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Comparative analysis of sperm freezability of sex-reversed female brook trout and sex-reversed female rainbow trout semen

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ABSTRACT

The aim of our study was to compare quality of fresh and cryopreserved semen of sex-reversed female rainbow trout and sex-reversed female brook trout. The effect of different final sperm concentrations in straws with a constant extender concentration and final glucose concentration on the sperm motility parameters of cryopreserved semen was tested. Furthermore, we examined the effect of post-thaw storage time on sperm motility parameters at optimal sperm and glucose concentrations. The effects of semen cryopreservation from sex-reversed female rainbow trout and sex-reversed female brook trout on sperm fertilizing ability at the sperm-to-egg ratios 500,000:1 and 1,000,000:1 after 0 and 60 min of post-thaw storage of semen were investigated. We have demonstrated that final sperm concentrations in straws as well as final glucose concentrations in extended semen influenced post-thaw sperm motility parameters in both species. The high post-thaw sperm motility was recorded for sperm concentrations within the range of 1.0–4.0 × 10^9 spermatozoa ml^{-1} for both species. Furthermore, the glucose concentration appeared to be very important for cryopreservation success and its effect was species-specific. The final glucose concentrations of 0.15 M and 0.19 M, for sex-reversed female rainbow trout and sex-reversed female brook trout, respectively, produced the highest results for sperm motility after cryopreservation. The post-thaw sperm motility was unaffected by 60 and 360 min after thawing for sex-reversed female rainbow trout and sex-reversed female brook trout, respectively. The fertilization rates of cryopreserved semen of sex-reversed female rainbow trout were high and did not differ between the investigated sperm:egg ratios after 0 and 60 min of post-thaw storage. However, fertilization rates of cryopreserved semen of sex-reversed females brook trout were lower and decreased after 60 min of post-thaw storage of semen at sperm: egg ratio 1,000,000:1. In conclusion, our results demonstrated differences in freezability between the semen of sex-reversed female rainbow trout and sex-reversed female brook trout. The semen of both species can be cryopreserved at the final sperm concentration of 3.0 × 10^9 spermatozoa ml^{-1}. However, the influence of final glucose concentration on cryopreservation success, as well as the duration of post-thaw storage time and fertilization rates, are species-specific. In our opinion, standardizing the procedure of semen cryopreservation presented in this study is a prerequisite for the development of repeatable procedures and the future implementation of cryopreserved semen from sex-reversed females into hatchery practice.

1. Introduction

The formation of female monoculture stocks of Salmonids is of interest of commercial aquaculture (Chiasson and Benfey, 2007; Donaldson, 1996; Haffray et al., 2009; Pandian and Sheela, 1995). It eliminates the premature development of males, and reduces early maturation and subsequent mortality in broodstock facilities, as well as reducing the number of necessary broodstock fish (Fitzpatrick et al., 2005). The sex-reversed females (masculinized females, neomales) obtained by the hormonal treatment of fertilized eggs or larvae, retain the female (XX) genotype, but have a male phenotype. For that reason, the spermatozoa produced in their testes possess only an X chromosome (Geffen and Evans, 2000). Sex-reversed females have less well-developed testes and there is usually a lack of sperm ducts. For that reason, the sperm do not undergo complete maturation, and have to be extracted directly from the testes by sacrificing the fish (Johnstone et al., 1979).

The cryopreservation of fish spermatozoa ensures the availability of gametes throughout the year, which is especially important for Salmonid fish farming to provide fish all-year-round. Moreover, this allows the transport of cryopreserved sperm from different fish farms and supports artificial fertilization by eliminating the problems

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associated with asynchronous reproductive activity between males and females (Cabrita et al., 2010; Kopeika and Kopeika, 2008). Moreover, it allows the optimization of reproduction, improving breeding and fish conservation programs (Lubzens et al., 1993; McAndrew et al., 1993). The use of cryopreserved spermatozoa will also reduce animal breeding costs, as the number of individuals in a fish farm needed to preserve the desired genetic variability can be reduced. For these reasons, due to differences in sperm quality, development of cryopreservation protocols is a particular challenge.

Existing knowledge about the quality of sex-reversed female semen is limited. Studies about the quality and cryopreservation of sperm were conducted only on sex-reversed female rainbow trout semen (Ciereszko et al., 2015; Dietrich et al., 2014). However, there is a gap in the knowledge about the semen of other species of sex-reversed females, especially brook trout. In our opinion, sex-reversed female brook trout deserve special attention due to their use in breeding to create commercial hybrids, such as 'tiger' (Bartley et al., 2000). For brook trout, information concerning neither fresh semen of sex-reversed females nor cryopreservation is available.

Development of cryopreservation technology involves semen collection, evaluation of initial sperm quality, cryoprotectants selection, cooling, thawing and evaluation of post-thaw sperm quality (Nynca et al., 2017). Due to commercialization of cryopreserved semen into hatchery practice, the standardization of cryopreservation procedures is highly desired. Recently, the standardized procedure for the cryopreservation of Salmonids semen of normal males was developed (Judycka et al., 2018a; Nynca et al., 2017) with significant species-specific differences in freezing protocols. The critical parameters which need to be evaluated for the successful cryopreservation of Salmonids spermatozoa were found to be optimal sperm concentration in straws and final glucose concentrations in the extended semen. Motility parameters of fresh and cryopreserved semen appeared to be useful for evaluation of effectiveness of cryopreservation. It is also important to determine the quality of post-thaw stored semen in order to evaluate handling time. Due to differences between the semen of sex-reversed females and normal males, it is necessary to carefully determine the sperm cryopreservation protocol of sex-reversed female semen, which was previously established for normal male Salmonids.

The aim of our study was to compare the quality of fresh and cryopreserved semen of sex-reversed female rainbow trout and sex-reversed female brook trout. The effect of different sperm concentrations in straws with a constant extender concentration on the sperm motility parameters of cryopreserved semen was tested. Additionally, we evaluated the effect of final glucose concentration in extended semen on post-thaw sperm motility. Furthermore, we examined the effect of post-thaw storage time on sperm motility parameters at optimal sperm and glucose concentrations. The effects of cryopreservation of semen from sex-reversed female rainbow trout and sex-reversed female brook trout on sperm fertilizing ability at the sperm-to-egg ratios 500,000:1 and 1,000,000:1 after 0 and 60 min of post-thaw storage of semen were investigated.

2. Material and methods

2.1. Source of milt

The experiments were carried out on sex-reversed female rainbow trout (1050 ± 150 g, 44 ± 4 cm) and sex-reversed female brook trout (1200 ± 180 g, 45 ± 5 cm for weight and length, respectively), which were born and raised in the Dąbie Fish Hatchery (Poland). The masculinization of rainbow trout was performed using 4-androsten-11β-ol-3,17-dione and in brook trout using 17α-methyltestosterone following the protocol described by Kuźmiński and Dobosz (2010). Milt was obtained *post mortem* by cutting the testes and gently squeezing them through double-layer gauze to remove any testicular tissue, and was collected individually in an open glass beaker (depth of 0.5 cm). Since the semen of sex-reversed females was characterized by different sperm quality values, the semen samples for experiments were selected from those which were characterized by sperm motility higher than 40%.

2.2. Measurement of sperm concentration and seminal plasma osmolality

The sperm concentration of fresh semen was measured using the computer-aided fluorescent microscopy NucleoCounter SP-100 (Chemometec, Allerød, Denmark), as described by Nynca and Ciereszko (2009). Briefly, semen was diluted 100-times with PBS, then 51-times with Reagent S100 and subsequently loaded into a disposable cassette containing propidium iodide. Data were processed and documented using SemenView software (Chemometec, Denmark). Seminal plasma was obtained by centrifuging of semen at 10,000*g* for 10 min. The supernatant was centrifuged again at the same conditions to ensure that no sperm cells were present in the seminal plasma. The seminal plasma osmolality was measured using a Minitübe Abfüll-u Labortechnik apparatus (Tiefenbach, Germany).

2.3. Effects of semen cryopreservation on sperm motility parameters

2.3.1. Cryopreservation protocol

Cryopreservation followed the previously described procedure using a glucose-methanol (GM) extender (Judycka et al., 2018a; Nynca et al., 2017). Semen mixed with cryoprotectant was loaded into 0.5 ml plastic straws (IMV Technologies, L'Aigle, France), which were placed on a floating rack and equilibrated for 15 min on ice. After equilibration, the straws were frozen 3 cm above liquid nitrogen (in the vapor of liquid nitrogen) for 5 min in a Styrofoam box with an isolating Neopor block (Minitübe GmbH, Tiefenbach, Germany) at cooling rate 35 °C/min and then placed in liquid nitrogen. The straws were then thawed by immersion in a water bath at 40 °C for 10 s.

2.3.2. EXP. 1 - Effects of sperm concentration in straws on post-thaw sperm motility parameters

Semen samples were cryopreserved as described in Section 2.3.1. Semen was diluted in GM extender (0.15 M glucose and 7.5% methanol for sex-reversed females rainbow trout and 0.19 M glucose and 7.5% methanol for sex-reversed females brook trout semen) to obtain a sperm concentrations in the straw of 0.5; 1.0; 2.0; 3.0; 4.0 × 10^9 spermatozoa ml^{-1} combined with a constant final extender concentration. Diluted semen was loaded into 0.5 ml plastic straws for each sperm concentration, equilibrated and frozen in liquid nitrogen. The samples were then processed as outlined in Section 2.3.1.

2.3.3. EXP. 2 - Effects of final glucose concentration in extended semen on post-thaw sperm motility parameters

Semen samples were cryopreserved as described in Section 2.3.1. Semen was diluted to obtain final glucose concentrations in the straw ranging from 0.11 to 0.23 M and 7.5% methanol combined with a constant final sperm concentration in the straw established earlier (3.0 × 10^9 spermatozoa ml^{-1}; Section 2.3.2). Diluted semen was loaded into 0.5 ml plastic straws for each glucose concentration, equilibrated and frozen in liquid nitrogen. The samples were then processed as outlined in Section 2.3.1.

2.3.4. EXP. 3 - Effects of semen post-thaw storage on sperm motility parameters

Semen samples were diluted in extender to optimal conditions (3.0 × 10^9 spermatozoa ml^{-1}, at final glucose concentration of 0.15 M for sex-reversed female rainbow trout and 0.19 M for sex-reversed female brook trout semen) and then cryopreserved as described in Section 2.3.1. The straws were then thawed by immersion in a water bath at 40 °C for 10 s. After thawing, semen samples were stored for 30, 60, 120, 240, 360 (sex-reversed female rainbow trout) and 480 min (sex-reversed female brook trout) at 4 °C using a thermo block.