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PROPERTIES AND CRYOPRESERVATION OF DANUBE SALMON (*Hucho hucho*) MILT

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A B S T R A C T. Motility and concentration of spermatozoa, as well as osmolality, protein content, activity of aspartate aminotransferase (AspAT), acid (AcP) and alkaline (AP) phosphatase of seminal plasma were determined in 8 samples of Danube salmon (*Hucho hucho*) fresh milt. Semen pooled from 6 males was cryopreserved in 8 extenders containing DMSO or glycerol as cryoprotectants. Extender containing 0.3 M glucose + 25 mM KCl + 10% egg yolk + 10% DMSO was the most suitable (87.5% of eyed eggs, as compared to control fertilization). Percentage of eyed eggs correlated with post-thaw spermatozoa motility.

Key words: CRYOPRESERVATION, DANUBE SALMON, FERTILIZATION, MOTILITY, SPERM

INTRODUCTION

Cryopreservation of fish sperm is becoming widespread. Experimental-scale cryopreservation of milt is successful mainly in some salmonids (Piironen 1993) showing promise for further application of this tool in breeding programmes. So far, little information is available on the properties of milt of Danube salmon (*Hucho hucho*), and an attempt to cryopreserve its sperm resulted in limited success (Stein & Bayrle 1978). The aims of this study were (i) to determine quality indicators and some biochemical parameters in an intact milt of Danube salmon, and (ii) to find an efficient extender for cryopreservation of its semen.

MATERIAL AND METHODS

Samples of milt were obtained from ripe Danube salmon spawners (7 - 9 years old) cultivated at the Inland Fisheries Institute, Salmonid Research Laboratory, Rutki, Poland. Sperm motility was estimated using a microscope (x 500) and expressed as a percentage of motile spermatozoa. Hatchery water with lixiviated $CaCO_3$ was used

TABLE 1

Constituents of extenders used in cryopreservation of Danube salmon milt.

Extender No.	Constituents	Reference
1	0.3 M glucose + 10% DMSO	Stoss and Reftsie (1983)
2	0.3 M glucose + 10% yolk + 10% DMSO	Alderson and MacNeil (1984)
3	0.3 M sucrose + 10% DMSO	
4	0.3 M sucrose + 10% yolk + 10% DMSO	
5	0.3 M glucose + 23 mM KCl + 10% yolk + 10% DMSO	
6	0.75 g NaCl; 0.2 g $NaHCO_3$; 0.038 g KCl; 0.1 g glucose; 100 ml H_2O + 20% yolk + 10% DMSO	Stein and Bayrle (1978)
7	0.3 M glucose + 20% glycerol	Piironen and Hyvarinen (1983)
8	0.3 M glucose + 10% yolk + 20% glycerol	

as an activating solution. Spermatozoa concentration was estimated using a cytometer (Bürker chamber) method. Total protein content (according to Lowry *et al.* 1951) and activity of aspartate aminotransferase (AspAT), acid phosphatase (AcP) and alkaline phosphatase (AP) was determined in seminal plasma according to the procedure described by Babiak *et al.* (1997). Osmolality of seminal plasma was also evaluated. The suitability of activating diluents (0.7% NaCl, 1% $NaHCO_3$, distilled water, hatchery water, hatchery water with lixiviated $CaCO_3$) was tested for estimation of the spermatozoa motility.

For cryopreservation, milt was pooled from 6 males (spermatozoa motility above 80%, spermatozoa concentration 7.98 x 10^9 ml^{-1}). Pooled sample was diluted (1 : 3) with extenders (Table 1) and dropped within 30 s on a dry ice (-79°C). After 5 min., frozen pellets were removed to liquid nitrogen (-196°C).

Prior to fertilization of eggs, pellets were thawed in a hatchery water containing $CaCO_3$ (warmed up to 30°C), then after 7 s, batches of eggs pooled from 4 females were inseminated (approximately 3 x 10^6 spermatozoa per egg) and divided into three incubation replicates. Control fertilization of eggs was made with the use of fresh sperm.

Post-thaw spermatozoa motility was assessed, and supernatant and spermatozoa AspAT and AcP activity determined according to the procedure described by Babiak *et al.* (1997).

Percentages of eyed eggs were subjected to angular transformation to obtain normal distribution instead of binomial (Babiak *et al.* 1997). Differences between the tran-

sformed mean values were estimated by multiple range test (Tukey HSD intervals). Pearson's correlation coefficient was used to evaluate correlations between the data.

RESULTS AND DISCUSSION

Among the tested activating diluents, the most suitable was hatchery water with lixiviated $CaCO_3$ (5.95 mg% Ca^{2+}, pH = 8.79). Values of quality and biochemical indicators of fresh semen of Danube salmon are given in Table 2. Within groups, the variation was lowest in total protein content, AspAT activity in seminal plasma, and spermatozoa motility.

High variation in seminal plasma osmolality (Table 2) might be due to possible contamination of the semen with urine (Rana 1995). This suggestion can be confirmed by AP activity in seminal plasma, which was not detected in the non-contaminated samples. High values of AP activity in seminal plasma were previously noticed in contaminated sperm of Danube salmon (Glogowski *et al*. 1994).

Survival to the eyed-egg stage in the case of eggs fertilized with cryopreserved sperm, post-thaw spermatozoa motility, and AspAT and AcP activities are given in Table 3. The most suitable extenders were No. 5 (0.3 M glucose + 25 mM KCl + 10% yolk + 10% DMSO), and No. 8 (0.3 M glucose + 10% yolk + 20% glycerol); their use resulted in 87.5% and 73.6% of eyed eggs respectively (as compared to the control fertilization: 29.4%). Relatively low fertilization rate of control group was similar to the results of a routine fertilization in this hatchery, which is not supplied with water containing $CaCO_3$. Percentage of eyed eggs correlated with post-thaw spermatozoa motility ($r = 0.87$; $N = 8$; $P < 0.005$). Post-thaw motility of spermatozoa correlated negatively with supernatant AcP activity ($r = -0.85$; $N = 8$; $P < 0.01$). This suggests a possibility of

TABLE 2

Quality and biochemical indicators of fresh semen of Danube salmon (N = 8).

	Spermatozoa concentration (10^9 ml^{-1})	Motility (%)	Osmolality (mOsm kg^{-1})	Protein (mg ml^{-1})	AspAT (U)	AcP (U)	AP (U)
mean	7.60	73.8	287	1.65	31.4	4.51	61.8
min.	5.22	40.0	245	1.51	28.1	1.65	7.5
max.	9.29	80.0	340	1.77	35.5	6.85	216.2
S.D.	1.50	14.1	27.3	0.11	2.4	1.75	76.5

TABLE 3

Post-thaw motility, activity of aspartate aminotransferase (AspAT) and acid phosphatase (AcP) in supernatants and spermatozoa of cryopreserved Danube salmon sperm, and percentage of eyed eggs (±S.E.) fertilized with cryopreserved milt (as compared to control fertilization, which resulted in 29.4% of eyed eggs). Values having the same superscript do not differ significantly from each other

Extender No.	Eyed eggs (%)	Post-thaw motility (%)	AspAT activity (mU/10^9 spermatozoa)		AcP activity (mU/10^9 spermatozoa)	
	(%)	(%)	supernatants	spermatozoa	supernatants	spermatozoa
1	1.5 ± 0.3^c	5	41.7	17.6	4.60	3.38
2	5.9 ± 1.2^c	5	37.4	18.7	4.45	3.85
3	1.7 ± 0.2^c	1	38.2	20.7	4.70	3.30
4	3.0 ± 0.3^c	1	37.4	18.7	4.80	3.45
5	87.5 ± 2.1^a	20	29.3	19.1	4.40	3.55
6	43.9 ± 1.8^b	20	46.4	15.4	3.90	2.95
7	51.2 ± 2.6^b	30	37.1	22.6	3.50	2.90
8	73.6 ± 1.1^a	50	29.3	23.4	4.15	3.35

applying quality and bochemical indicators in an assessment of cryopreservation efficiency.

This experimental-scale cryopreservation procedure shows some promise for hatchery operations, but low percentage of eyed eggs fertilized with fresh semen requires further investigations on an improvement of artificial propagation of Danube salmon in Northern Poland.

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STRESZCZENIE

WŁAŚCIWOŚCI I KRIOKONSERWACJA MLECZA GŁOWACICY (*Hucho hucho*)

W 8 próbach świeżego mlecza głowacicy (*Hucho hucho*) oznaczono ruchliwość i koncentrację plemników, osmolarność, zawartość białka, aktywność aminotransferazy AspAT oraz fosfatazy kwaśnej i zasadowej (AcP i AP). Następnie zmieszano nasienie 6 samców i poddano je kriokonserwacji w 8 rozcieńczalnikach zawierających DHSO oraz glicerol jako krioprotektory. Najlepszy okazał się rozcieńczalnik zawierający 0,3 M glukozę + 25 mM KCl + 10% żółtka jaja kurzego + 10% DMSO. Tak konserwowany mlecz dał 87,5% zaoczkowanej ikry w porównaniu do zapłodnienia kontrolnego. Procent zaoczkowanej ikry korelował z ruchliwością plemników po rozmrożeniu nasienia.

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