Viability of Najawa carp (*Cyprinus carpio* L.) sperm at 4°C temperature storage

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Abstract. The objectives of this study were to evaluate the sperm viability of Najawa carp (*Cyprinus carpio* L.) in cryopreservation pre-conditions at 4°C. The design used in this study was Complete Randomized Design with 4 treatments, BSS as a control, 10% DMSO, 0,2 M Sucrose, and 5% DMSO + 0,1 M Sucrose; each consist of three replications. The parameters observed were progressive motility of fresh sperm, diluted sperm before low temperature storage, and 2 hours; 3 hours; 4 hours; 5 hours; one day; one week; a month after 4°C storage. The data were analyzed by ANOVA. The data showed that there was no significant difference between treatment (P>0.05). The best viability was 40.56% of sperm motility which survive for one week, it was achieved by 5% DMSO + 0,1 M Sucrose.

1 Introduction

Najawa carp as potential Indonesian local products generally spawn at the beginning of the rainy season [1]. There is a possibility that sperm or eggs are not available during the breeding season. In many cases, the maturity of sperm and eggs not at the same time. Cryopreservation technology become important to provide sperm when the eggs are ready and store sperm when they have their best quality.

Cryopreservation is a technique to preserve sperm cells for a long storage period at very low-temperature storage. The success of cryopreservation is influenced by several factors such as the type and concentration of cryoprotectant used, the type and physiological state of the cell or material to be cryopreserved, freezing speed, and storage temperature. Sperm handling before cryopreservation (pre-conditioning) and after cryopreservation (post-cryopreservation) also become the critical factors.

Our previous study [2, 3, 4] showed that Najawa carp sperm could be preserved for a long time in a stable condition at liquid nitrogen (-196°C) by using a cryoprotectant such as DMSO and or sucrose. But, there was a significant decline in sperm motility during pre-conditioning, which stored in 4°C temperature storage. The declining of sperm motility reached 30-50% during pre-conditioning period. In this study, we tried to reveal the viability of Najawa carp sperm at 4°C temperature storage and to find the best solution to preserve najawa carp at 4°C temperature storage.

2 Method

2.1 Treatments:

1. Control (BSS)
2. 10% DMSO
3. 0.2 M sucrose
4. 5% DMSO + 0.1 M sucrose

Each treatment consists of 3 replications.

2.2 Extender solution and cryoprotectants:

Extender solution: Iwamatsu BSS [5] with pH modification (equivalent to an internal environment of carp sperm pH (6.8)).

Cryoprotectant: DMSO (permeating cryoprotectant) and sucrose (non-permeating cryoprotectant).

2.3 Sperm collection:

Sperm was collected from Najawa carp broodstock by squeezing method and diluted in the treatment solution in a ratio of 1:9.

2.4 Fresh sperm evaluation:

Fresh sperm was checked for color, pH, consistency, individual motility, mass movement, and sperm concentration. The standard motility used in this study was > 50%, the sperm motility ≤ 50% was not used for cryopreservation.

2.5 Cryopreservation:

Diluted sperm in the treatment solution was put in a 0.25 ml straw in the filling-sealing process and stored in a refrigerator (4ºC).

2.6 Sperm function evaluation:

Sperm motility was observed after diluted in the treatment solution and stored at 4ºC, i.e. 2 h, 3 h, 4 h, 5 h, 24 h, 1 week, and 1 month.

2.7 Data analysis:

The data were analyzed by using ANOVA with $p < 0.05$ level of significance.

3 Result and Discussion

3.1 Fresh sperm

Fresh sperm of Najawa carp directly observed after squeezing to evaluate the characteristic of the sperm before cryopreservation. Table 1 showed that Najawa fresh sperm had a milky white color, thick consistency, 67.77% motility, 3 (+++) mass movement, and 19.4 x 10^9 cells/ml. Based on the data, the fresh sperm had a very good quality [6].

Table 1. Fresh sperm characteristics of Najawa carp

Sperm parameters	Characteristics
Color	Milky white
pH	6.8
Consistency	Thick
Motility	67.77 %
Mass movement	3 (+++)
Consentration	19.4 x 10^9 cells/ml

3.2 Sperm motility after 4°C temperature storage

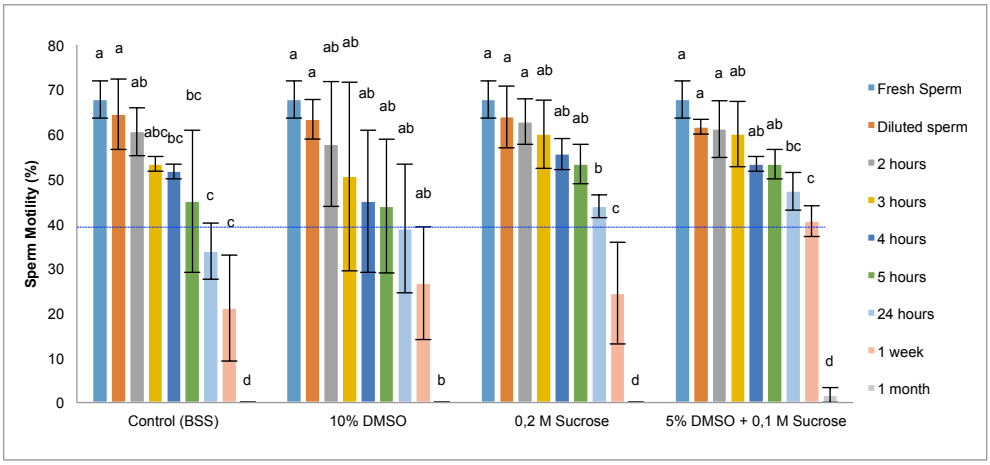

Figure 1. Sperm motility after 4°C temperature storage. Data were presented as mean ± SD. Different letters indicate statistically significant differences in sperm motility after the 4°C temperature storage period in the same treatment ($P < 0.05$).

Figure 1 showed sperm motility after 4°C temperature storage of control (BSS), 10% DMSO, 0.2 M sucrose, and 5% DMSO + 0.1 M sucrose. Control showed a significant decline of sperm motility at 4 hours after 4°C temperature storage and sperm motility was still categorized good (above 40%) until 5 hours after storage.

10% DMSO treatment did not show a significant decline of sperm motility until 1-week storage, but sperm motility was categorized good just until 5 hours after storage. The standard deviation of sperm motility in 10% DMSO treatment was so high, this data showed that the respond of the sperm cells to 10% DMSO was heterogeneous. DMSO could be toxic in a certain conditions, such as high DMSO concentration and room temperature storage [7].

0.2 M sucrose showed a significant decline of sperm motility at 24 hours after 4°C temperature storage and sperm motility was still categorized good until 24 hours after storage. 5% DMSO + 0.2 M sucrose showed significant decline of sperm motility at 24 hours after 4°C temperature storage and sperm motility was still categorized good until 1 week after storage.

The percentage of sperm motility was declined along with the 4°C storage period, different from liquid nitrogen storage (-196°C) which stable for the long term. At 4°C storage, the metabolism of the cells still occurs although it works slowly. The decline of sperm motility due to the toxicity level of the sperm environment that caused by the by-products of the metabolism such as lactic acid production and CO2, and also by the

reduction of energy supply. As shown in Figure 1, sucrose addition could delay the sperm motility at a longer storage period. We assumed that sucrose, which is disaccharide, hydrolyzed into monosaccharide molecules and then use as an energy source of the sperm cells.

This study is important to support sperm preservation, either for pre-conditioning of sperm cryopreservation or sperm delivery from one place to another which intended for aquaculture.

4 Conclusion

4.1. 4^{o}C temperature storage by using 0.2 M sucrose and 5 % DMSO + 0.1 sucrose delay the significant decline of Najawa carp sperm motility up to 24 h.

4.2. 4^{o}C temperature storage by using 5 % DMSO + 0.1 sucrose of Najawa carp sperm maintain more than 40% sperm motility up to one week.

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