STSM report

Zebrafish (*Danio rerio*) sperm cryopreservation was the topic of my research work in the Laboratory of Fish Physiology and Genomics, Rennes, France, where I spent 2 months. Through our work, we tried to refine the circumstances of cryopreservation.

We carried out some different experiments. At first, the quality of individual and pooled stripped sperm was compared. Also different extenders for sperm storage were tested: Hanks' Balanced Salt Solution - with 300 and 400 mOsm/kg osmolality, grayling extender - which is used in cyprinids sperm cryopreservation, and Leibovitz-15. Otherwise, cryopreservation with and without "holding" were tried: basically, Yang-method was used for cryopreservation (Yang et al, 2007), just a short, 6 minutes holding period was tried after the crystallization step, compared with the non-held samples. In the other hand, we tried to repeat the American method (Draper&Moens, 2009): Ginsburg Fish Ringer (Ginsburg 1963), powdered skim milk and 8% methanol were used for cryopreservation and the samples were splitted in 2 straws (10 µl final volume). Afterwards, samples were cryopreserved with splitting: Yang-method was used with "holding" and the samples were splitted in 2 straws according to the American-method. Sperm was cryopreserved with grayling extender: according to the Yang-method (30 µl sample volume) and also with splitting of the samples. Separation was explored: zebrafish were kept individually separately and the sperm quality was checked in every 2 days.

We found, that individual samples are better quality, than the pooled. The grayling extender with holding method is the most favourable for zebrafish sperm cryopreservation, combined with the Yang-method (30 µl sample volume). We could reach very nice results with this: the average fertilization rate with thawed sperm was 11.79%, and the maximum was 58.82% (N=28). In the future, we would like to carry out some further experiments and publish our results.

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