The effect of various concentration of quail egg yolk on spermatozoa motility of kancra fish (*Tor soro* Valenciennes, 1842) post cryopreservation

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Abstract. Kancra fish, also known as Batak fish, is often used in North Sumatera in traditional ceremonies. Nevertheless, overfishing has led to a decrease in its population. Cryopreservation is a strategy that might be effectively used to solve this problem. This study aimed to evaluate the effect of various concentrations of quail egg yolk on the spermatozoa motility of kancra fish after 48-hours of cryopreservation. The concentration of quail egg yolk were 0%, 5%, 10%, 15%, 20%, and 25%. The sperm of kancra fish were collected using the stripping method and was diluted with the quail egg yolk, fish ringer, and 10% methanol. It was then equilibrated for 10 minutes at a temperature of 5° C before being frozen at -10° C for 48 hours. Thawing was carried out at 40° C for 1 minute. The motility was analyzed using ANOVA and proceeded by Tukey test. The ANOVA test showed the use of various concentrations of quail egg yolk has a significant effect (p<0.05) on the spermatozoa motility of kancra fish after cryopreservation. The concentration of 10% of quail egg yolk showed the highest spermatozoa motility (85.10 ± 1.51%) after 48-hours of cryopreservation (p<0.05).

1. Introduction

One of the four types of tor fish in Indonesia is *Tor soro* [1], which is known as kancra fish in West Java, and batak fish in North Sumatra. *Tor soro* has a high cultural value, for instance, the Batak people use it in some traditional ceremonies such as marriage and childbirth [2]. It is consumable with high economic value. However, overfishing has led to a decrease in its population [3]. Therefore, adequate conservation is needed to prevent this fish from extinction.

Cryopreservation is one of the effective strategies to save endangered species [4]. It is a technique used to store genetic material at a low temperature for a long time. Cryopreservation is generally used for sperm preservation because it has higher resistance to low temperatures than the ovum or embryo [5]. Its main aim is to maintain sperm quality [6].

The cryoprotectant compatibility is one of the determinants of the success of cryopreservation [7], needed to protect cells from drastic temperature changes [8]. Egg yolk is a natural cryoprotectant that is often used in sperm cryopreservation, with that of chicken used in the study of *Barbonymus gonionotus* [9], *Chromobotia macracanthus* [10], and *Clarias gariepinus* [11]. The use of chicken egg yolk in *Barbonymus gonionotus* sperm cryopreservation tends to maintain sperm motility up to 96.10±3.31% [9].
Egg yolk from other bird species is also used with the use of quail resulting in better sperm motility in poitou jackass [12] and boar’s sperm [13]. Quail yolk eggs are also used in Cyprinus carpio sperm cryopreservation [14]. However, research on its use in sperm cryopreservation of kancra is yet to be conducted, therefore, this study aims to evaluate the effects of various concentrations of quail egg yolk on spermatozoa motility of kancra fish 48 hours post-cryopreservation.

2. Materials and methods

2.1. Semen collection
Semen was collected from adult kancra fish using the stripping method at the Installations for Freshwater Fish Genetic Resources, Ministry for Marine Affairs and Fisheries, Cijeruk, West Java, Indonesia. The physical and chemical characteristics of fresh sperm, such as color, volume, and pH analyzed.

2.2. Fish ringer preparation
The fish ringer was prepared by dissolving 3.25 g NaCl, 0.125 g KCl, 0.175 g CaCl\(_2\)·2H\(_2\)O and 0.1 g NaHCO\(_3\) in 500 ml of distilled water [15].

2.3. Activator dilution
Activators were prepared by dissolving 0.2633 g NaCl, 0.0373 g KCl, and 0.3634 g C\(_4\)H\(_{11}\)NO\(_3\) in 100 ml of distilled water [16].

2.4. Semen dilution
The semen was diluted using a solution consisting of quail egg yolk, 10% methanol, and fish ringer. The ratio diluted is 1:10 [16] with modification. Egg yolk concentration which is used in this study was: 0%, 5%, 10%, 15%, 20%, and 25%, respectively.

2.5. Equilibration and freezing
The sperm is equilibrated for 10 minutes at 5 °C [9] with modification and frozen at -10 °C for 48 hours.

2.6. Post-thaw parameters examined
Thawing is carried out at 40°C for 1 minute [9], which was followed by an evaluation of the spermatozoa motility per sample using a light microscope and digital eyepiece connected to a computer via Scopephoto 2.0.4.

2.7. The motility rate
Approximately 10 μl of the sample was placed on an Improved Nauber computation chamber and observed using a microscope with a magnification of 10x40. The following formula is used to calculate spermatozoa’s percentage:

\[
\frac{\text{Motility}}{100} = \frac{\sum \text{Spermatozoa motile}}{\sum \text{Spermatozoa total}} \times 100\% [17].
\]

2.8. Statistical analysis
Motility analysis was performed using ANOVA, with the Turkey test used to compare the averages. All statistical analyses were performed using SPSS Version 16.0 of 2007.

3. Results and discussion

3.1. Fresh sperm analysis
Fresh semen’s colour is milk-white, a pH level of 8.5 and volume of 1.5 ml per ejaculation. Motile sperm shows white colour (transparent). The percentage of fresh motile sperm is 88.91±1.41% (Table 1). These results are different from previous studies which produced fresh sperm with an average pH of 7.6 - 7.9, volume of 3.92 ml, and sperm motility of 76.67±5.37% [2].

Table 1. Fresh sperm profile.

| Volume (ml) | pH  | Colour     | Motility (%) |
|-------------|-----|------------|--------------|
| 1.5         | 8.5 | Milk-White | 88.91±1.41%  |

3.2. Sperm analysis after freezing

Spermatozoa motility at 48 hours post cryopreservation in 0% control and 5%, 10%, 15%, 20%, 25% quail egg yolk were: 61.13±1.94%, 81.18±1.77%, 85.10±1.51%, 78.83±1.96%, 73.07±2.77%, and 72.24±2.88% respectively as shown in table 2. The highest sperm motility (85.10 ± 1.51%) percentage is produced by 10% quail egg yolk while the lowest (61.13 ± 1.94) by 0% control. ANOVA test results showed that the administration of various concentrations of quail egg yolk (0%, 5%, 10%, 15%, 20%, 25%) was significantly different (P<0.05) to the percentage of motility spermatozoa of kancra fish, 48 hours post cryopreservation. Tukey test results showed a significant difference (P<0.05) in the control (0%) with 5%, 10%, 15%, 20%, and 25% treatments. However, the 10% treatment was significantly different (P <0.05) with a concentration of 15%, 20% and 25% (figure 1).

Table 2. Post cryopreservation sperm quality.

| Treatment | Motility (%) |
|-----------|--------------|
| 0%        | 61.13±1.94a  |
| 5%        | 81.18±1.77cd |
| 10%       | 85.10±1.51d  |
| 15%       | 78.83±1.96c  |
| 20%       | 73.07±2.77b  |
| 25%       | 72.24±2.88b  |

Note: Different letters in each column indicate significant difference (P <0.05).
Motility is a determinant and an imperative index of sperm quality and fertility [18]. This means that the higher the motility, the higher the fertility [19]. The good value of sperm motility is the condition of sperm to be able to be cryopreserved. Cryopreservable sperm is sperm with minimum motility value of 80% [20]. The result percentage of fresh sperm motility was 88.91±1.41%, which met the conditions for cryopreservation. The average percentage of sperm motility of the kancra fish at 48 hours post-cryopreservation was lower than the fresh sperm. Furthermore, the post-cryopreservation motility (85.10±1.51%) was 3.81% lower than the fresh sperm (88.91±1.41%). This decrease was lower compared to 0% treatment (61.13±1.94%) which had sperm motility decrease up to 27.78%. The treatments using quail egg yolk (5%, 10%, 15%, 20%, and 25%) were able to preserve sperm motility better than control treatment (0%).

The optimum concentration in maintaining post-cryopreservation spermatozoa motility is 10% of quail egg yolk which resulted in 85.10±1.51% motility. This is higher than the motility obtained from the use of 0% of quail egg yolk/control (61.13±1.94%). Egg yolk protects the outer surface of spermatozoa cell membranes to prevent damage [21]. Quail egg yolk contains phospholipids, cholesterol, and low-density lipoprotein (LDL) which tends to protect sperm from cold shock and ice crystal formation [22]. Furthermore, lipoproteins maintain stability and protect the spermatozoa membrane, with the Phospholipid fraction in lipoprotein forming a protective layer on the surface of spermatozoa, thereby, replacing the bilayer lost or damaged during the freezing process [23].

The use of egg yolk also increases the viscosity of semen plasma which inhibits the movement of spermatozoa capable of reducing energy use (ATP) and extending the duration of spermatozoa motility [24]. The sperm motility of 20% quail egg yolk (73.07±2.77%) and 25% (72.24±2.88%), respectively 12.03% and 12.86% lower than the optimum concentration (85.10±1.51%). High viscosity at concentrations of 20% and 25% tend to inhibit the movement of spermatozoa.

The sperm motility of kancra fish produced by 10% concentration (85.10 ± 1.51%) is higher than the total yolk egg yolk chicken + dimethyl sulfoxide (DMSO) 10% (83.33%) due to the differences in cryoprotectant combinations [2]. The ratio of polyunsaturated fatty acids to saturated fats in quail egg yolk is smaller than the egg yolk in chicken [25]. Better stability of saturated fatty acids provides better protection [13]. This is, however, in line with the previous studies which reported that quail egg yolk has better motile percentage and spermatozoa wave progression than chicken [12]. The results of this study were also higher than previous studies which examined the use of a combination of quail egg yolk + glycerol 5% (55.2 ± 2.1%) in *Cyprinus carpio* sperm [14] due to the differences in the cryoprotectants combination and species.

4. Conclusion
The combination of 10% quail egg yolk + 10% methanol is the optimum concentration needed to maintain the spermatozoa motility (85.10±1.51%) rate of kancra fish at 48 hours post-cryopreservation.

5. References
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