



A short review of discovery and development of fish sperm cryopreservation

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ARTICLE INFO	ABSTRACT
<p>Keywords: Cryopreservation History Depik Endemic species</p> <p>DOI: 10.13170/depik.10.1.18794</p>	<p>Global biodiversity, especially fish, has experienced a decline, this occurs as a result of over-exploitation, the presence of introduced fish species and climate change. This condition makes researchers look for solutions to overcome these problems by using cryopreservation techniques. The main purpose of cryopreservation is to store, maintain, and ensure the survival of genetic material, so that using cryopreservation techniques can maintain the viability and function of gamete cells immunologically, biologically and physiologically. The success of the cryopreservation technique has made this technique widely developed in various species of living organism including fish. This article summarizes and reviews the history of the development of cryopreservation of animal species with specific focus on fish.</p>

Introduction

Biodiversity is one of components supporting survival of human life, including for food, development of medicines, science, and even energy. However, global biodiversity experienced a decline in recent years. Presumably is caused by many factors such as specimens' excessive exploitation (legal and illegal), climate change, habitat destruction due to environmental pollution, and the presence of invasive species (Samedi, 2015; Muchlisin *et al.*, 2009). In addition, the uniformity of plant and animal varieties causing genetic erosion resulted in biodiversity crisis (Sutoyo, 2010). This condition attracts experts to find a solution to protect biodiversity and maintain ecological balance through preservation methods (Hagedorn *et al.*, 2018).

Theoretically, cryopreservation is a process when germplasm such as cells and tissues are preserved in low temperatures (-196°C) (Agarwal, 2011). Phillips and Lardy (1939) were the first to use egg yolks to protect bulls' sperm from coldshock. Egg yolks contain phospholipids and lipoproteins that protect cells from ice crystal formation. Salisbury *et al.* (1941) added sodium citrate in egg yolk media and cooling the samples at temperature of 5°C for 3 days. In 1949,

Polge *et al.* (1949) made an important discovery in modern cryobiology that glycerol enables to protect cells at low temperature. The use of glycerol was successful in freezing avian spermatozoa and became a revolution in cattle breeding industry (Pegg, 2002). In 1953, when dairy industry needed a long-term storage for cow spermatozoa, sperm cryopreservation became a very popular topic of study. Since then, cryopreservation methods developed rapidly and successfully in preserving various species including fish.

Research on artificial insemination in poultry was first carried out by Quinn and Burrows (1936) on Black Rosecomb sperm. While cryopreservation of human sperm was first reported by Sherman and Bunge (1953), until 1963, they continued to develop methods of preserving human spermatozoa by following various cryopreservation protocols and freeze-drying techniques. Finally, they found the standard method for human sperm preservation stored in liquid nitrogen at -196°C.

The early development of preservation method in vertebrate such as fish was carried out in a simple way, stored in formalin solution and then transferred to alcohol. This preservation method is commonly

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applied for conventional use and long-term storage. However, this technique reduces the quality of samples for molecular analysis (Hykin and McGuire, 2015). Additionally, preservation at 4-5°C has also been studied yet the quality of the sperm can only last up to 6 days (Eriani et al., 2008).

In the last two decades, there has been a rapid development in the science and technology of specimen preservation, where tissue samples are preserved using ethanol and frozen for DNA (genetic) analysis as phylogenesis purposes. However, specimens in long-term storage experience a decline in quality (Zimmermann et al., 2008; Hagedorn et al., 2018). Therefore, researchers are again exploring preservation method without causing additional damage during storage process.

In actual reference, cryopreservation was first recorded when Lazzaro Spallanzani (1776) attempted to preserve bull sperm using snow (Royere et al., 1996). Further scientific research on sperm cryobiology conducted by Polge et al. (1949) found that glycerol could protect poultry sperm during cryopreservation process (Pegg, 2002).

Sperm cryopreservation method was first applied to cattle and human sperm. The development of cattle sperm cryopreservation protocol was started in the 1930s by Phillips (1939), then developed by Bratton et al. (1955). Research on artificial insemination of poultry was first carried out by Quinn and Burrows (1936) on Black rosecomb spermatozoa. While cryopreservation of human sperm was first reported by Sherman and Bunge (1953). Until 1963, Sherman and Bunge continued to develop methods of preserving human sperm by seeking various cryopreservation protocols and freeze-drying techniques, resulting in storage technique for human sperm preservation is in liquid nitrogen with a temperature of -196°C.

History and Development of Fish Sperm Cryopreservation

The cryopreservation of fish spermatozoa was first reported by Blaxter (1953) who carried out a cross-breeding and spermatozoa storage of Herring *Clupea harengus* collected in spring and autumn. Blaxter refers to the research of Polge and Rowson (1952) who used carbon dioxide and 15% glycerol at a temperature of -79°C. Since then, many researches and publications have been published regarding cryopreservation of sperm in marine and freshwater fish. According to Hiemstra et al. (2005) there have been more than 200 species of freshwater and marine fish whose sperm cryopreservation methods have been recorded until 2005.

Fish cryopreservation has an immense value in protecting populations from extinction due to disease outbreaks, natural disasters, and overexploitation. Cryopreservation of fish spermatozoa also contribute to selective breeding and gene transfer (Agarwal, 2011). In terms of genetic transfer, fish cryopreservation facilitates sperm transport to distant locations. A number of fish sperm cryopreservation protocols have been reported, for instance; common carp *Cyprinus carpio* (Kurokura et al., 1984; Yavas and Bozkurt, 2014), African catfish *Clarias gariepinus* (Horvath and Urbanyi, 2001; Mahfudhah et al., 2020), rainbow trout *Oncorhynchus mykiss* (Cabrita et al., 2001), *Pangasius hypophthalmus* (Kwantong and Bart, 2003), *Mystus nemurus* (Muchlisin et al., 2004), brown trout *Salmo trutta* (Lahnsteiner et al., 2011), and *Siganus guttatus* (Larayanti, 2017).

In Indonesia, there are approximately 9 species of fish with high economic value that has been cryopreserved (Maulida et al., 2020), including *Channa striata* (Mangkunegara et al., 2019), *Chromobotia macracanthus* (Abinawanto et al., 2018), *Tor soro* (Zairin et al., 2005; Alifiani et al., 2020; Fatriani et al., 2020; Harjanti et al., 2020; Laeni et al., 2020; Pamungkas et al., 2020; Putri et al., 2020; Vardini et al., 2020; Wulandari et al., 2020), *Barbonymus gonionotus* (Abinawanto et al., 2013; Abinawanto et al., 2016; Abinawanto and Aisyah, 2018), *Osphronemus goramy* (Abinawanto et al., 2011; Abinawanto et al., 2012a; Abinawanto et al., 2012b; Abinawanto et al., 2015; Abinawanto and Putri, 2017; Abinawanto et al., 2017), *Osteochilus hasseltii* or *Osteochilus vittatus* (Sunarma et al., 2008; Sunarma et al., 2010), *Poropontius tawarensis* (Muthmainnah et al., 2019) and *Rasbora tawarensis* (Muchlisin et al., 2020).

Technique of Fish Sperm Cryopreservation

Based on existing publications, the first Indonesian researcher to conduct research on fish sperm cryopreservation was Muchlisin et al. (2004). The study was conducted at Universiti Sains Malaysia on baung fish (*Mystus nemurus*) testing 4 different cryoprotectants, namely DMSO, ethanol, and glycerol. These cryoprotectants were added separately into Ringer's solution as the extender. Results show that the addition of 10% methanol leads to the highest motility of baung spermatozoa (Muchlisin et al., 2004). In 2005, Zairin et al. carried out a research on batak fish *T. soro*, and Sunarma et al. (2008) on tawes *O. vittatus*. After 16 years of development, only 9 species of native Indonesian fish have been cryopreserved (Maulida et al., 2020). Therefore, the development of fish sperm

cryopreservation in Indonesia is considered relatively slow.

Extender and cryoprotectant play significant role in cryopreservation. The type of extender and cryoprotectant must be adapted to the characteristics of fish sperm cells (Muchlisin, 2005). The extender is a diluent to extend the volume of sperm and increases the fertilization ability of the sperm. Extenders are generally designed to match the physical and chemical composition of sperm cells (Agarwal, 2011). Common extender used for fish cryopreservation are Ringer's solution (Li et al., 1994; Pillai et al., 1994; Muchlisin et al., 2004; Pan et al., 2008; Muchlisin et al., 2018; Muchlisin et al., 2020), NaCl (Gwo, 1993; Glogowski et al., 2002; Maria et al., 2006), artificial seminal plasma (ASP) (Herranz et al., 2019; Le et al., 2011; Lim and Le, 2013), marine fish ringer (MFR) (Kiryakit et al., 2011; Widyaningsih et al., 2019; Afni et al., 2019), coconut water (Viveiros et al., 2010; Silva et al., 2012; Muchlisin et al., 2010; Muchlisin et al., 2015; Abinawanto and Putri, 2017).

Besides extender, study on cryoprotectant as protective substance for sperm cryopreservation has also been established. In general, there is two types of cryoprotectants, namely; intracellular (permeable) cryoprotectant and extracellular (impermeable) cryoprotectant. An intracellular cryoprotectant is able to penetrate the wall cell and enter inside the cell so that it can provide protection from the inside, while extracellular cryoprotectant cannot penetrate the cell wall so it only provides protection to cells from outside.

Various cryoprotective agents are used to minimize the damage to sperm cells during cryopreservation process. DMSO and glycerol are the most widely used cryoprotectant (Agarwal, 2011) as well as methanol (MeOH) (Gazali and Tambing, 2002; Yang et al., 2010; Hagedorn et al., 2012), and lipoproteins derived from milk, egg yolk and vegetable oil (Cerezales et al., 2010; Muchlisin et al., 2015; Yildiz, et al., 2013), DMF (Dimethylformamide) (Varela-Jr. et al., 2012; Perry et al., 2019; Yang et al., 2010), DMA (Dimethylacetamide) (Horvath and Urbanyi, 2001; Baulny et al., 1999; McNiven, 1993) methylglycol (Perry et al., 2019; Viveiros et al., 2009; Maria et al., 2006).

Universal protocols differ from one species to another. Therefore, protocol optimization is needed for each individual species although some general rules are applied for each fish species (Agarwal, 2011). Cryopreservation protocols should be adapted to the type of fish or their habitat, such as the snow trout (*S. richardsonii*) which is used as a model for

cryopreservation of freshwater fish sperm (Agarwal, 2011), methanol and glucose (Judyccka et al., 2015; Ciereszko et al., 2014; Horvarth et al., 2003). A few researchers in Indonesia develop different extenders such as honey (Mangkunegara et al., 2019; Sunarma, et al., 2010; Sulmartiwi, et al., 2011), and egg yolk (Muchlisin et al., 2015) as extracellular cryoprotectant. Honey are composed of fructose and glucose which are believed to be able to induce early motility and increase sperm fertilization (Muchlisin, 2005).

As technology and science have been advanced, current cryopreservation methods offer antioxidants to protect sperm cells during the cryopreservation process. Chemically, antioxidant compounds are electron-giving compounds (electron donors). Biologically, the notion of antioxidants is a compound reducing the negative impact of oxidants (Winarsi, 2007). Antioxidants are active to protect biological systems due to adverse reaction by reducing, extinguishing or suppressing free radical reactions (Eriani et al., 2018). Some of antioxidants have been tested in fish sperm cryopreservation such as glutathione in *P. tawarenensis* (Muthmainnah et al., 2019), superoxide dismutase in brown trout *Salmo trutta* (Lahnsteiner et al., 2011), Uric acid, ascorbic acid, α -tocopherol, methionine in Rainbow trout *Oncorhynchus mykiss* (Kutluyer et al., 2014).

Conclusion

Cryopreservation techniques were first introduced to poultry and cattle. On fish spermatozoa, cryopreservation was first reported by Blaxter (1953) on *Herring Clupea harengus*. In its development, studies on sperm cryopreservation develop to the addition of cryoprotectants and antioxidants. Approximately 200 fish species have been studied for cryopreservation techniques including 9 species from Indonesia.

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