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POST-THAW STORAGE OF SPERM FROM VARIOUS SALMONID SPECIES

Ákos Horváth[1]*, Catherine Labbé[2], Dušan Jesenšek[3], Gergely Bernáth[1], Dániel Kaczkó[1], Zoltán Bokor[1], Béla Urbányi[1]

[1] Department of Aquaculture, Szent István University, H-2100, Gödöllő, Hungary
[2] INRA, Fish Physiology and Genomics, Campus de Beaulieu, F-35000 Rennes, France
[3] Angling club of Tolmin, Trg 1. maja 7, 5220 Tolmin, Slovenia
* Corresponding author e-mail: Horvath.Akos@mkk.szie.hu

INTRODUCTION

Cryopreservation of salmonid sperm is thoroughly studied area of cryopreservation science, several methods that have been publihed were standardized and are used in aquaculture practice. There is no consensus, however, on the period of post-thaw storage of salmonid sperm without loss of fertilizing capacity. Two earlier reports suggest that storage for 2 minutes or 30 seconds following thawing significantly reduces fertilization success (Stoss and Holtz 1981, Lahnsteiner et al. 1996). This would render cryopreservation methods very difficult to apply in field conditions. The objective of this study was to investigate the the effect of post-thaw storage on the motility and fertilizing capacity of cryopreserved sperm in the Adriatic grayling *(Thymallus thymallus),* rainbow trout *(Oncorhynchus mykiss),* marble trout *(Salmo marmoratus)* and the brown trout *(Salmo trutta m. fario).*

MATERIALS AND METHODS

Sperm of the Adriatic grayling and the marble trout (5 individuals in both species) was collected at the Tolminka fish farm in Tolmin, Slovenia, that of the rainbow trout (6 individuals) was collected in Rennes, France while milt of brown trout (6 individuals) was obtained at the Lillafüred trout farm in Hungary. Sperm of the Adriatic grayling, marble trout and brown trout was diluted with an extender containing 200 mM glucose, 40 mM KCl, 30 mM Tris (pH 8.0) and 10% methanol (v/v final concentration) except for the marble trout where 10% DMSO and 10% methanol were tested. Sperm of the rainbow trout was diluted in a commercially available Cryofish extender supplemented with either 10% DMSO or 10% methanol. All samples were loaded into 0.5-ml straws and frozen in the vapor of liquid nitrogen. Following thawing sperm of the grayling was stored for 0, 2, 5, 10, 30, 60 and 120 minutes at room temperature while that of other species was stored for 0, 10 and 60 minutes before use for fertilization. Motility of sperm was determined for the marble and brown trout using CASA. The percentage of eyed and hatched eggs was calculated for each species.

RESULTS

In the Adriatic grayling, the percentage of eyed eggs in the control was 60±3% while the hatch rate was 48±3%. No significant decrease of fertilizing capacity was found between the percentages of eyed eggs (60±2% vs. 59±2%) and hatch rates (47±2% vs. 47±2%) of eggs fertilized with sperm stored for 0 or 60 minutes following thawing. In case of the rainbow trout, fertilization and hatch rates were generally low when DMSO was used as a cryoprotectant (between 19±9% – 1±1% of eyed eggs and 18±9% and 1±1% hatch) and the storage time prior to fertilization reduced even more the development rates. The use of methanol on the contrary yielded the highest percentage of eyed (45±4%) and hatched eggs (41±5%) after 10 minutes of post-thaw storage. A 60 min storage of the methanol frozen-thawed sample prior to fertilization allowed the same development rates as instant fertilization after thawing. All treatments were significantly different from the fresh control (62±4% at eyed stage and 60±4% at hatching).

In the marble trout, the use of methanol again yielded higher fertilization and hatch rates than DMSO at 0 and 10 minutes of post-thaw storage. The highest percentage of eyed (60±15%) and hatched (55±13%) eggs was observed when sperm was used for fertilization immediately following thawing, although significant difference was observed only between 0 and 60 minutes of post-thaw storage. The sperm of one individual of the tested 5 yielded similar results to the rainbow trout:

75% eyed eggs and 71% hatch at 0 minutes as well as 73% eyed eggs and 71% hatch at 10 minutes of post-thaw storage. Post-thaw motility of marble trout sperm remained low (11-15% in average) with very high individual variation.
In the brown trout, post-thaw motility values were surprisingly high: 55±21% at 0 minutes, 56±15% at 10 minutes and 53±12% at 60 minutes of post-thaw storage. Motility of fresh sperm was 88±7%. The quality of eggs was generally low as shown by the percentage of eyed eggs in the control (34±14%). Fertilization rate generally improved with post-thaw storage time (from 29±14% eyed eggs at 0 minutes to 42±19% at 60 minutes), however due to large individual variation no significant difference was found among the treatments or between the treatments and the control.

DISCUSSION AND CONCLUSIONS

Our results demonstrate that the cryopreserved sperm of salmonid species can be stored without a loss of fertilizing capacity for at least 10 minutes. The sensitivity of sperm from various species seems to be species-specific as shown by the relatively high fertilization success of grayling and brown trout sperm even after one hour of post-thaw storage. Change of DMSO for MeOH in rainbow trout sperm cryopreservation did allow a spectacular improvement of post-thaw sperm storage, possibly because of a better control over osmotic challenge. Controversial results with the marble trout can be attributed to the high individual variation, therefore, improvements of the currently used sperm collection, preparation and cryopreservation methods are needed. Results with the brown trout are even more difficult to explain, especially the high post-thaw motility rates. At the moment the only difference in sperm treatment of this species comparing to the other ones was that the extender used for cryopreservation was stored frozen until use, whereas in the other species refrigerated extenders (prepared approximately 1 month before use) were employed. Possible bacterial growth could have contributed to the high individual variation in the marble trout in comparison to the brown trout.

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