. Ichthyol. 24(4), 443–449.

Horváth, Á., Urbányi, B., Wang, C., Onders, R.J., Mims, S.D., 2010. Cryopreservation of paddlefish sperm in 5-mL straws. J. Appl. Ichthyol. 25(5), 715-719.

Horváth, Á., Asturiano, J.F., Rosenthal, H., 2012a. On the biology of fish gametes: summary and recommendations of the Third International Workshop, Budapest and Gödöllo, Hungary, 2011. J. Appl. Ichthyol. 28(6), 863–864.

Horváth, Á., Jesenšek, D., Csorbai, B., Bokor, Z., Raboczki, É., Kaczkó, D., Bernáth, G., Hoitsy, G.; Urbányi, B., Sušnik Bajec, S., Snoj, A., 2012b. Application of sperm cryopreservation to hatchery practice and species conservation: a case of the Post-thaw storage of sperm Adriatic grayling (*Thymallus thymallus*). Aquaculture 358–359, 213–215.

Horváth, Á., Labbé, C., Jesenšek, D., Hoitsy, G., Bernáth, G., Kaczkó, D. , Bokor, Z., Urbányi, B., 2015a. Post-thaw storage of sperm from various salmonid species. J. Appl. Ichthyol. 31(Suppl. 1), 119–124.

Horváth, Á., Bokor, Z., Bernáth, G., Csenki, Z., Gorjan, A., Herráez, M.P., Urbányi, B., Jesenšek, D., 2015b. Very low sperm–egg ratios result in successful fertilization using cryopreserved sperm in the Adriatic grayling (*Thymallus thymallus*). Aquaculture 435, 75-77.

Judycka, S., Szczepkowski, M., Ciereszko, A., Słowinska, M., Bodek, G., Dietrich, G.J., 2015. Characterization of Siberian sturgeon (*Acipenser baerii*, Brandt 1869)

sperm obtained out of season. J. Appl. Ichthyol. 31 (Suppl. 1), 34-40.

Kása, E., Vílchez, M.C., Morini, M., Peñaranda, D.S., Pérez, L., Asturiano, J.F., Urbányi, B., Horváth, Á., 2014. Vitrification of the sperm of European eel (*A. anguilla*): investigation of different protocols. Aquaculture Europe 14. Adding value (AE2014). European Aquaculture Society annual meeting. San Sebastián (Spain). Book of abstracts, p. 616–617.

Kása, E., Bernáth, G., Kollár, T., Żarski, D., Lujic, J., Marinovic, Z., Bokor, Z., Hegyi, Á., Urbanyi, B., Vílchez, M.C., Morini, M., Peñaranda, D.S., Pérez, L., Asturiano, J.F., Horváth, Á., 2015. Vitrification of fish sperm. 5th International Workshop on the Biology of Fish Gametes. Ancona (Italy). Book of abstracts, p. 106–107.

Kása, E., Bernáth, G., Kollár, T., Żarski, D., Lujić, J., Marinović, Z., Bokor, Z., Hegyi, Á., Urbányi, B., Vílchez, M.C., Morini, M., Peñaranda, D.S., Pérez, L., Asturiano, J.F., Horváth , Á. Development of sperm vitrification protocols for freshwater fish (Eurasian perch, *Perca fluviatilis*) and marine fish (European eel, *Anguilla anguilla*). Gen. Comp. Endocrinol., in press. Doi:10.1016/j.ygcen.2016.05.010

Kollár, T., Horváth, Á., Labesse, C., Milon, P., Csenki, Z., Kovács, R., Bernáth, G., Béres, T., Depincé, A., Urbányi, B., Labbé, C., 2015. Development of cryopreservation of zebrafish (*Danio rerio*) sperm. 5th International Workshop on the Biology of Fish Gametes. Ancona (Italy). Book of abstracts, p. 161–162.

Kopeika, E., Kopeika, J., Zhang, T., 2007. Cryopreservation of fish sperm. Methods Mol. Biol. 368, 203–217.

Kopeika, E., Kopeika, J., 2008. Variability of Sperm Quality after Cryopreservation in Fish, in: Alavi, S.M.H., Cosson, J., Coward, K., Rafiee, G. (Eds.), Fish spermatology. Alpha Science, Oxford, UK, pp. 347-396.

Labbé, C., Robles, V., Herráez, M.P., 2013. Cryopreservation of gametes for

aquaculture and alternative cell sources for genome preservation, in: Allan, G., Burnell, G. (Eds.), Advances in Aquaculture Hatchery Technology. Woodhead Publishing, pp. 76–116. ISSN: 0213-3911

Labbé, C., Robles, V., Herráez, M.P. Epigenetics in fish gametes and early embryo. Aquaculture (submitted).

Lahnsteiner, F., Berger, B., Horvath, A., Urbanyi, B., 2004. Studies on the semen biology and sperm cryopreservation in the sterlet, *Acipenser ruthenus* L. Aquacult. Res. 35, 519-528.

Lahnsteiner, F., 2009. The role of free amino acids in semen of rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*). J. Fish Biol. 75, 816–833.

Lanes, C.F.C., Okamoto, M., Cavalcanti P.V., Collares, T., Campos, V.F., Deschamps, J.C., Robaldo, R.B., Marins, L.F., Sampaio, L.A., 2008. Cryopreservation of Brazilian flounder (*Paralichthys orbignyanus*) sperm. Aquaculture 275, 361–365.

Linhart, O., Rodina, M., Cosson, J., 2000. Cryopreservation of sperm in common carp *Cyprinus carpio*: sperm motility and hatching success of embryos. Cryobiology 41(3), 241-250.

Liu, Q.H., Li, J., Xiao, Z.Z., Ding, F.H., Yu, D.D., Xu, X.Z., 2007. Use of computer-assisted sperm analysis (CASA) to evaluate the quality of cryopreserved sperm in red seabream (*Pagrus major*). Aquaculture 263(1-4), 20-25.

Liu, J., Cheng, K.M., Silversides, F.G., 2013. Production of Live Offspring from Testicular Tissue Cryopreserved by Vitrification Procedures in Japanese Quail (*Coturnix japonica*). Biol. Reprod. 88, 124.

López, D.I., Leal, M.C., Viveiros, A.T.M., 2015. Extender composition and osmolality affects post-thaw motility and velocities of piracanjuba *Brycon orbignyanus* (Valenciennes, 1850) (Characiformes) sperm. J. Appl. Ichthyol. 31 (Suppl. 1), 114-

118.

Lujić, J., Bernáth, G., Marinović, Z., Radojković, N., Simić, V., Ćirković, M., Urbányi, B., Horváth. Á. Fertilizing capacity and motility of tench *Tinca tinca* (L., 1758) sperm following cryopreservation. Aquacult. Res., in press. Doi: 10.1111/are.12865

Marco-Jiménez, F., Garzón, D.L., Peñaranda, D.S., Pérez, L., Viudes-de-Castro, M.P., Vicente, J.S., Jover, M., Asturiano, J.F., 2006b. Cryopreservation of European eels (*Anguilla anguilla*) spermatozoa: Effect of rate dilution, foetal bovine serum supplementation and cryoprotectants. Cryobiology 53, 51-57.

Maria, A.N., Viveiros, A.T.M., Orfão, L.H., Oliveira, A.V., Moraes, G.F., 2006. Effects of cooling and freezing on sperm motility of the endangered fish piracanjuba *Brycon orbignyanus* (Characiformes, Characidae) Anim. Reprod. 3(1), 55-60.

Marques, L.S., Bos-Mikich, A., Godoy, L.C., Silva, L.A., Maschio, D., Zhang, T., Streit Jr., D.P., 2015. Viability of zebrafish (*Danio rerio*) ovarian follicles after vitrification in a metal container. Cryobioloy 71, 367-373.

Martínez-Páramo, S., Pérez-Cerezales, S., Robles, V., Anel, L., Herráez, M.P., 2008. Incorporation of antifreeze proteins into zebrafish embryos by a non-invasive method. Cryobiology 56, 216–222.

Martínez-Páramo, S., Pérez-Cerezales, S., Gómez-Romano, F., Blanco, G., Sánchez, J.A., Herráez, M.P., 2009. Cryobanking as tool for conservation of biodiversity: effect of brown trout sperm cryopreservation on the male genetic potential. Theriogenology 71, 594–604.

Martínez-Páramo, S., Diogo, P., Dinis, M.T., Herráez, M.P., Sarasquete, C., Cabrita, E., 2012a. Sea bass sperm freezability is influenced by motility variables and membrane lipid composition but not by membrane integrity and lipid peroxidation. Anim. Reprod. Sci. 131(3-4), 211-218.

Martínez-Páramo, S., Diogo, P., Beirão, J., Dinis, M.T., Cabrita, E., 2012b. Sperm lipid peroxidation is correlated with differences in sperm quality during the reproductive season in precocious European sea bass (*Dicentrarchus labrax*) males. Aquaculture 358–359, 246–252.

Martínez-Páramo, S., Horváth, Á., Labbé, C., Zhang, T., Robles, V., Herráez, P., Suquet, M., Adams, S., Viveiros, A., Tiersch, T., Cabrita, E. Cryobanking of aquatic species. Aquaculture, in press. Doi: 10.1016/j.aquaculture.2016.05.042

Mattei, X., 1991. Spermatozoon ultrastructure and its systematic implications in fishes. Can. J. Zool. 69, 3038–3055.

Müller, T., Urbányi, B., Váradi, B., Binder, T., Horn, P., Bercsényi, M., Horváth, A., 2004. Cryopreservation of sperm of farmed European eel *Anguilla anguilla*. J. World Aquacult. Soc. 35,240–246.

Mylonas, C.C.; Duncan, N.J., Asturiano, J.F. Hormonal manipulations for the enhancement of spermiation in cultured fish and evaluation of sperm quality. Aquaculture, in press. Doi: 10.1016/j.aquaculture.2016.04.021

Near, T.J., Dornburg, A., Eytan, R.I., Keck, B.P., Smith, W.L., Kuhn, K.L., Moore, J.A., Price, S.A., Burbrink, F.T., Friedman, M., Wainwrigh, P.C., 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. Proc. Natl. Acad. Sci. USA 110(31), 12738–12743.

Nynca, J., Dietrich, G.J., Dobosz, S., Grudniewska, J., Ciereszko, A., 2014. Effect of cryopreservation on sperm motility parameters and fertilizing ability of brown trout semen. Aquaculture 433, 62-65.

Nynca, J., Dietrich, G.J., Grudniewska, J., Dobosz, S., Liszewska, E., Krzyś, M., Różyński, R., Ciereszko, A., 2015a. Efficient method for cryopreservation of European huchen (*Hucho hucho* L.) and grayling (*Thymallus thymallus* L.) semen.

Aquaculture 435, 146-151.

Nynca, J., Dietrich, G.J., Dobosz, S., Zalewski, T., Ciereszko, A., 2015b. Effect of post-thaw storage time and sperm-to-egg ratio on fertility of cryopreserved brook trout sperm. Theriogenology 83, 253-256.

Oliveira, A. V.; Viveiros, A. T. M.; Maria, A. N.; Freitas, R. T. F.; Izau, A. Z., 2007: Sucesso do resfriamento e congelamento de sêmen de pirapitinga *Brycon nattereri*. Arq. Bras. Med. Vet. Zootec. 59, 1509–1515. (In Brazilian).

O'Reilly, P., Doyle, R., 2007. Live Gene Banking of Endangered Populations of Atlantic Salmon, in: The Atlantic Salmon. Blackwell Publishing Ltd, pp. 425–469.

Peñaranda, D.S., Pérez, L., Gallego, V., Jover, M., Asturiano, J.F., 2009. Improvement of European eel sperm cryopreservation method by preventing spermatozoa movement activation caused by cryoprotectants. Cryobiology 59, 119-126.

Pérez, L., Asturiano, J.F., Martínez, S., Tomás, A., Olivares, L., Mocé, E., Lavara, R., Vicente, J.S., Jover, M., 2003. Ionic composition and physio-chemical parameters of the European eel (*Anguilla anguilla*) seminal plasma. Fish Physiol. Biochem. 28, 221–222.

Pérez, L., Peñaranda, D.S., Gallego, V., Asturiano, J.F., 2009. Testis development, sperm quality evaluation and cryopreservation in the European eel, in: van den Thillart, G., Dufour, S., Rankin, C. (Eds.), Spawning Migration of the European Eel. Springer The Netherlands, pp. 333–362.

Pérez-Cerezales, S., Martínez-Páramo, S., Beirão, J., Herráez, M.P., 2010. Evaluation of DNA damage as a quality marker for rainbow trout sperm cryopreservation and use of LDL as cryoprotectant. Theriogenology 74, 282–289.

Rideout, R.M., Trippel, E.A., Litvak, M.K., 2004. The development of haddock and Atlantic cod sperm cryopreservation techniques and the effect of sperm age on

cryopreservation success. J. Fish Biol. 65, 299–311.

Riesco M.F., Robles, V., 2013. Cryopreservation causes genetic and epigenetic changes in zebrafish genital ridges. PLoS ONE 8 (6), e67614.

Riesco, M.F., Martínez-Pastor, F., Chereguini, O., Robles, V., 2011. Evaluation of zebrafish (*Danio rerio*) PGCs viability and DNA damage using different cryopreservation protocols. Theriogenology 77, 122–130.

Robles, V., Cabrita, E., Cunado, S., Herráez, M.P., 2003. Sperm cryopreservation of sex-reversed rainbow trout (*Oncorhynchus mykiss*): parameters that affect its ability for freezing. Aquaculture 224, 203-212.

Robles, V., Cabrita, E., Fletcher, G.L., Shears, M.A., King, M.J., Herráez, M.P., 2005. Vitrification assays with embryos from a cold tolerant sub-arctic fish species. Theriogenology 64, 1633–1646.

Robles, V., Riesco, M.F., Psenicka, M., Saito, T., Valcarce, D.G., Cabrita, E., Herráez, M.P. Biology of teleost primordial germ cells (PGCs) and spermatogonia: Biotechnological applications. Aquaculture, in press. doi:10.1016/j.aquaculture.2016.03.004

Rosenthal, H., Asturiano, J.F., Linhart, O., Horváth, Á., 2010. On the biology of fish gametes: summary and recommendations of the Second International Workshop, Valencia, Spain, 2009. J. Appl. Ichthyol. 26. 621–622.

Rurangwa, E., Volckaert, F.A.M., Huyskens, G., Kime, D.E., Ollevier, F., 2001. Quality control of refrigerated and cryopreserved semen using computer-assisted sperm analysis (CASA), viable staining and standardized fertilization in African catfish (*Clarias gariepinus*). Theriogenology 55, 751-769.

Seki, S., Kouya, T., Tsuchiya, R., Valdez, D.M., Jin, B., Hara, T., Saida, N., Kasai, M., Edashige, K., 2008. Development of a reliable in vitro maturation system for

zebrafish oocytes. Reproduction 135, 285–292.

Seki, S., Kouya, T., Tsuchiya, R., Valdez Jr, D.M., Jin, B., Koshimoto, C., Kasai, M., Edashige, K., 2011. Cryobiological properties of immature zebrafish oocytes assessed by their ability to be fertilized and develop into hatching embryos. Cryobiology 62, 8–14.

Siripattarapravat, K., Pinmee, B., Chang, E.A.H., Muñoz, J.D., Kawakami, K., Cibelli, J.B., 2011. The influence of donor nucleus source on the outcome of zebrafish somatic cell nuclear transfer. Int. J. Dev. Biol. 54, 1679–1683.

Sørensen, S.R., Gallego, V., Pérez, L., Butts, I.A.E., Tomkiewicz, J., Asturiano, J.F., 2013. Evaluation of methods to determine sperm density for the European eel, *Anguilla anguilla*. Reprod. Domest. Anim. 48(6), 936–944.

Streit Jr., D.P., Fornari, D.C., Povh, J.A., de Godoy, L.C., Lopes de Oliveira, C.A., Kawakami de Resende, E., Ribeira Pereiro, R., 2013. Germplasm Banking and its Role on Development of the Genetic Improvement Program for Fish in Brazil, in: Martínez-Páramo, S., Oliveira, C., Dinis, M.T. (Eds.), 4th International Workshop on the Biology of Fish Gametes, Albufeira, Portugal, pp. 140–141.

Streit Jr., D.P., Godoy, L.C., Ribeiro, R.P., Fornari, D.C., Digmayer, M., Zhang, T., 2014. Cryopreservation of embryos and oocytes of South American fish species. In: Yamashiro, H. (Ed.), Recent Advances in Cryopreservation. Intech. Pp. 45-58.

Streit Jr., D., Marques, L., de Godoy, L.C., Silva, L., Maschio, D., Zhang, T., Bos-Mikich, A., 2015. Viability of Zebrafish (*Danio rerio*) Ovarian Follicles after Vitrification, in: Carnevali, O., Miccoli, A. (Eds.), 5th International Workshop on the Biology of Fish Gametes. Ancona, Italy, pp. 155–156.

Suquet, M., Dreanno, C., Fauvel, C., Cosson, J., Billard, R., 2000. Cryopreservation of sperm in marine fish. Aquacult. Res. 31, 231-243.

Suquet, M., Chereguini, O., Fauvel, C., 2009. Cryopreservation of sperm in turbot (*Psetta maxima*) in: Cabrita, E., Robles, V. Herráez, M.P. (eds)**.** Methods in Reproductive Aquaculture: Marine and Freshwater Species. Biology Series, CRC Press (Taylor and Francis group), 463-468 pp.

Szabó, G., Müller, T., Bercsényi, M., Urbányi, B., Kucska, B., Horváth, A., 2005. Cryopreservation of European eel (*Anguilla anguilla*) sperm using different extenders and cryoprotectants. Acta Biol. Hung. 56, 173–175.

Tanaka, S., Zhang, H., Horie, N., Yamada, Y., Okamura, A., Utoh, T., Mikawa, N., Oka, H.P., Kurokura, H., 2002. Long-term cryopreservation of sperm of Japanese eel. J. Fish Biol. 60, 139–146.

Thomson, L.K., Fleming, S.D., Aitken, R.J., De Iuliis, G.N., Zieschang, J.A., Clark, A.M., 2009. Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis. Hum. Reprod. 24, 2061–2070.

Tiersch, T.R., Yang, H., Jenkins, J.A., Dong, Q., 2007. Sperm cryopreservation in fish and shellfish. Soc. Reprod. Fertil. Suppl. 65, 493–508.

Tiersch, T.R., Green, C.C. (Eds.), 2011. Cryopreservation in Aquatic Species Cryopreservation, 2nd Edition. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Tiersch, T.R., Yang, H., Hu, E., 2011. Outlook for development of high-throughput cryopreservation for small-bodied biomedical model fishes. Comp. Biochem. Physiol. C. 154, 76–81.

Tsai, S., Rawson, D.M., Zhang, T., 2010. Development of in vitro culture method for early stage zebrafish (*Danio rerio*) ovarian follicles for use in cryopreservation studies. Theriogenology 74, 290–303.

Tsai, H.Y., Hamilton, A., Guy, D.R., Tinch, A.E., Bishop, S.C., Houston, R.D., 2015.

The genetic architecture of growth and fillet traits in farmed Atlantic salmon (Salmo salar). BMC Genet. 16, 51.

Urbányi, B., Horváth, Á., Kovács, B., 2004. Successful hybridization of *Acipenser* species using cryopreserved sperm. Aquacult. Int. 12, 47-56.

Valdebenito, I.I., Gallegos, P.C., Effer, B.R., 2015. Gamete quality in fish: evaluation parameters and determining factors. Zygote 23(2), 177–197.

Van Der Walt, L.D., Van Der Bank, F.H., Steyn, G.J., 1993. The suitability of using cryopreservation of spermatozoa for the conservation of genetic diversity in African catfish (*Clarias gariepinus*). Comp. Biochem. Physiol. A: Physiol. 106, 313–318.

Vandeputte, M., 2011. Selective Breeding – Lessons learned from terrestrial animals and status of aquaculture implementation. Plenary session. Aquaculture Europe 11. European Aquaculture Society annual meeting. Rhodes (Greece).

Varela Junior, A.S., Goularte, K.L., Alves, J.P., Pereira, F.A., Silva, E.F., Cardoso, T.F., Jardim, R.D., Streit Jr, D.P., Corcini, C.D., 2015. Methods of cryopreservation of Tambaqui semen, *Colossoma macropomum*. Anim. Reprod. Sci. 157, 71–77.

Vílchez, M.C., Morini, M., Peñaranda, D., Pérez, L., Depincé, A., Kása, E., Labbé, C., Horváth, Á., Asturiano, J.F., 2014. Comparison of two validated methods for cryopreserving European eel (*A. anguilla*) sperm: standardization as a target. Aquaculture Europe 14. Adding value (AE2014). European Aquaculture Society annual meeting. San Sebastián (Spain). Book of abstracts, p. 1418-1419.

Viveiros, A.T., Godinho, H.P., 2009. Sperm quality and cryopreservation of Brazilian freshwater fish species: a review. Fish Physiol. Biochem. 35, 137–150.

Viveiros, A.T.M., Nascimento, A.F., Orfão, L.H., Isau, Z.A., 2010. Motility and fertility of the subtropical freshwater fish streaked prochilod (*Prochilodus lineatus*) sperm cryopreserved in powdered coconut water. Theriogenology 74, 551–556.

Viveiros, A.T.M., Amaral, T.B., Orfão, L.H., Isau, Z.A., Caneppele, D., Leal, M.C., 2011. Sperm cryopreservation of tiete tetra *Brycon insignis* (Characiformes): effects of cryoprotectants, extenders, thawing temperatures and activating agents on motility features. Aquacult. Res. 42, 858–865.

Viveiros, A.T.M., Orfão, L.H., Nascimento, A.F., Corrêa, F.M., Caneppele, D., 2012. Effects of extenders, cryoprotectants and freezing methods on sperm quality of the threatened Brazilian freshwater fish pirapitinga-do-sul *Brycon opalinus* (Characiformes). Theriogenology 78, 361–368.

Viveiros, A.T.M., Gonçalves, A.C.S., Leal, M.C., 2015. Fresh, equilibrated and post-thaw sperm quality of *Brycon orbignyanus* (Valenciennes, 1850) and *Prochilodus lineatus* (Valenciennes, 1837) treated with either salmon GnRHa and domperidone or pituitary extract. Neotrop. Ichthyol. 13(1), 157-164.

Woods III, L.C., He, S., Jenkins-Keeran, K., 2009. Cryopreservation of striped bass *Morone saxatilis* spermatozoa in: Cabrita, E., Robles, V. Herráez, MP (eds)**.** Methods in Reproductive Aquaculture: Marine and Freshwater Species. Biology Series, CRC Press (Taylor and Francis group), 421-425 pp.

Yang, H., Carmichael, C., Varga, Z.M., Tiersch, T.R., 2007. Development of a simplified and standardized protocol with potential for high-throughput for sperm cryopreservation in zebrafish *Danio rerio*. Theriogenology 68, 128-136.

Yasui, G.S., Arias-Rodriguez, L., Fujimoto, T., Arai, K., 2009. A sperm cryopreservation protocol for the loach *Misgurnus anguillicaudatus* and its applicability for other related species. Anim. Reprod. Sci. 116, 335-345.

Yoshizaki, G., Fujinuma, K., Iwasaki, Y., Okutsu, T., Shikina, S., Yazawa, R., Takeuchi, Y., 2011. Spermatogonial transplantation in fish: A novel method for the preservation of genetic resources. Comp. Biochem. Phys. D. 6, 55–61.

Yue, G.H., 2014. Recent advances of genome mapping and marker-assisted selection in aquaculture. Fish Fish. 15, 376–396.

Zhang, T., Rawson, D., Pekarsky, I., Blais, I., Lubzens, E., 2007. Low-temperature preservation of fish gonad cells and oocytes, in: Babin, P., Cerdà, J., Lubzens, E. (Eds.), The Fish Oocyte. Springer Netherlands, pp. 411–436.

Zilli, L., Vilella, S., 2012. Effect of cryopreservation on bio-chemical parameters, DNA Integrity, protein profile and phosphorylation state of proteins of seawater fish spermatozoa, in: Katkov, I.I. (Ed.), Current Frontiers in Cryobiology. InTech, InTechOpen. pp. 391-414

Table 1. Studies on sperm cryopreservation of fish species (published from year 2000 on) indicating the cryoprotectant(s) used in each case and giving (when mentioned) the best results on post-thaw motility (% motile cells), viability (% live cells) and fertilization rate (%).

Species	Post-thaw			Cryoprotectants**	Reference
	Motility (% or arbitrary scale*)	Viability (%)	Fertilization (%)		
Acipenser baerii	-	-	6.0-29.6	10% Methanol	Glogowski et al., 2002
(Siberian sturgeon)	51	40	20-35	10% Methanol	Judycka et al., 2015
Acipenser brevirostrum	13	-	19-39	10% Methanol	Horváth et al., 2005
(Shortnose sturgeon)	15	5	39-40	5% Methanol	Horváth et al., 2008
Acipenser ruthenus (Sterlet)	33.5	-	9.5-32.7	7.5% Methanol	Lahnsteiner et al., 2004
Acipenser sturio (European sturgeon)	60-70	-	34	10% Methanol	Urbányi et al., 2004
Anguilla anguilla	36.6	-	-	10% DMSO	Asturiano et al., 2004
(European eel)	36	-	-	10% Methanol	Müller et al., 2004
	47	-	-	10% DMSO	Szabó et al., 2005
	-	63.2	-	10% Methanol	Marco-Jiménez et al., 2006
	51.9	58.26	-	10% DMSO	Asturiano et al., 2007
	~18-22	~30-35	-	10% DMSO	Garzón et al., 2008
	38	-	-	10% DMSO	Peñaranda et al., 2009
	-	-	33	10% DMSO	Asturiano et al., in press
Anguilla japonica (Japanese eel)	37-46.6	-	-	10% DMSO	Tanaka et al., 2002
Barbus grypus (Shabout)	41.3	-	36.1	10% DMSO	Doğu, 2012
Brycon insignis (Tiete tetra)	76-88	51-69	-	10% Methyl glycol	Viveiros et al., 2011
Brycon nattereri (Pirapitinga)	45-72	-	-	10% Methyl glycol	Oliveira et al., 2007
Brycon opalinus	18-88	10-80	-	10% Methyl glycol	Viveiros et al., 2012

(Pirapitinga-do-sul)					
Brycon orbignyanus (Piracanjuba)	26-66 28-63 42	- - -	- - -	10% Methyl glycol 10% Methyl glycol 10% Methyl glycol	Maria et al., 2006 López et al., 2015 Viveiros et al., 2015
Clarias gariepinus (African catfish)	13.7-44 70.7	- 52.1	82.2-86.7 -	10% DMSO 8% DMSO	Horváth and Urbányi, 2000 Rurangwa et al., 2001
Coregonus lavaretus (Whitefish)	27.5	-	-	10% Methanol	Ciereszko et al., 2013
Cyprinus carpio (Common carp)	69	-	56	10% DMSO	Linhart et al., 2000
Danio rerio (Zebrafish)	35 <20 ~60	- 40 -	13 - 15	8% Methanol 20%, DMF 8% Methanol	Yang et al., 2007 Asturiano et al., 2015 Kollár et al., 2015
Dicentrarchus labrax (European sea bass)	- 35.3	~35-48 69.9	- -	10% DMSO 10% DMSO	Cabrita et al., 2011 Martínez-Páramo et al., 2012a
Epinephelus marginatus (Dusky grouper)	36.8	22.5	65.1	10% DMSO	Cabrita et al., 2009c
Gadus morhua (Atlantic cod)	13-64 ~55 ~38-58 44.2	- - - -	9-69 - - 52.4	10% DMSO 10% PG 10% PG 10% 1,2 propanediol	DeGraaf and Berlinsky, 2004 Rideout et al., 2004 Butts et al., 2010 Butts et al., 2011
Hucho hucho (European huchen)	45	-	87-88	9% Methanol	Nynca et al., 2015a
Hippoglossus hippoglossus (Atlantic halibut)	80	-	97	10% DMSO 10% DMA 10% methanol	Babiak et al., 2008
Hypophthalmichthys molitrix (Silver carp)	30	-	-	16% DMSO	Dzuba and Kopeika, 2002
Lateolabrax maculatus (Spotted sea bass)	50-75	-	-	5-10% DMSO	Gwo, 2010
Maccullochella peelii (Murray cod)	51	63	11	10% Methanol	Daly et al., 2008

Melanogrammus aeglefinus (Haddock)	11-53 ~58	- -	0.33-53 ~85	10% DMSO 10% PG	DeGraaf and Berlinsky, 2004 Rideout et al., 2004
Misgurnus anguillicaudatus (Loach)	72	-	28-32	15% Methanol	Yasui et al., 2009
Morone saxatilis (Striped bass)	45 10	- 56.5	54 -	7.5% DMSO+75mM glycine	Woods III et al., 2009 Frankel et al., 2013
Mugil soiuy (Marine haarder)	90	-	-	16% DMSO	Dzuba and Kopeika, 2002
Oncorhynchus mykiss (Rainbow trout)	- 1-29 43-58 49.9 -	- - 60.3-77.3 - -	94.4 8-65 72.8-84 84.4 ~30-40	10% DMA 7% DMSO 7% DMSO 9% Methanol 10% Methanol	Babiak et al., 2002 Robles et al., 2003 Cabrita et al., 2001 Ciereszko et al., 2014 Horváth et al., 2015a
Pagrus major (Red seabream)	64.8	-	>90	15% DMSO	Liu et al., 2007
Paralichthys orbignyanus (Flounder)	2.5*	43	78	10% DMSO	Lanes et al., 2008
Perca fluviatilis (Eurasian perch)	43-90	-	-	10% Methanol	Bernáth et al., 2015
Polyodon spathula (Paddlefish)	85 -	- -	80 48	10% Methanol 5% Methanol	Horváth et al., 2006 Horváth et al., 2010
Prochilodus lineatus (Curimbatá)	75 73	- -	- -	10% Methyl glycol 10% Methyl glycol	Viveiros et al., 2010 Viveiros et al., 2015
Salmo marmoratus (Marble trout)	11-15	-	15-60	10% Methanol	Horváth et al., 2015a
Salmo salar (Atlantic salmon)	8.2	-	58.7	10% Methanol	Dziewulska et al., 2011
Salmo trutta m. fario (Brown trout)	53-56 73.8	- -	29-42 >90%	10% Methanol 7.5% Methanol	Horváth et al., 2015a Nynca et al., 2014
Salvelinus fontinalis (Brook trout)	56.8	-	36.5	7.5% Methanol	Nynca et al., 2015b
Scaphyrinchus albus	57-70	-	79-85	10% Methanol	Horváth et al., 2005

(Pallid sturgeon)					
Scophthalmus maximus (Turbot)	75	-	68	10% DMSO	Suquet et al., 2009
Sparus aurata (Gilthead seabream)	~65 58.3-70 ~30-55 68	- 22.7-86.9 ~45-60 87	- 75.6 - -	5% DMSO 5% DMSO 5% DMSO 5% DMSO	Fabbrocini et al., 2000 Cabrita et al., 2005 Cabrita et al., 2011 Gallego et al., 2012
Tinca tinca (Tench)	-	-	85	10% Methanol	Lujić et al., in press
Tor tambroides (Malaysian Mahseer)	54.9-69.4	-	13.4-36.8	10% DMSO	Chew et al., 2010
Tor douronensis (Malaysian Mahseer)	74	-	-	10% DMSO	Chew et al., 2010
Thymallus thymallus (Grayling)	- - ~30 68	- - - -	51.1 59-60 ~60 ~50	10% Methanol 10% Methanol 10% Methanol 9% Methanol	Horváth et al., 2012b Horváth et al., 2015a Horváth et al., 2015b Nynca et al., 2015a

*** Dimethyl-acetamide (DMA); Dimethyl-formamide (DMF); Dimethyl-sulfoxide (DMSO); Propylene glycol (PG)*

Progress, challenges and perspectives on fish gamete cryopreservation: A mini-review

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Highlights

-Freezing protocols have been developed for many fish species

-Gamete cryopreservation has applications in aquaculture, biotechnology or cryobanking

-The lack of standardization limits the industrial use of fish gamete cryopreservation

-PGCs, spermatogonia or diploid cells are alternatives for fish genome preservation

-The improvement of techniques for sperm quality evaluation is required