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Influence of Ceolomic Fluid on Sperm Motility Characteristics in Angel Fish (*Pterophyllum scalare schultze*) During Spawning Season and its Chemical Composition

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Abstract: The aim of the present study was to determine the concentration of the major inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-), organic composition (glucose and total protein) and pH of ovarian fluid in Angel fish (*Pterophyllum Scalare Schultze*) and to determine if the composition of these fluids influences sperm motility trait (percentage and duration of motility). The effects of the composition of ceolomic fluid and sperm function are not well understood in teleostean fish species. In this purpose, sperm motility traits in 3 medium (ovarian fluid, fresh water + ovarian fluid and fresh water alone) were tested. The ovarian fluid was composed of sodium 133.1±3.8 mM L^{-1}, potassium 2.4±0.28 mM L^{-1}, calcium 0.56±0.28 mM L^{-1}, magnesium 0.65±0.23 mM L^{-1}, chloride 135.4±3.92 mM L^{-1}, total protein 3.3±0.46 gdL^{-1}, glucose 3.57±0.48 mM L^{-1} and pH 8.22±2.9. The results suggested that sperm movement duration was significantly higher in ovarian fluid than fresh water and fresh water + ovarian fluid during spawning season. The percentage of motile spermatozoa did not differ significantly among ovarian fluid, fresh water + ovarian fluid and fresh water in spawning periods. As a conclusion, the results of this study recommend the use of ovarian fluid; it is useful as an activation medium to improving sperm motility parameters of Angel fish.

Key words: Angel fish · Sperm motility · Ceolomic fluid · Spawning season

INTRODUCTION

The Angel fish (*Pterophyllum Scalare Schultze*) is a freshwater fish. Most Angel fish breed in captivity, particularly in pond settings. Breeding usually happens after a significant temperature change, often in spring. Males chase females, prompting them to release their eggs by bumping and nudging them.

High quality gametes are necessary for successful aquaculture programs. In species with external fertilization, sperm typically remain inactive until they are released by the male, but may show enhanced motility in the presence of the ovarian fluid (also called ceolomic fluid) that is typically released with eggs by the female during reproductive season [1]. It has been shown that ceolomic fluid influences sperm motility in fish [2]. Fish ovarian fluid has a unique composition regarding the presence of the organic and inorganic components which support the viability of spermatozoa [3-6]. Ovarian fluid also contains various nutrients, metabolites and hormones [7-9]. Some author have been shown that protein and carbohydrate fractions of ovarian fluid prolonged the motility of *Oncorhynchus mykiss*) sperm [10] but, Na^+ and K^+ levels have statistically significant positive and negative relations, respectively, with the percentage of motile cells [11]. Koya *et al.* [12], found that Elkhorn sculpin *Alcichthys alcicornis* (Herzenstein) sperm stayed active for >60 min in ovarian fluid, much longer than in other media tested, including sea water. They also observed that sperm swam faster in the vicinity of eggs and suggested that this may have been due to ovarian fluid on the egg surface. It has been already claimed that the motility parameters of the spermatozoa (such as the duration, intensity and the percentage of motility) are higher when the motility of spermatozoa were triggered in the ovarian fluid [13].

Though the mechanism by which ovarian fluid enhances sperm motility remain unknown [14], it is clear that ovarian fluid generally increases the sperm movement duration in externally fertilizing fish. There have been few investigations of the relation between composition of ovarian fluid and sperm motility such as Brown trout, *Salmo trutta caspius* [15, 16].

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The objective of the present study was to examine effects of the presence of ceolomic fluid of Angel Fish (*Pterophyllum Scalare Schultze*) with respect to the concentrations of the major inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-), organic composition (protein and glucose) and pH. We investigated whether composition of ovarian fluid influenced sperm motility characteristic (sperm movement duration and percentage of motile spermatozoa).

MATERIALS AND METHODS

Female and male Angel Fish were obtained from a reared hatchery at Sari, Iran. To stimulate fish for spawning injected intraperitoneally: 0.5 ml kg^{-1} Ovaprim (sGnRHa+Dompridon). Milt samples were collected during the 2010 spawning season (February, March, April and May) from 31 sexually mature two-years old male Angel Fish (Total Length: 17.6±1.85 cm, Total Weight: 60.43±8.48 g). Fish were dried to avoid activation of sperm by water, urine and blood and then milt was collected by applying gentle bilateral abdominal pressure. Ovarian fluid was also collected from mature two-years old female Angel fish during the 2010 spawning season (n= 12 February, n= 12 March, n= 12 April and n= 12 May). The ovarian fluid was then pipetted gently out of the egg batch and into screw–cap tubes with minimal head space to minimize air equilibration. Ovarian fluids were centrifuged at 3000 rpm for 8 min. The pH of ovarian fluids was immediately determined using a laboratory pH meter (pH meter, Iran T.S. co 462) and samples were frozen at -200C until the analysis moment. Two mineral (Ca^{2+} and Mg^{2+}) and two biochemical parameters (total protein and glucose) of the ovarian fluid were measured spectrophotometrically method (WPA-S2000-UV/VIS Cambridge - UK). The concentration of Na^+ and K^+ were determined with flame photometer (Jenway PFP 7, England) (standard kits from Parsazmoon, Tehran, Iran).

The effect of ovarian fluid on sperm motility trait was examined. Therefore, sperm motility trait in three solutions (fresh water from the reared hatchery, ovarian fluid + fresh water from the reared hatchery and ovarian fluid alone) was tested in each month during breeding season. One µl of milt was thoroughly mixed (for approximately 5 s) with 1000 µl of above solutions and then pipetted onto a glass slide. Samples reviewed at 200× on a negative phase-contrast microscope (Leica USA) and the motility was presented as the percentage and duration of motility. The duration of sperm motility was measured immediately after initiation of sperm activation until 100 % spermatozoa were immotile and expressed as sperm movement duration. Only forward moving sperm were judged motile, those simply vibrating or turning on their axes was considered immotile.

Data analysis of variance (ANOVA) was done with Duncan test for the comparison of mean values resulting from the various treatments at a significance level of $P<0.05$. Before analysis by ANOVA, data was used for normality of data distribution and homogeneity of variance. The data of sperm motility durations showed a normal distribution and were then analyzed with ANOVA with subsequent Duncan test for the comparison of mean values resulting from the various treatments at a significant level of $P< 0.05$. Results are presented as mean±SD. Statistical analyses were performed with SPSS 16 for windows statistical package.

RESULTS AND DISCUSSION

Ionic and biochemical compositions of the ovarian fluid of Angel Fish are presented in Table 1.
Values with the same superscript letter at the same row are not significantly different (P?0.05).

A higher duration of sperm motility was observed after triggering the motility in ovarian fluid and mixture of ovarian fluid with fresh water compared to that observed in freshwater during spawning season (Fig. 1) ($P< 0.05$), but no significant difference was observed between ovarian fluid and the mixture of ovarian fluid and fresh water ($P> 0.05$). The maximum and minimum sperm movement duration (59.23±10.17 and 27.31±8.5) in ovarian fluid (February) and fresh water (March) were recorded respectively.

The dynamics of the percentage of motile spermatozoa as a function of time post activation is shown in Figure 2. The percentage of motile sperm had not significant changes (P> 0.05) in ovarian fluid compared to those of ovarian fluid + fresh water and the fresh water alone.

Table 1: The ionic and biochemical compositions of the ovarian fluid in Angel Fish

Parameters	February	March	April	May
Sodium (mM L^{-1})	133.4±4.4^a	134±2.1^a	129±5.2^a	136±3.6^a
Potassium (mM L^{-1})	2.3±0.1^a	2.7±0.42^a	2.1±0.24^a	2.5±0.36^a
Calcium (mM L^{-1})	0.51±0.2^a	0.49±0.36^a	0.54±0.42^a	0.52±0.32^a
Magnesium (mM L^{-1})	0.68±0.20^a	0.65±0.26^a	0.66±0.15^a	0.64±0.32^a
Chloride (mM L^{-1})	136.3±4.2^a	131.7±5.4^a	135.4±3.6^a	138.2±2.4^a
Glucose (mM L^{-1})	3.6±0.42^a	3.2±0.76^a	3.7±3.6^a	3.8±0.23^a
Total protein (g dL^{-1})	3.25±0.86^a	3.68±0.41^a	3.1±0.23^a	3.4±0.36^a
pH	8.2±0.1^a	8.3±0.2^a	8.2±0.2^a	8.2±0.1^a

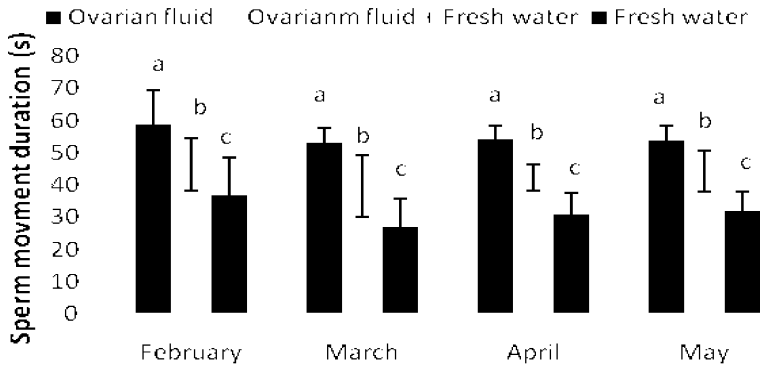

Fig. 1: Duration of sperm motility in *Pterophyllum Scalare Schultze* after triggering sperm motility in ovarian fluid, ovarian fluid+ fresh water and fresh water alone; significant differences are indicated with different superscripts.

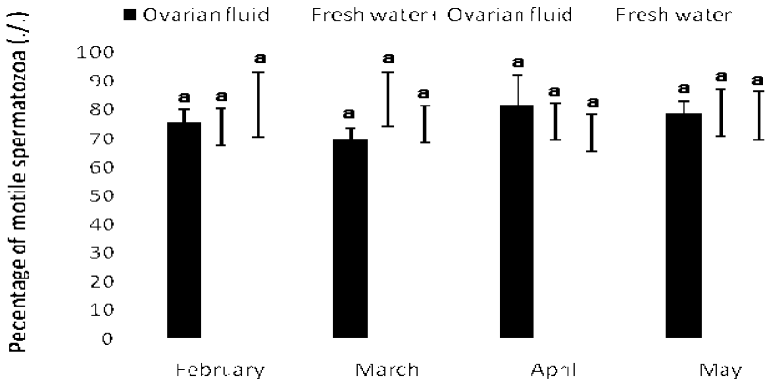

Fig. 2: Percentage of motile spermatozoa in *Pterophyllum Scalare Schultze* after triggering sperm motility in ovarian fluid, ovarian fluid + fresh water and freshwater alone. Values with the same superscript are not significantly different.

To our knowledge, this is the first study showing the composition of the ovarian fluid in Angel fish. In teleost fish, sperm motility is one of the biomarkers used for assessment of sperm quality [17]. The present results are showed that ovarian fluid enhances sperm movement duration (Fig. 1). It has already been demonstrated that sperm motility is a key factor determining the fertilizing ability of sperm [18].

Billard [19] claimed that some substances in ovarian fluid or seminal plasma protect sperm. The protein and carbohydrate fractions of ovarian fluid [20] or pH enhance the motility [21]. Litvak & Trippel [22] observed that sperm of Atlantic cod swam 30% faster in ovarian fluid at 30 s post-activation than in salt water. In addition, some chemical constituents of ovarian fluid influence ATP metabolism such that the rate and duration of energy production are increased [23]. In our recent work, we found that duration of sperm motility was higher in ovarian fluid than fresh water. This may be due to composition of female ovarian fluid [24] or some chemical constituents of ovarian fluid influence ATP metabolism such that the rate and duration of energy production is increased [25, 26]. More work is needed to identify the cellular processes involved [27].

In our study, although ovarian fluid enhanced sperm movement duration, there was no effect on percentage of motile spermatozoa. Also Elofsson *et al*. [28] showed that percentage of motile spermatozoa in stickleback presented no difference between sperm diluted in sea water alone or sea water with addition of ovarian fluid in agreement with our results. Recent studies have shown that the ovarian fluids of Angel Fish females enhance the sperm motility characteristics that are similar to previous researches [29]. This study suggested that ovarian fluid or (ovarian fluid + fresh water) can be used for increasing sperm movement. It is also useful for the triggering of sperm motility in Angel fish and enhances the fertilizing ability of sperm.

CONCLUSION

This study suggested that ovarian fluid or ovarian fluid + fresh water can be used for increasing sperm movement. It is also useful for the triggering of sperm motility in Angel Fish and enhances the fertilizing ability of sperm.

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