Cryopreservation of Brown Trout (*Salmo trutta macrostigma*) and Ornamental Koi Carp (*Cyprinus carpio*) Sperm

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1. Introduction

Cryopreservation is considered as one component in an effective strategy to save endangered species by facilitating the storage of their gametes in gene banks (Gausen, 1993; Akcay et al. 2004). Cryopreservation offers several benefits that in this way stocks can be protected from being totally eliminated due to sudden disease outbreak, natural utilization in hatcheries' production and laboratory experiments can be ensured. Stocks can be maintained more economically and experimental materials for advanced studies, such as gene transfer, can be made more accessible (Chao & Liao, 2001; Tekin et al. 2003).

Cryopreservation techniques involve addition of cryoprotectants, freezing and thawing of sperm samples, all of which may result in some damage to the spermatozoa and may decrease egg fertilization rate (Kopeika et al. 2003). Therefore, before cryopreservation of sperm, a through evaluation of different extender solutions, cryoprotectants, straw sizes and thawing rates is essential to develop optimum cryopreservation protocols for various species (Yavas & Bozkurt, 2011).

The species-specific cryopreservation procedure needs a suitable extender, as undiluted semen is not suitable for long-term preservation. Similarly, addition of optimum amount of cryoprotectant reduces cell damages associated with dehydration, cellular injuries and ice crystal formation (Leung, 1991). Although cryoprotectants help to prevent cryoinjuries during freezing and thawing, they can become toxic to cells when the exposure time and concentration are more (Tekin et al. 2007). In addition, type of cryoprotectants is also very specific to many species. There are no universal extenders and cryoprotectants available that can be used across species.

On the other hand, motility is the most commonly used parameter to evaluate sperm quality in fishes (Billard et al. 1995). This parameter is acceptable so that spermatozoa must be motile to achieve fertilization. Furthermore, sperm motility is an important component of a cryopreservation program in order to prevent poor sperm quality semen samples prior to freezing and to estimate the fertility of the stored sperm after thawing (Akçay et al., 2004;

Bozkurt, 2008). Thawing temperature and duration are also critical factors in the survival of cryopreserved sperm cells (Morris, 1981). Optimal freezig/thawing procedures have not been reported for *Salmo trutta macrostigma* sperm. So, in the present study three different thawing temperatures and thawing durations were also tested related to motility.

For this reason, there is a need to improve techniques on gamete storage and evaluation of sperm quality to facilitate optimization of controlled reproduction in fish (Alavi & Cosson, 2005). Important parameters for cryopreservation include type of extenders and cryoprotectants, dilution ratios, freezing/thawing rates and fertilization rates (Bozkurt et al. 2005).

Salmo trutta macrostigma is a salmonid species occurring in inland water habitats of Southern Europe, Western Asia, Northern Africa and Anatolia (Geldiay & Balik, 1988). It is also critically endangered fish species in inland waters because of illegal fishing, overfishing, and other environmental changes, including hydroelectric plants and pollution. For this reason a biological conservation program has been considered for *Salmo trutta macrostigma* in Turkey. On the other hand, ornamental koi carp is evaluated by its colour and have been used in the selecive propagation programs. These brightly colored koi carps are the result of selective breeding of wild carp. Over centuries a range of pleasing colors, patterns and shapes have been developed for this valuable species. Therefore, reliable methods for brown trout and koi carp sperm cryopreservation could benefit both aquaculture application and conservation of biodiversity.

Therefore, the present study was conducted in order to examine the effect of ionic extenders combined with different cryoprotectants at different ratios and to test the effect of different thawing temperatures and thawing periods on the post-thaw sperm quality of brown trout (*Salmo trutta macrostigma*) and koi carp (*Cyprinus carpio*) and development of a cryopreservation protocol for sperm of this commercially valuable two species.

2. Materials and methods

2.1 Broodstock management

The experiments were carried out spawning season of the brown trout (*Salmo trutta macrostigma*) and koi carp (*Cyprinus carpio*). In the pre-spawning period the mature bown trouts were kept seperately in small ponds under constant environmental conditions. The water temperature ranged 8-10°C during the spawning period. During the experiment, fish were kept under natural photoperiod. Mean water temperature and dissolved oxygen of the broodstock ponds were 8.7±2.46°C and 9.2±7.2 ppm respectively.

The koi carp broodstock was collected from wintering ponds by seining and transported into the hatchery 48 h prior to gamete collection. In the hatchery, male and female broodfish were held seperetely in shadowed tanks (V=1000 L) supplied with continuously (2.5 L min^{-1}) well-aerated water of 24°C. Brown trout and koi carp broodstock were not fed during the experiments.

2.2 Gamete collection

Sperm was collected by gently hand-stripping without anesthesia from mature 10 brown trout males. For koi carp cryopreservation experiments, semen was collected from 5

anesthetized (0.1 g/l MS 222) males by manual abdominal stripping 12 h after a single injection of 2 mg/kg of carp pituitary extract (CPE) at 20-22 °C water temperature. Eggs were collected by hand stripping 10-12 h after a double injection of 3.5 mg/kg of CPE. The first injection, 10% (0.35 mg/kg) CPE was given 10 h before the second (3.15 mg/kg).

For sperm collection, the urogenital papilla's of mature male fishes were carefully dried and sperm was hand-stripped directly into test tubes. Following sperm collection, the tubes containing sperm were placed in a styrofoambox containing crushed ice (4°C). Contamination of sperm with water, urine or faeces was carefully avoided. Sperm was transported to the laboratory within 15 min. For collection of eggs from koi carps, females were wiped dry, stripped by gentle abdominal massage and the eggs from each female were collected in a dry metal bowl. Eggs were checked visually and only those lots of homogenous shape, colour and size were used in the fertilization experiments.

2.3 Determination of fresh sperm quality parameters

Motility was estimated subjectively using light microscope (Olympus, Japan) with a x400 magnification. Samples were activated by mixing 1 µl of sperm with 20 µl activation solution (0.3% NaCl) on a glass slide. The percentage of motility was defined as the percentage of spermatozoa moving in a forward motion every 20% motile increment (i.e., 0, 20%, 40%, 60%, 80%, and 100%) (Vuthiphandchai & Zohar, 1999). Motility measurements were performed within 15 s. after activation. Sperm cells that vibrated in place were not considered to be motile. Sperm motility was estimated with three replicates of samples. For cryopreservation experiments, samples below 80% motile spermatozoa were discarded. Duration of sperm motility was determined using a sensitive chronometer (sensitivity: 1/100 s) by recording the time following addition of the activation solution to the sperm samples.

Spermatozoa density was determined according to the haemacytometric method. Sperm was diluted at ratio of 1:1000 with Hayem solution (5g Na_2SO_4, 1g NaCl, 0.5g $HgCl_2$, 200 mL bicine) and density was determined using a 100 µm deep Thoma haemocytometer (TH-100, Hecht-Assistent, Sondheim, Germany) at 400x magnification with Olympus BX50 phase contrast microscope (Olympus, Japan) and expressed as spermatozoa $x10^9$ mL^{-1} (three replicates). Counting chambers were always kept in a moist atmosphere for at least 10 min before cell counting. Sperm pH was measured using indicator papers (Merck, 5.5-9) within 30 min of sampling.

2.4 Experiment 1 - Brown trout (*Salmo trutta macrostigma*)

Collected sperm from 10 males that showing >80 motility was pooled into equal aliquots according to the required semen volume and sperm density to eliminate effects of individual variability of gamete donors. Semen and extenders were kept at 4°C prior to dilution. Pooled semen was diluted at 1:3 ratio (semen/extender) with extender containing 4.68 g l- NaCl, 2.98 g l- KCl, 0.11 g l- $CaCl_2$ and Trizma-HCl 3.15 g l- in distilled water; pH 9.0 (Billard & Cosson, 1992). The extender contained methanol and egg yolk at ratios of 5%, 10% and 15% separately. Dilution of semen with extender resulted in sperm concentrations of around 2.5x10⁹ cells/ml extender that was enough to avoid damage due to sperm

compression during freezing and thawing (Lahnsteiner, 2000). Following sperm suspension was equilibrated for 10 min at 4°C.

Within 1 h after sperm collection, the diluted semen samples were drawn into 0.25mL plastic straws (IMV, France). The open end of straws were sealed with polyvinyl alcohol (PVA). Following, the straws were placed on a styrofoam rack that floating on the surface of liquid nitrogen in a styrofoam box. The straws were frozen in liquid nitrogen vapour 4 cm above of the liquid nitrogen surface (temperature of styroframe surface was about -140°C) for 10 min. Following, the straws were plunged into the liquid nitrogen (-196°C) and stored for several days. For thawing, straws were thawed at 30°C for 10 s by gentle agitation in water bath. Thawed sperm was activated using pond water.

On the other hand, post-thaw sperm quality tests were carred out to evaluate motility rate and duration of motility. For this aim, sperm motility rate and duration of motility values following cryopreservation in the same ionic extender containing 15% egg yolk were determined. Sperm was thawed at 25°C, 35°C or 45°C for 5s, 15s or 25s and activated in 0.3% NaCl and 1% $NaHCO_3$.

2.5 Experiment 2 - Ornamental koi carp (*Cyprinus carpio*)

Collected semen from the 5 males that showing >80 motility was pooled into equal aliquots according to the required semen volume and sperm density needed to eliminate effects of individual variability of the donors. Semen and extenders were kept at 4°C, then diluted at a ratio of 1:3 (semen/extender) with 3 different extenders containing 10% DMSO. Extender 1 contained 5.8 g/L NaCl, 0.2 g/L KCl, 0.22 g/L $CaCl_2$, 0.04 g/L $MgCl_26H_2O$, 2.1 g/L $NaHCO_3$, 0.04 g/L $NaH_2PO_4.2H_2O$, 3.75 g/L glycine (Ravinder, et al. 1997). Extender 2 contained 300 mM glucose and 10% egg yolk pH:8 (Tekin et al. 2003) and extender 3 contained 4.68 g/L NaCl, 2.98 g/L KCl, 0.11 g/L $CaCl_2$, 3.15 g/L Tris-HCl, pH:9 (Billard & Cosson, 1992).

The diluted samples were drawn into 0.25 ml plastic straws (IMV, France) and were sealed with polyvinyl alcohol (PVA). Having been diluted, the samples were equilibrated for 10 min at 4°C. After equilibration, the straws were placed on a styrofoam rack that floated on the surface of liquid nitrogen in a styrofoam box. The straws were frozen in liquid nitrogen vapour 3 cm above the surface of liquid nitrogen (-140°C) for 10 min. After 10 min the straws were plunged into the liquid nitrogen (-196°C) and stored for several days. For thawing, the straws were removed from liquid nitrogen and immersed in 30°C water for 10 seconds. Thawed sperm was activated using 0.3% NaCl and observed under microscope for determination of spermatozoa motility and motility durations.

For fertilization experiments, pooled eggs from 3 mature females were used to determine fertilization rates. Egg samples (about 100 eggs) were inseminated in dry Petri dishes with fresh sperm or frozen sperm immediately after thawing at a spermatozoa:egg ratio of 1×10^5: 1. Eggs were inseminated by the dry fertilization technique using a solution of 3 g urea and 4 g NaCl in 1 L distilled water. The sperm and eggs were slightly stirred for 30 min, washed with hatchery water (24°C; 9 mg/l O_2), and gently transferred to labeled Zuger glass incubators with running water (24°C) where they were kept until hatching (3-4 d). Living

and dead eggs were counted in each incubator during incubation and dead eggs were removed. When the fertilized eggs developed to embryos at the gastrula stage, the fertilization rate (number of gastrula stage embryos/number of total eggs) was calculated.

2.6 Statistical analysis

Results are presented as means±SE. Differences between parameters were analyzed by repeated analysis of variance (ANOVA). Significant means were subjected to a multiple comparison test (Duncan) for post-hoc comparisons at a level of α=0.05. All analyses were carried out using SPSS 10 for Windows statistical software package.

3. Results

3.1 Fresh sperm quality parameters

In brown trout fresh semen volumes were rather variable and ranged from 9 to 17 ml and mean volume was 12.6±4.28 mL. Motility values were ranged from 75% to 90%. Samples that motility values were below than 80% were not used for the cryopreservation experiments. The mean motility value of fresh sperm samples were 84.5±7.59%. Mean spermatozoa movement duration (s), sperm density $x10^9$/mL and pH values were achieved as 57.4±3.8 s, 24.8±4.62 $x10^9$/mL and 7.28±2.46 respectively.

In koi carp mean fresh semen volume, spermatoza motility, motility duration, spermatozoa density and pH values of the collected fresh milt samples were determined as 6.2±4.7 ml, 85.4±2.4%, 125.2±3.5 s, 22.8 x 10^9 mL^{-1} and 7.4±3.7, respectively.

3.2. Experiment 1 - Brown trout (*Salmo trutta macrostigma*)

Post-thaw motility of sperm cryopreserved in ionic extender containing two different cryoprotectants at three different ratios is shown in Table 1. There were significant effect of cryoprotectants on motility rates. Sperm samples cryopreserved in the extenders containing egg yolk yielded greater post-thaw motility rates than methanol containing extenders. Sperm frozen with extender containing 15% egg yolk had the highest post-thaw motility. Differences between the post-thaw motility values were significant (P<0.05).

Methanol (5%)	Methanol (10%)	Methanol (15%)	Egg Yolk (5%)	Egg Yolk (10%)	Egg Yolk (15%)
10.6±4.57Af	15.2±5.80Ae	17.4±4.72Ae	40.5±3.27Aab	42.3±6.1Aa	45.3±4.27Aa
7.5±2.69Ae	9.6±3.37Be	12.3±5.24Ad	30.6±2.86Bb	35.4±4.17Abab	40.2±5.36Aba
5.4±2.73Ae	7.2±3.79Be	10.5±4.27Abd	25.7±4.69BCbc	30.2±5.29Bb	37.8±8.29Ba

Means followed by different superscripts (lowercase for lines and uppercase for columns within the same sperm feature) are different (p<0.05). (mean±SE, n=3).

Table 1. Post-thaw motility (%) of brown trout sperm cryopreserved with different cryoprotectants.

It was observed that a decrease in motility duration occurred following cryopreservation. The longest post-thaw motility longevity was also achieved with extender containing 15%

egg yolk as 54.2±3.46 s. Differences between the means of motility durations were significant (P<0.05). (Table 2).

Methanol (5%)	Methanol (10%)	Methanol (15%)	Egg Yolk (5%)	Egg Yolk (10%)	Egg Yolk (15%)
20.3±2.57Ad	24.5±2.47Acd	28.6±3.46Ac	40.2±1.29Aa	42.6±4.57Aa	46.4±2.38Ba
15.23±4.39Abf	19.6±4.39Abef	23.4±3.47Abe	37.2±3.45Ac	45.3±5.39Ab	54.2±3.46Aa
12.3±4.17Bd	15.7±1.28Bd	20.4±8.25Bcd	20.4±4.59Bcd	25.3±2.48Bc	32.5±5.27Cb

Means followed by different superscripts (lowercase for lines and uppercase for columns within the same sperm feature) are different (p<0.05). (mean±SE, n=3).

Table 2. Post-thaw longevity (s) of brown trout sperm cryopreserved with different cryoprotectants.

Sperm motility rate (Figure 1) and longevity of motility (Figure 2) values following cryopreservation in the ionic extender containing 15% egg yolk were determined. Sperm was thawed at 25°C, 35°C or 45°C for 5s, 15s or 25s and activated in 0.3% NaCl and 1% $NaHCO_3$.
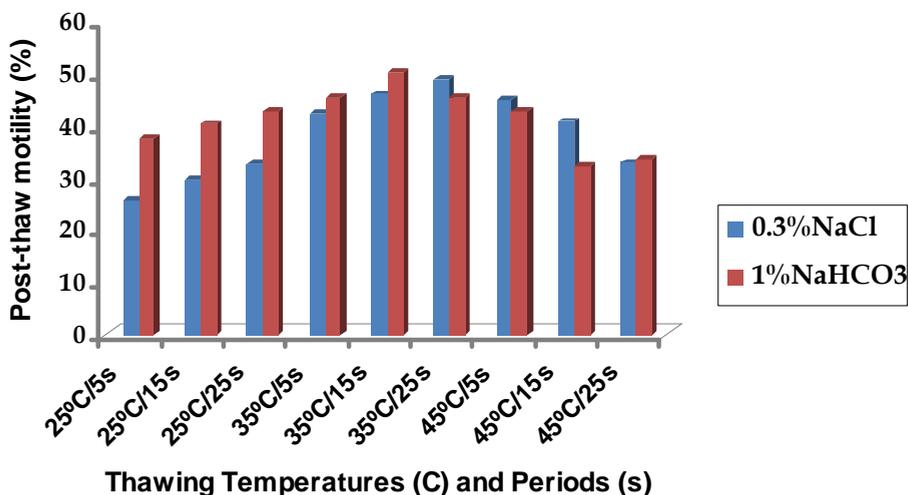

Fig. 1. Post-thaw motility (%) of brown trout sperm thawed at different degrees, periods and activating agents.

Post-thaw sperm motility rates were affected by thawing rates and activation agents and ranged from 25% to 50%. Also, the activating agents affected the duration of motility. All sperm samples triggered in 1% $NaHCO_3$ were motile for a longer period (32-57 s) compared with samples triggered in 0.3% NaCl (24-53 s). Differences between the post-thaw motility and longevity values were significant (P<0.05).
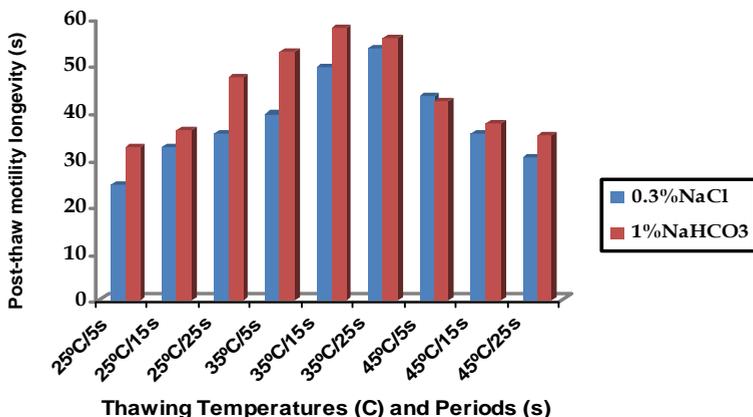
Fig. 2. Post-thaw motility longevity (s) of brown trout sperm thawed at different degrees, periods and activating agents.

3.3 Experiment 2 - Ornamental koi carp (*Cyprinus carpio*)

Effect of three different extenders containing 10% DMSO on the post-thaw motility and movement duration, fertilization and hatching rates of koi carp are shown in Table 3. Mean post-thaw motility of koi carp sperm was 75.3±6.4% while the best motility was determined as 85%. The overall mean fertilization rate was determined as 99.2±0.72 while the best fertilization rate was determined as 100%. The highest hatching rate was determined as 50% in all experimental groups. Motility features and hatching rates of cryopreserved koi carp sperm was statistically different between the experimental groups (p<0.05).

Extenders	Post-thaw motility (%)	Post-thaw motlity duration (s)	Fertilization rates (%)	Hatching rates (%)
E1	75.2±0.4[a]	27.5±1.2[a]	99.6±0.5	42.5±1.9[b]
E2	78.6±0.7[b]	32.9±0.4[b]	99.7±0.5	46.2±0.7[c]
E3	72.3±0.2[a]	25.2±0.6[a]	98.3±1.2	37.4±0.2[a]
Control	-	-	99.8±0.2	86.2±0.4[d]

Means followed by different superscripts are different (p<0.05). (mean±SE, n=3).

Table 3. Effect of different extenders on post-thaw motility, fertilization and hatching rates of koi carp sperm.

4. Discussion

Successful cryopreservation of fish spermatozoa depends on a range of factors including the collection of high quality sperm, equilibration conditions, choice of cryoprotectant medium, cooling/thawing regimes, and conditions for fertilization. Even though some general rules can be applied to any fish species, optimization of the protocol is needed for each individual species (Kopeika et al. 2007). Several factors have affected post-thaw quality of cryopreserved sperm from both brown trout (*Salmo trutta macrostigma*) and ornamental koi carp (*Cyprinus carpio*). The results obtained in the present study contribute significantly

improve the development protocol of sperm cryopreservation in brown trout and ornamental koi carp at large scale.

4.1 Brown trout (*Salmo trutta macrostigma*)

The results of the present study demonstrate for the first time cryopreservation of brown trout (*Salmo trutta macrostigma*) sperm. In the present study, post-thaw sperm quality was initially evaluated on the basis of sperm motility score and duration of motility for brown trout. For this aim, the effect of two cryoprotectants and three thawing temperatures on the post-thaw sperm quality of brown trout were assessed.

Motility is induced after the spermatozoa released into the aquatic environment during natural reproduction or after transfer to an activation medium during controlled reproduction (Alavi & Cosson, 2006). When salmonid spermatozoa are released into water they have a brief period of sperm activity between 20 and 40s (Morisawa & Morisawa, 1986). A better knowledge of the characteristics of fresh sperm motility is necessary to evaluate sperm quality in commercial hatcheries before artificial reproduction and in laboratories before experiments. Preliminary examination of fresh sperm was carried out in order to determine the relationship between sperm motility and seminal plazma composition of *Salmo trutta macrostigma* sperm (Bozkurt et al. 2011a).

Comparison of different cryoprotectant recipes and freeze-thaw protocols are difficult when each treatment tested for the ability of sperm to fertilise eggs. Cryoprotectants can suppress most cryoinjuries when used higher concentrations but at the same time it can become toxic to the cells. Therefore, a suitable concentration was needed for the development of a cryopreservation protocol. Methanol has been used successfully for sperm cryopreservation in African catfish (*Clarias gariepinus*) (Burchell) (Steyn & Van Vuren, 1987), tilapia (*Sarotherodon mossambicus*) (Peters) (Rana & McAndrew, 1989), bagrid catfish (*Mystus nemurus*) (Muchlisin et al., 2004) and salmonid fish (Lahnsteiner et al., 1996). Mansour et al. (2006) showed that 10% methanol was more effective as a cryoprotectant for Arctic char spermatozoa than 10% DMA or 10% DMSO when used with a glucose diluent. However, the effects of higher levels of methanol cryoprotectant were not investigated. In the present study, brown trout semen in an extender containing 15% egg yolk resulted in the highest overall percentage of sperm motility.

In addition, penetrating cryoprotectants could affect the percentage of motile sperm. In salmonids, some authors reported that higher post-thaw motility from methanol than from DMSO and other cryoprotectants (Mansour et al. 2006). In the present study, methanol and egg yolk have statistically significant effect on the percentage of sperm motility. On the other hand, it should be noted that egg yolk achieved better results than methanol for cryopreservation of brown trout sperm. Extender containing glucose, egg yolk and DMSO described by Alderson and McNeil (1984) gave good results in cryopreservation experiments with large straws. Baynes and Scott (1987) also reported that egg yolk is a valuable component in extenders for salmonid sperm cryopreservation. Furthermore, the addition of egg yolk to the medium interferes with the good visualization of spermatozoa during the motility rate analysis. With this in mind, we have tested several extender/cryoprotectant combinations with the addition of egg yolk that preserve sperm during storage and yet allow good visualization during motility analysis.

On the other hand, thawing temperature also play an important role in the post-freeze semen quality of fish (Wayman et al., 1998). Generally, thawing rates should be high to avoid recrystallization (Lahnsteiner, 2000). Significant post-thaw motility was determined when brown trout sperm was thawed at temperature of 35°C in the present study. According to the results of the present study, it was shown that higher temperatures are necessary to recover membrane stability or metabolism of spermatozoa. Also it appears that either recrystallization and ice crystal formation during thawing were reduced or avoided by this thawing procedure, or enzymatic activities were the best reactivated (Lahnsteiner, 2000). Although thawing from -196°C to 4°C is generally considered as critical phase because of potential recrystallization, the process was similar for all species. Furthermore, the two activating agents (0.3% NaCl and 1% NaHCO$_3$) tested did not affect post-thaw motility rates or quality motility score, although, in general, higher scores were observed when 1% NaHCO$_3$ was used in the present study. Duration of motility was significantly higher when 1% NaHCO$_3$ was used as an activating agent.

4.2 Ornamental koi carp (*Cyprinus carpio*)

The main purpose of the current experiment was to develop an appropriate protocol for ornamental koi carp (*Cyprinus carpio*) sperm cryopreservation to increase sperm availability outside the breeding season. By banking male gametes when they are abundant, most efforts can be devoted to raising healthy female broodstock and obtaining good quality eggs within a short captivity culture period. Through cryopreservation, a sperm repository can also be established for all males in captivity or from the wild. Such repository is important to maintain the genetic diversity to avoid inbreeding or loss of heterozygosity for captive breeding programs as well as possible future stock enhancement in the wild (Cabrita et al., 2009).

In the present experiment, koi carp males gave sperm characterized by good spermatozoa density and percent of motility. Such sperm should be used for cryopreservation experiments when considering minimization of artificial selection and sperm competition during hatchery operations in order to maintain the greatest biodiversity (Campton, 2004).

During the cryopreservation process, one of the important issue is the use of cryoprotectants, which role is to prevent cell damage during the freezing and thawing steps. Several cryoprotectants have been used for fish sperm cryopreservation, including methanol, ethylene glycol and dimethyl sulphoxide (DMSO); however, DMSO is reported to be the most efficient to cryopreserve fish spermatozoa (Anel & Cabrita, 2000) due mainly to its small molecular size, which allows it to enter and exit the spermatic cell easily (Tiersch et al. 1998).

The best fertilization rate obtained with extender II with 99.7% eyeing rate in koi carp. These results can be explained by the presence of 10% DMSO as a cryoprotectant in this extender. It can be concluded that DMSO has higher permeability by permeating into cell, causing reduced ice crystal formation for koi carp sperm. On the other hand, Lahnsteiner et al., (1996) used 10% methanol, 10% DMSO, 10% DMA, 5% glycerol and mixture of 5% DMSO and 1% glycerol for semen cryopreservation of the grayling (*Thymallus thymallus*) and the Danube salmon (*Hucho hucho*), which methanol showed the highest fertilization rates in relation to control 95.3% and 91.1% for grayling and Danube salmon, respectively.

Cryopreservation protocol carried out in the present study with a 1:100,000 egg: spermatozoa ratio, almost the same fertilization efficiency was obtained whether frozen or fresh semen was

used. This may be due to differences in extender, cryoprotectant, equilibration, egg quality, or protocol. In the present study, the interaction between the percentage of motile post-thaw sperm and fertilizing capacity was highly positive, similar to results in common carp (Linhart et al., 2000), African catfish (Rurangwa et al., 2001) and grass carp (Bozkurt et al., 2011b).

On the other hand, a wide range of temperatures used to thawed cryopreserved sperm with temperatures from refrigeration (4°C) to 80°C were reported (Lahnsteiner et al., 2000). A fast thawing temperature decreases the recrystallization effect in the spermatic cells and therefore diminishes the membrane damage (Tiersch et al.1998). Higher temperature such as 30°C were also used to thaw cyprinid semen in several studies (Stoss & Hotz, 1983) that similar with the pesent study.

5. Conclusion

It can be concluded that the cryopreservation protocol developed in this study is rather effective and brown trout (*Salmo trutta macrostigma*) and ornamental koi carp (*Cyprinus carpio*) sperm can be successfully cryopreserved. It seems that cryopreservation of brown trout sperm with ionic extenders containing 15% egg yolk is rather effetive on post-thaw sperm quality. In addition, based on the results obtained from this study, it is possible to suggest that sperm cryopreserved with ionic extender containing 10% DMSO packed in 0.25 mL volume straws and thawed at 30°C are the most suitable conditions to retain the sperm quality in koi carp having optimal sperm motility, duration of motility as well as high fertility percentages close to the values obtained with fresh sperm.

This study can help establish a frozen sperm bank for the conservation of genetic material of the brown trout and koi carp. On the other hand, additional research is needed on the effects of cryoprotectants, protective agents and freezing technique in cryopreservation on malformations, survival and condition of progeny produced with cryopreserved spermatozoa of brown trout and koi carp.

6. Acknowledgements

7. References

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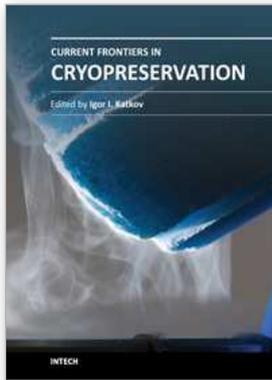
Current Frontiers in Cryopreservation

Edited by Prof. Igor Katkov

Almost a decade has passed since the last textbook on the science of cryobiology, Life in the Frozen State, was published. Recently, there have been some serious tectonic shifts in cryobiology which were perhaps not seen on the surface but will have a profound effect on both the future of cryobiology and the development of new cryopreservation methods. We feel that it is time to revise the previous paradigms and dogmas, discuss the conceptually new cryobiological ideas, and introduce the recently emerged practical protocols for cryopreservation. The present books, "Current Frontiers in Cryobiology" and "Current Frontiers in Cryopreservation" will serve the purpose. This is a global effort by scientists from 27 countries from all continents and we hope it will be interesting to a wide audience.

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