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Cryopreserving European eel (*A. anguilla*) sperm: comparison of two methods for standardization

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Summary

During the last years two groups of research have been working in parallel to develop protocols to cryopreserve European eel sperm. Each group established their own protocols in Spain (Asturiano et al., 2003; Peñaranda et al., 2009) and Hungary (Müller et al., 2004; Szabó et al., 2005). These methods are completely different in terms of cryoprotectant, volumes, freezing media, grade for dilution, etc., making clear the need of standardization (which is a usual situation in fish species).
Recent experiments being part of the PRO-EEL project (www.pro-eel.eu) proved the efficacy of the "Spanish method" by producing larvae of this species using previously frozen-thawed sperm (Asturiano et al., 2013), although the results should be improved because of the low fertilization rates. On the other hand, the Hungarian team was able to generate hybrid larvae using semen of European eel and Japanese eel eggs (Müller et al., 2012), evidencing the validity of the "Hungarian method".
In the framework of the AQUAGAMETE COST Action (aquagamete.webs.upv.es), a series of joint experiments were carried out in order to standardize eel cryopreservation procedures. Males were matured with weekly injections of hCG and after 10 weeks of treatment sperm samples were extracted. Sperm motility was determined after activation with sea water, and only those having more than 68% of motile cells were selected for the experiment. Sperm

samples were frozen using both protocols. In general, sperm was diluted with a mixture of extender and cryoprotectant, loaded into straws and frozen in the vapor of liquid nitrogen. Straws were thawed by immersion into water bath for a given period of time.
Fresh and post-thawed samples were evaluated using sperm motility and morphometry parameters (using CASA and ASMA software, respectively). Moreover, because sperm cryopreservation in fish often depends on the use of permeating cryoprotectants bearing chemically reactive methyl group, we wanted to decipher whether eel sperm DNA methylation pattern was affected by the different cryopreservation protocols. The degree of DNA methylation was evaluated in fresh and frozen-thawed samples using the restriction enzyme assay and assessing methylation by image analysis and by LUMA.
The “Hungarian method” caused the higher sperm motility results post-thawing. Spermatozoa morphometry analyses and epigenetics evaluation are still in course.
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