Initial evaluation of the motility and viability of African catfish *Clarias gariepinus* spermatozoa cryopreserved using glutathione

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Abstract. This study aimed to determine the optimum concentration of the glutathione in cryopreservation of African catfish *Clarias gariepinus* spermatozoa. The study was conducted at the Ujung Batee Brackish Aquaculture Center (BPBAP), Aceh Besar, Indonesia. Five concentrations of glutathione were tested in this study using the completely randomized design. These concentrations of glutathione were 0.0, 0.5, 1.0, 1.5 and 2.0 mgL⁻¹. Every treatment was performed with four replications. The results showed that the glutathione gave the significant effect on the sperm motility (P < 0.05), but did not give the significant effect on the sperm viability (P > 0.05). The average percentage of sperm motility after thawing was P0 (39.25%), P1 (28.50%), P2 (39.25%), P3 (34.00%) and P4 (40.50%), while the average percentage of sperm viability was P0 (84.37%), P1 (81.00%), P2 (91.62%), P3 (81.87%) and P4 (92.00%). The highest value of motility and viability were recorded in the in treatment P4 (2.0 mgL⁻¹ glutathione). However, these values were not different significantly with concentration of 1.0 mgL⁻¹. Therefore, it is concluded that the optimum concentration of the glutathione for the best sperm motility and viability is 1.0 mgL⁻¹.

1. Introduction

African catfish *Clarias gariepinus* is one of the commercial freshwater fish, and this is a popular cultured species worldwide [1][2]. However, the production of the fish has some obstacles, such as a limited supply of high quality and quality of larvae. This is because of the limitation of high-quality male broodstock. According to Abidin et al. [3] the quality of the larvae strongly depends on the broodstock quality. On the other hand, in artificial breeding of catfish, the male must be sacrificed which results in a decreasing number of male broodstock in the hatchery. The other constraint is asynchronous gonad maturity between male and female [4]. Therefore, sperm cryopreservation is one of the methods to overcome these problems [5][6][7][8].

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Cryopreservation ensures the availability of spermatozoa for artificial breeding and its use is efficient, economical, and effective [9][10][11]. However, cryopreservation has the disadvantage of causing damage to the plasma membrane due to freezing spermatozoa which can reduce the quality of spermatozoa [12][13]. Decreasing the quality of frozen spermatozoa is caused by increased production of Reactive Oxygen Species (ROS) [14]. The ROS occurs during the cryopreservation process which increases morphological damages which affects the decrease in sperm motility and viability post thawed [15].

The quality of spermatozoa can be maintained by adding antioxidants to the sperm diluent. One commonly used antioxidant is glutathione. Glutathione is an antioxidant compound that can reduce the occurrence of lipid peroxidation reactions in the plasma membrane of spermatozoa cells [16]. Lahnstainer et al. [17] reported that the addition of 1 mmol/L glutathione in sperm cryopreservation of trout (Salvelinus fontinalis) resulted in a fertility rate of 76.3%. Muthmainnah et al. [8] evaluated the Seurukan fish Osteochilus vittatus spermatozoa using glutathione, and they found that the best concentration was 40 mg L⁻¹. However, the application of glutathione in African catfish spermatozoa C. gariepinus has never been studied. Therefore, the aim of the present study was to evaluate the effect of glutathione on the sperm motility and viability of African catfish C. gariepinus.

2. Materials and methods

2.1. Experimental design

The completely randomized design (CRD) with five treatments and four replications was used in this study. The tested treatment is the difference in glutathione concentration in sperm dilution as follows: 0.0, 0.5, 1.0, 1.5 and 2.0 mg L⁻¹. The used diluents is Ringer's solution [18] [5] and 20% Fetal Bovine Serum (FBS) [19], while cryoprotectant was 15% Dimethyl Sulfoxide (DMSO) [20] [21]. The study was conducted at the Brackish Water Aquaculture Center (BPBAP) Ujung Batee, Aceh Besar.

2.2. Broodfish and sperm collection

A total of five male broodstock African catfish Clarias gariepinus with the bodyweight 1000 g each were purchased from the local market. Every male broodstock was injected intramuscularly with Ovaprin at a dosage of 0.3 ml/kg body weight to stimulate spermination. The spermination process occurred within 10 h after hormone injection. Then the males were anesthetised with cold water for 10 min and then sacrificed. The gonads were taken and cleaned with a tissue paper. Then the gonads were cut off and the sperm was released into a basic jar for temporary storage in an icebox set 4°C. Furthermore, fresh sperm quality was examined.

2.3. Dilution of spermatozoa

The Ringer solution was used as an extender; this solution was prepared based on Muchlisin et al.,[5], for 50 ml Ringer solution needs 0.375 g NaCl, 0.01 g KCL, 0.01 g CaCl₂, and 0.01 g NaHCO₃. The Ringer was distributed into five jars (vol. 10), and every jar was stocked with 6.5 ml of Ringer. Then a total of 2 ml of fetal bovine serum (FBS) and 1.5 ml DMSO were added into the Ringer in every of jar, resulting in a concentration of 20 and 15% of FBS and DMSO, respectively. Then 0.5 ml of sperm were added into the jar resulted in a dilution ratio of 1:20 (sperm:dilution). Then the diluted sperm from every jar was distributed into four cryotubes (as replication). Every cryotube was filled with 2 ml of diluted sperm. Then 0.005 mg L⁻¹ glutathione was added to every cryotube to obtain a concentration of 0.5 mg L⁻¹, 0.01 mg L⁻¹ ml glutathione for 1.0 mg L⁻¹, 0.015 mg L⁻¹ ml glutathione for 1.5 mg L⁻¹ and 0.02 mg L⁻¹ glutathione for 2.0 mg L⁻¹.

2.4. Freezing and thawing

Samples were kept in the icebox for 5 min at 4 - 5°C for the equilibration process. Then, the cryotubes were evaporated at a distance of 6 cm above the surface of liquid nitrogen (-79°C) for 5 min, then
immersed into liquid nitrogen at a temperature of -196°C for two weeks. After two weeks, the cryotube samples were taken one by one and then soaked in a water bath (40°C) for 5 min [7].

2.5. Percentage of motility and viability of spermatozoa

The percentage of sperm motility can be observed by dripping a drop of sperm with two drops of water homogenized on the prepared glass and then covered with a cover glass. Observations were carried out under abinocular stereo microscope with 400× magnification. The assessment was carried out in five different fields of view [22]. Two drops of eosin staining were mixed with two drops of sperm on a glass preparation then a total of 200 spermatozoa were chosen randomly to analysis the sperm viability. The spermatozoa viability is characterized by a white head, while dead spermatozoa are marked with a redhead. The percentage of live spermatozoa was calculated based on the ratio between the number of spermatozoa living with the total number of observed spermatozoa. Five field of view were chosen randomly to analyse the sperm motility and viability based on the ratio between the number of living spermatozoa.

2.6. Data analysis

The data of sperm viability and motility were subjected to one-way ANOVA and followed by Duncan’s multiple range test using SPSS ver 25.0.

3. Results and Discussion

The direct observation showed that the sperm had pH 7. The resultsshowed that the sperm viability range between 81 to 92% and sperm motility range between 28.5 to 40.5% (Table 1). The ANOVA test showed that glutathione gave a significant effect on the sperm viability and motility (P<0.05), where the highest sperm viability and sperm motility were recorded at glutathione concentration of 2.0 mg L⁻¹.

Table 1. Percentage of sperm motility and viability of African catfish *Clarias gariepinus* after freezing.

| Glutathione Concentration (mg L⁻¹) | Sperm motility (%) | Sperm viability (%) |
|-----------------------------------|--------------------|--------------------|
| 0 (Control)                       | 39.25 ± 4.43       | 84.38 ± 2.14       |
| 0.5                               | 28.50 ± 4.43       | 81.00 ± 5.74       |
| 1.0                               | 39.25 ± 5.16       | 91.63 ± 11.90      |
| 1.5                               | 34.00 ± 1.73       | 81.88 ± 3.03       |
| 2.0                               | 40.50 ± 5.74       | 92.00 ± 1.35       |

The mean values with different superscripts in the same column differ p (< 0.05) significantly.

Motility is one of the important parameters to assess the sperm quality; this parameter is also used to estimate the survival and life span of the spermatozoa. The living spermatozoa had fast, moderate, or slow movement. The type of motility can be divided into strict forward, spiral, or circular [23]. The sperm motility with the glutathione concentration of 2 mg L⁻¹ was not significantly different from control. However, the sperm motility in this treatment was significantly different from the control. This indicates that the addition of glutathione gave a positive effect on sperm viability. However, the sperm motility and viability in this treatment were not significantly different with glutathione concentration of 1.0 mg L⁻¹. However, a lower concentration of glutathione (0.5 mg L⁻¹) resulted in low sperm motility and viability. This is probably due to at low concentration; the glutathione is not effective at protecting the ROS [24]. Therefore, we assumed that 1 mg L⁻¹ is the optimum concentration of glutathione for African catfish spermatozoa.

The concentration requirement of glutathione for African catfish spermatozoa is lower compared to Seurukan *Osteochilus vattatus* spermatozoa where the optimum concentration was 40 mg L⁻¹ with the higher motility was 48.88% [8]. For comparison, Lahnstainer *et al.*, [25] reported that the application
of 2 mmol L\(^{-1}\) of glutathione in brown trout *Salmo trutta* sperm resulted in the best motility of 46.4%. In addition, Lahnstainer *et al.* [17] recorded sperm motility of 28.7% with the addition of 3 mmol L\(^{-1}\) of glutathione in *Salvelinus fontinalis* sperm. These reports showed that the motility of African catfish spermatozoa using glutathione at low concentration (1 mgL\(^{-1}\)) result almost the same as Muthmainnah *et al.*, [8] and Lahnstainer *et al.*, [25]. Even higher than in *S. fontinalis* as reported by Lahnstainer *et al.*, [25].

The study showed that the viability value was higher than the motility. This is probably due to classification, where immotile sperm does not necessarily mean dead. These immotile sperm may still be a live, and they may have not yet activated themselves, and therefore these sperm does not absorb the cosin. In some cases, the immotile sperm can still fertilize the egg because eggs produce hormones or enzymes which can stimulate the movement of the sperm.

As mentioned above, that the sperm motility of this study was higher than in seurukan fish *O. vitattus* as reported by Muthmainnah *et al.* [8]. This is probably due to our study using FBS. It is presumed that FBS could strengthen the positive effect of glutathione during cryopreservation. According to Garzon *et al.*, [26] FBS contains protein, hormone, and lipid. These compounds act as a protective agent against temperature shock during freezing and thawing.

4. Conclusion

It is concluded that glutathione gave a positive effect on sperm quality, and therefore, glutathione gave a significant effect on sperm motility and viability. The optimum concentration of glutathione for African catfish sperm was 1 mg L\(^{-1}\).

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