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Cryopreservation of the sperm of some freshwater teleosts

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Summary. The sperm of 7 freshwater fishes was cryopreserved using the pellet technique of Nagase (1964). The fertilization rate in the rainbow trout was about 80 p. 100 when the sperm was equilibrated for 15 mins before deep freezing. Under the same conditions, the fertilization rate in the brown trout was about 70 p. 100. All the other species included in the experiments also showed high activity of frozen and thawed spermatozoa, but the fertilization rate was low or zero. It was more difficult to cryopreserve the sperm of cyprinid fishes than the sperm of salmonid fishes, although the activity of the thawed spermatozoa was the same. There are few papers describing experiments and fertilization rates with cryopreserved cyprinid sperm, and further experimentation is thus necessary.

Introduction.

Some of the genetic progress in the breeding of domestic animals can only be obtained by applying the technique of sperm-cryopreservation. This technique would also open the way to new opportunities in the propagation of cultivated freshwater fishes if their sperm could be preserved.

The present investigations were aimed at developing a technique for deep freezing the sperm of freshwater fishes living under the climatic conditions of West Germany.

Materials and methods.

The subjects under investigation during the breeding season of 1976/77 were the rainbow trout (*Salmo gairdneri* Richardson), the brown trout (*Salmo trutta forma* fario L.), the brook trout (*Salvelinus fontinalis* Mitchill), the danube salmon (*Hucho hucho* L.), the grayling (*Thymallus thymallus* L.), the pike (*Esox lucius* L.) and the carp (*Cyprinus carpio* L.).

The sperm was taken by hand stripping from the males and collected in syringes. In the period between collection and cryopreservation the syringes were stored in pond water in order to maintain a constant temperature.

One drop of the sample of each male was examined microscopically before and after dilution with water and dilution medium. Only samples without activity before

dilution and high activity after dilution with water were used for cryopreservation experiments.

The sperm was cryopreserved according to the technique described by Nagase (1964). After dilution the sperm was dropped on carbon ice and stored in liquid nitrogen. The extender always contained 10 p. 100 DMSO. The dilution ratio was 1 : 3 and the size of the pellets 0.2 ml. For thawing, 3 of the pellets were put in 10 ml of a 1 p. 100 $NaHCO_3$ solution. The thawing process was accelerated by rapid shaking. Immediately after thawing the pellets were poured over the eggs or examined microscopically. The sperm was thawed after a storage period of 7 days. According to the results of earlier experiments (Stein, 1976 ; Stein and Lamina, 1976) we tested two different extenders without equilibration time and with an equilibration time of 15 and 20 min. because we found that the equilibration time differs depending on the extender and the species.

Extender 1 : 750 mg NaCl, 200 mg $NaHCO_3$, 53 mg Na_2HPO_4, 23 mg $MgSO_4.7\,H_2O$, 38 mg KCl, 46 mg $CaCl_2.2\,H_2O$, 100 mg glucose, 500 mg glycine, 100 ml H_2O, 20 ml egg yolk (= V2).

Extender 2 : 750 mg NaCl, 200 mg $NaHCO_3$, 38 mg KCl, 100 mg glucose, 100 ml H_2O, 20 ml egg yolk (= V2e).

TABLE 1

Results of fertilization experiments of the breeding season 1976/77

Species	Dilution medium	Equil. time	n	Fertil. rate ± SD*	Number of eggs/sample	Spermatozoa/egg	
						frozen	fresh
Rainbow trout	V2	0	15	78,1 ± 6,9	500	$4\text{-}6.10^6$	
	V2e	15	26	81,9 ± 3,8	—	—	
	control		30	87,9 ± 17,3	—		$6\text{-}10.10^6$
Brown trout	V2	0	15	41,2 ± 17,6	—	—	
	V2e	15	5	77,0 ± 6,5	—	—	
	control		20	90,2 ± 9,3	—	—	
Grayling	V2	0	6	46,8 ± 19,0	1 500	$1\text{-}2.10^6$	
	V2	20	9	55,0 ± 15,4	—	—	
	control		6	95,3 ± 2,4	—		$2\text{-}3.10^6$
Brook trout	V2	0	4	27,4 ± 7,3	500	$4\text{-}6.10^6$	
Danube salmon	V2	0	2	22,5 ± 1,6	500	$4\text{-}6.10^6$	
Pike	V2 (650)	0	2	25,0 ± 2,4	5 000	$0{,}8.10^6$	
Carp	V2	0	2	—	20 000	$0{,}4.10^6$	
	control		1	81,0	—		1.10^6

* SD : standard deviation.

In the pike the NaCl content of Extender 1 was reduced to 650 mg/100 ml because there was a better motility.

The F-test was used to test the significance between different treatments.

Results.

The most interesting results of the fertilization experiments of the breeding season 1976/77 are summarized in table 1.

There was alway high motility in the dilution medium before and after freezing. More than 70 p. 100 of the spermatozoa were motile. The duration of the motility of the thawed spermatozoa was about 30 s. In the rainbow trout the difference in the fertilization rate between V2 and V2e with equilibration is statistically significant ; in the brown trout the difference is highly significant. The difference between equilibrated and non-equilibrated sperm in the grayling is not significant.

There was high motility in the thawed sperm of the carp too, but no fertilizing capacity.

In the brook trout, danube salmon and pike we could not obtain fresh eggs and fresh sperm at the same time, so control tests were impossible.

Discussion.

In the rainbow trout and brown trout the fertilization rate was improved by applying a new extender and an equilibration time of 15 minutes. As compared to our earlier experiments it is now possible to cryopreserve the sperm of the rainbow trout with a fertilization rate of 80 p. 100 and with small variance. In the brown trout the new technique succeeded with a significantly higher fertilization rate but there is still a difference between the two trout species. This difference is very marked in V2 without equilibration time. The number of experiments with the sperm of the brook trout, the danube salmon and the pike was low because we had no more males and females. But the experiments corroborated our earlier results showing that it is possible to cryopreserve the sperm of these species with a relatively low fertilization rate. Further improvement is certainly possible but it is difficult to procure the material.

We could not do more experiments on the grayling either to demonstrate a possible significant increase of the fertilization rate with equilibrated sperm. In the thawed semen of the carp there was high motility, but no fertilizing capacity. The eggs were possibly affected by the thawing solution or the extender. It seems to be very difficult to cryopreserve the sperm of cyprinid fishes. Thus, there are only two papers describing successful fertilization tests with cryopreserved sperm in cyprinid fishes (Moczarski, 1976, 1977).

Conclusion.

The experiments point out that the sperm of salmonid and esocid fishes can be cryopreserved using the technique of Nagase (1964). In the rainbow trout a practical application of this technique would bring a relatively small loss of fertilizing capacity.

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Résumé. La congélation du sperme de 7 téléostéens d'eau douce a été expérimentée en utilisant la technique de Nagase (1964). Avec 15 mn d'équilibration, les taux moyens de fécondation obtenus après décongélation ont été de 80 p. 100 chez *Salmo gairdneri* et environ 70 p. 100 chez *Salmo trutta forma* fario. Le sperme des autres espèces s'est révélé motile, mais peu fécondant, après décongélation. La conservation du pouvoir fécondant du sperme après congélation semble plus difficile à obtenir chez les Cyprinidés que chez les Salmonidés

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