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SHORT-TERM PRESERVATION OF EUROPEAN CATFISH (*Silurus glanis* L.) MILT

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A B S T R A C T. Milt and testes were obtained from European catfish (*Silurus glanis*) and refrigerated. Viability of stored spermatozoa was prolonged by keeping them in plastic bags, inflated with pure oxygen and moistened with water. Further prolongation of storage time was achieved in samples diluted with immobilizing solution. Most successful was preservation of testes under oxygene atmosphere: more than 80% of activated spermatozoa were motile after 29 days and 50% motility was observed on 37th day of storage.

Key words: EUROPEAN CATFISH, FISH, PRESERVATION, *SILURUS GLANIS*, SPERM

INTRODUCTION

Storage of the fish sperm for short periods of time can be applied to hatchery operations, especially in situations where breeders need temperature and/or hormone stimulation. Difficulties in egg collection from female spawners of European catfish (*Silurus glanis* L.) often cause postponing of the time of spawning. In such cases, storage of catfish milt allows efficient egg fertilization irrespectively of delays in female maturation.

Short-term preservation techniques have been developed in some species, mainly salmonids (for review see Scott & Baynes 1980; Stoss 1983). European catfish sperm maintains full fertilization ability up to 13 days of storage at 4 - 6°C (Marian & Krasnai 1987; Linhart *et al*. 1991). The purpose of this study was to develope a method of efficient storage of the catfish sperm during the spawning period for aquaculture application.

MATERIAL AND METHODS

Two males were injected with carp pituitary extract at single doses of 4.5 mg/kg of fish. Ripe donors were anaesthetized in a solution of 2-phenoxyethanol (1 : 1000) and sacrificed. Testes were removed according to the method described by Siwicki & Jeney (1986). After removal testes were cut into pieces and milt was obtained.

Motility of spermatozoa was assessed in 0.3% NaCl activating solution under a microscope (x 500) and expressed as a percentage of motile sperm (Terner 1986; Babiak *et al.* 1995). After estimation of pre-treatment spermatozoa motility (more than 90% of motile spermatozoa), milt and pieces of testes were refrigerated at 1 - 2°C. Experimental groups were set as follows:

A - undiluted milt, air atmosphere;

B - undiluted milt, moistened, oxygen atmosphere;

C1 - milt diluted (1 : 1) with Saad immobilizing solution (NaCl 200 mM, Tris 30 mM, pH 7; Saad *et al*. 1988, cited by Linhart *et al*. 1993) with addition of antibiotics (final concentration of antibiotics: 125 Units penicillin + 0.125 mg streptomycin + 0.250 mg neomycin per 1 mL; Sigma Chemical Co.);

C2 - as in C1 above, supplemented weekly with antibiotics at concentration of 625 U penicillin + 0.625 mg streptomycin + 1.25 mg neomycin per 1 mL;

C3 - as in C1 above, supplemented weekly with antibiotics at concentration of 1667 U penicillin + 1.667 mg streptomycin + 3.333 mg neomycin per 1 mL;

D1 - pieces of testes in testicular fluid;

D2 - pieces of testes, with addition of 5 ml of immobilizing solution with antibiotics (1000 U penicyllin + 1 mg streptomycin + 2 mg neomycin).

All samples were kept in separate glass beakers in refrigerator; milt (both undiluted and diluted) was in a thin layer, not exceeding 5 - 6 mm. Except for experimental group A, beakers were placed in a plastic tray filled with water. The tray was put into a black plastic bag, which was inflated with pure oxygen and sealed. The oxygen was replenished every 24 - 48 hours.

RESULTS AND DISCUSSION

Spermatozoa in undiluted milt in an open beaker (experimental group A) lost their movement ability after 48 hours, in contrast to samples held in closed oxygenated

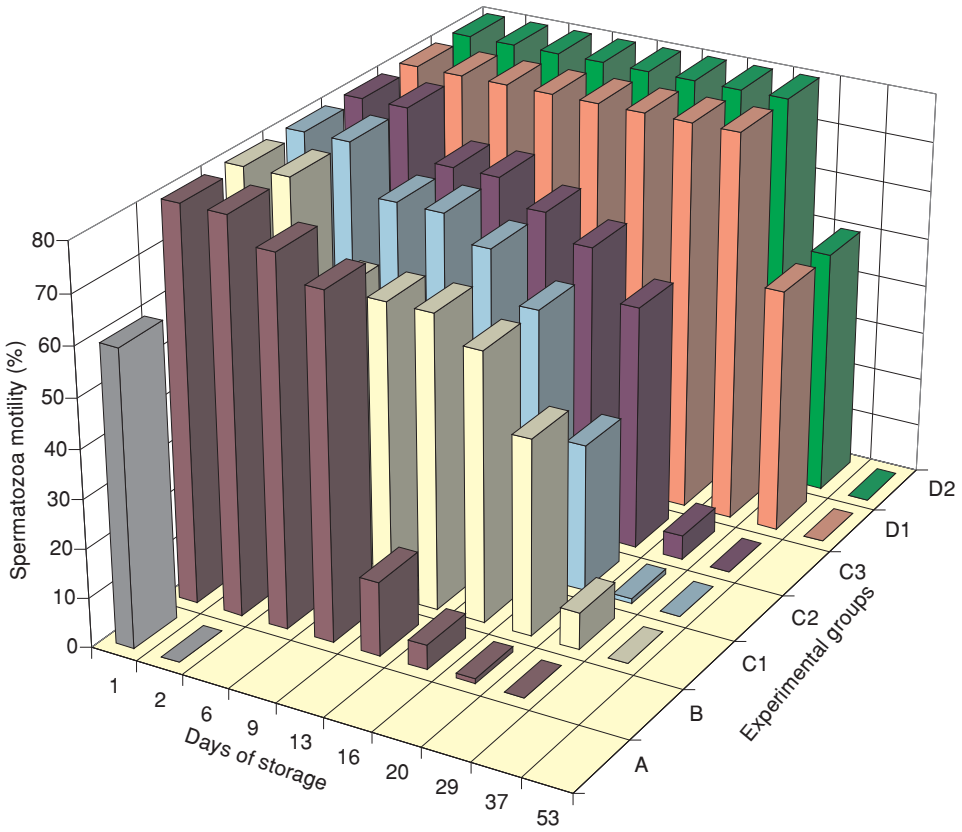

Fig. 1. Motility of European catfish (*Silurus glanis*) spermatozoa during succesive days of storage in a refrigerator (1 - 2°C). A - undiluted milt in air atmosphere; B - undiluted, oxygenated milt in moist atmosphere; C1 - milt diluted in immobilizing solution supplemented with single dose of antibiotics (oxygen, moist atmosphere); C2 - milt diluted in immobilizing solution supplemented weekly with antibiotics (oxygen, moist atmosphere); C3 - milt diluted in immobilizing solution supplemented weekly with antibiotics (other doses than in C2, oxygen, moist atmosphere); D1 - testes in testicular fluid (oxygen, moist atmosphere); D2 - testes in immobilizing solution (oxygen, moist atmosphere).

bags (Fig. 1). The presence of water in tray decreased, but did not eliminate the effect of desiccation. Undiluted and oxygenated milt (experimental group B) became almost completely desiccated after two weeks of storage. Dilution with Saad immobilizing solution proved to be more efficient in prevention of desiccation (experimental groups C1, C2, C3). Testes, submerged in testicular fluid (D1) or in Saad solution (D2), were also well protected against desiccation.

In spite of addition of antibiotics (penicillin + streptomycin + neomycin solution) the presence of microorganisms in samples was observed in diluted, oxygenated milt (C1, C2, C3) since 6th day of storage, similarly like in experimental group B (undiluted, oxygenated milt). Microorganisms in testes (D1, D2) appeared after 13 days of preservation. Antibiotics, supplemented weekly (C2, C3, D2), did not seem to improve sterile conditions, as compared to experimental groups with single adition (C1) or with no addition of antibiotics (D1).

Among all experimental groups, preservation of testes was superior to other treatments. Spermatozoa from chilled and oxygenated testes (D1, D2) maintained their viability for 29 days (Fig. 1). After 37 days of milt storage, spermatozoa motility decreased to 50%.

According to Stoss (1983), the main factors affecting the storage of milt are: temperature, gaseous exchange, sterile conditions and prevention of desiccation. In short-term preservation of European catfish sperm, desiccation was a most probable reason of lack of motile spermatozoa after second day of storage (Fig. 1, experimental group A). Keeping undiluted milt in oxygenated sealed bags with water (experimental group B) prolonged spermatozoa viability to at least 9 days (60% of motility). Hulata & Rothbard (1979) showed that the two-days storage of undiluted sperm of common carp (*Cyprinus carpio*) in refrigerator did not affect spermatozoa fertilizing ability. Also, after 2 days of storage at 4°C milt of European catfish retained full fertilizability (Marian & Krasznai 1987). Sperm of Atlantic sturgeon maintained viability for at least 5 days after storage in a bag inflated with oxygen (DiLauro *et al.* 1994). These results suggest that refrigerator storage of undiluted milt of some non-salmonid species in refrigerator is sufficient for the period of 1 - 2 days. Prolongation for further several days can be achieved by keeping the sperm under oxygen atmosphere in a sealed and moistened bag.

Dilution of the fish sperm in media that do not activate motility of spermatozoa may be beneficial for the storage (Stoss 1983). Immobilizing diluents prevent desiccation and enable use of antibiotics without risk of untimely spermatozoa activation. Chilled storage of diluted semen secures viability of spermatozoa for up to several weeks (Clemens & Hill 1969; Moore 1987; Palmer *et al*. 1994). Using non-aqueous diluent (fluorocarbon), McNiven *et al*. (1993) preserved rainbow trout sperm for 37 days. Linhart *et al*. (1991) diluted sperm of European catfish in Saad immobilizing solution with addition of antibiotics; after 13 days of storage they obtained fertilization rates similar to controls. This agrees well with our results: motility of diluted sperm

(Fig. 1; groups C1, C2, C3) was high (more than 60%) till the 13th day of experiment, then a decrease in percentage of motile spermatozoa occured.

The viability of spermatozoa was prolongated by the storage of testes. Guest *et al*. (1976) stored for 9 weeks macerated pieces of testis of channel catfish (*Ictalurus punctatus*) and motility of spermatozoa could still be induced. In this study preserved testes of European catfish retained full viability of spermatozoa for a month of storage. In hatchery operations, such a period of time is quite sufficient, but there is a need for further investigations on fertilization ability of stored sperm for its application in breeding programs.

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STRESZCZENIE

KRÓTKOOKRESOWE PRZECHOWYWANIE MLECZU SUMA (*Silurus glanis* L.)

Mlecz i jądra suma (*Silurus glanis* L.) przechowywano w lodówce w temperaturze 1 - 4°C. Żywotność przechowywanych plemników została wydłużona poprzez umieszczenie prób w plastikowych torbach napełnionych tlenem i nawilżonych. Dalsze wydłużenie czasu przechowywania osiągnięto poprzez dodanie do prób roztworu immobilizującego, który nie aktywował ruchu plemników, natomiast zapobiegał wysychaniu prób. Najbardziej efektywne okazało się przechowywanie jąder w atmosferze tlenu: po 29 dniach przechowywania ruchliwość plemników wyniosła ponad 80%. Po 37 dniach przechowywania stwierdzono 50% ruchliwych plemników. Ta prosta i niewymagająca specjalistycznego sprzętu metoda, umożliwiająca przechowywanie jąder suma przez 1 miesiąc, może być użyteczna przy sztucznym rozrodzie tej ryby.

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