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COMPARISON BETWEEN THE EFFECT OF EGG YOLK-BASED EXTENDER AND *ALOE VERA* (*ALOE BARBADENSIS*)-BASED EXTENDER ON RED TILAPIA (*OREOCHROMIS NILOTICUS*) SPERM QUALITY

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ABSTRACT

This study is aimed to determine the efficiency of *Aloe vera* extract as a cryoprotectant for chilling of fish semen compared to the conventional egg yolk-based extender (EYBX). A sample of 13 adult tilapia fishes was used for semen collection. All semen collected was pooled and the total volume of semen was divided into five aliquots where each of them was added with tris stabilizer (control), EYBX, 10%, 20% and 30% *Aloe vera*-based extender (AVBX) respectively. The samples were chilled at 4°C and the sperm quality was evaluated at different time intervals for 45 hours. There was a significant difference ($p < 0.05$) between EYBX and AVBX in both parameters with EYBX showing superior sperm preserving ability. However, AVBX showed the potential for semen preservation as well with 10% AVBX for best sperm viability while 30% AVBX offered the best result in sperm motility score.

Keywords: semen extender; *Aloe vera*; red tilapia; sperm chilling.

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1. INTRODUCTION

1.1. Egg Yolk-Based Extender (EYBX)

Egg yolk is one of the most widely used cryoprotectant that is incorporated into semen extender for sperm. The conventional egg yolk-based extender (EYBX) has showed satisfactory results for terrestrial animals, irrespective of domestic animal [1] or wild life animal [2]. However, egg yolk runs a higher risk of bacterial contamination [3] and its structural property that contains granular material can also interfere with microscopic observations or biochemical assays because of the granular material that resembles the size and shape of the sperm [4].

1.2. *Aloe Vera*-Based Extender (AVBX)

Aloe vera appears to be a more sanitary substitute to avian egg yolk as it contains some biological active substances that can also act as the conventional cryoprotectants [5]. Previous studies [6-8] have already shown that *Aloe vera* is potential natural product that can be used as protectant in preservation of sperm for land animals. Since there is no research has been conducted yet by using *Aloe vera* on preserving fish sperm, this study appeared to be the first to document the comparative research related with *Aloe vera* as an alternative to egg yolk for the preservation of fish sperm.

2. RESULTS AND DISCUSSION

The average sperm concentration of pooled semen in three replicates ranged from 1.5 billion sperms per ml to 2.3 billion of sperms per ml. The data obtained are relevant to previous studies [9-10] as the data are in the same magnitude of a billion (10^9). The mean initial sperm viability was 83.30 ± 1.18% while the motility score was 8 ± 0.5.

Table 1. The values (means ± SEM) for sperm quality of red tilapia (*Oreochromis niloticus*) semen diluted in Tris stabilizer (control), egg yolk-based extender (EYBX) and *Aloe vera*-based extender (AVBX) after 45 hours of chilling at 4°C (n = 13)

Treatment	Sperm Motility Score	Sperm Viability (%)
Control	1 ± 0^{b}	57.66 ± 1.28^{b}
EYBX	4 ± 0.5^{a}	68.69 ± 1.10^{b}
10% AVBX	3 ± 0^{a}	67.23 ± 0.56^{b}
20% AVBX	3 ± 0^{a}	57.01 ± 1.71^{b}
30% AVBX	4 ± 0^{a}	57.78 ± 1.31^{b}

[a,b] Means with different superscripts within a column were significantly different ($p < 0.05$)

The end result of comparison (Table 1) showed that there is no significant difference between EYBX and AVBX in preserving tilapia semen quality after 45 hours of chilling. However, EYBX yielded a better result compared to AVBX. It was confirmed that low-density lipoproteins (LDL) in the egg yolk (EY) have a cryoprotective action [11]. *Aloe vera* which does not contain any lipoproteins [5] could be the most probable reason why it is not as effective as EY in maintaining the sperm quality. Nevertheless, its ability to preserve semen quality is still undeniable. Since no studies had ever documented about the use of *Aloe vera* in preserving fish sperm, assumption can be made that successful preservation of fish semen can be achieved preferably by using EYBX.

Table 2. Comparison of the values (means ± SEM) for sperm quality of red tilapia (*Oreochromis niloticus*) semen diluted in three concentrations of *Aloe vera*-based extender (AVBX) with different storage time until 35 hours at 4°C (n = 13)

Time (Hours)	Sperm Motility Score			Sperm Viability (%)		
	10% AVBX	20% AVBX	30% AVBX	10% AVBX	20% AVBX	30% AVBX
0	8 ± 0.5[a]	8 ± 0.5[a]	8 ± 0.5[a]	83.30 ± 1.18[a]	83.30 ± 1.18[b]	83.30 ± 1.18[a]
3	8 ± 0.5[a]	8 ± 0.5[a]	8 ± 0.5[a]	82.49 ± 1.23[a]	81.72 ± 1.08[a]	81.40 ± 1.21[a]
9	6 ± 0[a]	6 ± 0.5[b]	7 ± 0.5[b]	80.82 ± 1.22[a]	77.99 ± 0.81[a]	78.30 ± 2.41[a]
18	5 ± 0[a]	5 ± 0[a]	6 ± 0.5[b]	77.27 ± 0.84[b]	72.58 ± 1.33[b]	72.52 ± 3.66[b]
30	4 ± 0[a]	4 ± 0[a]	5 ± 0[a]	72.26 ± 0.33[b]	65.90 ± 1.69[b]	65.41 ± 2.89[b]
45	3 ± 0[a]	3 ± 0[a]	4 ± 0[a]	67.23 ± 0.56[b]	57.01 ± 1.71[b]	57.78 ± 1.31[b]

[a,b] Means with different superscripts within a column were significantly different ($p < 0.05$)

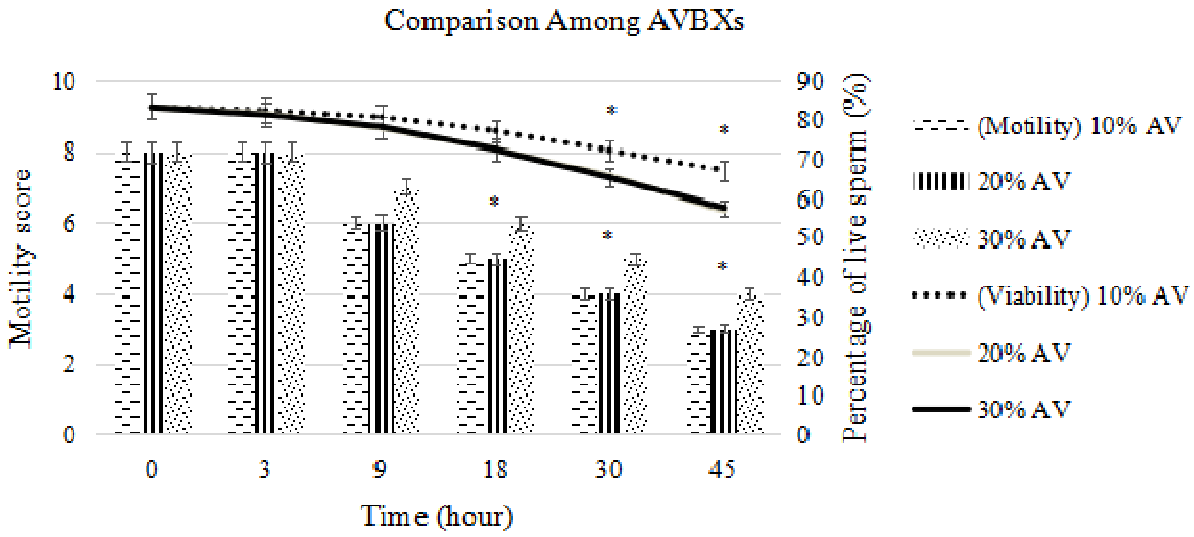

Fig.1. Comparison of sperm quality among different concentrations of AVBX

The percentages of live sperm for 20% and 30% AVBX did not showed statistical significance in comparison with each other. (Table 2) This result just made 10% AVBX to stand out among the three concentrations as it had higher live sperm percentage. At the same time, it means that the sperm "treated" in both 20% and 30% AVBX deteriorate more after 30 hours of storage when compared to 10% AVBX. This result is contradicted to [8]. They obtained a result of 66.9 ± 4.2% for 20% AVBX and 67.6 ± 2.7% for 10% AVBX, which means that higher concentration of *Aloe vera* gives higher sperm viability. However, that study was directed to conservation of collared peccaries semen.

The chemistry of *Aloe vera* content was revealed that the phenolics and aloins of *Aloe vera* were shown to disrupt membranes by weakening hydrophobic interactions between hydrocarbon chains in the phospholipid bilayers [5]. Since the survivability of spermatozoa depend very much on the structure of membrane, this could be the reason why higher concentration of *Aloe vera* resulted in poorer sperm viability in fish semen. Thus, the 10% AVBX which contains lower concentration of phenolics and aloins gave the highest sperm viability.

In terms of motility, 30% AVBX stands out among the three concentrations, which indicates that higher concentration of *Aloe vera* give better sperm motility. This is because *Aloe vera* contains various polysaccharides [12], which served as an energy source for the sperms and therefore increased their movement. It is also known that it contains folic acid and zinc that act as antioxidants [13], thus improving the semen quality by reducing semen apoptosis [14]. Furthermore, deviation of data from previous study can also be due to other factors. The physical and chemical composition of *Aloe*s differs depending on the species, climate and growing conditions of the plant [15]. Thus, not all *Aloe vera* used would give the same result.

3.1. Preparation of Semen Extender

Tris stablilizer was first prepared, followed by the addition of either egg yolk to produce EYBX or *Aloe vera* to make up AVBX. 20% EYBX was prepared as the standard EYBX [16] in preserving fish semen. On the other hand, AVBX was prepared in three different separated concentrations, which contained 10%, 20% and 30% of *Aloe vera* gel added in the mixture of tris stabilizer [8].

3.2. Collection of Milt

A sample of 13 matured and healthy fishes around five months of age with body weight about 250 grams were held in an indoor tank with controlled room temperature of 30°C and water temperature between 23°C to 29°C. To collect fish milt, a fish was taken out from the tank and the eyes were covered with a piece of black cloth. The region around the genital opening was cleaned with a paper and gentle abdominal pressure was applied to squeeze the milt out from the genital papilla of fish.

3.3. Assessment of Sperm Quality

The quality of sperm was assessed with two major parameters namely semen mass motility and sperm viability. Sperm viability is determined through calculating the live-dead sperm ratio with the help of Eosin-nigrosin stain and a microscope. Semen mass motility determination was done by estimating the percentage of motile sperm in a semen sample, and evaluated the quality by assigning motility score based on the latest motility scoring system [17].

4. CONCLUSION

Aloe vera can be concluded as a potential natural product that can be incorporated into the semen extender. 10% AVBX offered the highest percentage of live sperm whereas 30% AVBX gave the best sperm motility. Therefore, it is recommended that, the potential of using *Aloe vera* as semen extender to be further investigated in order to obtain the optimum concentration of *Aloe vera* extract. This study can also be further extrapolated to the use of *Aloe vera* in cryopreservation for valuable fish species.

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