Preservation of common carp (*Cyprinus carpio*) sperm using 0.9% NaCl and ringer's lactate solution

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Abstract. This study aims to determine the effect of the preservation of common carp (*Cyprinus carpio*) sperm using 0.9 % NaCl solution, Ringer's lactate, and 0.9 % NaCl+Ringer's lactate on the sperm's viability and motility. The male fish with a weight of 178.32 g and a total length of 20.3 cm were stripped to obtain the sperm. A total of 10 µl of sperm was diluted in 910 µl for each solution and stored at 5 ºC for 7 days. The observation of the sperm's viability and motility was done daily and the data was analyzed descriptively. After 7 days of storage, we obtained the best result in the 0.9% NaCl solution, namely with a 17.56 % sperm viability and 31 sec duration of motility.

1. Introduction

Common carp (*Cyprinus carpio*) are a type of fish that has important economic value; many farmers therefore try to increase their production. To increase production, two methods that are often carried out by farmers are natural spawning and artificial spawning. The main factor in the activity of increasing production requires a superior broodstock that has ripe gonads; this is so then the fry will be produced in both good quality and quantity. To produce a superior broodstock takes a long time and is expensive, therefore the existence of a superior broodstock must be utilized optimally. During the reproductive season, superior male broodstock sperm can be stored so then when it is needed, it can be directly used without having to use mature male gonads again. Gonads are genitals that are owned by every individual, both male and female; in males, they are in the form of testes, which are organs that produce sperm. In females, they are in the form of ovaries as the producers of eggs [1].

Sperm has the advantage of being able to be stored for a long time and it can be used at any time after that as needed [2]. Sperm storage is needed because naturally, the lifespan of freshwater fish spermatozoa is very short after leaving the testicles. The life span of sperm of common carp in fresh water is only 30-60 seconds [3]. Normally, the lifespan of sperm after coming out and into the water is only about 1-2 minutes [1].

Sperm storage depend on several factors such as ion concentration (K^+, Na^+, Ca^{2+}, Mg^{2+}), osmotic pressure, pH, temperature, and dilution [4] [5]. Sperm storage requires a diluent that can protect the sperm from low temperatures and provide a source of energy during the storage process. Without this diluent, the sperm will be damaged and die during storage. The diluents can supply nutrients and are isotonic in nature, which can maintain the osmotic pressure and appropriate electrolyte balance so then the sperm can survive. The 0.9% NaCl solution and Ringer's lactate solution was used as a cement diluent because it contains electrolyte elements that can maintain the osmotic and isotonic pressure of the plasma cement [6]. This study aims to determine the effect on the preservation of common carp

(*Cyprinus carpio*) sperm using 0.9 % NaCl solution, Ringer's lactate, and 0.9 % NaCl+Ringer's lactate on the sperm's viability and motility.

2. Material and methods

2.1 *Fish*

The selection of the fish aimed to focus on male common carp with already ripe gonads based on the size and weight of their body. The male fish with a weight of 178.32 g and a total length of 20.3 cm were stripped to obtain their sperm.

2.2 *Treatment design and data analysis*

This study used three treatments without repeating the preservation. The preservation of the sperm was done in a 0.9% Nacl solution, Ringer's lactate solution, and a mixture of 0.9% NaCl + Ringer's lactate (1: 1). A total of 10 µl of sperm was diluted in 910 µl for each solution and stored at 5 ºC for 7 days. The observation of the sperm's viability and motility was done after 7 days and the data was analyzed descriptively.

2.3 *Data collection*

The parameters observed in this study were the sperm's viability and motility. The sperm's viability was observed by making a preparations review using an eosin 2% staining solution. Fish milt was taken by as much as 1 µl and then homogenized with 999 µl eosin 2% above the object glass. The observation of the sperm's viability was done with a compound Eclipse E200-LED light microscope connected to a video monitor with 1000x magnification. The process was carried out for no more than 15 seconds. Sperm viability was determined from all of the sperm cells that were clear or that did not enter stained; the sperm that were dead were all stained. The formula for calculating the sperm viability was done by counting out the living sperm divided by the number of sperm that was observed. The result was then multiplied by 100%.

The sperm motility assessment was done by taking 1 µl of the sperm and placing it on the object glass. The object glass was then covered with a cover glass and observed by adding aquadest. The examination of motility (movement) was done using a compound Eclipse E200-LED light microscope connected to a video monitor with 100x and 400x magnification. The duration of the sperm's motility was observed in conjunction with the determination of the sperm's motility score. The observation of the duration of the sperm's motility was done by observing the duration of the sperm moving until it did not move anymore.

3. Results and discussion

After 7 days in storage, we obtained the viability and motility duration of common carp sperm diluted in 0.9 % NaCl solution, namely 17.56 % and 31 sec. For the Ringer's lactate solution, it was 9.46 % and 18 sec, and the 0.9% NaCl+Ringer's lactate solution results were 5.92% and 16 sec (Table.1).

Table 1. The viability and motility duration of common carp (*Cyprinus carpio*) sperm diluted in the 0.9% NaCl solution, Ringer's lactate solution and 0.9% NaCl+Ringer's lactate solution after 7 days of storage

Treatment	Viability (%)	Motility duration (sec)
0.9% NaCl	17.56	31
Ringer's lactate	9.46	18
0.9% NaCl+Ringer's lactate	5.92	16

The addition of the 0.9% NaCl solution and ringer lactate solution to the stored milt is thought to maintain the energy level and osmotic pressure so then the motility and progression can be maintained [5]. In addition, both the 0.9% NaCl solution and ringer lactate solution can delay the formation of

coagulants in milt. This can increase the milt velocity so as to shorten motility and to reduce sperm progression [6].

Based on the results, the best solution that obtained the best sperm viability and highest sperm motility was 0.9% NaCl. This is presumably because the solution was more isotonic than the other two other solutions; it was therefore able to maintain the electrolyte equilibrium of the sperm liquid which causes higher viability and a better duration of motility after being stored for 7 days.

4. References

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