Antioxidant status in fresh and cryopreserved sperm from gilthead sea bream (Sparus aurata)

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INTRODUCTION

Cryopreservation may induce oxidative stress on the sperm membrane, causing irreversible damage such as changes in membrane fluidity, enzymatic activity and further peroxidative insult affecting to ONA and other cellular structures. These findings are associated with a reduction in sperm motility, viability and fertilizing ability, due to a significant generation of reactive species (ROS) by spermatozoa (Aitken et al., 1989a,b; de Lamirande and Gagnon, 1992). Superoxide dismutase (SOD), glutathione reductase (GSR) and glutathione peroxidase (GPX) are the main proactive enzymes, acting as scavenging agents that remove reactive species once formed. In fish spermatozoa there are no previous studies on the presence and activity of these enzymes, or how cryopreservation could affect their functionality. It is known that during freezing/thawing spermatozoa suffer different kind of stress that may be responsible for ROS production. Moreover, damage on sperm function can be minimized by addition of antioxidants to semen prior to cryopreservation (Bucak et al., 2007). thus the evaluation of total oxidants in plasma samples may give some indication on further post-thaw sperm quality.

The aim of the present work was to analyse total antioxidant levels (TAS) and the activity of glutathione peroxidase (GPX), glutathione reductase (GSR) and superoxide dismutase (SOD) in fresh and cryopreserved sperm.

MATERIALS ANO METHOOS

Sperm collection and freezing

Sperm was collected by stripping from 50 males, which were maintained at ICMAN facilities under photoperiod control. Immediately alter collection, half of each sample was cryopreserved using 5% DMSO in 1 % NaCI solution. Diluted sperm (1:6) was loaded into 0.5 ml straws and frozen in liquid nitrogen vapour (2 cm above the LN, surface during 10 min). Samples were stored in a liquid nitrogen container until use.

Sperm processíng

Fresh sperm was centrifuged (6000xg, 15 min, 4 ºC) to separate seminal plasma and cells. Seminal plasma was stored at -80 °C. Spermatozoa were diluted (1:1) in 0.01 M PBS plus 0.1 % Triton and frozen in liquid nitrogen. Two cycles of freezing/thawing were used to lyse the cells. Cell lysates were diluted (1:3) in 0.01 M PBS and stored at -80 ºC. Cryopreserved samples were thawed (30 s at 25 ºC) and processed in that same manner.

Enzymatic assays *and total antioxidant status (TAS)*

The activity of glutathione peroxidase (GPX), glutathione reductase (GSR) and superoxide dismutase (SOD) and Total antioxidant status (TAS) were determined using specific determination kits (RANDOX, Crumlin, UK) modifying the protocols provided by the manufacturer, for fish sperm.

RESULTS

Activity of all antioxidant enzymes was detected in seminal plasma. However only superoxide dismutase and glutathione peroxidase were detected in spermatozoa extracts. No significant differences ($p<0.05$) were detected between fresh and cryopreserved samples ranging the values for SOD between 2.5±0.51 and 2.6±0.49 U mg^{-1} for fresh and cryopreserved spermatozoa. Superoxide dismutase values were 1.6±0.12 U ml^{-1} and 1.8±0.06 U ml^{-1} in fresh and cryopreserved seminal plasma, respectively. Both glutathione reductase and peroxidase were detected in fresh and cryopreserved seminal plasma (3.60-127.60 and 4.71-88.86 U ml^{-1}, respectively), however no detectable values of GSR were found in spermatozoa extracts. Total antioxidant status in fresh seminal

plasma was 0.1±0.16 mmol l^{-1}. No detectable values were registered in cryopreserved samples.

DISCUSSION AND CONCLUSIONS

Our results demonstrate that both seminal plasma and spermatozoa showed activity for SOD, GPX, been GSR only present in seminal plasma. None of them was affected by cryopreservation . As was demonstrated in human sperm (Storey, 1997). seminal plasma offers an additional protection against ROS due to the presence of other antioxidant species, hereby detected by TAS. The lack of total antioxidant status may render the sperm more susceptible to further oxidative damage alter cryopreservation, which may indicate the need of adding some antioxidants to the extender media.

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