Aquaculture 486 (2018) 90–97

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Optimal sperm concentration in straws and final glucose concentration in extender are crucial for improving the cryopreservation protocol of salmonid spermatozoa

Sylwia Judycka[a,*], Joanna Nynca[a], Ewa Liszewska[a], Stefan Dobosz[b], Joanna Grudniewska[b], Andrzej Ciereszko[a]

[a] Department of Gametes and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland
[b] Department of Salmonid Fish Research, Inland Fisheries Institute, Rutki, 83-300 Żukowo, Poland

ARTICLE INFO

Keywords:
Spermatozoa
Sperm motility parameters
Salmonids
Cryopreservation
Sperm concentration

ABSTRACT

Ensuring the precise conditions during cryopreservation relies on constant sperm concentration in straws with constant cryoprotectant concentrations, which are essential for standardizing cryopreservation protocols. The aim of our study was to test the effect of different sperm concentrations in straws with constant extender concentration on sperm motility parameters of cryopreserved semen of brown trout (*Salmo trutta* m. *fario*), sea trout (*Salmo trutta* m. *trutta*), brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*). At the same time, the usefulness of different straw sizes (0.25 and 0.5 ml) for cryopreservation of the semen of these species was examined. Additionally, the effect of the final glucose concentration at a constant sperm concentration on post-thaw sperm motility was evaluated. The final sperm concentration in straws at a constant cryoprotectant concentration influenced the post-thaw sperm motility parameters of salmonids. The optimal sperm concentration with high post-thaw sperm motility was 2.0×10^9 spermatozoa ml^{-1} for brook trout, 3.0×10^9 spermatozoa ml^{-1} for brown trout and sea trout and 4.0×10^9 spermatozoa ml^{-1} for Atlantic salmon. The use of 0.5 ml straws led to higher or similar values of post-thaw sperm motility than the 0.25 ml straws. The final glucose concentration of 0.15 M produced the highest results of sperm motility after cryopreservation for all tested species. Our results demonstrated that the influence of sperm concentration in straws on cryopreservation success is species-specific within salmonids. On the other hand, the effect of glucose concentration did not appear to be species-specific. In our opinion, standardization of the semen cryopreservation protocol presented in this study is a prerequisite for the development of a repeatable procedure and hence the future implementation of cryopreserved semen in hatchery practice.

1. Introduction

The cryopreservation of fish spermatozoa allows the optimization of reproduction, improving breeding and fish conservation programs. Moreover, it ensures the availability of gametes throughout the year, allowing the transport of gametes from different fish farms and supporting artificial fertilization by eliminating problems of asynchronous reproductive activity between males and females (Cabrita et al., 2010; Kopeika and Kopeika, 2008; Lubzens et al., 1993; McAndrew et al., 1993). The use of cryopreserved spermatozoa can 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, improving protocols for cryopreservation of spermatozoa, particularly standardizing the freezing and thawing process, is highly desired.

To our knowledge, most freezing protocols of fish spermatozoa are based on a dilution ratio of semen to extender (Cabrita et al., 2010; Martínez-Páramo et al., 2017); thus, the final sperm concentration is variable. Semen concentration varies among species, stocks, and even within samples from the same animal, depending on the collection period during the reproductive season (Cabrita et al., 2010). Therefore, the sperm concentration in straws after dilution with an extender differs between individuals and this could cause differences in post-thaw sperm quality. For this reason, there is a need to standardize cryopreservation protocols by establishing an optimal and constant sperm concentration in straws at a constant cryoprotectant concentration. Recently, a successful attempt has been made to standardize the cryopreservation protocol for rainbow trout semen (Nynca et al., 2017).

* Corresponding author.
E-mail address: s.judycka@pan.olsztyn.pl (S. Judycka).

https://doi.org/10.1016/j.aquaculture.2017.12.019
Received 4 May 2017; Received in revised form 8 December 2017; Accepted 12 December 2017
Available online 12 December 2017
0044-8486/

However, it is unknown at present if the values of sperm concentration standardized for rainbow trout semen can be applied to other salmonid fish.

Simple extenders based on glucose as the external cryoprotectant have been used successfully for the cryopreservation of salmonid semen (Dietrich et al., 2016; Dziewulska et al., 2011; Gwo et al., 1999). However, in these mentioned studies, the glucose concentrations were variable (180, 200, 300 mM) and different dilution ratios were used (1:3, 1:5) for spermatozoa cryopreservation (Horváth et al., 2012; Mansour et al., 2006; Nynca et al., 2012; Tekin et al., 2003). These variable glucose concentrations (resulting in osmolality changes) could affect the results of sperm motility after cryopreservation. The standardization of cryopreservation procedures regarding glucose concentration is very important, since small changes in the osmolality of the extender can seriously influence post-thaw sperm quality (Judycka et al., 2016a).

The aim of our study was to test the effect of different sperm concentrations in straws with a constant extender concentration on the sperm motility parameters of cryopreserved semen of brown trout (*Salmo trutta* m. *fario*), sea trout (*Salmo trutta* m. *trutta*), brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*). At the same time, we examined the utility of different straw sizes (0.25 and 0.5 ml) for the cryopreservation of semen of the mentioned species. Additionally, we evaluated the effect of the final glucose concentration at constant sperm concentration on post-thaw sperm motility.

2. Material and methods

2.1. Source of milt

The experiments were carried out during the natural spawning period on sexually mature males of four salmonid species (brown trout (1327 ± 115 g, 45 ± 6 cm), sea trout (400 ± 45 g, 25 ± 4 cm), brook trout (1131 ± 45 g, 37 ± 7 cm) and Atlantic salmon (902 ± 83 g, 36 ± 4 cm, for weight and length, respectively)), which were born and raised in the Inland Fisheries Institute in Olsztyn, Department of Salmonid Fish Research in Rutki (Poland). Fish were housed in 3 m^3 concrete ponds supplied with water from the Radunia River, with oxygen saturation levels maintained at 85–95% and temperatures of 3–6 °C during spawning. Before milt collection, males were anesthetized using 1 ppm Propiscin (IFI, Żabieniec, Poland). Milt was obtained by gentle abdominal massage, with special care to avoid blood, urine, or fecal contamination, and was collected individually in an open glass beaker (0.5 cm depth). Samples with visible contamination were discarded. Approval was given by the Animal Experiments Committee in Olsztyn, Poland.

2.2. Measurement of sperm concentration, viability and seminal plasma osmolality

Sperm concentration and viability were measured using a Muse Cell Analyzer (Millipore, Billerica, MA, USA) according to Nynca et al. (2016). Briefly, semen samples were first diluted 100 times with PBS, then 30 times with PBS. Twenty microliters of the diluted sample was mixed with 380 µl of Muse Count and Viability Assay Reagent (Millipore, USA) in a 1.5 ml screw-cap microfuge tube, and was incubated for 5 min in the dark at room temperature. Samples were introduced to the system by microcapillary. Data were generated with the Muse™ Count and Viability Software Module (Millipore, USA), simultaneously providing viable cell count, total cell count and sample viability (%). The osmotic pressure of seminal plasma was measured using a Minitüb Abfüll-u Labortechnik apparatus (Tiefenbach, Germany).

2.3. Effects of cryopreservation on sperm motility parameters

2.3.1. Cryopreservation protocol

Cryopreservation followed the previously described procedure using a glucose-methanol (GM) extender (Nynca et al., 2017). Semen mixed with cryoprotectant was loaded into 0.25 or 0.5 ml plastic straws (IMV Technologies, L'Aigle, France), which was placed on a 3 cm adjustable 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 (Minitüb GmbH, Tiefenbach, Germany) and were then placed in liquid nitrogen. The straws were then thawed by immersion in a water bath at 40 °C for 5 s (for 0.25 ml straws) or 10 s (for 0.5 ml straws).

2.3.2. Effects of sperm concentration in straws and volume of straws on sperm motility parameters of equilibrated and frozen/thawed semen

The experiments were conducted in November 2016. Semen samples were cryopreserved as described in Section 2.3.1. Semen was diluted in extender to obtain final sperm concentrations in the straw (0.5; 1.0; 1.5; 2.0; 3.0; 4.0; 6.0; 8.0; 10.0 × 10^9 spermatozoa ml^{-1} for brown trout and sea trout and 1.0; 1.5; 2.0; 3.0; 4.0; 5.0; 6.0 × 10^9 spermatozoa ml^{-1} for brook trout and Atlantic salmon) combined with a constant final extender concentration (0.15 M glucose and 7.5% methanol). Briefly, we added water to a GM glucose-methanol extender (10% glucose and 50% methanol) stock first. Then, after mixing, we added the appropriate volume of semen. In such conditions, no activation of sperm motility occurred. The dilution of semen with extender to obtain final sperm concentrations in the straw (0.5–10.0 × 10^9 spermatozoa ml^{-1}) is presented only for the exemplary sperm concentration 23 × 10^9 spermatozoa ml^{-1} (Table 1). We did not adjust the final seminal plasma volume. Diluted semen was loaded into 0.25 ml and 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. For brook trout, sea trout and Atlantic salmon

Table 1
Example of dilution of brown trout semen (23 × 10^9 spermatozoa ml^{-1}) with extender to obtain final sperm concentrations in the straw (0.5–10.0 × 10^9 spermatozoa ml^{-1}).

Fresh sperm concentration (× 10^9 ml^{-1})	Final sperm concentration in straw (× 10^9 ml^{-1})	Dilution rate	Semen volume (ml)	H_2O volume (ml)	Volume (ml) of GM stock	Final volume (ml) of semen diluted with extender
23	0.5	46.0	0.22	5.58	4.2	10
23	1.0	23.0	0.43	5.37	4.2	10
23	1.5	15.3	0.65	5.15	4.2	10
23	2.0	11.5	0.87	4.93	4.2	10
23	3.0	7.7	1.30	4.50	4.2	10
23	4.0	5.8	1.74	4.06	4.2	10
23	6.0	3.8	2.61	3.19	4.2	10
23	8.0	2.9	3.48	2.32	4.2	10
23	10.0	2.3	4.35	1.45	4.2	10

GM stock – glucose-methanol stock containing 10% glucose (27 ml) and 50% methanol (15 ml).