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Sea bass sperm freezability is influenced by motility variables and membrane lipid composition but not by membrane integrity and lipid peroxidation

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ABSTRACT

Cryopreserved sperm quality depends on the characteristics of fresh sperm. Thus, it is necessary to establish a group of variables to predict the cryopreservation potential of the fresh samples with the aim of optimizing resources. Motility, viability, lipid peroxidation and lipid profile of European sea bass (*Dicentrarchus labrax*) sperm were determined before and after cryopreservation to establish which variables more accurately predict the sperm cryopreservation potential in this species. Cryopreservation compromised sperm quality, expressed as a reduction of motility ($46.5 \pm 2.0\%$ to $35.3 \pm 2.5\%$; $P < 0.01$) and viability ($91.3 \pm 0.7\%$ to $69.9 \pm 1.6\%$; $P < 0.01$), and produced an increase in lipid peroxidation (2.4 ± 0.4 to 4.0 ± 0.4 μmoles MDA/mill spz; $P < 0.01$). Also, significant changes were observed in the lipid composition before and after freezing, resulting in a reduction in the cholesterol/phospholipids ratio (1.4 ± 0.1 to 1.1 ± 0.0; $P < 0.01$), phosphatidylcholine ($47.7 \pm 0.8\%$ to $44.2 \pm 0.8\%$; $P < 0.01$) and oleic acid ($8.7 \pm 0.2\%$ to $8.3 \pm 0.2\%$; $P < 0.05$) in cryopreserved sperm, as well as an increase in lysophosphatidylcholine ($4.4 \pm 0.3\%$ to $4.8 \pm 0.3\%$; $P < 0.01$) and C24:1n9 fatty acid ($0.5 \pm 0.1\%$ to $0.6 \pm 0.1\%$; $P < 0.05$). Motility, velocity, cholesterol/phospholipids ratio, monounsaturated fatty acids and the n3/n6 ratio were positively correlated ($P < 0.05$) before and after freezing, whereas, viability and lipid peroxidation were not correlated. Motility and the cholesterol/phospholipids (CHO/PL) ratio were negatively correlated ($P < 0.05$) with each other and the CHO/PL ratio was positively correlated ($P < 0.05$) with lipid peroxidation. Therefore, the results demonstrated that motility and plasma membrane lipid composition (CHO/PL) were the most desirable variables determined in fresh samples to predict cryo-resistance in European sea bass sperm, taking into account the effect of both on cryopreserved sperm quality.

1. Introduction

In general, cryopreservation has been widely used for reproductive practices, germplasm conservation and genetic improvement of resources in several species of mammals (Watson and Fuller, 2001). However, despite its application to preserve the genetic profile of threatened species (He et al., 2011; Martínez-Páramo et al., 2009) or strains with biotechnological interest (Robles et al., 2009), cryopreserved sperm is scarcely used for routine fertilization practices. Factors such as reduced motility and fertilization ability, embryo development failure or reduced offspring survival and quality (Cabrita et al., 2010; Pérez-Cerezales et al., 2010, 2011) limit the use of cryopreserved fish sperm. However, this may be counteracted

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by improving cryopreservation protocols and adding certain compounds that protect cells during freezing–thawing (Cabrita et al., 2001; Martínez-Páramo et al., 2012) or by a more precise selection of samples prior to freezing (Cabrita et al., 2011). There are several reports relating post-thaw sperm quality to the initial features of fresh samples. Therefore, during recent years, numerous variables (motility, viability, cell concentration, osmolality, pH, enzymatic activity, membrane composition and antioxidant activity) have been used to characterize sperm to establish which samples can be cryopreserved (Groison et al., 2010; Lahnsteiner et al., 2010; Rurangwa et al., 2004). This procedure would guarantee the reduction of risks and damage associated with the use of bad quality samples.

Motility is the most common variable used for determining sperm quality (Cosson et al., 2008). Also, in many cases, a correlation between sperm motility and its ability to fertilize eggs has been established for some species (Dietrich et al., 2005; Ottesen et al., 2009). In general, sperm motility decreases after cryopreservation, and fish sperm contains different spermatozoa subpopulations according to motility pattern, which may be differentially affected by the cryopreservation protocol (Beirão et al., 2011). Plasma membrane composition and integrity are key factors affecting sperm functionality (Am-in et al., 2011; Argov et al., 2007; Lahnsteiner et al., 2009). During freezing and thawing, the formation of ice crystals together with osmotic shock, promote cell breakages that reduce the percentage of viability (Asturiano et al., 2007). Moreover, several authors have demonstrated that the cryopreservation process modifies the spermatozoa plasma membrane lipid profile (Cerolini et al., 2001; Chakrabarty et al., 2007), inducing changes in phospholipids and cholesterol organization, which modify cellular homeostasis, leading to losses in sperm function.

Different studies have shown that the variables used to characterize sperm quality respond differently to the cryopreservation process depending on the species (Rurangwa et al., 2004). Therefore some variables determined in fresh sperm may predict the capacity for sperm cryopreservation in one species but fail to do so in another. Motility predicted sperm cryopreservation potential in several mammalian species such as goat and boar (Dorado et al., 2010; Flores et al., 2009) and in some fish species such as Atlantic cod (Butts et al., 2011). In other studies, a positive correlation occurred between early changes in sperm membrane integrity and post-thaw quality of boar spermatozoa (Peña et al., 2007). Furthermore, Zilli et al. (2004) determined the β-D-glucuronidase activity and the ATP concentration in sea bass sperm and considered that fertilization ability in both fresh and cryopreserved sperm could be reliably predicted by determining these biochemical parameters. Taking into account that sperm freezability depends on initial sperm quality, it is of paramount importance to define a group of variables that accurately predict which males are "good" or "bad" sperm donors for cryopreservation or which samples may have more chances of resisting cryodamage. Moreover the possibility of defining key quality variables that ensure the characterization of sperm quality in frozen samples can contribute to a faster analysis, avoiding time-consuming screening of quality descriptors in frozen samples.

In the present work, sperm from European sea bass (*Dicentrarchus labrax*) was characterized before and after cryopreservation, to determine which of the variables tested were useful for forecasting sperm cryopreservation potential in this species. Thus, motility, viability, lipid peroxidation and lipid profile of European sea bass sperm were determined with the aim of establishing possible correlations between sperm quality before and after freezing.

2. Materials and methods

2.1. Sperm collection, cryopreservation and thawing

European sea bass males, supplied by the Aqualvor fishfarm (Odiaxere, Lagos, Portugal), were used for this experiment. Sperm was collected during the natural reproductive cycle, from the beginning of November to the end of February to obtain samples of different quality. A total of 32 pools containing sperm from 10 to 12 males (mean weight 840 ± 132 g) were obtained.

Immediately after extraction, sperm was diluted (1:6, v/v) in a non-activating mineral medium (NAM: 59.8 mM NaCl, 1.5 mM KCl, 12.9 mM $MgCl_2$, 3.5 mM $CaCl_2$, 20 mM $NaHCO_3$, 0.4 mM glucose and 1% (w/v) BSA, pH 7.7) to avoid motility activation (Fauvel et al., 1998). Diluted sperm was maintained at 4 °C for further analyses of motility, viability, level of lipid peroxidation and lipid extraction.

For cryopreservation, 10% DMSO (final concentration; v/v) was added to the diluted sperm and, following the protocol described by Martínez-Páramo et al. (2012). The mixture was immediately loaded into 0.5 ml straws (I.M.V., France) and frozen for 15 min at 6.5 cm above a liquid nitrogen surface, and then immersed in the nitrogen and stored in a container until used. They were thawed at 35 °C for 15 s.

2.2. Determination of quality variables

2.2.1. Sperm motility

Sperm motility was analyzed in fresh and cryopreserved sperm using computer-assisted sperm analysis (CASA). Sperm placed in a Makler chamber (0.5 μl of diluted sperm; 1:6, v/v in NAM) was activated with 20 μl of artificial sea water (513.3 mM NaCl, 10.7 mM KCl, 11.7 mM $CaCl_2$, 54.8 mM $MgSO_4$ and 11.6 mM $NaHCO_3$), and immediately, digitalized images obtained using an 10× negative phase contrast objective in a light microscope (Nikon E200, Tokyo, Japan) were recorded with a Basler camera (Basler Afc, Ahrensburg, Germany) at 10, 20, 30 and 45 s post-activation. Images were processed with ISAS software (Proiser, Valencia, Spain) to determine total spermatozoa motility (TM, %), progressive motility (PM, %), velocity (VCL and VSL, μm/s) and linearity index (LIN, %).

2.2.2. Cell viability

Cell viability was determined before and after cryopreservation using the double fluorescent dye SYBR-green and propidium iodide (PI) (Invitrogen, Spain). Sperm diluted 1:6 in NAM was re-diluted 1:1000 in the same