OUT OF SEASON QUALITY ASSESMENT AND CRYOPRESERVATION OF EURASIAN PERCH (*PERCA FLUVIATILIS*) SPERM

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PROCENA KVALITETA I METODE KRIOPREZERVACIJE SPERME GRGEČA (*PERCA FLUVIATILIS*) UZORKOVANE VAN SEZONE

Apstrakt

Od svih vrsta koje su introdukovane u evropsku akvakulturu, grgeč (*Perca fluviatilis*) najviše obećava. Mogučnost mrešćenja van sezone je jedan od najbitnijih faktora u veštačkom mrestu bilo koje vrste. Gajenje grgeča (u severnoj i zapadnoj Evropi) se uglavnom obavlja u recirkulacionim sistemima, gde je mogućnost proizvodnje u toku cele godine ključni faktor da bi se zadovoljile potrebe tržišta (Migaud et al. 2002). Krioprezervacija sperme je efikasan način smanjenja troškova koji nastaju držanjem matica i pruža dobar kvalitet gameta tokom cele kalendarske godine. (Cabrita et al. 2010). Matice grgeča (Perca fluviatilis) su izlovljavane u periodu od oktobra do novembra 2014. 13 mužjaka (težina: 39-137 g) su čuvani u vodi čija je temperatura iznosila 6-16°C. Ispuštanje sperme je indukovano hormonima, korišćenjem 500 IU^{-1} kg hCG (humanog horionskog gonadotropina). Sperma je sakupljena 1. i 6. dana nakon ubrizgavanja hormona. Parametri pokretljivosti spermatozoida sveže i odmrznute sperme bez ubrizgavanja hormona (Wo), nakon 1. (1da) i nakon 6. (6da) dana ubrizgavanja hormona su kvantifikovani CASA sistemom. Ukupna zapremina sperme nakon istiskanja je izmerena u svim tretmanima. Sperma grgeča bez i sa injektiranog hormona nakon 1. i 6. dana je prezervirana u skladu sa prethodno definisanim protokolima. Za prezervaciju je korišćen zamrzivač sa automatskim programom hlađenja (od 7.5 ºC do -160 ºC, stopa hlađenja: 56 ºC/min) (Bernáth et al. 2015). Sperma sa najvećom prosečnom zapreminom je istisnuta u 6da grupi riba (1611 ± 1428µl). Prosečna zapremina sperme je bila značajno niža u grupi Wo (58 ± 82µl) u odnosu na grupu 6da. Prosečna za-

premina sperme u grupi 1da (64 ± 49µl) se nije statistički razlikovala od druge dve grupe. Progresivna pokretljivost spermatozoida u sveže istisnutoj spermi je bila slična pokretljivosti nakon hormonalne stimulacije (Wo: 79 ± 10%, 1da: 54 ± 26%, 6da: 75 ± 11%). Ista tendencija je zabeležena u slučaju brzine nepravilnog kretanja (VCL) spermatozoida (Wo: 149 ± 24 µm/s, 1da: 137 ± 23 µm/s, 6da: 145 ± 40 µm/s), kao i pravolinijskog kretanja (STR) spermatozoida (Wo: 76 ± 7%, 1da: 80 ± 1%, 6da: 80 ± 8%) u sveže istisnutoj spermi. Slične vrednosti progresivne pokretljivosti, VCL-a i STR-a su izmerene u krioprezerviranim uzorcima nakon odleđivanja sperme. Ipak, progresivna pokretljivost je značajno redukovana u grupi 6da nakon krioprezervacije (11 ± 7%) u poređenju sa sveže istisnutom spermom u grupama Wo i 6da. Pokretljivost u odleđenoj spermi nije značajno opala u grupama Wo (18 ± 8%) i 1da (14 ± 5%), dok je značajno smanjenje primećeno za parametar VCL u grupi 6da, nakon odleđivanja (70 ± 11 µm/s) u odnosu na sve grupe gde je sperma sveže isceđena. Značajno smanjenje u parametru VCL je primećeno u grupi Wo (88 ± 25 µm/s) nakon krioprezervacije u poređenju sa grupama Wo i 6da kada je sperma sveže istisnuta. Parametar VCL u grupi 1da nakon odleđivanja (101 ± 15 µm/s) se nije promenio u odnosu na sveže isceđene grupe. Vrednosti STR-a su bile jako visoke nakon odleđivanja u svim krioprezerviranim grupama (Wo: 90 ± 5%, 1da: 92 ± 2%, 6da: 88 ± 4%). Značajna razlika je primećena između grupe 1da, posle odleđivanja i sveže istisnute sperme grupe Wo. Ovi rezultati su pokazali da je hormonalna stimulacija uspešno sprovedena kod mužjaka grgeča u cilju indukovanja proizvodnje sperme van sezone parenja.

Abstract

Eurasian perch (*Perca fluviatilis*) is a promising species among those that were recently introduced into European aquaculture. Out-of-season spawning is a remarkable factor in artificial propagation of every species. The production of Eurasian perch is mainly (Northern and Western Europe) maintained in recirculating systems where all year long production is a key factor in the satisfaction of current market demands (Migaud et al. 2002). Cryopreservation of sperm could be an efficient tool to reduce the costs of broodstock management and provide good quality gametes all year round (Cabrita et al. 2010). A broodstock of wild caught Eurasian perch (*Perca fluviatilis*) males was established from October to November 2014. The 13 males (bodyweight: 39-137 g) were kept at the same water temperature in the range of 6-16°C (according to the hatchery temperature). Spermiation was hormonally stimulated using 500 IU^{-1} kg hCG (human chorionic gonadotropin). Sperm was stripped 1 day and 6 days after injection according to the experimental design. Motility parameters of fresh and thawed sperm without injection (Wo), 1 day (1da) and 6 days (6da) after injection were measured using a CASA system. The total volume was estimated in all treated freshly stripped groups. Perch sperm was cryopreserved without injection, 1 day after and 6 days after injection according to our previously developed cryopreservation protocol. A controlled rate freezer with a cooling program (from 7.5 ºC to -160 ºC, cooling rate: 56 ºC/min) was used (Bernáth et al. 2015). The largest volume of sperm was stripped 6 days after injection (1611 ± 1428µl). Average sperm volume was significantly lower in Wo (58 ± 82µl) compared to 6da. Total volume of sperm at 1da did not differ significantly from the other groups (64 ± 49µl). Progressive motility of freshly stripped perch sperm was similar after hormonal stimulation (Wo: 79 ± 10%, 1da: 54 ± 26%, 6da: 75 ± 11%). The same tendency was observed in the case of curvilinear velocity (VCL) of

spermatozoa (Wo: 149 ± 24 µm/s, 1da: 137 ± 23 µm/s, 6da: 145 ± 40 µm/s) and straightness (STR) of sperm movement (Wo: 76 ± 7%, 1da: 80 ± 1%, 6da: 80 ± 8%) in freshly stripped sperm.A similar progressive motility, VCL and STR was measured after thawing among cryopreserved groups. However, progressive motility was significantly reduced after cryopreservation in the group 6da (11 ± 7%) compare to fresh Wo and 6da (see above). Post-thaw motility did not decrease significantly in Wo (18 ± 8%) and 1da (14 ± 5%). A significant reduction was observed after thawing in VCL 6da (70 ± 11 µm/s) compared to all fresh groups. A significantly decreased VCL was recorded in Wo (88 ± 25 µm/s) after cryopreservation compared to fresh Wo and 6da. Post-thaw VCL in 1da (101 ± 15 µm/s) did not change in comparison to freshly stripped groups. STR was quite high after thawing in all cryopreserved groups (Wo: 90 ± 5%, 1da: 92 ± 2%, 6da: 88 ± 4%). A significant difference was observed between thawed 1da and fresh Wo. Hormonal stimulation was succesfully used in the out-of-season induction of spermiation in male Eurasian perch. Eurasian perch sperm can be cryopreserved out-of-season, as well.

The work was supported by the projects EUREKA_HU_12-1-2012-0056, 8526-5/2014/TUDPOL of the Ministry of Human Resources of Hungary awarded to Szent István University and the GOP-1.1.1- 11.2012-0306.

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