Fertilizing ability of frozen-thawed sperm of trout in relation with rearing temperature and dietary lipid origin : what about membrane fluidity and polar lipid composition

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WORKSHOP ON GAMETE AND EMBRYO STORAGE AND CRYOPRESERVATION IN AQUATIC ORGANISMS;

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ABSTRACTS

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FERTILIZING ABILITY OF FROZEN-THAWED SPERM OF TROUT IN RELATION WITH REARING TEMPERATURE AND DIETARY LIPID ORIGIN : WHAT ABOUT MEMBRANE FLUIDITY AND POLAR LIPID COMPOSITION

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A factorial experiment was designed to investigate the influence of low rearing temperature (8°C) and high rearing temperature (18°C) with a linoleic fatty acid rich diet (corn oil) and a n-3 fatty acid rich diet (cod liver oil) on fertilizing ability of frozen-thawed trout sperm. The biochemical and biophysical modifications of the sperm plasma membrane induced by these rearing conditions were assessed. Juvenile trout (one year old and 100 g mean weight) were submitted to one of the temperatures and one of the diets. The experiment was two years during which they went twice through gonadal development and reached twice sexual maturity. Milt was collected throughout the first spawning season. A part was frozen (Maisse *et al.* 1988, *Aquat. Living Res. 1 45-51*). Fatty acid analyses inside each phospholipid classes of the plasma membrane was performed on the remaining semen sample. Sperm from the second spawning season was collected once for biophysical analyses : membrane fluidity was recorded by electron spin resonance (ESR) using spin-labeled phospholipids with a short (C5) b-chain bearing a nitroxide radical (Seigneuret et Devaux 1984, *Proc. Natl. Acad. Sci. USA 81 3751-3755*).

Semen from the 18°C adapted trout displayed a two fold greater fertilizing ability after freeze-thawing than the 8°C ones although motility was the same before cryopreservation. The diet had no influence on sperm quality and the fertilization rates were not significantly modified by either linoleate or n-3 fatty acid enrichments. Fatty acid analysis of each phospholipid revealed that PC had the lower UFA/SFA ratio (= 1,4) with 36 % of 16:0 and PS the higher (= 4) with 26 % of 18:1 n-9 and 26 % of 22:6 n-3. The fatty acid composition of PE, PI (phosphatidyl inositol), PS and PC reflected well the features of the experimental diets mainly with 20:4 n-6 being two fold higher in corn oil-milt and 20:5 n-3 being two fold higher in fish oil-milt. The rearing temperature of the broodstock influenced mainly the 18:0 levels, higher in 18°C-milt than in 8°C-milt. In PE, 16:0 and 20:5 n-3 were higher in 8°C-milt than in 18°C-ones but in minor proportions. Neither diet nor rearing temperature of the broodstock seemed to affect the fluidity of the sperm plasma membrane as assessed with labeled phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS) or sphingomyeline (SM). However, fluidity assessed with labeled cholesterol seemed slightly lower when mesured at 8°C on 8°C-milt than when mesured at 18°C on 18°C-milt. We estimated that 90 % of the membrane PS and 85 % of the membrane PE were localized on the inner leaflet of the membrane while 85 % of PC and 100 % of SM were on the outer leaflet. Labeled PC and SM are in a more packed environment than labeled PS leading to the observation that the outer membrane leaflet is more rigid than the inner one. These characteristics are not influenced by diet or temperature.

In conclusion, we pointed out that 18°C-milt tolerance to cryopreservation is two fold better than 8°C-milt although neither fluidity nor phospholipid fatty acid compositions displayed major differences between the two temperatures. When measured by ESR, no "homeoviscous adaptation" appeared between the two broodstock sperm. We propose that, at the opposite of metabolically active cells, the sperm cell, which has a very low metabolic activity, does not restructure its plasma membrane. Such a restructuring is energy-consuming and would be without adaptative advantage for the gamete. The biological factors involved in the improved freeze-thaw tolerance we observed for 18°C milt are then not yet elucidated. We also described sperm plasma membrane with the outer leflet more fluid, enriched in PE and PS, in MUFA and PUFA while the outer leflet is more rigid, enriched in PC and in SFA. The high differences induced by diet on fatty acid composition did not affect fluidity or sperm freeze-thaw resistance.