REVIEWS IN Aquaculture

Reviews in Aquaculture (2020) **12**, 382–391 doi: 10.1111/raq.12323

Potential biomarkers of DNA quality in cryopreserved fish sperm: impact on gene expression and embryonic development

Elías Figueroa[1,2], Manuel Lee-Estévez[3], Ivan Valdebenito[1], Jorge G. Farías[3] and Jaime Romero[2]

1 Núcleo de Investigación en Producción Alimentaria, Departamento de Ciencias Agropecuarias y Acuícolas, Universidad Católica de Temuco, Temuco, Chile
2 Laboratorio de Biotecnología, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, Chile
3 Departamento de Ingeniería Química, Facultad de Ingeniería y Ciencias, Universidad de La Frontera, Temuco, Chile

Correspondence
Jaime Romero, Laboratorio de Biotecnología, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, Chile. Email: jromero@inta.uchile.cl

Received 17 July 2018; In Revised form 22 November 2018; accepted 25 November 2018.

Abstract

Sperm DNA quality and gene expression are crucial for early embryo development. Abnormal genomic processes can cause irreversible damage to totipotent cells, thereby altering the capacity for cell differentiation. Cryopreservation is a complex procedure that exposes cells to extreme conditions; it is therefore important to determine whether cryopreservation affects genomic stability. Despite this, few studies have focused on the effects of cryopreservation on the genomic stability of fish sperm. Thus, information is lacking to determine how the cryopreservation of fish sperm affects embryo quality and subsequently aquaculture. The aim of this review was to compile background information pertaining to the study of genomic stability in cryopreserved sperm. In this way, we hope to characterize more clearly the embryonic development of fish that are of biological and economic interest. We also discuss the potential use of antioxidants in cryopreservation to help preserve DNA integrity, gene expression and embryo quality. There is a current need to evaluate the use of potential biomarkers of genomic integrity to establish the effects of cryopreservation on the DNA quality of fish gametes and embryos.

Key words: biomarkers, cryopreservation, DNA integrity, embryos, gene expression, spermatozoa.

Introduction

Fish play a key role in the world's food supply and consumption has grown steadily during the last five decades (FAO/WHO 2014, 2016). Aquaculture production has also increased, due to demand from consumers and the depletion/restriction of wild capture fisheries, now accounting for 50% of all fish consumed globally; this industry is expected to become the main source of fish and seafood by 2030 (Henchion *et al.* 2017). The development of protocols for sperm preservation in salmonids and other commercially valuable fish species is fundamental for improving the productivity of global aquaculture. In this context, cryopreservation is a well-established technique used to preserve gametes and improve reproduction efficiency, and is widely used in aquaculture with acceptable results (Cabrita *et al.* 2010). It has been used to increase the longevity of spermatozoa for periods extending to years (Betsy & Kumar 2014); however, it can cause extensive cellular and molecular damage, not yet studied in full, leading to a reduction in sperm quality and an increased rate of malformed embryos (Herráez *et al.* 2017). It has been shown that cryopreservation alters structural and physiological components of cells, specifically affecting plasma membrane integrity, mitochondrial membrane potential ($\Delta\Psi m$) and enzyme activity; while production of free radicals (ROS) increases and ATP concentrations are disrupted (Figueroa *et al.* 2017). All of these have been shown to affect the integrity, motility and fertilization capacity of post-thawed spermatozoa (Figueroa *et al.* 2017; Magnotti *et al.* 2018). On the other hand, several works have demonstrated that sperm cryopreservation generates damage to DNA in mammals (Hu *et al.* 2008) and fish (Pérez-Cerezales *et al.* 2009). Since the main purpose of spermatozoa is

the transmission of paternal genetic information to the next generation, the preservation of genome integrity must be prioritized when establishing cryopreservation protocols.

Although it is not yet clear how this damage is produced, the cryopreservation process has been shown to involve an increase in ROS such as superoxide (O_2^{-}), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), hypohalous acids (HOX), nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^{-}$). These generate abasic sites, cross-linking and structural lesions in the DNA double strand, which modify base locations and cause DNA fragmentation (Aitken & De Iuliis 2007; Pérez-Cerezales *et al.* 2009). Furthermore, some studies have shown differing susceptibilities of sperm nuclear genes to cryodamage (Cartón-García *et al.* 2013; González-Rojo *et al.* 2014), affecting both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). These negative effects do not always decrease sperm functionality and fertilization capacity; however, they may affect cellular and molecular processes post-fertilization and generate defective embryos (Pérez-Cerezales *et al.* 2011; Herráez *et al.* 2017; Fernández-Díez & Herráez 2018). Studies in sheep and human gametes and embryos have shown that both DNA integrity and gene expression are negatively affected by cryopreservation. Moreover, studies in fish indicate that cryopreservation causes damage to certain regions of nDNA and mtDNA, however further studies are needed to fully characterize this damage (Cartón-García *et al.* 2013).

The negative effects of cryopreservation on sperm DNA may be repaired during embryonic growth and development; however, any loss of DNA integrity and changes in gene expression caused by cryopreservation could be related to alterations in morphogenesis and cell differentiation at the developmental level of the brain, ears, eyes and kidneys in both mammals and fish (Lin *et al.* 2008; González-Rojo *et al.* 2014; Kopeika *et al.* 2015). In fish, cryopreservation has also been shown to shorten telomeres, a region of non-coding DNA found at the ends of chromosomes, possibly affecting embryonic development and causing teratogenicity in offspring (Pérez-Cerezales *et al.* 2011).

In mammals, embryos produced by sperm with damaged DNA are generally not able to survive past the blastocyst stage, and suffer alterations in the transcription of genes involved in early growth and development (Menezo *et al.* 2010; Gawecka *et al.* 2013). Specifically, the transcription of genes encoding insulin-like growth factors (*Igf*), growth factors (*Gh*) and insulin (*Ins*) has been shown to be affected (Pérez-Cerezales *et al.* 2011), leading to multiple abortions during early embryo development. In addition, these genes associated with cell growth are related to cancer development, ageing, abnormal growth and abnormal behaviour in adult individuals (Aitken *et al.* 2009; González-Rojo *et al.* 2014; Kopeika *et al.* 2015). Since the integrity and correct replication of DNA, as well as proper gene expression, are crucial for embryonic development, abnormal genomic processes can cause irreversible damage to totipotent cells and the embryo. Cryopreservation is a complex process involving physical and chemical factors that expose cells to extreme conditions. Therefore, it is important to study the extent to which cryopreservation adversely affects genomes, and the mechanisms involved. Despite this, studies focusing on this subject in fish spermatozoa and embryo quality are limited and sparse; hence the aim of this review was to compile background information pertaining to the study of DNA quality in cryopreserved fish spermatozoa, and its impact on gene expression and embryonic development, as well as identifying possible targets for future research.

Characteristics of sperm DNA

Chromatin integrity in spermatozoa has been shown to play an important role during embryonic development, and a close relationship has been established between the stability of chromosome structural organization and the fertilizing capacity of spermatozoa (Evenson *et al.* 1999). Moreover, sperm chromatin is reported to play a more important role in early embryonic development than during fertilization (Speyer *et al.* 2010). The DNA in the spermatozoa of most vertebrates is highly compacted in combination with protamines, providing better protection to the genetic material. During mammalian spermatogenesis, most histones are replaced by transition proteins, which are further substituted by protamines (Miller *et al.* 2010). This process, however, is not homogenous, then different regions of chromatin are finally packed in one of three different schemes: (i) DNA linked to histones with a nucleosomal organization (HDNA), representing up to 15% of chromatin; (ii) DNA linked to protamines (PDNA) adopting a toroids form characteristic of sperm nuclei; and (iii) a small fraction of DNA located between nucleosomes and toroids and bound to sperm nuclear matrix (Ward 2010; González-Rojo *et al.*2014). This differential compartmentalization of paternal genes leads to different accessibility, and has been shown to be related to early or late transcription during embryonic development. On the other hand, the packaging of sperm genome in teleost fish varies widely; some species use only histone, while others use protamine or protamine-like proteins in DNA compaction. These variations have been studied to understand their sensitivity to environmental effects, including exposure to toxins or radiation, ageing, cold storage, cryopreservation or changes in thermal regime during spermiogenesis (Saperas *et al.* 1997; Pérez-Cerezales *et al.* 2009). In some species, such as *Oncorhynchus mykiss* (Christensen *et al.* 1984), the chromatin is arranged with protamines, while in other species DNA is associated with somatic-like histones to form nucleosomes, as in

Danio rerio and *Sparus aurata* (Kurtz *et al.* 2009; Wu *et al.* 2011). Yet other species, such as *Mullus surmuletus*, have chromatin organized using both protamines and histones (Saperas *et al.* 1994).

The genes participating in early embryonic development are usually located in hypomethylated arrays associated with histones, meaning that they are readily accessible for early transcription (Ward 2010; Wu *et al.* 2011). These special characteristics have been observed in human sperm in studies of *Hox loci* (Hammoud *et al.* 2009). Additionally, these genes in particular are more exposed than others to harmful agents, and therefore are more susceptible to cryodamage. In line with this, differences in the level of oxidative damage in mouse sperm DNA have been found to target specific chromatin domains with lower compaction, associated with histones and bound to the nuclear matrix, due to oxidative lesions following treatment with H_2O_2 (Noblanc *et al.* 2013). Thus, it can be said that chromatin arrangement and interaction with proteins are related to the susceptibility of DNA to cryodamage by oxidative stress.

Studies in frozen sperm of *Sparus aurata*, whose DNA is homogenously compacted with histones, revealed lesions in two nuclear genes related to embryonic growth and development (*igf1* and *Gh*) (Cartón-García *et al.* 2013); however, they were less susceptible to DNA damage than spermatozoa of *Salmo trutta* and *O. mykiss* (Cabrita *et al.* 2005; Martínez-Páramo *et al.* 2009; Pérez-Cerezales *et al.* 2009). Chromatin groups, all homogenously compacted with histones, include genes that regulate embryonic development; these sets of genes are similar in humans and mice (Wu *et al.* 2011). Nevertheless, limited information exists regarding candidate genes or genetic biomarkers that could be used for evaluation of cryopreservation effects on spermatozoa genes associated with early development of fish embryos.

The methodologies traditionally used for evaluating chromatin integrity, such as the comet assay, sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD) and terminal deoxynucleotidyl transferase dUTP nick end labelling assay (TUNEL), analyse different aspects of the complete nuclear genome state, but they are not sufficiently sensitive to detect damage in specific genes (González-Rojo *et al.* 2014; Figueroa *et al.* 2016b). At present, microarray analysis, real-time PCR analysis (qPCR), next-generation sequencing (NGS) and bioinformatics analysis are the available technologies capable of detecting genomic variation, including single nucleotide polymorphisms, point mutations, insertions, deletions, inversions and translocations. They are powerful tools for defining the characteristics of individual cells and identifying differences between them (Bäumer *et al.* 2018).

Effects of cryopreservation on DNA quality and gene expression

It has been demonstrated in human, monkey, boar, ram, mouse and fish that cryopreservation damages the DNA of sperm, altering the expression of the genes involved in early and late embryo development (Ron-el *et al.* 1991; Donnelly *et al.* 2001; Hammadeh *et al.* 2001; Labbe *et al.* 2001; Zilli *et al.* 2003; Fraser & Strzezek 2004; Peris *et al.* 2004; Li *et al.* 2007; Yildiz *et al.* 2007). These alterations in gene expression patterns have been associated with abnormal segmentation patterns, alterations in organogenesis and embryonic malformations, causing increased mortality in fish embryos and hatched larvae (Kopeika *et al.* 2004; Park *et al.* 2006; Bobe & Labbé 2010; Labbé *et al.* 2017; Martínez-Páramo *et al.* 2017). In addition, DNA damage is related to an increase in mutagenic alterations in genes associated with embryonic development (Valcarce *et al.* 2013; Kopeika *et al.* 2015).

DNA fragmentation is a classic approximation parameter for evaluating DNA quality in the frozen spermatozoa of fish that are of biological and economic interest for aquaculture (Labbe *et al.* 2001; Zilli *et al.* 2003; Cabrita *et al.* 2005; Dietrich *et al.* 2005; Beirão *et al.* 2008; Pérez-Cerezales *et al.* 2009; Riesco *et al.* 2011; Figueroa *et al.* 2018). Damage to sperm chromatin has been shown to have greater effects on early embryonic development than on the fertilization process (Speyer *et al.* 2010) due to the role that sperm chromatin plays in the first steps of embryonic development (Delbès *et al.* 2010; Ward 2010). For example, Pérez-Cerezales *et al.* (2010) reported in *O. mykiss* sperm with 10% fragmented DNA that oocyte fertilization and production of viable larvae are possible, with survival values above 60%. In another study of the same species, increased abortion rates and differential expression of genes related to larval growth and development were found when sperm with fragmented DNA was used (Pérez-Cerezales *et al.* 2011).

In salmonids in general, sperm DNA fragmentation during freezing exceeds 20%, more than in other teleost species, but this could decrease depending on the oocyte repair mechanism during the fertilization and embryo development processes described in mammals and fish (Cabrita *et al.* 2005; Zhou *et al.* 2016; Huang *et al.* 2017). However, this repair capacity is limited. Yamauchi *et al.* (2007) observed in mice that the zygote was unable to repair 50 kb fragments of sperm with fragmented DNA. In the case of fish, oocytes have a high capacity to repair damaged DNA, with up to 10% repair of fragmented sperm chromatin estimated during the process of fertilization and embryonic development (Fernández-Díez *et al.* 2015, 2016).

The main mechanisms responsible for sperm DNA fragmentation during cryopreservation are the production

of ROS and reactive nitrogen species (RNS) (Aitken *et al.* 2012; Figueroa *et al.* 2017). Oxidation weakens the guanine and adenine bonds at position 8 (8-oxodG and 8-oxodA) adjacent to the ribose unit, allowing the formation of abasic sites or thymine dimers that destabilize and break down DNA structure (Dianov & Parsons 2007; Pérez-Cerezales *et al.* 2009; González-Rojo *et al.* 2014), affecting the expression of genes associated with the fertilizing capacity of spermatozoa, such as *bdnf* (brain-derived neurotrophic factor) and *hspa8* (heat shock protein 8) (Najafi *et al.* 2017; Riesco *et al.* 2017). In addition, other significant effects have been reported in sperm mitochondria of mammals and fish that are susceptible to damage by cryopreservation, altering the mitochondrial function and mtDNA integrity (Yakes & Van Houten 1997; Cartón-García *et al.* 2013; Figueroa *et al.* 2016a, 2018). This is presumably due to the absence of chromatin organization and the generation of secondary ROS caused by alterations in the electron transport chain; these could be the main causes of the greater vulnerability of mitochondrial DNA to damage (Figueroa *et al.* 2017).

Currently, damage to spermatic DNA derived from the effects of ROS is evaluated through classic techniques such as 8-oxodG and 8-oxodA detection. It can be quantified by flow cytometry with specific probes or with a Western blot of 8-Oxoguanine glycosylase (OGG1), allowing the repair expression of OGG1 enzymes in the spermatozoa to be identified and evaluated (Martínez-Páramo *et al.* 2009; Shaliutina *et al.* 2013; Farias *et al.* 2018). Another technique in which progress has been made in detecting damage in specific genes or regions of the genome is reverse transcription polymerase chain reaction (RT-PCR) (Cawthon 2002; Bennetts & Aitken 2005; Fernandez-Gonzalez *et al.* 2008; Pérez-Cerezales *et al.* 2009). A complementary analysis would be the evaluation of telomere length using real-time PCR analysis (qPCR). It has been reported that telomere regions remain associated with histones and situated near the periphery of the nucleus (Gineitis *et al.* 2000; Wykes & Krawetz 2003). This fact could be associated with higher susceptibility to cryodamage caused by ROS.

In frozen fish sperm, the number of lesions in specific genes has been quantified with quantitative PCR (qPCR). This analysis is based on the ability to detect DNA lesions (abasic sites, cross-linking, double lesions, modification of nitrogenous bases, chain breaks and DNA fragmentation) that delay and block the advance of polymerase in the DNA template, causing a decrease in the number of amplified products and a displacement in the threshold cycle (Ct) (Cartón-García *et al.* 2013). This method, used in studies involving the cryopreservation of human sperm, has been applied to detect lesions in genes that are key to the fertilization and early development of human embryos. Additionally, lesions have been quantified in specific genes in the primordial germ cells (PGCs) of zebrafish (Cartón-García *et al.* 2013; Riesco & Robles 2013; González-Rojo *et al.* 2014; Herráez *et al.* 2017).

In recent years, the evaluation of DNA quality in spermatozoa has gained special attention as an important quality biomarker in *Oncorhynchus mykiss, Salmo salar, Acipenser ruthenus, Acipenser gueldenstaedtii* and *Acipenser baerii* during storage and cryopreservation. In addition, the incorporation and evaluation of genes of interest have improved the quality of these markers of spermatic and embryonic quality, for example of the *Hox* (*HoxA3a-1, HoxB5bi, HoxC4a-2, HoxD4ai, HoxD9aii*), *Sox2* and *Eif1b* genes which are responsible for the regulation of morphogenesis and cell differentiation during early embryonic development; these have been shown to be susceptible to oxidative stress induced by cryopreservation (Table 1) (Pérez-Cerezales *et al.* 2011; González-Rojo *et al.* 2014). According to González-Rojo *et al.* (2014), the *Hox, Sox2 and Eif1b* genes would be good candidate genes as biomarkers of genotoxic damage (UV irradiation, hydrogen peroxide treatment and cryopreservation) in *O. mykiss* spermatozoa. The gene expression of other candidate genes, such as *Igf1* and *Gh* which are related to embryonic growth and development, should be analysed to determine the relationship between these genes and the quality of the fish embryo (Cartón-García *et al.* 2013).

Semen antioxidant system components are present in seminal plasma and within the spermatozoa; however, lower availability of antioxidant molecules in the cell cytoplasm limits the antioxidant activity in this compartment (Lahnsteiner & Mansour 2010; Shiva *et al.* 2011). On the other hand, seminal plasma antioxidant activity decreases when semen is diluted with cryoprotective media prior to freezing, since concentration of both, enzymatic and non-enzymatic antioxidants, is reduced. This generates higher vulnerability to oxidative stress, leading to structural alterations of spermatozoa during cryopreservation (Figueroa *et al.* 2017). Several recent studies, though, have shown that in mammals and fish, supplementing cryoprotective media with antioxidants successfully reduces the effects of ROS on spermatozoa, exhibiting different degrees of protection depending on species (Jeong *et al.* 2009; Cabrita *et al.* 2011; Figueroa *et al.* 2018).

Embryo survival and quality

Measures of fish embryo survival, or the ability of fertilized eggs to develop successfully, can be used to evaluate the quality of gametes produced. Survival is usually evaluated at different stages (early/late), that is, in the first cell divisions of the embryo, during organogenesis, or in more

Table 1 Genetic biomarkers of interest for the study of the effect of cryopreservation of semen at the sperm, mitochondrial and embryonic levels in fish

Cell	Target Genes	Role	Species	Author
Genomic sperm DNA	*hspa 8*	Sperm maturity, fertility, spermatogenesis		
	bdnf	Fertility		
	kita	Sperm factor at fertilization, TR-KIT expression appears to correlate particularly with sperm DNA integrity		
	lepa	Sperm physiology and energy metabolism		
	myca	Capacitation		
	acvr 1/Ihcgr			Pérez-Cerezales *et al.,* 2011.
	bik	Potential male fertility markers	*D. rerio*	
	dmrtl		*O. mykiss*	Cartón-Garcia *et al.,* 2013.
	fshb			
	hsdl7b4		*S. aurata*	Guerra et al., 2013.
	gh	Cell growth, reproduction and regeneration		González-Rojo *et al.,* 2014
	igfl	Regulation of neuronal development		
	hox a3a-l			
	hox b5bi	Embryonic development and organogenesis		
	hox c4a-2			
	hoxd 9aii			
	SOX	Regulation of embryonic development and cell differentiation		
	eiflb	Early development		
	tert	Role in cellular senescence in response to stress and damage occurred in a cell		
Mitochondrial sperm DNA	*cyt b*	Electron transport and the generation of ATP		
	co i			Cartón-Garcia *et al.,* 2013
			S. aurata	
	atpSgl		*O. mykiss*	
	tp5g3			Fernández-Diez *et al.*, 2015
	atp5s	ATP production		
	cmc1			
	sdhafl			
Embryonic and larvae genomic DNA	*igfl*			
	igf2			
	igfr la			
	igfr lb			
	sox 2			
	sox 3			
	wntl6	Regulation early/late development and growth		
	id2			
	ube2b			Gabillard *et al.*, 2003
	kif3a			
	angptl			Caruso et al., 2008.
	dadl			
	hlx			
	ghl		*C. auratus*	Pérez-Cerezales *et al.,* 2011
	gh2		*D. rerio*	Marandel *et al,* 2013
	gfr4	Growth of bones, soft tissues and viscera	*O. mykiss*	
	glgl			Riesco and Robles, 2013
	gflr			
	Ptn			Fernández-Diez *et al.*, 2015
	ins 1	Regulation of carbohydrate and lipid metabolism		
	ins 2			
	tert	Role in cellular senescence in response to stress and damage occurred in a cell		
	hsp 70	Survival in situations of thermal shock or osmotic stress		
	hsp 90			

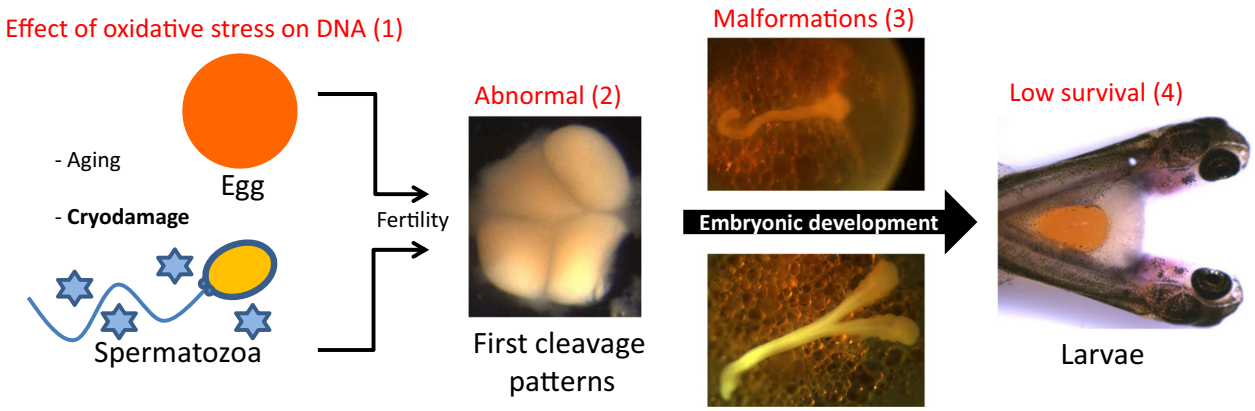

Figure 1 Effects of *in vitro* gamete management techniques and their relationship with the quality of embryonic development in fish: (1) Effects of cellular ageing (increased production of reactive oxygen species (O_2^-) and lipoperoxidation) and cryodamage (✶) on the integrity of genomic DNA in gamete quality (fertilizing capacity); (2) and (3) effect of altered genomic DNA in the expression of genes that control early (first cleavages) and late (organogenesis) embryonic development; (4) Possible activation of lethal genes that increase changes in phenotype expression, increasing teratogenic levels and mortality of larvae produced in aquaculture.

advanced phases such as during hatching and reabsorption of the yolk sac. This monitoring can be extremely valuable to evaluate embryonic development and how it relates with the quality of the gametes (fertilization capacity) produced in experimental and productive conditions in aquaculture (Kopeika *et al.* 2007; Valdebenito *et al.* 2017).

The appearance of embryonic or larval malformations can be a useful tool for characterizing the development potential of fertilized gametes during *in vitro* handling (Figure 1). In *O. mykiss*, it has been shown that the conditions of reproductive fish which produce low quality gametes induce some specific types of malformations in the offspring (Bonnet *et al.* 2007). This phenomenon could increase when cryopreserved gametes are used. For example, in *Clarias gariepinus,* an increase in the rate of malformations was observed in larvae that were produced with cryopreserved spermatozoa (Horvath & Urbanyi 2000); however, not all species are affected equally – see the example of *O. mykiss* reported by Labbe *et al.* (2001). One explanation for the discrepancies between species is that some species may have more resistant DNA than others (Cabrita *et al.* 2014; Fernández-Díez *et al.* 2015; Figueroa *et al.* 2017; Magnotti *et al.* 2018). It is also possible, in some cases, that DNA defects in the sperm may be repaired by the oocyte repair system after fertilization, as in the case of *Logurnus fossilis* (Kopeika *et al.* 2004).

It is clear, nonetheless, that DNA integrity and sperm quality are crucial for the survival and proper development of fish embryos. In addition to the traditional evaluations therefore (for example of sperm DNA fragmentation), there is a need to identify and study specific genes susceptible to cryodamage, or those in which further transcription is altered as result of freezing processes, in order to find specific markers for predicting and optimizing the reproduction efficiency of fish species of biological and commercial value.

Perspectives

Currently, there is little information regarding the effects of fish sperm genomic quality on embryo quality, hence further studies are necessary in order to gain new insights into the genes involved in early embryo development which might be prone to damage or altered expression, leading to mortality or malformations. The object of this review was to provide available information to support future studies and identify possible targets for further investigation, especially those related to cryopreservation-induced damage. Particular attention should also be paid to gene expression after fertilization and in early embryonic development. This knowledge is necessary to optimize cryopreservation protocols and to establish biomarkers of DNA quality (candidate genes) associated with the control and traceability of embryonic development in fish. Identification of these predictive indicators or biomarkers of gamete quality would have major applications both in research and in industry. The development of this area of research will contribute to general scientific knowledge and will provide standardized tools to guarantee the successful use of cryopreservation for fish of interest for aquaculture.

Acknowledgements

This work was supported by the Fund for the Promotion of Scientific and Technological Development of CONICYT, FONDECYT/POSTDOCTORAL (No. 3180765) and FONDECYT/REGULAR (No. 1171129 and 1180387) Chile.

References

Aitken RJ, De Iuliis GN (2007) Origins and consequences of DNA damage in male germ cells. *Reproductive Biomedicine Online* **14**: 727–733.

Aitken RJ, De Iuliis GN, McLachlan RI (2009) Biological and clinical significance of DNA damage in the male germ line. *International Journal of Andrology* **32**: 46–56.

Aitken RJ, Jones KT, Robertson SA (2012) Reactive oxygen species and sperm function in sickness and in health. *Journal of Andrology* **33**: 1096–1106.

Bäumer C, Fisch E, Wedler H, Reinecke F, Korfhage C (2018) Exploring DNA quality of single cells for genome analysis with simultaneous whole-genome amplification. *Scientific Reports* **8** (1): 7476.

Beirão J, Cabrita E, Soares F, Herráez MP, Dinis MT (2008) Cellular damage in spermatozoa from wild-captured *Solea senegalensis* detected with comet analysis and annexin-V. *Journal of Applied Ichthyology* **24**: 508–513.

Bennetts LE, Aitken RJ (2005) A comparative study of oxidative DNA damage in mammalian spermatozoa. *Molecular Reproduction and Development: Incorporating Gamete Research* **71** (1): 77–87.

Betsy CJ, Kumar JSS (2014) Role of cryopreserved fish spermatozoa as a biotechnological tool in enhancing fish production. *International Journal of Fisheries and Aquatic Studies* **1** (3): 22–25.

Bobe J, Labbé C (2010) Egg and sperm quality in fish. *General and Comparative Endocrinology* **165**: 535–548.

Bonnet E, Fostier A, Bobe J (2007) Characterization of rainbow trout egg quality: a case study using four different breeding protocols, with emphasis on the incidence of embryonic malformations. *Theriogenology* **67**: 786–794.

Cabrita E, Robles V, Rebordinos L, Sarasquete C, Herraez MP (2005) Evaluation of DNA damage in rainbow trout (Onco*rhynchus mykiss*) and gilthead sea bream (*Sparus aurata*) cryopreserved sperm. *Cryobiology* **50**: 144–153.

Cabrita E, Sarasquete C, Martinez-Páramo S, Robles V, Beirão J, Pérez-Cerezales S *et al.* (2010) Cryopreservation of fish sperm: applications and perspectives. *Journal of Applied Ichthyology* **26**: 623–635.

Cabrita E, Ma S, Diogo P, Martínez-Páramo S, Sarasquete C, Dinis MT (2011) The influence of certain aminoacids and vitamins on post-thaw fish sperm motility, viability and DNA fragmentation. *Animal Reproduction Science* **125**: 189–195.

Cabrita E, Martínez-Páramoa S, Gavaia PJ, Riesco MF, Valcarce DG, Sarasquete C *et al.* (2014) Factors enhancing fish sperm quality and emerging tools for sperm analysis. *Aquaculture* **432**: 389–401.

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

Cawthon RM (2002) Telomere measurement by quantitative PCR. *Nucleic Acids Research* **30**: 1–6.

Christensen ME, Rattne JB, Dixon GH (1984) Hyperacetylation of histone H4 promotes chromatin decondensation prior to histone replacement by protamines during spermatogenesis in rainbow trout. *Nucleic Acids Research* **12**: 4575–4592.

Delbès C, Hales BF, Robaire B (2010) Toxicants and human sperm chromatin integrity. *Molecular Human Reproduction* **16**: 14–22.

Dianov GL, Parsons JL (2007) Co-ordination of DNA single strand break repair. *DNA Repair* **6**: 454–460.

Dietrich GJ, Szpyrka A, Wojtczak M, Dobosz S, Goryczko K, Żakowski Ł *et al.* (2005) Effects of UV irradiation and hydrogen peroxide on DNA fragmentation, motility and fertilizing ability of rainbow trout (*Oncorhynchus mykiss*) spermatozoa. *Theriogenology* **64**: 1809–1822.

Donnelly ET, McClure N, Lewis SE (2001) Cryopreservation of human semen and prepared sperm: effects on motility parameters and DNA integrity. *Fertility and Sterility* **76**: 892–900.

Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E (1999) Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. *Human Reproduction* **14**: 1039–1049.

FAO/WHO (2014) The State of World Fisheries and Aquaculture 2014. Opportunities and Challenges. FAO/WHO, Rome. 243 pp.

FAO/WHO (2016) *The State of World Fisheries and Aquaculture 2016. Contributing to Food Security and Nutrition for All*. FAO/WHO, Rome. 200 pp.

Farias JG, Zepeda A, Castillo R, Figueroa E, Ademoyero OT, Pulgar VM (2018) Chronic hypobaric hypoxia diminishes the expression of base excision repair OGG1 enzymes in spermatozoa. *Andrologia* **50** (2): e12876.

Fernández-Díez C, Herráez MP (2018) Changes in transcriptomic profile of trout larvae obtained with frozen sperm. *Aquaculture* **492**: 306–320.

Fernández-Díez C, González-Rojo S, Montfort J, Le Cam A, Bobe J, Robles V *et al.* (2015) Inhibition of zygotic DNA repair: transcriptome analysis of the offspring in trout (*Oncorhynchus mykiss*). *Reproduction* **149** (1): 101–111.

Fernández-Díez C, González-Rojo S, Lombó M, Herráez MP (2016) Impact of sperm DNA damage and oocyte-repairing capacity on trout development. *Reproduction* **152**: 57–67.

Fernandez-Gonzalez R, Moreira PN, Perez-Crespo M, Sánchez-Martin M, Ramirez MA, Pericuesta E *et al.* (2008) Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. *Biology of Reproduction* **78**: 761–772.

Figueroa E, Valdebenito I, Merino O, Ubilla A, Risopatron J, Farias JG (2016a) Cryopreservation of Atlantic salmon *Salmo salar* sperm: effects on sperm physiology. *Journal of Fish Biology* **89**: 1537–1550.

Figueroa E, Valdebenito I, Farias JG (2016b) Technologies used in the study of sperm function in cryopreserved fish spermatozoa. *Aquaculture Research* **47**: 1691–1705.

Figueroa E, Valdebenito I, Zepeda AB, Figueroa CA, Dumorne K, Castillo RL *et al.* (2017) Effects of cryopreservation on

mitochondria of fish spermatozoa. *Reviews in Aquaculture* **9**: 76–87.

Figueroa E, Farias JG, Lee-Estevez M, Valdebenito I, Risopatrón J, Magnotti C *et al.* (2018) Sperm cryopreservation with supplementation of α-tocopherol and ascorbic acid in freezing media increase sperm function and fertility rate in Atlantic salmon (*Salmo salar*). *Aquaculture* **493**: 1–8.

Fraser L, Strzezek J (2004) The use of comet assay to assess DNA integrity of boar spermatozoa following liquid preservation at 5 degrees C and 16 degrees C. *Folia Histochemica et Cytobiologica* **42**: 49–55.

Gabillard JC, Duval H, Cauty C, Rescan PY, Weil C, Le Bail PY (2003) Differential expression of the two GH genes during embryonic development of rainbow trout Oncorhynchus mykiss in relation with the IGFs system. *Molecular Reproduction and Development* **64**: 32–40.

Gawecka JE, Marh J, Ortega M, Yamauchi Y, Ward MA, Ward WS (2013) Mouse zygotes respond to severe sperm DNA damage by delaying paternal DNA replication and embryonic development. *PLoS ONE* **8**: e56385.

Gineitis AA, Zalenskaya IA, Yau PM, Bradbury EM, Zalensky AO (2000) Human sperm telomere-binding complex involves histone H2B and secures telomere membrane attachment. *Journal of Cell Biology* **151**: 1591–1597.

González-Rojo S, Fernández-Díez C, Guerra SM, Robles V, Herraez MP (2014) Differential gene susceptibility to sperm DNA damage: analysis of developmental key genes in trout. *PLoS ONE* **9** (12): e114161. https://doi.org/10.1371/journal.pone.0114161.

Guerra SM, Valcarce DG, Cabrita E, Robles V (2013) Analysis of transcripts in gilthead seabream sperm and zebrafish testicular cells: mRNA profile as a predictor of gamete quality. *Aquaculture* **406–407**: 28–33.

Hammadeh ME, Greiner S, Rosenbaum P, Schmidt W (2001) Comparison between human sperm preservation medium and TEST-yolk buffer on protecting chromatin and morphology integrity of human spermatozoa in fertile and subfertile men after freeze- thawing procedure. *Journal of Andrology* **22**: 1012–1018.

Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT (2009) Distinctive chromatin in human sperm packages genes for embryo development. *Nature* **460**: 473–478.

Henchion M, Hayes M, Mullen AM, Fenelon M, Tiwari B (2017) Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods* **6** (7): 53. https://doi.org/10.3390/foods6070053.

Herráez MP, Ausió J, Devaux A, González-Rojo S, Fernández-Díez C, Bony S *et al.* (2017) Paternal contribution to development: sperm genetic damage and repair in fish. *Aquaculture* **472**: 45–59.

Horvath A, Urbanyi B (2000) The effect of cryoprotectants on the motility and fertilizing capacity of cryopreserved African catfish *Clarias gariepinus* (Burchell 1822) sperm. *Aquaculture Research* **31** (3): 317–324.

Hu JH, Li QW, Jiang ZL, Li WY (2008) Effects of different extenders on DNA integrity of boar spermatozoa following freezing–thawing. *Cryobiology* **57**: 257–262.

Huang CJ, Wu D, Jiao XF, Khan FA, Xiong CL, Liu XM *et al.* (2017) Maternal SENP7 programs meiosis architecture and embryo survival in mouse. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1864**: 1195–1206.

Jeong YJ, Kim MK, Song HJ, Kang EJ, Ock SA, Mohana Kumar BM (2009) Effect of alpha-tocopherol supplementation during boar semen cryopreservation on sperm characteristics and expression of apoptosis related genes. *Cryobiology* **58**: 181–189.

Kopeika J, Kopeika E, Zhang T, Rawson DM, Holt WV (2004) Effect of DNA repair inhibitor (3-aminobenzamide) on genetic stability of loach (*Misgurnus fossilis*) embryos derived from cryopreserved sperm. *Theriogenology* **61** (9): 1661–1673.

Kopeika E, Kopeika J, Zhang T (2007) Cryopreservation of fish sperm. *Methods in Molecular Biology* **368**: 203–217.

Kopeika J, Thornhill A, Khalaf Y (2015) The effect of cryopreservation on the genome of gametes and embryos: principles of cryobiology and critical appraisal of the evidence. *Human Reproduction Update* **21**: 209–227.

Kurtz K, Saperas N, Ausio J, Chiva M (2009) Spermiogenic nuclear protein transitions and chromatin condensation. Proposal for an ancestral model of nuclear spermiogenesis. *Journal of Molecular Evolution* **44** (4): 422–431.

Labbe C, Martoriati A, Devaux A, Maisse G (2001) Effect of sperm cryopreservation on sperm DNA stability and progeny development in rainbow trout. *Molecular Reproduction and Development* **60** (3): 397–404.

Labbé C, Robles V, Herráez MP (2017) Epigenetics in fish gametes and early embryo. *Aquaculture* **472**: 93–106.

Lahnsteiner F, Mansour NA (2010) A comparative study on antioxidant systems in semen of species of the Percidae, Salmonidae, Cyprinidae, and Lotidae for improving semen storage techniques. *Aquaculture* **307**: 130–140.

Li Zl, Ql Lin, Liu RJ, Xie WY, Wf Xiao (2007) Reducing oxidative DNA damage by adding antioxidants in human semen samples undergoing cryopreservation procedure. *Zhonghua Yi Xue Za Zhi* **87**: 3174–3177.

Lin MH, Lee RKK, Li SH, Lu CH, Sun FJ, Hwu YM (2008) Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. *Fertility and Sterility* **90** (2): 352–359.

Magnotti C, Cerqueira V, Lee-Estevez M, Farias JG, Valdebenito I, Figueroa E (2018) Cryopreservation and vitrification of fish semen: a review with special emphasis on marine species. *Reviews in Aquaculture* **10** (1): 15–25.

Marandel L, Labbe C, Bobe J, Jammes H, Lareyre JJ, Le Bail PY (2013) Do not put all teleosts in one net: focus on the sox2 and pou2 genes. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **164**(2): 69–79.

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

Martínez-Páramo S, Horváth Á, Labbé C, Zhang T, Robles V, Herráez P *et al.* (2017) Cryobanking of aquatic species. *Aquaculture* **472**: 156–177.

Menezo Y, Dale B, Cohen M (2010) DNA damage and repair in human oocytes and embryos: a review. *Zygote* **18**: 357–365.

Miller D, Brinkworth M, Iles D (2010) Paternal DNA packaging in spermatozoa: more than the sum of its parts? DNA, histones, protamines and epigenetics. *Reproduction* **139** (2): 287–301.

Najafi A, Amidi F, Sedighi Gilani MA, Moawad AR, Asadi E, Khanlarkhni N *et al.* (2017) Effect of brain-derived neurotrophic factor on sperm function, oxidative stress and membrane integrity in human. *Andrologia* **49**: e12601.

Noblanc A, Damon-Soubeyrand C, Karrich B, Henry-Berger J, Cadet R, Saez F *et al.* (2013) DNA oxidative damage in mammalian spermatozoa: where and why is the male nucleus affected? *Free Radical Biology and Medicine* **65**: 719–723.

Park SY, Kim EY, Cui XS, Tae JC, Lee WD, Kim NH *et al.* (2006) Increase in DNA fragmentation and apopto- sis-related gene expression in frozen-thawed bovine blastocysts. *Zygote* **14**: 125–131.

Pérez-Cerezales S, Martínez-Paramo S, Cabrita E, Martínez-Pastor F, Herráez MP (2009) Evaluation of oxidative DNA damage promoted by storage in sperm from sex-reversed rainbow trout. *Theriogenology* **71**: 605–613.

Pérez-Cerezales S, Martínez-Páramo S, Beirão J, Herráez MP (2010) Evaluation of DNA damage as a quality marker for rainbow trout sperm cryopreservation and use of LDL as cryoprotectant. *Theriogenology* **74** (2): 282–289.

Pérez-Cerezales S, Gutiérrez-Adán A, Martínez-Paramo S, Beirão J, Herráez MP (2011) Altered gene transcription and telomere length in trout embryo and larvae obtained with DNA cryodamaged sperm. *Theriogenology* **76**: 1234–1245.

Peris SI, Morrier A, Dufour M, Bailey JL (2004) Cryopreservation of ram semen facilitates sperm DNA damage: relationship between sperm andrological parameters and the sperm chromatin structure assay. *Journal of Andrology* **25**: 224–233.

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

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

Riesco MF, Oliveira C, Soares F, Gavaia PJ, Dinis MT, Cabrita E (2017) *Solea senegalensis* sperm cryopreservation: new insights on sperm quality. *PLoS ONE* **12** (10): e0186542. https://doi.org/10.1371/journal.pone.0186542.

Ron-el R, Nachum H, Herman A, Golan A, Caspi E, Soffer Y (1991) Delayed fertilization and poor embryonic development associated with impaired semen quality. *Fertility and Sterility* **55**: 338–344.

Saperas N, Ausio J, Lloris D, Chiva M (1994) On the evolution of protamines in bony fish: alternatives to the "retroviral horizontal transmission" hypothesis. *Journal of Molecular Evolution* **39** (3): 282–295.

Saperas N, Chiva M, Pfeiffer DC, Kasinsky HE, Ausio J (1997) Sperm nuclear basic proteins (SNBPs) of agnathans and chondrichthyans: variability and evolution of sperm proteins in fish. *Journal of Molecular Evolution* **44** (4): 422–431.

Shaliutina A, Hulak M, Gazo I, Linhartova P, Linhart O (2013) Effect of short-term storage on quality parameters, DNA integrity, and oxidative stress in Russian (*Acipenser gueldenstaedtii*) and Siberian (*Acipenser baerii*) sturgeon sperm. *Animal Reproduction Science* **139**: 127–135.

Shiva M, Gautam AK, Verma Y, Shivgotra V, Doshi H, Kumar S (2011) Association between sperm quality, oxidative stress, and seminal antioxidant activity. *Clinical Biochemistry* **44**: 319–324.

Speyer BE, Pizzey AR, Ranieri M, Joshi R, Delhanty JDA, Serhal P (2010) Fall in implantation rates following ICSI with sperm with high DNA fragmentation. *Human Reproduction.* **25**: 1609–1618.

Valcarce DG, Carton-García F, Riesco MF, Herráez MP, Robles V (2013) Analysis of DNA damage after human sperm cryopreservation in genes crucial for fertilization and early embryo development. *Andrology* **1**: 723–730.

Valdebenito I, Cosson J, Contreras P, Sánchez JC, Oliveira RPS, Risopatrón J *et al.* (2017) Spermatological research of experimentally farmed Patagonian blenny (*Eleginops maclovinus*) (*Perciformes: Eleginopsidae*) in Chile. *Aquaculture Research* **48** (8): 4197–4204.

Ward SW (2010) Function of sperm chromatin structural elements in fertilization and development. *Molecular Human Reproduction* **16**: 30–36.

Wu SF, Zhang H, Cairns BR (2011) Genes for embryo development are packaged in blocks of multivalent chromatin in zebrafish sperm. *Genome Research* **21** (4): 578–589.

Wykes SM, Krawetz SA (2003) The structural organization of sperm chromatin. *Journal of Biological Chemistry* **278**: 29471–29477.

Yakes FM, Van Houten B (1997) Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proceedings of the National Academy of Sciences of the United States of America* **94**: 514–519.

Yamauchi Y, Shaman JA, Boaz SM, Ward WS (2007) Paternal pronuclear DNA degradation is functionally linked to DNA replication in mouse oocytes. *Biology of Reproduction* **77**: 407–415. https://doi.org/10.1095/biolreprod.107.061473.

Yildiz C, Ottaviani P, Law N, Ayearst R, Liu L, McKerlie C (2007) Effects of cryopreservation on sperm quality, nuclear DNA integrity, in vitro fertilization, and in vitro

embryo development in the mouse. *Reproduction* **133**: 585–595.

Zhou X, Wang L, Du Y, Xie F, Li L, Liu Y *et al.* (2016) Efficient generation of gene-modified pigs harboring precise orthologous human mutation via CRISPR/Cas9-induced homology-directed repair in zygotes. *Human Mutation* **37**: 110–118.

Zilli L, Schiavone R, Zonno V, Storelli C, Vilella S (2003) Evaluation of DNA damage in *Dicentrarchus labrax* sperm following cryopreservation. *Cryobiology* **47**: 227–235.