Chapter

Cryopreservation Studies in Aquaculture from Past to Present: Scientific Techniques and Quality Controls for Commercial Applications

Aygül Ekici, Güneş Yamaner and Menekşe Didem Demircan

Abstract

In this section, cryopreservation of fish genetic resources, which is one of the important applications to ensure the sustainability of genetic resources of freshwater fish species, is discussed. At the same time, information is provided about the possible sources of contamination that may be encountered during cryopreservation applications. In this context, the results of sperm, egg, and embryo cryopreservation studies of fish and their success and failure in applications were evaluated in addition to the process from past to present. Information is given about the contamination that may develop depending on the applications in the process of cryopreservation and dissolving processes, as well as the studies carried out to eliminate extracellular disease agents. In the section, in addition to the evaluation of the results of scientific studies, commercial companies that commercially carry out gamete cryopreservation applications are also included. The contamination that may develop depending on the applications in the process of cryopreservation and thawing processes, as well as the studies carried out to eliminate extracellular disease agents are mentioned.

Keywords: sperm, egg, germ cell, storage, contamination

1. Introduction

Developing gamete conservation programs will be invaluable in future commercial fish reproductive studies. Gamete conservation is an important tool for fish reproduction and is very important for aquaculture. It may be widely used in breeding laboratories. The growing interest in improving technology has led to an increase in the number of studies on this subject. In particular, sperm has an important place in cryopreservation studies and is still performed both in laboratory studies and in culture applications [1, 2]. Although the freezing of male sperm dates back to the 1600s, the successful method of artificial insemination at the end of the 1950s, with the need for long-term storage of sperm. In 1953, once the method of freezing sperm was successful with the

herring (*Clupea harengus*) that achieved approximately 80% cellular motility upon thawing [3]. After that, the growing interest in improving preservation technology has increased the number of studies on sperm cryopreservation. Therefore, cryopreserved sperm can now be used in routine fish reproduction and aquaculture practices [4].

Before achieving standardization in the sperm cryopreservation procedure, aquaculture conditions, broodstock management, standardization of the feeding regime, and the production of disease-free lineages are essential for the success of standardization. In the next stage, providing appropriate biosecurity conditions in the laboratory where sperm cryopreservation process will be performed will be an important step that increases the success and brings it closer to standardization. Once a standard procedure has been established, for example, the kits used in DNA and RNA isolation, standardization can be achieved by creating fish species-specific sperm cryopreservation kits.

Although sperm cryopreservation studies have focused on Cyprinid, Sturgeon Salmonids, and Catfish species that are intensively cultured, there are many cases of successful freezing studies with other various fish species [5–7]. Since a review on cryopreservation of fish sperm was carried out before [8], the work in this present section will be focused on more recent improvements in fish (carp, sturgeon, eel, Salmonid, and catfish) sperm cryopreservation with the extender (+cryoprotectant)/additive material and freezing/thawing procedure, mostly.

2. Cryopreservation of freshwater fish sperm

Populations of aquatic species are threatened by anthropogenic influences, overfishing and poaching, destruction of spawning habitats, as well as an increase in water temperature caused by climate change. Today, fish farms carry out production activities by preserving live fish. However; in addition, because of problem with water resources, gametes, genetic factors, disease-related factors, operational problems, system failure, environmental problems, and the survival chances of these creatures become difficult.

High volume and high-quality sperm quantity must be obtained for the commercialization of sperm cryopreservation applications. The successful use of cryopreserved sperm in gene banks in fertilization studies requires that the preserved material is of an acceptable quality/viability and quantity [9]. A company based in Norway provides the aquaculture industry with cryopreserved sperm of 16 fish species, including 8 species belonging to the Salmonidae family [10]. In addition, sperm preservation, maturation, and cryopreservation solutions specific to aquatic species are commercially available on the market [11]. There are also some solutions developed by various commercial companies for use in tissue and cell freezing.

In sperm cryopreservation studies, the goal is to achieve the highest fertilization rate with frozen sperm. However, the failure to achieve similar results in repeated experiments in sperm cryopreservation studies, using of this application in aquaculture studies is limited [12]. Standardization is the biggest problem in sperm cryopreservation, but it will be useful to understand the molecular mechanisms by using new-generation technologies to ensure standardization.

2.1 Carp sperm cryopreservation

The common carp (*Cyprinus carpio*) is one of the largest farmed freshwater fish in the world, with its production reaching 4363.3 thousand tons in 2020 [13]. In addition

to being of commercial interest, it is also a model organism within Teleostei, and is one of the first cultured fish [14]. The sperm cryopreservation of carp was developed several decades ago, and cryopreservation of sperm has often been investigated in carp due to its economic (commercial) and recreational value. However, Carp have undergone selective breeding, resulting in different strains. Cryopreservation can be used to secure sperm from the desired common carp strains in sperm banks and for sperm transport [8, 15]. Therefore, there is a growing interest in methodological and practical innovation in the cryopreservation of carp sperm. Even though the protocols for cryopreservation of carp sperm have been developed [16–19], reduction in sperm viability and motility is still observed in cryopreserved sperm. Therefore, before the cryopreservation of sperm, a thorough evaluation of different extender solutions with cryoprotectant, additives material (cyclodextrin, ext.,) and cooling or thawing rates are essential to develop optimum cryopreservation protocols for the carp.

An extender is a chemical compound used in sperm cryopreservation studies and includes inorganic chemicals. The extender has to be similar to blood and sperm seminal plasma in terms of the inorganic chemicals it contains in order for sperm cells to maintain their viability in vitro preservation. Also, the extender functions as nutrients, pH regulators, and seminal plasm osmotic pressure. Therefore, the extender used to freeze the sperm of freshwater fish should be specific to the species. Since the sperm activation mechanism of each species is different, the extenders used vary. Cryoprotectants are added for protection and stored in a cryogen that can produce very low temperature due to its varied state (e.g., liquid nitrogen at −196°C) [8].

A number of efficient cryo-medium context has been developed for carp sperm. One of the most important steps in the successful cryopreservation of carp sperm is selecting the cryoprotectant in the extender during the process. Various cryoprotectants such as glycerol, DMSO, methanol, and DMA have been used as cryoprotectants for fish sperm. Some studies show that DMSO works best as cryoprotectant in carp sperm compared to glycerol and methanol. In another study conducted with carp, it was emphasized that the use of DMSO at a rate of 5–20% was effective in the sperm of carp [16–19]. Another cryoprotectant used to freeze carp sperm is the yolk of an egg. It has been reported that duck egg yolk, which is used as an extracellular cryoprotectant in cryopreservation sperm of carp, increases motility after thawing and the fertilization rate compared to chicken egg yolk [20].

In addition to the studies investigating the effectiveness and success of cryoprotectants in the freezing process of carp sperm, there are studies that revealing the oxidative stress caused by dilution with cryoprotectants during/before the cryopreservation process. Seminal plasma protects sperm cells against oxidative stress. Dilution of sperm cells during cryopreservation reduces the seminal plasma content, which makes sperm cells more sensitive to oxidative stress [21]. Therefore studies have focused on reducing the effect of oxidative stress in the cell. The fact that amino acids have antioxidant properties and are present in high concentrations in seminal plasma has made amino acids an important component in sperm cryopreservation studies [21–24]. It has been shown that the use of L-cysteine in the process of sperm freezing in carp has a positive effect on motility and viability of sperm cells [25]. It has been stated that the use of Cysteine at 20 mM in the freezing medium of carp sperm process makes a significant difference in motility and motility time [26]. Another additive used is cholesterol-laden cyclodextrin (CLC) to reduce cell damage during the freezing process. In the freezing of carp sperm, the use of 1.5 mg of CLC in the extender was found to have the best cryoprotective effect in maintaining sperm motility, duration, and viability of sperm cells [27].

In addition to the cryoprotectant studies mentioned above, one of the factors affecting the freezing process and is the density of the sperm before freezing. It has been reported that sperm concentration is often ignored before freezing, and the sperm and extender are diluted in a certain volume-to-volume ratio [28, 29]. On the other hand, it has been shown that optimization of sperm concentration plays a crucial role in a number of aquaculture species, in particular pikeperch (*Sander lucioperca*) [30], European perch (*Perca fluviatilis*) [9], and Salmonid [31]. However, it was observed that there was no significant difference between sperm samples' motility and velocity after thawing when different sperm concentrations were specially adjusted before freezing in carp sperm [32].

Besides studies on the effect of chemical and non-chemical components used in freezing sperm, studies on the freezing process are varied. In adding to studies on the freezing procedure with liquid nitrogen and liquid nitrogen vapor used for a long time, studies are also performed with programmable freezing devices. In studies using liquid nitrogen vapor and liquid nitrogen, the heights of straws to liquid nitrogen vapor and straws volumes were studied. In Ref. [33], tested different freezing rates by modifying the height (2–6 cm) above the surface of liquid nitrogen, where the straws were placed. The highest fertilization was observed when samples were frozen at 2 cm above the level of liquid nitrogen and 10 min freezing time (74 ± 7%).

2.2 Sturgeon sperm cryopreservation

Sturgeons are the oldest freshwater fishes, having evolved around 200–250 million years ago [34]. The high economic value of sturgeon, mainly because of their caviar, the failure to manage the caviar trade, and unsustainable fishing (in the seas and river), along with serious habitat fragmentation have led to a significant decline of wild sturgeon populations [35, 36]. Due to this decrease in natural stocks, all sturgeon species have been included in the list of endangered species under CITES (Convention on International Trade in Endangered Species) since 1997 for population restoration; however, thanks to aquaculture, the sturgeon still maintains its place in the valuable product category in fish markets worldwide [37]. Sturgeon culture is developing and increasing in order to meet the need for products obtained from sturgeon and, in addition, support natural stocks. Another method of protection of natural stocks, as already described, is the cryopreservation of gametes or embryos. Especially fish sperm cryobanking is considered a potentially powerful tool in aquaculture for endangered species [38].

Gamete cryoperservation in sturgeon has been given more importance because the species is one of the extinct species. And in sturgeon species, especially sperm freezing, biological, mechanical, and biochemical factors affecting sperm cryopreservation have been studied extensively in order to increase post-thawing motility. Since there is literature [8] on this subject before, current research topics of recent years are given in this section.

The most commonly used cryoprotectant for cryopreservation sperm of sturgeon is methanol. In various species of sturgeon, the lowest fertilization rate obtained using this cryoprotectant 6% (Siberian sturgeon, *Acipenser baeri*); the highest was reported as 40% (Shortnose sturgeon, *A. brevirostrum*) [39–41]. In Russian sturgeon (*A. gueldenstaedtii*), the use of 10% methanol in sperm cryopreservation causes a decrease in acrosin activity and an increase in DNA damage; however, compared to the solution in which methanol was not used, it was reported that this cryoprotectant protects sperm cells during cryopreservation process [42]. In sperm cryopreservation

in sturgeon, in addition to intracellular cryoprotectant substances, there are studies in which cryoproctants such as sugars, egg yolk, and vegetable oil, which are called extracellular, are tested [43]. In the study conducted with Persian (*A. persicus*) and Beluga (*Huso huso*) sturgeon sperm; the addition of different disaccharides such as maltose, trehalose, lactose, and lactulose along with 9% methanol solution has been reported to have an effect on motile sperm cells, except for lactulose. The authors also reported that the cryopreserved sperm using each of the four disaccharides could be stored for at least 30 min without losing sperm motility [44]. In addition to the studies reporting that the protective effect of disaccharides during sperm freezing is due to the high molecular weight they have [44], there are studies reporting that this protective effect is entirely due to the chemical structure of the disaccharides [45, 46]. When the use of sucrose or trehalose alone and in combination with different concentrations was tested for cryopreservation of Dabry's sturgeon (*Acipenser dabryanus*) sperm, low concentration sucrose plus trehalose ($S_{15}T_{15}$) solution was reported to be the optimal solution. Also, it was reported that mixing of the extender with sucrose, lactose, or trehalose alone or with pairwise mixtures revealed that a mixture of lactose and trehalose (L15T15) gave the best results for both Chinese sturgeon (*A. sinensis*) and Dabry's sturgeon [47].

Along with cryoprotectants that protect the viability of sperm cells, various dilution agents that provide the dilution of the sperm and reduce the cell damage seen in the freezing process are also used. Another substance added in cryomedium in the freezing of sturgeon sperm is antioxidant substances. These substances, which reduce oxidative stress in the sperm cell during the freezing process and therefore increase sperm quality after thawing, are ascorbic acid, catalase, glutathione, and cysteine [48]. Although the protective effect of using catalase (25 U/mL), glutathione (0.25–0.5 mg/mL), and ascorbic acid (0.5 mg/mL) in freezing the sperm of three species of sturgeon (*A. dabryanus*, *A. sinensis*, and *Acipenser baerii*) on sperm cells have been mentioned; it has been reported that the three antioxidants should not be used together [49].

In order to protect sperm cells from cryodamage in sperm cryopreservation in many fish species, including sturgeon, it is recommended to use various proteins, enzymatic or non-enzymatic antioxidants, and antifreeze proteins in the cryopreservation procedure [50]. However, the effect of antifreezing protein on sperm cells during freezing in sterlet sturgeon was examined and it was found that a significant decrease in motility rate and velocity of curvilinear (VCL) was observed in cryopreserved spermatozoa with/without supplementation of 10 g AFPI compared to fresh spermatozoa. And also the results showed that in partial changes in the ultrastructural compartments, weakening of the midpiece and rupture of the plasma membrane of the flagellum were seen. The author believes that this damage is not due to oxidative stress that can occur in cryopreserved sperm; expressed that there is physical damage that occurs during the formation of ice crystals during freezing process [51].

It is a known fact that after cryopreservation of the sperm, the motility in the sperm cells decreases and if these sperm samples are used in the fertilization study, a low fertilization rate will be obtained [52]. All the studies carried out so far have been aimed at increasing sperm viability/motility, that is, the fertility of sperm, after thawing. However, in the sperm cryopreservation study using methanol in Russian sturgeon, the motility value obtained after thawing sperm samples was found low (18–25%); in the fertilization study conducted with the same sperm samples, fertilization percentage was obtained as 72.67% [53].

Compared to other species, sturgeon sperm is one of the species with low sperm density due to the mixing of sperm with urine and the originality of the maturation

process of sperm. This low concentration of sperm in sturgeon fish is especially important for the optimum dilution rate in sperm cryopreservation studies where the dilution rate is important. It has been reported that the percentage postthawed sperm motility in Sterlet sturgeon (*A. ruthenus*) depends on the sperm concentration in the samples. While the highest motility after thawing in the study was found in the frozen sperm samples at concentrations of 0.2–1 × 10^9 spz/mL; the sperm concentration of 3 × 10^9 spz/mL, which is higher than the natural sperm concentration in the sterlet, has been reported as suitable for use in cryopreservation procedures as sperm fertilization ability remains at a high level despite a significant decrease in sperm motility percentage. And these findings support the conclusion that high utilization of sperm concentration before freezing may be useful for reducing the volume of sperm retained during freezing and reducing the sample volume required for artificial insemination [54].

In addition to sperm concentration, there are studies investigating the effect of various sperm volumes during freezing. From this point of view, some research was carried out to examine the effect of various volumes (0.5, 0.75, 1.5, and 2 ml) and also the possibility of using the method of vitrification of sperm under deep low-temperature cooling in Russian and Siberian sturgeon. In this study, it was observed that the highest percentage of motility and motility duration was in samples frozen in 0.5 ml Eppendorf tubes. Also, in the study, the following was reported, when cryopreservation of seminal fluid in larger test tubes (0.75, 1.5, and 2 ml), the results were slightly worse [55].

One of the newest issues being investigated in the freezing of sturgeon sperm is the use of ultrasonic waves, which allows the creation of optimum conditions so that the sperm can be preserved at a low temperature. The new methodological approach to low-temperature preservation of fish germ cells using acoustic-mechanical effect offers great opportunities to create new effective deep-freezing methods. The report on the acoustic-mechanical effect on sterlet sperm, it was showed that different parameters of time, frequency, and wavelength can have both positive and negative effects on the reproductive qualities of thawed sperm. It was observed that an increase in the exposure time above 2 min and a frequency up to 5 kHz and a change in the wavelength lead to severe cell damage after defrosting [56].

One of the other current issues studied in sperm cryopreservation in sturgeon is the effect of organotin components (OTs) on fresh and frozen sperm. It has been reported that the accumulation of OTs in the gonad in Russian sturgeon is a stress factor affecting the cells in the cryopreservation process and also this buildup may cause in vitro oxidative stress in sturgeon sperm, reduce gamete quality, and affect fertilization success [57].

2.3 Eel sperm cryopreservation

Eels are species that contain species of economic importance for fisheries and aquaculture, and have reduced natural stocks such as sturgeons. Since the 1980s, natural stocks have been reported to have decreased by 90% for the populations of European eel (*Anguilla anguilla*) and Japanese eel (*A. japonica*) due to climate change, habitat degradation, pollution, parasite infection, and overfishing [58]. All two temperate eel species have been included in the Red List of the International Union for Conservation of Nature (IUCN) as threatened due to population decline, with *A. japonica* categorized as "Endangered" [59] and *A. anguilla* included as "Critically Endangered" [60], which is the highest category before extinction rating. As mentioned earlier, one of the most common methods of protection in endangered species and species where there are difficulties in the reproductive cycle, such as eels,

is cryopreservation. Sperm freezing in eels was first tried in Japanese eel in the 2000s [61]. And after this study, a lot of studies have been done on freezing the sperm of eels, including the sperm of European eels, with the development of protocols being prioritized [62].

The protocol first developed for sperm freezing of eels was the protocol in which DMSO was used as a cryoprotectant in both species. In cryopreservation study using DMSO, post-thawed motility was 45% and above in Japanese eel; in European eels, it has been reported as 35% and above [61]. Although the post-hawed motility value is considered high; because sperm cells frozen using DMSO gives low rates of fertilization rate, and at the same time, with recent studies, DMSO causes epigenetic changes in eel sperm; the use of methanol instead of DMSO as a cryoprotectant substance in the freezing of eel sperm has become widespread. And studies have shown that the use of methanol as a cryoprotectant in the freezing of both Japanese and European eel sperm has led to an increase in motility values after thawed [61, 63]. In another study conducted on European eel; with the use of 5% egg yolk with methanol (10%) as cryoprotectant, it was reported that the motility after thawing was higher than 50%. And this value was found to be significantly higher than the values obtained by using 10% methanol used as a control group in the study [64].

In a study with Japanese eel; artificial seminal plasma (in mM; 149.3 NaCl, 15.2 KCl, 1.3 $CaCl_2$, 1.6 $MgCl_2$, and 20 $NaHCO_3$, buffered with 20 mM TAPS-NaOH at pH 8.1) and methanol (in 1:100 ratio) and 10% methanol in v/v final concentration were tested as extenders and cryoprotectants for sperm freezing. As a result of the fertilization study, although the embryos obtained with cryopreserved sperm had a low survival rate and a high malformation rate; it has been reported that this freezing procedure can be used successfully [65]. In another study conducted on a Japanese eel, three different cryoprotectants were tried to freeze sperm cells stored by creating artificial seminal plasma. At the end of the study, the most successful result was obtained by using 10% and 15% MeOH, in addition to the combination of 5% MeOH and 5% DMA; however, DMSO in artificial seminal plasma has been reported to have no cryopreservation properties and is toxic to sperm [66].

The latest protocols for sperm cryopreservation of European and Japanese eel use methanol as cryoprotectant and they have been adapted to large volumes. In the case of the protocol for Japanese eel sperm, successful fertilization has been achieved and with similar survival rates as with fresh sperm. Moreover, the morphology of the larvae produced with cryopreserved sperm was similar to larvae produced from fresh sperm. In the case of the protocol for European eel sperm, the latest protocol has not been tested for fertilization trials yet, but the motility of frozen–thawed sperm obtained was over 50%, which is the highest ever obtained in this species [67].

2.4 Salmonid sperm cryopreservation

The Salmonidae family consists of important species produced in the world, and total Salmonidae production accounts for <1.8% of the total share of global production [68]. There is an increase in water temperature due to global climate change. Salmonid populations distributed in cold waters are the most studied taxonomic group due to their low tolerance to fluctuations in water temperature. These temperature fluctuations are also thought to affect their reproductive performance [69]. In order to support natural stocks and be used in aquaculture, sperm cryopreservation studies are carried out intensively on Salmonid species. Moreover, the wide distribution of fish species belongs to the Salmonidae family in the world and the fact that

they have been produced for many years has made the species of this family suitable for cryopreservation studies. In the sperm cryopreservation studies conducted to date, post-thawing motility parameters and fertilization rates may vary due to reasons such as differences in freezing procedures, genetic differences in species, and differences in culture conditions. For these reasons, the inability to achieve standardization is one of the most important problems in this field.

The issues summarized below about cryopreservation studies; it will help to understand why standardization on fish-species-specific basis cannot be achieved.

Sperm motility parameters after thawing straws containing cryopreserved sperm; fertilization and hatching rate, straws volume, chemicals, cryoprotectants, spermatozoa density, and reproductive season can be affected by factors. One of the first steps in starting cryopreservation is the choice of materials to be used. In cryopreservation of fish sperm, 0.25 or 0.5 mL straws are usually used. In the selection of the straws to be used; it should be ensured that it is in a volume that will not reduce the motility rate after thawing and will allow the fertilization process to be done easily. When using straws of 0.5 mL; it is preferred because sperm motility parameters after thawing are high and save time during fertilization (*Salmo salar*, [70]; *Salmo trutta* m. *trutta*, *S. salar*, *Salvelinus fontinalis*, S.t. m. *fario*, [31]). In addition to the straws volume change, the glucose rate used in the extender (as it changes the osmotic pressure value) affects sperm motility [31]. In the extender of sperm cryopreservation of salmonid species, sucrose, trehalose, and glucose [71–73] are used, however, mostly glucose is preferred [22, 31, 70, 71, 74]. Sperm concentration in the straws also affects sperm motility parameters. However, this sperm concentration may even differ between species belong to the Salmonidae family. In rainbow trout ($0.5–1.0 \times 10^9$ spz/mL) [75]; sperm concentration in straw, where the survival rate after thawing is the highest, is significantly lower than in other Salmonid species ($2.0, 3.0, 4.0 \times 10^9$ spz/mL; *S. fontinalis,* S. *trutta*, *S. salar*, respectively) [31]. Due to cryopreservation process, cells are subjected to stress due to imbalances in low temperature and osmotic pressure. In order to reduce this stress on the cell and to protect sperm cells from freezing effects, various cryoprotectant (can/cannot penetrate into the cell) agents are used. In 2017, in ref. [76] listed cryoprotectant agents under the headings "Alcohols and Derivatives; Sugars and Sugar alcohols; Polymers, Sulfoxides, and Amides; Amines".

Although the success in fertilization and motility rates with cryopreserved fish sperm to date has been achieved with 10% DMSO [38]; in *S. salar*, the motility and fertilization success rate is higher in 10% methanol than 10% DMSO [77]. Although the egg yolk used in the extender creates difficulty in use due to its viscous structure after thawing, it is a popular cryoprotectant (not penetrate into the cell) used for freezing and storing sperm of various species. The addition of egg yolk and sucrose to the extender together with cryoprotectant agents with penetrating properties into the cell significantly improves sperm quality [78]. Various antioxidants are used in the extender to prevent lipid peroxidation and Reactive Oxygen Species (ROS) activity that may occur during cryopreservation [22, 79, 80]. The addition of α-tocopherol and ascorbic acid to extender lead to a decrease in membrane lipoperoxidation and O2 – i production of *S. salar* spermatozoa, thereby increasing the fertilization capacity [80]. The addition of ascorbic acid to the extender in *S. salar* increases the cell integrity and sperm function of spermatozoa [80]. A decrease in sperm motility after thawing can be associated with membrane permeability and DNA damage. ROS, which affects sperm motility in cryopreservation studies, may also induce lipid peroxidation in the membrane. This can lead to the induction of cell apoptosis [81]. In salmonids, there is a positive correlation between the mitochondrial membrane permeability of

cryopreserved sperm and fertilization [70]. The initiation of sperm motility and the duration of motility depend on the ATP provided by mitochondria in most fish [82]. Therefore, any damage to the mitochondria has a negative effect on motility [70] and can limit the motility and fertilization potential of spermatozoa [83].

Potassium ion has an important place in cryopreservation studies because it has the ability to inhibit sperm motility of Salmonid species based on this inhibitory property, the addition of potassium ions to the diluent has a species-specific effect on Salmonid sperm motility after thawing. Although potassium ion negatively affects the percentage of motility after thawing on the species *O. mykiss*, *S. trutta*, *S. fontinalis*; it showed a positive effect on sperm *Coregonus lavaretus*. This effect of the potassium ion is thought to be due to the osmotic pressure, not the concentration used [84]. This has created a similar situation, such as glucose's ability to change osmotic pressure [31]. In sperm during reproductive season and outside the reproductive season, sperm parameters may differ after thawing in sex-reversed rainbow trout [85], however, it is also stated that sperm collection season does not affect post-thawing motility [71]. These different results in the studies depend on the difference in the extender contents used in cryopreservation, the differences in fish strains [71], and the interaction between the extender and the cryoprotectant substance [86].

One of the goals of cryopreservation applications is that this application is commercialized. For this reason, the creation of a cheap and easily prepared cryomedium will be one of the most important factors in the spread of the application. In addition, starting from the studies aimed at spreading the use of natural products in many areas today, non-chemical methods that have the opportunity to be standardized in sperm cryopreservation can be turned to. This allows the use of minimal chemicals in cryomedium to be emphasized. A prototype of the magnetic field that already exists in nature can be created in the laboratory with the help of a magnet, and the motility parameter values of sperm cells can be increased [87]. It has been suggested that the magnetic field may have an effect on the permeability of the sperm cell [88]. Magnetized sperm or water can be used in cryopreservation trials.

2.5 Catfish sperm cryopreservation

Catfish, which are accepted to have more than 3000 species in the world, show a rapid development among cultured species. However, due to the increase in feed and fuel prices, the production of these fish is also adversely affected. In order to increase the production of these species, hybrid species (with specialty fast growth, disease resistance, and efficient growth rate) are obtained. Since the small number of male fish is asynchronous and the killing of fish is mandatory for sperm collection, sperm cryopreservation provides an important opportunity for these species [89]. Studies have also been carried out to increase sperm motility values and fertilization capacity after cryopreservation in catfish. The rate and temperature of thawing straws after cryopreservation are also parameters that affect sperm motility. In *Ictalurus furcatus*, it is the thawing temperatures that give positive results in motility parameters of 7 and 20 s at 40°C and 40 s at 20°C when using 0.5 mL straws [89, 90]. When straws were thawed for 20 s at 20°C or 40°C, it was observed that the sperm motility results after thawing were similar [89]. In addition to the use of small volumes of straw, catfish sperm cryopreservation was performed in large volumes (1 L) bags using dairy cryopreservation technology, and this method was successfully adapted to catfish [90]. Like studies with salmonid species, egg yolk has been used for sperm cryopreservation in catfish. In *Clarias gariepinus*, 10% egg yolk prevented sperm cell

damage during cryopreservation and thawing processes. It also showed protection against adverse environmental conditions such as temperature, pH and osmotic pressure changes, and against the accumulation of harmful substances caused by the toxicity of cryoprotectant substances. Although fertilization and hatching rates can be achieved in *C. garipionus* without the use of intracellular cryoprotectant agents, this rate is low compared to the use of intracellular cryoprotectant agents such as DMSO [91]. In the extender (365–385 mOsm/kg) in which 10% egg yolk was used with glucose or NaCl in *Silurus triostegus*, cryopreservation was successful in the evaluation of motility parameters after thawing. Necrotic cells were observed in the use of glucose-containing extenders (325 mOsm/kg) with low osmotic pressure [92]. In order to develop standardized protocols for sperm cryopreservation, knowing the sperm concentration is important for the viability rate to be obtained after thawing [89]. This, in turn, can greatly improve the effectiveness of cryopreservation sperm use during the fertilization process. In most of the cryopreservation studies of catfish sperm, motility after dissolution was similar to 1×10^8 spz/mL if 1×10^9 cell/mL was used. It was observed that sperm solutions became viscous at a concentration of 1.7×10^9 spz/mL [89]. Cryopreservation; in the processes of cooling, freezing, and thawing, some biophysical and chemical events occur, such as osmotic changes, dehydration and rehydration, changes in cell volume, formation of ice crystals, and toxicity from cryoprotectant. Sperm cells, which have different characteristics specific to the species, are sensitive to these changes. Therefore, consensus should be achieved between species-specific cryopreservation protocols. An increasing number of studies explaining methods of cryopreservation of sperm in many species are proving this diversity [12]. Cryopreservation has been shown to have detrimental effects on the plasma membrane, mitochondria, chromatin structure, osmotic control, and spermatozoa motility [93, 94]. The cryopreservation process can lead to apoptosis and mitochondrial dysfunction [95], and studies have been carried out at the molecular level in recent years to determine the effect of cryoinjury [96, 97]. After thawing, sperm motility parameters in most species, including *I. furcatus*, show a decrease. This reduction in motility parameters can reduce fertilization potentials and three times higher oxidative stress level has been determined. It indicates that sperm quality may deteriorate after cryopreservation due to a 4-fold increase in the DNA fragmentation level of sperm after thawing [96]. One of the effects of cryopreservation on sperm cells is the increase of apoptotic cells [98, 99]. In order to reduce these effects, "amide" has been used in recent years as a cryoprotector for the protection of sperm [100].

Cryopreservation increases the oxidative stress level and DNA fragmentation of the sperm and thus decreases the sperm kinematic parameter values. Transcriptome analyses are also performed to determine the cryodamage caused by cryopreservation in sperm cells. In these analyses, upregulated genes were identified in sperm samples after thawing and an increase in oxidative phosphorylation activities leading to excessive production of ROS associated with cell death was detected. Despite these negative results, the presence of the potential of sperm to fertilize eggs after thawing is expressed in the fact that compensatory processes occur in the gene expression of sperm cells after thawing to offset these harmful effects (MnSOD, induction to control ROS production; correction of misfolded proteins; apoptosis, functions related to amide biosynthesis) [96]. Sperm cryopreservation can affect several biological processes, including apoptosis, spermatogenesis, mitochondrial activity, ROS production, amide biosynthesis, protein folding, and degradation [96]. Therefore, the effect of cryopreservation is quite complex, with both harmful and compensatory effects on

sperm quality. In addition to the level of gene expression, cryopreservation can also affect DNA methylation, which has been identified as 1266 differentially methylated genes in sperm methyloma [97].

3. Germ cell cryopreservation

Freezing and storage of gametes is used to protect endangered species as well as to ensure sustainability in aquaculture applications. While only sperm cryopreservation is performed in fish, germ cell cryopreservation, which has the feature of differentiation to both gamete types, can be successfully performed. Although cryopreservation and long-term storage of sperm is a technique that has been practiced for a long time, it is impossible to ensure the continuity of generation by using sperm alone. Therefore, germ cell cryopreservation offers an important opportunity in achieving the above-mentioned goals [101].

Germline stem cells isolated from immature gametes can be intraperitoneal transplantation of immunologically immature newly hatched larvae into the body cavity [102]. In addition to the larvae, germline stem cells can be transferred to broodstock fish and embryos [103]. Germline stem cells, transferred to the larvae, migrate to the genital ridge, multiply and initiate spermatogenesis or oogenesis. Cryopreservation of germ cells is performed for transplantation. For this purpose, in cryopreservation germ cells; slow freezing and vitrification methods are used. After transplantation of germ cells, frozen by both methods, to the larvae, there is no significant difference in the rate of migration to the genital ridge and their reproduction compared to the control group [104]. In addition to cryopreservation of all testis tissue, germ cell isolation can be performed from immature gonads after dissection of fish. Freezing of all testicular tissue; while it is made using immature gonads that are frozen in a freezer and stored without the help of exogenous cryoprotectant [105], it can also be done using cryoprotectant [106].

Germ cell isolation in fish can be performed from all cryopreserved testicular tissue as well as from immature gonads. The differentiation of spermatogonia to the ova after the transfer of spermatogonia isolated from cryopreservation testis tissue to the female recipient fish provides a definitive solution to continuity of the species. As a result of isolation from rainbow trout testis tissue in the absence of cryopreservation and the presence of dead fish, transplantation efficiency was found to be 90.61 ± 5.26%, 82.22% ± 11.76%, 73.33% ± 3.33%, and 6.68 ± 6.66%, respectively [107].

In the cryopreservation of oogonia; DMSO, methanol, glycerol, ethylene glycol, and egg yolk are used as cryoprotectants. Between these, the use of DMSO, which is a cryoprotectant that has the ability to penetrate into the cell, gives the best rate on both motility and fertilization rate. Although egg yolk does not have penetrating properties, success has been observed in its use with lactose [108]. DMSO; has been identified as the cryoprotectant substance with the most successful results for cryopreservation of spermatogonial stem cells of *Oncorhynchus mykiss*, type A spermatogonia of *I. furcatus* [109], ovary of *C. carpio* [110], and oogonia [108].

In sperm cryopreservation, evaluation of motility parameters without determining the post-thawing observation and hatching rate will be incomplete in terms of determining the success of the experiment. In addition, the development of germ cell cryopreservation procedures without germ cell transplantation does not yield results. Since spermatogonia/oogonia has sexual plasticity (the ability to produce both eggs and sperm), these mitotic germ cells can be stored by freezing. This application will

be an alternative to sperm cryopreservation as well as freezing and storing fish eggs or embryos [111]. Reducing the complicated steps in germ cell cryopreservation as much as possible (reducing the use of chemicals, applying some of the experimental stages in fish farms, and easy transferability of samples) will accelerate the conservation of species, which is an urgent global problem.

4. Cryopreservation of fish embryo

It is an important issue to use gametes obtained by aquaculture to support natural stocks and, to make cryopreservation techniques that provide long-term protection of these gametes available in all species and reproductive cells of species. Sperm cells, due to its small size and greater durability during freezing, have given more successful results than other reproductive cells, and these features have made sperm the most researched cell in the cryopreservation of fish gamete. Freezing sperm cells is successfully practiced in many fish species, and a protocol has been established for almost cultured fish [38]. However, still, egg freezing has not been successful because of its features such as dehydration problems, large volume, and different membrane permeability. For this reason, many studies have focused on freezing fish oocytes and ovarian follicle. The reasons for the increase in oocytes and ovarian follicle cryopreservation studies are that these cells have a small volume, high membrane permeability, membrane systems are simpler and less sensitive to freezing [112, 113].

The cryopreservation of embryo, which allows the storage of both the female and male genomes, has been a challenging subject of cryopreservation studies for many years. In terms of aquaculture, successful fish embryo cryopreservation will significantly facilitate the establishment and management of genetic selection programs in fish farms [38]. Fish embryos have a low surface-to-volume ratio. It also has a large volume of yolk and a low rate of membrane permeability. In addition to these features of fish embryos, their high sensitivity to low temperatures has made it difficult to use and achieve success in cryopreservation studies [113, 114]. However, despite all these difficulties, there are studies on embryo freezing in fish. Studies have been carried out on chilling and cryopreservation of embryos in 20 different teleost fish [115–117]. The first study on cryopreservation fish embryos (slow-freezing method) was tried and successfully recorded in carp fish embryos in 1989 [118], however, a complete standard has not been established yet.

One of the important issues in the cryopreservation of fish embryos is the toxicity and penetration of the cryoprotectant to be used. In addition to the freezing procedure in the cryopreservation of fish embryos, there are also studies on the selection of cryoprotectants to be used [38]. In an embryo-freezing study with zebrafish, known as model fish, methanol was found to be more effective cryoprotectant compared to DMSO and Ethanediol [119]. In another embryo freezing study using vitrification in zebrafish, it was reported that the embryos survived for 3 h after thawing [120]. In Ref. [121], it was observed that continuity in the development seen only 2.96% seven-band grouper (*Epinephelus septemfasciatus*) embryos, postvitrification. In a recent review study on fish embryo freezing, the issues that need to be developed in relation to the vitrification method that is widely used and tried in embryo freezing are systematically given. In this review, the issues that need to be considered for the development of vitrification protocol are listed as cryoprotectant toxicity, developmental stage of the embryo, and the conditions at the time the embryo to be frozen is treated with cryoprotectant and vitrification [122]. Most of the studies focused

on fish embryo vitrification within the area of toxicity of vitrification solutions. In 2006, it was reported that Japanese flounder (*Paralichthys olivaceus*) embryos were successfully cryopreserved by using vitrification method [123]. Fish embryos show different sensitivity to cryoprotectant permeability at each developmental stage. With this feature that embryos have, some studies have been conducted using various storage methods to determine cryoprotectant flux or concentration at different stages during embryo development [122]. In study conducted on carp, it was reported that the hatching rate was 41% if sucrose was used to protect carp embryos in −4°C. In the same study, it was reported that the use of sucrose and methanol together gave the best results [124]. Recently, in the embryo freezing study, where two different cryoprotectants (DMSO and Methanol) were tested in carp, it was stated that the use of two cryoprotectant substances together had a protective effect by keeping the embryos at −2°C for 1 day and the average larval survival rate was 12.38% [125]. Another study showed that 9.7% of the embryos continued their lives for 2 h by freezing the embryos in liquid nitrogen (−196°C) in *Epinephelus moara* [126]. In a study with carp, using Modified Haga's solution; the toxicity of DMSO and glycerol and its effect on the survival rate of embryos were examined. In the same study where the effect of embryo freezing on the survival rate of the application of different developmental stages was investigated, it was stated that carp embryos were successfully frozen (−196°C) [127].

In cryopreservation of fish embryo studies, the highest success achieved so far has been seen in Persian sturgeon (*A. persicus*) with a hatching rate of 45.45%. In this study, where the vitrification technique was applied, DMSO was selected as a cryoprotectant [128]. Another study whose results were successfully stated was the one which propylene glycol was injected into the zebrafish embryos in freezing and the samples were frozen in liquid nitrogen with a rate of 90,000°C/min. In this study, the survival rate of the thawing embryos was found to be 10% after 24 h post-thawing [129].

Despite all the studies and efforts made on the freezing of fish embryos, unlike fish sperms and germ cells, the work done to prevent crystallization and biological damage during freezing/thawing of fish embryos is still a challenging topic. However, recent studies with cryopreservation primordial germ cells look promising when storing both female and male genomes. Such studies can be used in cryopreservation studies until an undisputed result is obtained in terms of embryo freezing in fish and serve the purpose of cryopreservation.

5. Prevention of disease transmission

5.1 Contamination during the fish gamete cryopreservation and thawing process

The cryopreservation method is one of the assisted reproductive techniques (ART) and has been in use for many years to serve needs such as infertility treatment or genetic improvement and preservation or transportation in living things. Performing this method under aseptic conditions is one of the important factors in the preservation of gametes without microbial contamination and resulting in successful fertilization [130, 131].

The main sources of contamination encountered in cryopreservation studies are (1) Dewar, (2) Cell, (3) Liquid Nitrogen (LN), and (4) Handling (extender, supply, etc.) [131–134]. When the LN container is opened, the upper part begins to cool, and the water in the air turns into ice crystals with a high electrostatic charge, catching the

microorganisms in the air. It is known that these ice crystals fall into the LN container and cause the accumulation of microorganisms in masses at the bottom and can combine with cell residues in the environment. In a study conducted on LN container that was used continuously for 7 and 12 years, it was shown that the contamination intensity did not depend on the year in use, but the microbial diversity was different in each of them [134].

Bacteria are single-celled organisms that have been cryopreserved for extensive research since 1950 [135]. Bacteria are not affected by cold when stored in suitable conditions specific to the species and can reproduce when thawed. For long-term storage at −20°C causes the death of perishable bacteria [136], while bacteria are usually stored in a −80°C freezer with little loss, longer and more appropriate storage is provided with liquid nitrogen and vapor phase (140–196°C) [137]. Cryopreservation is also used for other microorganisms such as viruses and fungi for research purposes [138]. In gamete cryopreservation studies, cryoprotectant agents are added to the extender to protect the cell from freezing. The cryoprotectants and nutritional elements used to store microorganisms at −196°C are very similar to the solutions used to store sperm cells. Therefore, while the gamete cells are frozen, microorganisms can be frozen together with the cells unintentionally [139–141].

Also, infected gamete samples can contaminate other pathogen-free samples when stored in the same LN container. Especially in human sperm storage conditions, cross-contamination poses a major problem. Storing the sperm of an individual carrying the disease and the sperm samples of an individual who is not diseased in the same LN container causes a healthy parent to have a diseased child. It has been shown in studies that liquid nitrogen can contaminate the samples to be placed in contaminated with bacteria and viruses [142].

As we mentioned above, during the transfer of LN used in cryopreservation, microorganisms present in atmospheric air can contaminate liquid nitrogen [134, 143]. Although the fact that many microorganisms in the air are not fish pathogens does not seem to be an important problem in terms of disease transmission, the presence of bacteria in the environment where sperm is present is an important factor that impairs its quality [144]. In other words, microorganisms use the nutrients and oxygen in the environment, which are necessary for the survival of the sperm, as well as changing the pH of the environment [8], and this affects the quality of the sperm and changes its fertilization capacity [145]. In addition, the most important problem will be the use of gamete cells contaminated with the microorganism in fertilization, the transmission of pathogens to the embryo, and the emergence of diseased offspring [146].

5.2 Disease transmission via gametes to embryo

Research that has been conducted on sperm and embryo has found a relationship with pathogens such as viral, bacterial, fungal, and parasitic organisms [147]. It has been reported that some of them are in the seminal plasma, attach to the sperm cells, but do not enter the cell, and attach to the egg from the zona pellucida [142, 148].

To summarize briefly, the literature on vertical transmission of fish pathogens; It has been proved that the infected pancreatic necrosis (IPN) virus is present in the ovarian fluid and can be attached to the salmonid sperm and transmitted vertically to the embryo [149–152]. Viral hemorrhagic septicemia virus (VHSV) and Infectious hematopoietic necrosis virus (IHNV) have been isolated from the seminal and ovarian fluid of salmonid species [153, 154]. In addition, some bacteria have been shown by staining sperm samples, and *Aeromonas* sp., *Pseudomonas*

sp., and *Flavobacterium* spp. have been detected in the seminal plasma of trout using culture methods [144, 155, 156]. *Renibacterium salmoninarum* is bind to tail of sperm and has been found intra-ovum of rainbow trout [146]. In a study showing that *Piscirickettsia salmonis* can be found in the seminal fluid of trout, it was shown that vertical transmission is also possible [157].

5.3 Eliminate non-cellular disease agents from gametes

It has long been known that semen can contain a variety of viruses, and good safety protection systems and methods have been established in many laboratories to reduce virus particles from semen obtained from humans with viral diseases [158]. Viral Infection is recognized as a possible cause of male infertility in humans [159]. Among HIV-infected men, vertical transmission to infants has been reported when in vitro fertilization is performed after sperm washing [160].

However, as far as we know, there is no study on the inhibition of the transmission of viruses from sperm to embryo in fish. It is known as general information that the viral titer decreases after the freezing–thawing process [146], so the viral load in the sperm can be lowered by cryopreservation.

5.4 Antibiotics

Although antibiotics are used to eliminate bacterial contamination in sperm freezing, it should also be considered that they are harmful to sperm [8, 161]. Although there are studies in which antimicrobial biomolecules (resveratrol, curcumin, etc.) are used instead of antibiotics, these have not yet taken the place of antibiotics [162].

5.5 Sperm washing and cell separating methods

Density gradient centrifuge method is a method that allows cells to be separated according to their size. Although this method is frequently used for sperm washing in mammals, that is, for removing microorganisms, its applications in fish are generally based on separating quality sperm cells from all other foreign substances [163]. Although the "swim-up" method, which is one of the sperm washing methods, is also used in mammalian sperm cells, this method does not work in fish since motile sperm must be used in this method [155].

During the process of sperm washing, freezing, and thawing, in order to obtain a high concentration of quality spermatozoa, it is critical that we reduce and remove any risk or chance of pathogen contamination during the preservation of frozen sperm in tanks. While sperm washing is usually performed before cryopreservation of mammalian sperm [164], in sperm washing studies applied in fish, since 2010, the washing process is performed after thawing [165–167]. In some species, it is used to separate seminal plasma from cells by centrifugation before freezing [168]. In the study in Ref. [168], firstly, seminal plasma was separated by centrifugation, and then live sperm cells were separated from the dead by magnetic selective. As a result of this study, it was possible to obtain better quality and functional sperm.

Studies using sperm washing method in fish have increased, especially in recent years. In the first study on this subject, in which frozen sperm from carp were used, it was determined that the sperm washed using the percoll gradient method had high motility, and the spermatozoa, which were immobile and whose membrane was damaged during the freezing–thawing process, were also removed. As a result, it is stated

that the use of this technique will allow easier and higher quality spermatozoa to be obtained compared to other biotechnological cell separation methods [169]. Another study conducted in sturgeon includes the evaluation of sperm motility parameters and sperm characteristics after gradient centrifugation method of control and frozen sperm samples [165]. In a freeze–thaw washing study performed with testicular sperm, the fertilization rate was found to be higher than the control [170]. In the only study in which sperm washing was used to remove bacterial load, it was observed that gradient centrifugation method reduced bacterial load, although it caused a decrease in motility rates in rainbow trout sperm [155]. Again, according to the results of the study by the same authors on the relationship between newly completed sperm washing and fertilization success, low motility after washing did not significantly change the fertilization success and survival rate (unpublished data).

In studies using the density gradient centrifugation method, percoll was the most widely used gradient-forming chemical [169, 170]. Sil-Select Plus TM [155] and AllGrad® 90% [167], which are commercial solutions produced in humans, have been tried in fish and successful results have been obtained.

5.6 Disinfection of gametes and embryo

When the sperm are treated with a disinfectant, the sperm cell loses its vitality. However, iodine and anti-fungal treatment of eggs with formaldehyde is routinely and repeatedly used during the eyed stage of salmonids [146, 171]. Disinfection of eggs with iodophor prevents some viruses (VHSV, etc.) and bacteria (*Flavobacterium psychrophilum*, *Yersinia ruckeri*, etc.) from passing into the embryo, while it cannot provide protection for others (IPNV, *R. salmoninarum*, etc.) [146]. According to the literature, no studies were found regarding the presence or solution of contamination in frozen embryos or eggs (perhaps because embryo and egg cryopreservation is a new subject).

5.7 Suggestions

In cryopreservation facilities, it has been reported that the microbial load should be controlled before freezing sperm in order to prevent cross-contamination in liquid nitrogen [172]. Freezing sperm from brood stocks that are not infected or have the least pathogen in their sperm will be the best solution. This can be possible with continuous health checks and monitoring of fish. The use of vaccinated brood stocks to prevent the spread of the disease with high mortality would be excellent for sustainability. Sperm cells of brood stocks infected with a possible pathogen can be separated from the pathogen with the sperm washing method and then frozen. This is also promising technique in obtaining quality sperm. In addition, continuous disinfection and use of tanks, UV sterilization of liquid nitrogen used in small volumes, sterilization of all materials used, and sealing the ends of the straws by burning will prevent possible contamination [133].

Establishing special sperm collection laboratories for cryopreservation would also be a good solution to prevent contamination. The use of a common laboratory with professional staff, where special Biosafety measures are taken and hygiene rules are followed, will be beneficial for every fish farm [131]. There are cryobanks where gametes are stored in different countries, but since the number is not sufficient, it would be beneficial to create an easily accessible cryobank for each country [38].

Cryostorage facilities are expensive and may not be affordable or readily available due to their constant need for electrical power and liquid nitrogen production [173]. Additionally, monitoring liquid nitrogen levels is a time-consuming and expensive process that requires constant visual inspection. Therefore, cryostorage systems have recently been said to be prone to failures leading to undesired sample losses [174]. Liquid nitrogen is seen as a pathogenic transmissible agent, and its production is also a high carbon footprint procedure [173]. The most convenient way to prevent LN contamination is to use filtered (sterile) LN. However, this application is not suitable for use in practice due to its high cost. It is recommended to find a cheaper, simpler, greener alternative [173]. Another alternative is to use ultra-low freezers at −150°C, which has increased production today, instead of liquid nitrogen tanks, and it is thought that the risk of nitrogen vapor contamination can be reduced [175]. And even in a recent study in zebra fish, an ultrafreezer of −150°C was used for sperm cryopreservation and successful result was achieved [176].

It has also been reported that freeze-dry technology can eliminate the difficulties and contamination risk of cryo-storage using liquid nitrogen in today's technology [173, 174]. In fact, some mammalian spermatozoa have been studied with this method [173, 174, 177]. In these studies, sperm motility is not important and efforts are made to prevent DNA damage of sperm. Therefore, this method can be used for large mammals in the intracytoplasmic sperm injection (ICSI) method, but it does not seem possible to apply it to animals with multiple eggs such as fish [178]. In the literature, there has not yet been a study in which this technology is used in fish species.

Although the OIE has a "Health code" for terrestrial animals on the prevention of the risk of transmission of cryopreserved sperm-borne disease, there is no such information in the "Aquatic Animal Health Code" [171]. In the future, it will be useful to come up with standard methods for aquatic organisms.

6. Conclusions

Cryopreservation applications are one of the important assisted reproductive techniques that can be used to maintain the vitality of an organism. When the subject is to ensure the sustainability of a living thing, external factors such as its own biological, physiological, genetic, and epigenetic characteristics as well as its feeding regime, living environment, and presence of pollutants are also effective. For this reason, standardization of all these differences while conducting research is very difficult (perhaps impossible with current practices) and requires long time-consuming studies. However, in order to eliminate the negative effects of cryopreservation on the cell, the standardization of the factors mentioned above, as well as the laboratory environment, ensures the quality of the cells to be obtained. In reproductive biotechnology studies, laboratory studies should be carried out under sterile conditions after obtaining eggs and sperm from the fish broodstock.

Ensuring the importance shown in aseptic laboratory environment also allows the cells obtained to increase the viability rate of the offspring, as well as to be healthy. Fish farms carrying out these practices should be certified and sample entry from non-certified farms should not be allowed. A living organism is in constant contact with its environment and is constantly under this influence. Breeding and fish health practices are subjects that cannot be considered independently of each other. In this context, if it is desired to ensure the continuity of high quality, fast growing, and healthy generations, it is not possible for these two fields to be independent from each other.

Although the concern and necessity of this issue have been stated in the studies, the use of sterile environments has not been actively provided yet. Informing the people working in fish production farms on this subject and organizing meetings/seminars that emphasize the importance of the subject and organizing trainings/courses would be beneficial. In addition, these practices may be made compulsory by various regulations.

Author details

Aygül Ekici*, Güneş Yamaner and Menekşe Didem Demircan
İstanbul University, Faculty of Aquatic Sciences, Department of Aquaculture and Fish Diseases, İstanbul, Turkey

*Address all correspondence to: aekici@istanbul.edu.tr

IntechOpen

References

[1] Carolsfeld J, Harvey B, Godinho HP, Zaniboni-Filho E. Cryopreservation of sperm in Brazilian migratory fish conservation. Journal of Fish Biology. 2003;**63**:472-481. DOI: 10.1046/j.1095-8649.2003.00170.x

[2] Hu E, Tiersch T.R. Development of high-throughput cryopreservation for aquatic species. In: Tiersch T.R, Green C.C, editors. Cryopreservation in Aquatic Species. 2nd ed. World Aquaculture Society, Baton Rouge, LO; 2011. p. 995-1003

[3] Blaxter JHS. Sperm storage and cross-fertilization of spring and autumn spawning herring. Nature. 1953;**172**:1189-1190. DOI: 10.1038/1721189b0

[4] Labbé C, Robles V, Herráez MP. Cryopreservation of gametes for aquaculture and alternative cell sources for genome preservation. In: Allan G, Burnell G, editors. Advances in Aquaculture Hatchery Technology. Woodhead Publishing; Sawston, Cambridge, UK. 2013. pp. 76-116. DOI: 10.1533/9780857097460.1.76

[5] Cabrita E, Robles V, Herráez M.P, editors. Methods in Reproductive Aquaculture: Marine and Freshwater Species. CRC Press, Boca Raton, FL, USA 2008. 549 p. ISBN: 13: 978-0-8493-8053-2

[6] Tiersch TR. Process pathways for sperm cryopreservation research, application, and commercialization. In: Tiersch TR, Green C, editors. Cryopreservation in Aquatic Species. 2nd ed. Baton Rouge, LO: World Aquaculture Society; 2011. pp. 646-671

[7] Kopeika E, Kopeika J, Zhang T. Cryopreservation of fish sperm. Methods in Molecular Biology. 2007;**368**:203-217. DOI: 10.1007/978-1-59745-362-2_14

[8] Jawahar KTP, Betsy J. Cryopreservation of fish gametes: An overview. In: Betsy J, Kumar S, editors. Cryopreservation of Fish Gametes; Springer. 2020. pp. 151-175. DOI: 10.1007/978-981-15-4025-7

[9] Judycka S, Żarski D, Dietrich M.A, Palińska-Żarska K, Karol H, Ciereszko A. Standardized cryopreservation protocol of European perch (*Perca fluviatilis*) semen allows to obtain high fertilization rates with the use of frozen/thawed semen. Aquaculture2019;498:208-216. DOI: 10.1016/j.aquaculture.2018.08.059

[10] www.cryogenetics.com [Accessed: September 7, 2022]

[11] www.imv-technologies.com [Accessed: September 7, 2022]

[12] Asturiano JF, Cabrita E, Horváth Á. Progress, challenges and perspectives on fish gamete cryopreservation: A mini-review. General and Comparative Endocrinology. 2017;**245**:69-76. DOI: 10.1016/j.ygcen.2016.06.019

[13] The State of World Fisheries and Aquaculture.2022. Available from: https://www.fao.org/fishery/en/culturedspecies/cyprinus_carpio/en [Accessed: September 6, 2022]

[14] Balon EK. Origin and domestication of the wild carp, *Cyprinus carpio*: From Roman gourmets to the swimming flowers. Aquaculture. 1995;**129**:3-48. DOI: 10.1016/0044-8486(94)00227-F

[15] Lubzens E, Rothbard S, Hadani A. Cryopreservation and viability of spermatozoa from the ornamental Japanese carp. Israeli Journal of Aquaculture. 1993;**45**:169-174

[16] Gwo JC, Kurokura H, Hirano R. Cryopreservation of spermatozoa from rainbow trout, common carp and marine puffer. Bulletin of the Japanese Society of Scientific Fisheries. 1993;**59**:777-782. DOI: 10.2331/suisan.59.777

[17] Linhart O, Rodina M, Cosson J. Cryopreservation of sperm common carp *Cyprinus carpio*: Sperm motility and hatching success of embryos. Cryobiology. 2000;**2000**(41):241-250. DOI: 10.1006/cryo.2000.2284

[18] Horváth Á, Miskolczi E, Urbányi B. Cryopreservation of common carp sperm. Aquatic Living Resources. 2003;**16**:457-460. DOI: 10.1016/S0990-7440(03)00084-6

[19] Warnecke D, Pluta HJ. Motility and fertilizing capacity of frozen/thawed common carp (*Cyprinus carpio* L.) sperm using dimethyl-acetamide as the main cryoprotectant. Aquaculture. 2003;**215**:167-185. DOI: 10.1016/S0044-8486(02)00371-X

[20] Havlar E, Bozkurt Y. Protective effects of different egg yolk sources on cryopreservation of scaly carp (*Cyprinus carpio*) sperm. Acta Aquatica Turcica. 2022;**18**(3):393-402. DOI: 10.22392/actaquatr.1085283

[21] Cabrita E, Ma S, Diogo P, Martínez-Páramo S, Sarasquete C, Dinis MT. The influence of certain amino acids and vitamins on post-thaw fish sperm motility, viability and DNA fragmentation. Animal Reproduction Science. 2011;**125**:189-195. DOI: 10.1016/j.anireprosci.2011.03.003

[22] Ekici A, Baran A, Yamaner G, Özdas ÖB, Sandal AI, Güven E. Effects of different doses of taurine in the glucose-based extender during cryopreservation of rainbow trout (*Oncorhynchus mykiss*) semen. Biotechnology & Biotechnological Equipment. 2012;**26**:3113-3115. DOI: 10.5504/BBEQ.2012.0041

[23] Rani KU, Munuswamy N. Effect of DNA damage caused by cryopreservation of spermatozoa using a modified single cell gel electrophoresis in the freshwater catfish *Pangasianodon hypophthalmus* (Fowler, 1936). Journal of Coastal Life Medicine. 2014;**2**:515-519. DOI: 10.12980/JCLM.2.2014J15

[24] Valdebenito I, Moreno C, Lozano C, Ubilla A. Effect of L-glutamate and glycine incorporated in activation media, on sperm motility and fertilization rate of rainbow trout (*Oncorhynchus mykiss*) spermatozoa. Journal of Applied Ichthyology. 2010;**26**:702-706. DOI: 10.1111/j.1439-0426.2010.01555.x

[25] Kledmanee K, Taweedet S, Thaijongruk P, Chanapiwat P, Kaeoket K. Effect of L-cysteine on chilled carp (*Cyprinus carpio*) semen qualities. The Thai Journal of Veterinary Medicine. 2013;**43**:91-97

[26] Öğretmen F, İnanan BE, Kutluyer F, Kayim M. Effect of semen extender supplementation with cysteine on postthaw sperm quality, DNA damage, and fertilizing ability in the common carp (*Cyprinus carpio*). Theriogenology. 2015;**83**(9):1548-1552. DOI: 10.1016/j.theriogenology.2015.02.001

[27] Yıldız C, Yavaş I, Bozkurt Y, Aksoy M. Effect of cholesterol-loaded cyclodextrin on cryosurvival and fertility of cryopreserved carp (*Cyprinus carpi*o) sperm. Cryobiology. 2015;**70**(2):90-194. DOI: 10.1016/j.cryobiol.2015.01.009

[28] Horvath A, Bokor Z, Bernath G, Csenki Z, Gorjan A, Herraez MP, et al. Very low sperm–egg ratios result in successful fertilization using cryopreserved sperm in the Adriatic grayling (*Thymallus thymallus*).

Aquaculture. 2015;**435**:75-77. DOI: 10.1016/j.aquaculture.2014.09.032

[29] Molnár J, Bokor Z, Várkonyi L, Izsák T, Füzes-Solymosi E, Láng ZL, et al. The systematic development and optimization of large-scale sperm cryopreservation in northern pike (*Esox lucius*). Cryobiology. 2020;**94**:26-31. DOI: 10.1016/j.cryobiol.2020.05.003

[30] Judycka S, Dietrich MA, Żarski D, Karol H, Palińska-Żarska K, Błażejewski M, et al. Towards standardization of the cryopreservation procedure of cultured pikeperch (*Sander lucioperca*) semen. Aquaculture. 2021;**538**:736-539. DOI: 10.1016/j.aquaculture.2021.736539

[31] Judycka S, Nynca J, Liszewska E, Dobosz S, Grudniewska J, Ciereszko A. Optimal sperm concentration in straws and final glucose concentration in extender are crucial for improving the cryopreservation protocol of salmonid spermatozoa. Aquaculture. 2018;**486**:90-97. DOI: 10.1016/j.aquaculture.2017.12.019

[32] Pataki B, Horváth Á, Mészáros G, Kitanović N, Ács A, Hegyi Á, et al. Adjustment of common carp sperm concentration prior to cryopreservation: Does it matter? Aquaculture Reports. 2022;**24**:101-109. DOI: 10.1016/j.aqrep.2022.101109

[33] Irawan H, Vuthiphandchai V, Nimrat S. The effect of extenders, cryoprotectants and cryopreservation methods on common carp (*Cyprinus carpio*) sperm. Animal Reproduction Science. 2010;**122**(3-4):236-243. DOI: 10.1016/j.anireprosci.2010.08.017

[34] Chebanov MS, Galich EV. Sturgeon hatchery manual. FAO Fisheries and Aquaculture Technical Paper. No. 558. Ankara, FAO. 2011; pp 303

[35] Scarnecchia DL, Lim Y, Ryckman LF, Backes KM, Miller SE, Gangl RS, et al. Virtual population analysis, episodic recruitment, and harvest management of Paddlefish with applications to other Acipenseriform fishes. Reviews in Fisheries Science & Aquaculture. 2014;**221**:16-35. DOI: 10.1080/10641262.2013.830592

[36] Wu H, Chen J, Xu J, Zeng G, Sang L, Liu Q, et al. Effects of dam construction on biodiversity: A review. Journal of Cleaner Production. 2019;**221**:480-489. DOI: 10.1016/j.jclepro.2019.03.001

[37] Bronzi P, Rosenthal H. Present and future sturgeon and caviar production and marketing: A global market overview. Journal of Applied Ichthyology. 2014;**30**(6):1536-1546. DOI: 10.1111/jai.12628

[38] Martínez-Páramo S, Horváth Á, Labbé C, Zhang T, Robles V, Herráez P, et al. Cryobanking of aquatic species. Aquaculture. 2017;**472**:156-177. DOI: 10.1016/j.aquaculture.2016.05.042

[39] Glogowski J, Kolman R, Szczepkowski M, Horváth Á, Urbányi B, Sieczyński P, et al. Fertilization rate of Siberian sturgeon (*Acipenser baeri*, Brandt) milt cryopreserved with methanol. Aquaculture. 2002;**211**(1-4):367-373. DOI: 10.1016/S0044-8486(02)00003-0

[40] Horváth Á, Wayman WR, Urbányi B, Ware KM, Dean JC, Tiersch TR. The relationship of the cryoprotectants methanol and dimethyl sulfoxide and hyperosmotic extenders on sperm cryopreservation of two North-American sturgeon species. Aquaculture. 2005;**247**(1-4):243-251. DOI: 10.1016/j.aquaculture.2005.02.007

[41] Horváth Á, Wayman WR, Dean JC, Urbányi B, Tiersch TR, Mims SD, et al.

Viability and fertilizing capacity of cryopreserved sperm from three North American acipenseriform species: A retrospective study. Journal of Applied Ichthyology. 2008;**24**(4):443-449. DOI: 10.1111/j.1439-0426.2008.01134.x

[42] Huang X, Zhang T, Zhao F, Feng G, Liu J, Yang G, et al. Effects of cryopreservation on acrosin activity and DNA damage of Russian sturgeon (*Acipenser gueldenstaedtii*) semen. CryoLetters. 2021;**42**(3):129-136

[43] Tiersch TR, Green CC, editors. Cryopreservation in Aquatic Species. 2nd ed. Baton Rouge, LO: World Aquaculture Society; 2011. pp. 1-17

[44] Golshahi K, Aramli MS, Nazari RM, Habibi E. Disaccharide supplementation of extenders is an effective means of improving the cryopreservation of semen in sturgeon. Aquaculture. 2018;**486**:261-265. DOI: 10.1016/j.aquaculture.2017.12.045

[45] Malo C, Gil L, Gonzalez N, Cano R, de Blas I, Espinosa E. Comparing sugar type supplementation for cryopreservation of boar semen in egg yolk based extender. Cryobiology. 2010;**61**:17-21. DOI: 10.1016/j.cryobiol.2010.03.008

[46] Gómez-Fernández J, Gómez-Izquierdo E, Tomás C, Mocé E, de Mercado E. Effect of different monosaccharides and disaccharides on boar sperm quality after cryopreservation. Animal Reproduction Science. 2012;**133**(1-2):109-116. DOI: 10.1016/j.anireprosci.2012.06.010

[47] Xi MD, Li P, Du H, Qiao XM, Liu ZG, Wei QW. Disaccharide combinations and the expression of enolase3 and plasma membrane Ca^{2+} ATPase isoform in sturgeon sperm cryopreservation. Reproduction in Domestic Animals. 2018;**53**(2):472-483. DOI: 10.1111/rda.13134

[48] Ubilla A, Valdebenito I. Use of antioxidants on rainbow trout *Oncorhynchus mykis*s (Walbaum, 1792) sperm diluent: Effects on motility and fertilizing capability. Latin American Journal of Aquatic Research. 2011;**39**(2):338-343. DOI: 10.3856/vol39-issue2-fulltext-1

[49] Li P, Xi M.D, Du H, Qiao X.M, Liu Z.G, Wei Q.W. Antioxidant supplementation, effect on post-thaw spermatozoan function in three sturgeon species. Reproduction in Domestic Animals 2018;53:2:287-295. DOI:10.1111/rda.13103

[50] Figueroa E, Lee-Estevez M, Valdebenito I, Watanabe I, Oliveira RPS, Romero J. Effects of cryopreservation on mitochondrial function and sperm quality in fish. Aquaculture. 2019;**511**:634190. DOI: 10.1016/j.aquaculture.2019.06.004

[51] Dadras H, Golpour A, Rahi D, Lieskovská J, Dzyuba V, Gazo I, et al. Cryopreservation of Sterlet, *Acipenser ruthenus* spermatozoa: Evaluation of quality parameters and fine ultrastructure. Frontiers in Marine Science. 2022;**78**(32):78. DOI: 10.3389/fmars.2022.783278

[52] Boryshpolets S, Dzyuba B, Rodina M, Alavi SMH, Gela D, Linhart O. Cryopreservation of Sterlet (*Acipenser ruthenus*) Spermatozoa Using Different Cryoprotectants. Journal of Applied Ichthyology. 2011;**27**:1147-1149. DOI: 10.1111/j.1439-0426.2011.01866.x

[53] Igna V, Telea A, Florea T, Popp R, Grozea A. Evaluation of Russian sturgeon (*Acipenser gueldenstaedtii*) semen quality and semen cryopreservation. Animals. 2022;**12**(16):2153. DOI: 10.3390/ani12162153

[54] Nascimento J.P, Horokhovatskyi Y, Kholodnyy V, Rodina M, Dzyuba V, Stechkina T, Dzyuba B. Optimization of sterlet sperm concentration for cryopreservation. Aquaculture 2021;540:736682. DOI:10.1016/j.aquaculture.2021.736682.

[55] Krasilnikova A, Ponomareva E, Shvedova S, Magomedov M, Rudoy D, Olshevskaya A. The volume of the sample as a factor of survival of sturgeon spermatozoa after cryopreservation. E3S Web of Conferences. 2020;**210**:07010. DOI: 10.1051/e3sconf/202021007010

[56] Ponomareva EN, Belaya MM, Firsova AV, Krasilnikova AA. Influence of acoustic-mechanical impact on the reproductive qualities of sturgeon sperm during cryopreservation. Doklady. Biochemistry and Biophysics. 2022;**505**(1):170-172. DOI: 10.1134/S1607672922040081

[57] Kolyada M, Osipova V, Berberova N, Pimenov Y. The effect of tin compounds on the lipid peroxidation level of Russian sturgeon fresh and cryopreserved sperm. Environmental Research, Engineering and Management. 2020;**76**(2):34-42. DOI: 10.5755/j01.erem.76.2.23407

[58] Eels IWGo, Report of the 2011 session of the joint EIFAAC/ICES working group on eels. 2011:24

[59] Jacoby D, Gollock, M. Anguilla japonica. The IUCN Red list of threatened species. 2014:Version 2014.3.a

[60] Jacoby D, Gollock, M. Anguilla anguilla. The IUCN red list of threatened species, 2014.Version 2014.3.b

[61] Tanaka S, Zhang H, Horie N, Yamada Y, Okamura A, Utoh T, et al. Long-term cryopreservation of sperm of Japanese eel. Journal of Fish Biology. 2002;**60**(1):139-146. DOI: 10.1111/j.1095-8649.2002.tb02393.x

[62] Herranz-Jusdado JG, Gallego V, Morini M, Rozenfeld C, Pérez L, Müller T, et al. Eel sperm cryopreservation: An overview. Theriogenology. 2019;**133**: 210215. DOI: 10.1016/j.theriogenology.019.03.033a

[63] Herranz-Jusdado JG, Gallego V, Marina M, Rozenfeld C, Pérez L, Kása E, et al. Comparison of European eel sperm cryopreservation protocols with standardization as a target. Aquaculture. 2019;**498**:539-544. DOI: 10.1016/j.aquaculture.2018.09.006b

[64] Herranz-Jusdado JG, Gallego V, Rozenfeld C, Marína M, Pérez L, Asturiano JF. European eel sperm storage: Optimization of short-term protocols and cryopreservation of large volumes. Aquaculture. 2019;**506**:42-50. DOI: 10.1016/j.aquaculture.2019.03.019c

[65] Müller T, Matsubara H, Kubara Y, Horváth Á, Asturiano JF, Urbányi B. Japanese eel (*Anguilla japonica* Temminck & Schlegel, 1846) propagation using cryopreserved sperm samples. Journal of Applied Ichthyology. 2017;**33**(3):550-552. DOI: 10.1111/jai.13316

[66] ICC K, Hamada D, Tsuji YA, Okuda D, Nomura K, Tanaka H, et al. Sperm cryopreservation of Japanese eel, *Anguilla japonica*. Aquaculture. 2017;**473**:487-492. DOI: 10.1016/j.aquaculture.2017.03.011

[67] Nomura K, ICC K, Iio R, Okuda D, Kazeto Y, Tanaka H, et al. Sperm cryopreservation protocols for the large-scale fertilization of Japanese eel using a combination of large-volume straws and low sperm dilution ratio. Aquaculture. 2018;**496**:203-210. DOI: 10.1016/j.aquaculture.2018.07.007

[68] FAO. The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Italy: Food and Agriculture Organization of the United Nations; Rome, Italy. 2020. p. 244 Available from: www.fao.org [Accessed: 2022-09-07]

[69] Huang M, Ding L, Wang J, Ding C, Tao J. The impacts of climate change on fish growth: A summary of conducted studies and current knowledge. Ecological Indicators. 2021;**121**:106976. DOI: 10.1016/j.ecolind.2020.106976

[70] Figueroa E, Valdebenito I, Merino O, Ubilla A, Risopatrón J, Farias JG. Cryopreservation of Atlantic salmon *Salmo salar* sperm: Effects on sperm physiology. Journal of Fish Biology. 2016;**89**(3):1537-1550. DOI: 10.1111/jfb.13052

[71] Ciereszko A, Dietrich GJ, Nynca J, Krom J, Dobosz S. Semen from sex-reversed rainbow trout of spring strain can be successfully cryopreserved and used for fertilization of elevated number of eggs. Aquaculture. 2015;**448**:564-568. DOI: 10.1016/j.aquaculture.2015.06.039

[72] Nynca J, Judycka S, Liszewska E, Dobosz S, Grudniewska J, Fujimoto T, et al. Utility of different sugar extenders for cryopreservation and post-thaw storage of sperm from Salmonidae species. Aquaculture. 2016;**464**:340-348. DOI: 10.1016/j.aquaculture.2016.07.014

[73] Di Iorio M, Esposito S, Rusco G, et al. Semen cryopreservation for the Mediterranean brown trout of the Biferno River (Molise-Italy): Comparative study on the effects of basic extenders and cryoprotectants. Scientific Reports. 2019;**9**:9703. DOI: 10.1038/s41598-019-45006-4

[74] Bozkurt Y, Seçer N, Tekin E, Akçay E. Cryopreservation of rainbow trout (*Oncorhynchus mykiss*) and mirror carp (*Cyprinus carpio*) sperm with glucose based extender. Süleyman Demirel Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi. 2005;**1**:21-25

[75] Nynca J, Judycka S, Liszewska E, Dobosz S, Ciereszko A. Standardization of spermatozoa concentration for cryopreservation of rainbow trout semen using a glucose methanol extender. Aquaculture. 2017;**477**:23-27. DOI: 10.1016/j.aquaculture.2017.04.036

[76] Elliott GD, Wang S, Fuller BJ. Cryoprotectants: A review of the actions and applications of cryoprotective solutes that modulate cell recovery from ultra-low temperatures. Cryobiology. 2017;**76**:74-91. DOI: 10.1016/j.cryobiol.2017.04.004

[77] Yang H, Hu E, Buchanan JT, Tiersch TR. A strategy for sperm cryopreservation of Atlantic salmon, *Salmo salar*, for remote commercial-scale high-throughput processing. Journal of the World Aquaculture Society. 2018;**49**(1):96-112. DOI: 10.1111/jwas.12431

[78] Lahnsteiner F, Berger B, Weismann T, Patzner R. The influence of various cryoprotectants on semen quality of the rainbow trout (*Oncorhynchus mykiss*) before and after cryopreservation. Journal of Applied Ichtyology. 1996;**12**(2):99-106. DOI: 10.1111/j.1439-0426.1996.tb00070.x

[79] Kutluyer F, Kayim M, Öğretmen F, Büyükleblebici S, Tuncer PB. Cryopreservation of rainbow trout *Oncorhynchus mykiss* spermatozoa: Effects of extender supplemented with different antioxidants on sperm motility, velocity and fertility. Cryobiology. 2014;**69**:462-466. DOI: 10.1016/j.cryobiol.2014.10.005

[80] Figueroa E, Elías-Farias JG, Lee EstevezM, Valdebenito I, Iván-Risopatron J,

Magnotti C, et al. Sperm cryopreservation with supplementation of a-tocopherol and ascorbic acid in freezing media increase sperm function and fertility rate in Atlantic salmon (*Salmo salar*). Aquaculture. 2018;**493**:1-8. DOI: 10.1016/j.aquaculture.2018.04.046

[81] Wang B, Van Veldhoven PP, Brees C, Rubio N, Nordgren M, Apanasets O, et al. Mitochondria are targets for peroxisome-derived oxidative stress in cultured mammalian cells. Free Radical Biology & Medicine. 2013;**5**:882-894. DOI: 10.1016/j.freeradbiomed.2013.08.173

[82] Cosson MP, Cosson J, André F, Billard R. cAMP/ATP relationship in the activation of trout sperm motility: Their interaction in membrane-deprived models and in live spermatozoa. Cell Motility and the Cytoskeleton. 1995;**31**(2):159-176. DOI: 10.1002/cm.970310208

[83] Gallego V, Pérez L, Asturianoa JF, Yoshida M. Relationship between spermatozoa motility parameters, sperm/egg ratio and fertilization and hatching rates in pufferfish (*Takifugu niphobles*). Aquaculture. 2013;**416**(417):238-243. DOI: 10.1016/j.aquaculture.2013.08.035

[84] Judycka S, Nynca J, Liszewska E, Dobosz S, Zalewski T, Ciereszko A. Potassium ions in extender differentially influence the post-thaw sperm motility of salmonid fish. Cryobiology. 2016;**73**:248-256. DOI: 0.1016/j.cryobiol.2016.07.002

[85] Robles V, Cabrita E, Cuñado S, Herraez MP. Sperm cryopreservation of sex-reversed rainbow trout (*Oncorhynchus mykiss*): Parameters that affect its ability for freezing. Aquaculture. 2003;**224**:203-212. DOI: 10.1016/S0044-8486(03)00221-7

[86] Tekin N, Secer S, Akcay E, Bozkurt Y, Kayam S. Effects of glycerol additions on post-thaw fertility of frozen rainbow trout sperm, with an emphasis on interaction between extender and cryoprotectant. Journal of Applied Ichthyology. 2007;**23**(1):60-63. DOI: 10.1111/j.1439-0426.2006.00792.x

[87] Ahmed MH, Keskin İ, Özkorucuklu S, Ekici A. A study on effect of using magnetized water in dilution the milt of Black Sea Trout (*Salmo trutta labrax*) on sperm motility. Aquaculture Research. **53**(17):6324-6332. DOI: 10.1111/are.16104

[88] Formicki K, Szulc J, Korzelecka-Orkisz A, Tański A, Kurzydłowski JK, Grzonka J, et al. The effect of a magnetic field on trout (*Salmo trutta* Linnaeus, 1758) sperm motility parameters and fertilisation rate. Journal of Applied Ichthyology. 2015;**31**:136-146. DOI: 10.1111/jai.12737

[89] Hu E, Yang H, Tiersch TR. High-throughput cryopreservation of spermatozoa of blue catfish (*Ictalurus furcatus*): Establishment of an approach for commercial-scale processing. Cryobiology. 2011;**62**(1):74-82. DOI: 10.1016/j.cryobiol.2010.12.006

[90] Lang RP, Riley KL, Chandler JE, Tiersch TR. The use of dairy protocols for sperm cryopreservation of blue catfish (*Ictalurus furcatus*). Journal of the World Aquaculture Society. 2003;**34**:66-75. DOI: 10.1111/j.1749-7345.2003.tb00040.x

[91] Muchlisin ZA, Nadiah WN, Nadiya N, Fadli N, Hendri A, Khalil M, et al. Exploration of natural cryoprotectants for cryopreservation of African catfish, *Clarias gariepinus*, Burchell 1822 (Pisces: Clariidae) spermatozoa. Czech Journal of Animal Science. 2015;**60**:10-15. DOI: 10.17221/7906-CJAS

[92] Şahinöz E, Aral F, Doğu Z, Koyuncu İ, Yüksekdağ Ö.

Cryopreservation of Mesopotamian catfish (*Silurus triostegus* H., 1843) spermatozoa: Effects of diluents and osmotic pressure on spermatozoa DNA damage, rate and duration of motility. Iranian Journal of Fisheries Sciences. 2020;**19**(5):2293-2307

[93] Watson P, Holt W. Principles of Cryopreservation. Cryobanking the Genetic Resource: Wildlife Conservation for the Future. Boca Raton, FL: CRC Press; 2001. pp. 23-46

[94] Watson PF, Morris GJ. Cold shock injury in animal cells. Symposia of the Society for Experimental Biology. 1987;**41**:311-340

[95] Cabrita E, Martínez-Páramo S, Gavaia PJ, Riesco MF, Valcarce DG, Sarasquete C, et al. Factors enhancing fish sperm quality and emerging tools for sperm analysis. Aquaculture. 2014;**432**:389-401. DOI: 10.1016/j.aquaculture.2014.04.034

[96] Wang H, Montague HR, Hess HN, Zhang Y, Aguilar GL, Dunham RA, et al. Transcriptome analysis reveals key gene expression changes in Blue Catfish sperm in response to cryopreservation. International Journal of Molecular Sciences. 2022;**23**(14):7618. DOI: 10.3390/ijms23147618

[97] Niu J, Wang X, Liu P, Liu H, Li R, Li Z, et al. Effects of cryopreservation on sperm with cryodiluent in viviparous Black Rockfish (*Sebastes schlegelii*). International Journal of Molecular Sciences. 2022;**23**(6):3392. DOI: 10.3390/ijms23063392

[98] Martin G, Sabido O, Durand P, Levy R. Cryopreservation induces an apoptosis-like mechanism in bull sperm. Biology of Reproduction. 2004;**71**:28-37. DOI: 10.1095/biolreprod.103.024281

[99] Riesco MF, Oliveira C, Soares F, Gavaia PJ, Dinis MT, Cabrita E. *Solea senegalensis* sperm cryopreservation: New insights on sperm quality. PLoS One. 2017;**12**:e0186542. DOI: 10.1371/journal.pone.0186542

[100] Acosta IB, Corcini CD, Gheller SMM, Brito CR, Goulart TLS, Varela Junior AS. Effect of amide on semen cryopreservation of curimba (*Prochilodus lineatus*). CryoLetters. 2020;**41**:1-5

[101] Yoshizaki G, Yazawa R. Application of surrogate broodstock technology in aquaculture. Fisheries Science. 2019;**85**:429-437. DOI: 10.1007/s12562-019-01299-y

[102] Okutsu T, Shikina S, Kanno M, Takeuchi Y, Yoshizaki G. Production of trout offspring from triploid salmon parents. Science (New York, N.Y.). 2007;**317**(5844):1517. DOI: 10.1126/science.1145626

[103] Lacerda SMSN, Costa GMJ, Campos-Junior PHA, et al. Germ cell transplantation as a potential biotechnological approach to fish reproduction. Fish Physiology and Biochemistry. 2013;**39**:3-11. DOI: 10.1007/s10695-012-9606-4

[104] Octavera A, Yoshizaki G. Production of Chinese rosy bitterling offspring derived from frozen and vitrified whole testis by spermatogonial transplantation. Fish Physiology and Biochemistry. 2020;**46**(4):1431-1442. DOI: 10.1007/s10695-020-00802-y

[105] Lee S, Seki S, Katayama N. Production of viable trout offspring derived from frozen whole fish. Scientific Reports. 2015;**5**:16045. DOI: 10.1038/srep16045

[106] Lee S, Bang WY, Yang HS, Lee DS, Song HY. Production of juvenile masu

salmon (*Oncorhynchus masou*) from spermatogonia-derived sperm and oogonia-derived eggs via intraperitoneal transplantation of immature germ cells. Biochemical and Biophysical Research Communications. 2021;**535**:6-11. DOI: 10.1016/j.bbrc.2020.12.021

[107] Yang F, Ichida K, Yoshizaki G. Gametogenesis commencement in recipient gonads using germ cells retrieved from dead fish. Aquaculture. 2022;**552**:737952 DOI: 10.1016/j.aquaculture.2022.737952

[108] Abualreesh M, Myers JN, Gurbatow J, Johnson A, Xing D, Wang J, et al. Development of an effective cryopreservation protocol for Blue Catfish Oogonia. North American Journal of Aquaculture. 2021;**83**:336-345. DOI: 10.1002/naaq.10203

[109] Abualreesh MJN, Myers J, Gurbatow A, Johnson D, Xing J, Wang S, et al. Development of a spermatogonial stem cell cryopreservation protocol for Blue Catfish. Cryobiology. 2020;**97**:46-52. DOI: 10.1016/j.cryobiol.2020.10.010

[110] Franěk R, Tichopád T, Steinbach C, Xie X, Lujić J, Marinović Z, et al. Preservation of female genetic resources of common carp through oogonial stem cell manipulation. Cryobiology. 2019;**87**:78-85. DOI: 10.1016/j.cryobiol.2019.01.016

[111] Yoshizaki G, Ichikawa M, Hayashi M, Iwasaki Y, Miwa M, Shikina S, et al. Sexual plasticity of ovarian germ cells in rainbow trout. Development. 2010;**137**:1227-1230. DOI: 10.1242/dev.044982

[112] Isayeva A, Zhang T, Rawson DM. Studies on chilling sensitivity of zebrafish (*Danio rerio*) oocytes. Cryobiology. 2004;**49**:114-122. DOI: 10.1016/j.cryobiol.2004.05.005

[113] Zhang T, Rawson DM. Studies on chilling sensitivity of zebrafish (*Brachydanio rerio*) embryos. Cryobiology. 1995;**32**:239-246. DOI: 10.1006/cryo.1995.1023

[114] Hagedorn M, Kleinhans FW, Wildt DE, Rall WF. Chill sensitivity and cryoprotectant permeability of dechorionated zebrafish embryos, *Brachydanio rerio*. Cryobiology. 1997;**34**:251-263. DOI: 10.1006/cryo.1997.2002

[115] Fornari DC, Ribeiro RP, Streit DJ, Godoy LC, Neves PR, de Oliveira D, et al. Effect of cryoprotectants on the survival of cascudo preto (*Rhinelepis aspera*) embryos stored at −8°C. Zygote. 2014;**22**:58-63. DOI: 10.1017/S0967199411000517

[116] Liu XH, Zhang T, Rawson DM. Effect of cooling rate and partial removal of yolk on the chilling injury in zebrafish (*Danio rerio*) embryos. Theriogenology. 2001;**55**:1719-1731. DOI: 10.1016/S0093-691X(01)00515-5

[117] Pessoa NO, Galvão JAS, de Souza Filho FGM, de Sousa MLNM, Sampaio CMS. Cooling of pirapitinga (*Piaractus brachypomus*) embryos stored at −10°C. Zygote. 2015;**23**(3):453-459. DOI: 10.1017/S0967199414000057

[118] Zhang XS, Zhao L, Hua TC, Chen XH, Zhu HY. A study on the cryopreservation of common carp, *Cyprinus carpio* embryos. Cryo-Letters. 1989;**10**:271-278

[119] Zhang T, Rawson DM, Morris BJ. Cryopreservation of prehatch embryo of zebrafish. Aquatic Living Resources. 1993;**6**:145-153. DOI: 10.1051/alr:1993014

[120] Connolly MH, Paredes E, Mazur P. A preliminary study of osmotic dehydration in zebrafish embryos:

Implications for vitrification and ultra-fast laser warming. Cryobiology. 2017;**78**:106-109. DOI: 10.1016/j.cryobiol.2017.08.004

[121] Tian YS, Jiang J, Song LN, Chen ZF, Zhai JM, Liu JC, et al. Effects of cryopreservation on the survival rate of the seven-band grouper (*Epinephelus septemfasciatus*) embryos. Cryobiology. 2015;**71**(3):499-506. DOI: 10.1016/j.cryobiol.2015.10.147

[122] de Carvalho AFS, Ramos SE, de Carvalho TSG, de Souza YCP, Zangeronimo MG, Pereira LJ, et al. Efficacy of fish embryo vitrification protocols in terms of embryo morphology – A systematic review. CryoLetters. 2014;**35**:361-370

[123] Edashige K, Valdez DM Jr, Hara T, Saida N, Seki S, Kasai M. Japanese flounder (*Paralichthys olivaceus*) embryos are difficult to cryopreserve by vitrification. Cryobiology. 2006;**53**:96-106. DOI: 10.1016/j.cryobiol.2006.04.002

[124] Ahammad MM, Bhattacharyya D, Jana BB. The hatching of common carp (*Cyprinus carpio* L.) embryos in response to exposure to different concentrations of cryoprotectant at low temperatures. Cryobiology. 2003;**44**:114-121. DOI: 10.1016/S0011-2240(02)00012-3

[125] Keivanloo S, Sudagar M, Mazandarani M. Evaluating the suitability of cryopreservation solutions for common carp (*Cyprinus carpio*) embryos stored at −2°C. Iranian Journal of Fisheries Sciences. 2019;**18**(4):1036-1045

[126] Tian T, Chen Z, Tang J, Duan H, Zhai J, Li B, et al. Effects of cryopreservation at various temperatures on the survival of kelp grouper (*Epinephelus moara*) embryos from fertilization with cryopreserved sperm. Cryobiology. 2017;**75**:37-44. DOI: 10.1016/j. cryobiol.2017.02.007

[127] Serhat E, Ozgur A. Cryopreservation of common carp (*Cyprinus carpio*) embryos using different cryoprotectant. Survey in Fisheries Sciences. 2022;**8**(2):1-10. DOI: 10.18331/SFS2022.8.2.1

[128] Keivanloo S, Sudagar M. Cryopreservation of Persian sturgeon (*Acipenser persicus*) embryos by DMSO-based vitrificated solutions. Theriogenology. 2016;**85**(5):1013-1018. DOI: 10.1016/j.theriogenology.2015.11.012

[129] Khosla K, Wang Y, Hagedorn M, Qin Z, Bischof J. Gold nanorod induced warming of embryos from the cryogenic state enhances viability. ACS Nano. 2017;**11**(8):7869-7878. DOI: 10.1021/acsnano.7b02216

[130] Bielanski A. Disinfection procedures for controlling microorganisms in the semen and embryos of humans and farm animals. Theriogenology. 2007;**68**:1-22. DOI: 10.1016/j.theriogenology.2007.03.025

[131] Thibier M, Guerin B. Hygienic aspects of storage and use of semen for artificial insemination. Animal Reproduction Science. 2000;**62**:233-251. DOI: 10.1016/S0378-4320(00)00161 5

[132] Bielanski A, Bergeron H, Lau PCK, Devenish J. Microbial contamination of embryos and semen during long term banking in liquid nitrogen. Cryobiology. 2003;**46**:146-152. DOI: 10.1016/S0011-2240(03)00020-8

[133] Joaquim DC, Borges ED, Viana IGR, Navarro PA, Vireque AA. Risk of contamination of gametes and embryos during cryopreservation and measures to prevent cross-contamination. BioMed Research

International. 2017;**2017**:1840417. DOI: 10.1155/2017/1840417

[134] Morris GJ. The origin, ultrastructure, and microbiology of the sediment accumulating in liquid nitrogen storage vessels. Cryobiology. 2005;**50**:231-238. DOI: 10.1016/j.cryobiol.2005.01.005

[135] Steel KJ, Ross HE. Survival of freeze dried bacterial cultures. The Journal of Applied Bacteriology. 1963;**26**:370-375. DOI: 10.1111/j.1365-2672.1963.tb04787.x

[136] Pandya SP, Doshi H, Codipilly CN, Fireizen Y, Potak D, Schanler RJ. Bacterial stability with freezer storage of human milk. Journal of Perinatal Medicine. 2021;**49**:225-228. DOI: 10.1515/jpm-2020-0131

[137] Perry SF. Freeze-drying and cryopreservation of bacteria. In: Day JG, Pennington MW. (eds) Cryopreservation and Freeze-Drying Protocols. Methods in Molecular Biology™, Humana Press, Totowa, NJ. 1995;**38**:21-30. DOI: 10.1385/0-89603-296-5:21

[138] Yamashita H, Mori K, Nakai T. Protection conferred against viral nervous necrosis by simultaneous inoculation of aquabirnavirus and inactivated betanodavirus in the sevenband grouper, *Epinephelus septemfasciatus* (Thunberg). Journal of Fish Diseases. 2009;**32**:201-210. DOI: 10.1111/J.1365-2761.2008.01017.X

[139] Bielanski A, Vajta G. Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units. Human Reproduction. 2009;**24**:2457-2467. DOI: 10.1093/HUMREP/DEP117

[140] Garcia A, Sierra MF, Friberg J. Survival of bacteria after freezing of human semen in liquid nitrogen. Fertility and Sterility. 1981;**35**:549-551. DOI: 10.1016/S0015-0282(16)45499-3

[141] Smith D, Ryan M. Implementing best practices and validation of cryopreservation techniques for microorganisms. Scientific World Journal. 2012;**2012**:805659. DOI: 10.1100/2012/805659

[142] Bielanski A, Nadin-Davis S, Sapp T, Lutze-Wallace C. Viral contamination of embryos cryopreserved in liquid nitrogen. Cryobiology. 2000;**40**:110-116. DOI: 10.1006/cryo.1999.2227

[143] Fountain D, Ralston M, Higgins N, Gorlin J, Uhl L, Wheeler C, et al. Liquid nitrogen freezers: A potential source of microbial contamination of hematopoietic stem cell components. Transfusion. 1997;**37**:585-591. DOI: 10.1046/j.1537-2995.1997.37697335152.x

[144] Ciereszko A, Własow T, Dobosz S, Goryczko K, Glogowski J. Blood cells in rainbow trout *Oncorhynchus mykiss* milt: Relation to milt collection method and sampling period. Theriogenology. 2004;**62**:1353-1364. DOI: 10.1016/j.theriogenology.2004.02.003

[145] Rurangwa E, Kime D, Ollevier F, Nash J. The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture. 2004;**234**:1-28. DOI: 10.1016/j.aquaculture.2003.12.006

[146] VKM, Rimstad E, Basic D, Brun E, Colquhoun D, Olesen NJ, Bøe KE, Gjøen T, Godfroid J, Janczak AM, Madslien K, Olsen RE, Øverli Ø, Agdestein A. The risk of transmission of infectious disease through trade of cryopreserved milt. Scientific Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety. VKM report 2019:02, ISBN:

978-82-8259-318-2, ISSN: 2535-4019. 2019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

[147] Hare WCD. Diseases transmissible by semen and embryo transfer techniques. OIE Technical Bulletin. 1985;**4**:1-117

[148] Brackett BG, Baranska W, Sawicki W, Koprowski H. Uptake of heterologous genome by mammalian spermatozoa and its transfer to ova through fertilization. Proceedings of the National Academy of Sciences. 1971;**68**:353-357. DOI: 10.1073/pnas.68.2.353

[149] Mulcahy D, Pascho RJ. Adsorption to fish sperm of vertically transmitted fish viruses. Science. 1984;**225**:333-335

[150] Mulcahy D, Pascho RJ. Vertical transmission of infectious haematopoietic virus in sockeye salmon, *Oncorhynchus nerka* (Walbaum): Isolation of virus from dead eggs and fry. Journal of Fish Diseases. 1985;**8**:393-396

[151] Ahne W. Presence of infectious pancreatic necrosis virus in the seminal fluid of rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases. 1983;**6**(4):377-377

[152] Smail DA, Munro ES. Isolation and quantification of infectious pancreatic necrosis virus from ovarian and seminal fluids of Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases. 2008;**31**(1):49-58. DOI: 10.1111/j.1365-2761.2007.00866.x

[153] Eaton WD, Hulett J, Brunson R, True K. The first isolation in North America of infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) in coho salmon from the same watershed. Journal of Aquatic Animal Health. 1991;**3**:114-117. DOI: 10.1577/1548-8667(1991)003

[154] Meyers TR, Thomas JB, Follett JE, Saft RR. Infectious hematopoietic necrosis virus: Trends in prevalence and the risk management approach in Alaskan sockeye salmon culture. Journal of Aquatic Animal Health. 1990;**2**:85-98. DOI: 10.1577/1548-8667(1990)002

[155] Ercan MD, Ekici A. Reducing bacterial density in the semen of rainbow trout (*Oncorhynchus mykiss*) by applying gradient centrifugation and swim-up washing methods. Aquaculture Research. 2016;**47**:3845-3851. DOI: 10.1111/are.12835

[156] Yildirim S, Özer S. The existence of Flavobacterium spp. at rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) hatcheries in Caglarca village, Mersin-Turkey. Journal of Fisheries. 2010;**4**:112-122. DOI: 10.3153/jfscom.2010010

[157] Larenas JJ, Bartholomew J, Troncoso O, Fernández S, Ledezma H, Sandoval N, et al. Experimental vertical transmission of *Piscirickettsia salmonis* and in vitro study of attachment and mode of entrance into the fish ovum. Diseases of Aquatic Organisms. 2003;**56**:25-30. DOI: 10.3354/DAO056025

[158] Wu Y, Zhang X, Wang Z, Xia X. Can we cryopreserve the sperm of COVID-19 patients during the pandemic? Frontiers in Endocrinology (Lausanne). 2022;**13**:753267. DOI: 10.3389/FENDO.2022.753267

[159] Batiha O, Al-Deeb T, Al-zoubi E, Alsharu E. Impact of COVID-19 and other viruses on reproductive health. Andrologia. 2020;**52**:e13791. DOI: 10.1111/AND.13791

[160] Zafer M, Horvath H, Mmeje O, Van Der Poel S, Semprini AE, Rutherford G, et al. Effectiveness of semen washing to prevent human immunodeficiency virus (HIV) transmission and assist pregnancy in HIV-discordant couples: A systematic review and meta-analysis. Fertility and Sterility. 2016;**105**:645-655. e2. DOI: 10.1016/j.fertnstert.2015.11.028

[161] Ekici A, Baran A, Ozdas OB, Sandal AI, Yamaner G, Guven E. The effect of streptomycin on freezing rainbow trout (*Oncorhynchus mykiss*) sperm. Israeli Journal of Aquaculture – Bamidgeh. 2014;**66**:1-6. DOI: 10.46989/001c.20749

[162] Duracka M, Lukac N, Kacaniova M, Kantor A, Hleba L, Ondruska L, et al. Antibiotics versus natural biomolecules: The case of in vitro induced bacteriospermia by *Enterococcus faecalis* in rabbit semen. Molecules. 2019;**24**:4329. DOI: 10.3390/MOLECULES24234329

[163] Rivers N, Daly J, Daly J, Temple-Smith P. New directions in assisted breeding techniques for fish conservation. Reproduction, Fertility, and Development. 2020;**32**:807-821. DOI: 10.1071/RD19457

[164] Meacham RB. Practical considerations in the preparation of sperm prior to cryopreservation. Journal of Andrology. 2005;**26**(4):451. DOI: 10.2164/jandrol.05061

[165] Horokhovatskyi Y, Dietrich MA, Lebeda I, Fedorov P, Rodina M, Dzyuba B. Cryopreservation effects on a viable sperm sterlet (*Acipenser ruthenus*) subpopulation obtained by a Percoll density gradient method. Rutherford S, editor. PLoS One. 2018;13:e0202514. DOI: 10.1371/journal.pone.0202514

[166] Marinović Z, Šćekić I, Lujić J, Urbányi B, Horváth Á. The effects of cryopreservation and cold storage on sperm subpopulation structure of common carp (*Cyprinus carpio* L.). Cryobiology. 2021;**99**:88-94. DOI: 10.1016/J.CRYOBIOL.2021.01.007

[167] Pérez-Atehortúa M, Giannotti Galuppo A, Batista Rodrigues R, de Souza FT, dos Santos TN, Rodrigues de Freitas T, et al. The use of differential separation and density gradient with AllGrad 90% after thawing improves the sperm quality of South American catfish (*Rhamdia quelen*). Aquaculture. 2022;**553**:738072. DOI: 10.1016/j.aquaculture.2022.738072

[168] Valcarce DG, Herráez MP, Chereguini O, Rodríguez C, Robles V. Selection of nonapoptotic sperm by magnetic-activated cell sorting in Senegalese sole (*Solea senegalensis*). Theriogenology. 2016;**86**:1195-1202. DOI: 10.1016/j.theriogenology.2016.04.010

[169] Li P, Dzyuba B, Hulak M, Rodina M, Boryshpolets S, Li Z-H, et al. Percoll gradient separation of cryopreserved common carp spermatozoa to obtain a fraction with higher motility, velocity and membrane integrity. Theriogenology. 2010;**74**:1356-1361. DOI: 10.1016/j.theriogenology.2010.06.005

[170] Bravo W, Dumorné K, Beltrán Lissabet J, Jara-Seguel P, Romero J, Farías JG, et al. Effects of selection by the Percoll density gradient method on motility, mitochondrial membrane potential and fertility in a subpopulation of Atlantic salmon (*Salmo salar*) testicular spermatozoa. Animal Reproduction Science. 2020;**216**:106344. DOI: 10.1016/j.anireprosci.2020.106344

[171] OIE. Aquatic Animal Health Code, 22nd edition. 2019. ISBN 978-92-95108-96-7. World Organisation For Animal Health, Paris, France

[172] Uhrig M, Ezquer F, Ezquer M. Improving cell recovery: Freezing and thawing optimization of induced pluripotent stem cells. Cell. 2022;**11**:799. DOI: 10.3390/CELLS11050799/S1

[173] Saragusty J, Anzalone DA, Palazzese L, Arav A, Patrizio P, Gosálvez J, et al. Dry biobanking as a conservation tool in the Anthropocene. Theriogenology. 2020;**150**:130-138. DOI: 10.1016/J.THERIOGENOLOGY.2020.01.022

[174] Comizzoli P, Loi P, Patrizio P, Hubel A. Long-term storage of gametes and gonadal tissues at room temperatures: The end of the ice age? Journal of Assisted Reproduction and Genetics. 2022;**39**:321-325. DOI: 10.1007/S10815-021-02392-X

[175] Bajerski F, Nagel M, Overmann J. Microbial occurrence in liquid nitrogen storage tanks: A challenge for cryobanking? Applied Microbiology and Biotechnology. 2021;**105**:7635-7650. DOI: 10.1007/S00253-021-11531-4

[176] Diogo P, Martins G, Quinzico I, Nogueira R, Gavaia PJ, Cabrita E. Electric ultrafreezer (−150°C) as an alternative for zebrafish sperm cryopreservation and storage. Fish Physiology and Biochemistry. 2018;**44**:1443-1455. DOI: 10.1007/S10695-018-0500-6

[177] Keskintepe L, Pacholczyk G, Machnicka A, Norris K, Curuk MA, Khan I, et al. Bovine blastocyst development from oocytes injected with freeze-dried spermatozoa. Biology of Reproduction. 2002;**67**:409-415. DOI: 10.1095/BIOLREPROD67.2.409

[178] Mayer I. The role of reproductive sciences in the preservation and breeding of commercial and threatened teleost fishes. In: Comizzoli P, Brown JL, Holt WV, editors. Reproductive Science in Animal Conservation. Cham: Springer International Publishing; 2019. pp. 187-224