

# Comunicación Oral

## Protocol optimization for sea bass sperm cryopreservation and assessment of post-thawing dilution to prolong sperm usefulness in aquaculture Mediterranean species

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Our study aimed to optimize the sperm cryopreservation protocol of sea bass (*Dicentrarchus labrax*) and evaluate the post-thawing sperm dilution in species of interest for Mediterranean aquaculture. Sea bass sperm samples (n=6) were cryopreserved using a modified Non-Activating Media (NAM) extender with or without 5% Glucose (Glu), and 10% of Me2SO or MeOH as cryoprotectant. In a second experiment, sea bass sperm samples (n=7) were cryopreserved using a modified NAM extender and Me2SO. In both experiments, an aliquot of post-thawed sperm was diluted in NAM or NAM + Bovine Serum Albumin (BSA), or not diluted (control). Senegalese sole (*Solea senegalensis*) sperm samples (n=13) were cryopreserved. The post-thawed sperm was diluted in Mounib or NAM media, or not diluted (control). European eel (*Anguilla anguilla*) sperm samples (n=12) were cryopreserved. An aliquot of post-thaw sperm was diluted in P1 or P1 + BSA media, or not diluted (control).

Kinetic parameters were checked after thawing: sea bass (0, 2.5 and 6 h), Senegalese sole (0 and 3 h), and European eel (0, 24, 48, 72 and 96 h). The total motility (MOT - %), progressive motility (MOTp - %), the velocities curvilinear (VCL -  $\mu\text{m/s}$ ), straight line (VSL -  $\mu\text{m/s}$ ) and average path (VAP -  $\mu\text{m/s}$ ), were evaluated using a CASA-Mot software. The highest post-thawing kinetic parameters of sea bass sperm were observed when Me2SO or Me2SO+Glu were used and post-thawed sperm was diluted in NAM+BSA. The post-thaw sperm dilution increased kinetic parameters and prolonged sperm capacity compared to undiluted (control) samples. The post-thawing dilution did not increase the quality of the Senegalese sole sperm at 0 h. However, after

3 h, the spermatozoa diluted in NAM maintained the VCL. The European eel post-thaw sperm diluted in P1 maintained the MOT at 48 h and the velocities at 96 h after thawing.

The combination of Me2SO+Glu can be used to cryopreserve sea bass sperm, and MeOH should be avoided. Sea bass post-thawing sperm dilution increases sperm kinetic parameters and prolongs its use at 2.5 h. Senegalese sole post-thaw sperm dilution did not affect the sperm kinetic parameters. European eel post-thaw diluted in NAM can be used without losing quality for 48 h after thawing.

