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## The influence of ovarian fluid on the sperm physiology of *Rutilus kutum*

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### Abstract

Motility parameters of the spermatozoa in most fish species spawning in fresh water like *Rutilus kutum* lasts for a short time after activation. Ovarian fluid significantly influenced sperm motility (motility duration period) and percent motility (progressive forward motile sperm). Both of these variables generally increased as the concentration of ovarian fluid increased from 33% to 50%, respectively. It is concluded that ovarian fluid enhances sperm movement in this species at appropriate level and thus has the potential to influence fertilization capacity.

**Keywords:** Reproduction, Sperm physiology, Ovarian fluid, *Rutilus kutum*

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## Introduction

In externally fertilizing fish species, ovarian fluid is typically released with eggs by the female during reproductive season. Ovarian fluid have shown to be effective in enhancing sperm motility in fish (Turner and Montgomerie, 2002; Dietrich *et al.*, 2008). Ellis and Jones (1939) stated that presence of ovarian fluid once fertilization started could counteract a negative physiological effect on spermatozoa. Ovarian fluid has a unique composition regarding the presence of the organic and inorganic components which support the viability of spermatozoa (Morisawa *et al.*, 1983; Lahnsteiner *et al.*, 1993). It also contains various nutrients, metabolites and hormones (Lahnsteiner *et al.*, 1995; Ingermann *et al.*, 2002). Many authors believe that sperm motility and viability is increased by ovarian fluid that can be attributed mainly to the ion balance and pH of the fluid (Lahnsteiner *et al.*, 2002; Wojtczak *et al.*, 2007). Some authors have shown that Na<sup>+</sup> and K<sup>+</sup> levels have statistically significant positive and negative relationships, respectively, with the percentage of motile cells (Khara *et al.*, 2012; Halimi *et al.*, 2014). It is claimed that some motility parameters (i.e. duration, intensity and the percentage of motility) are higher when spermatozoa is triggered in the ovarian fluid (Billard, 1992; Turner and Montgomerie, 2002). Prolonged mobility periods have been found in the freshwater and marine water fish such as brown trout *Salmo trutta fario* (Lahnsteiner, 2002) three-spined stickleback, *Gasterosteus*

*aculeatus* (Elofsson *et al.*, 2003a) and marine sculpin *Hemilepidotus gilberti* (Hayakawa and Munehara, 1998). Ovarian fluid did not enhance the mobility period in some species such as fifteen-spined sticklebacks, *Spinachia spinachia* (Elofsson *et al.*, 2003b) and sockeye salmon, *Oncorhynchus nerka* (Macfarlane *et al.*, 2002). The aim of the current study was to determine the chemical compositions of ovarian fluid and evaluate the effect of different percent's of ovarian fluid in improving sperm quality parameters of *R. kutum*.

## Materials and methods

Female and male mature species were obtained in Tagan River with assistance from Rajae Fish Production and Culture Center (Semeskande) during spawning season. Semen samples were collected from 10 sexually mature males when their ages were 3 years old (TL: 42.4±2.99 cm, TW: 815±137.54 g). Abdominal area was wiped to avoid activation of sperm by water, urine and blood, and then semen was collected by applying gentle bilateral abdominal pressure. The samples were kept on ice until use for insemination. Ovarian fluid was also collected from 10 mature females when their ages were 4 years old during the spawning season (TL: 47.5±3.37 cm, TW: 1010±322.15 g). 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 (Rosengrave *et al.*, 2009). No anesthesia was used when handling the fish. Immediately after ovarian

collection, the osmolality and pH of ovarian fluid were measured with an osmometer (Melting Point Osmometer Nr 961003, Roebbling Company, Berlin, Germany) and pH meter, (Iran T.S.co 462), respectively. The ovarian fluid was centrifuged at 3000 rpm for 8 min. Then supernatants were separated and frozen at  $-20^{\circ}\text{C}$  until analysis (Rosengrave *et al.*, 2009). Two minerals ( $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$ ) and three biochemical parameters (glucose, total protein and cholesterol) were measured using spectrophotometric method (WPA-S2000-UV/VIS Cambridge - UK). The concentration of  $\text{Na}^{+}$  and  $\text{K}^{+}$  were determined with flame photometer (Jenway PFP 7, England) (standard kits from Parsazmoon, Tehran, Iran). The sperm was diluted with different percent of ovarian fluid as follows; 33 : 67, 50 : 50, 67 : 33, 75 : 25, 80 : 20 and 85 : 15 (ovarian fluid: sperm). Sperm motility traits were tested in these concentrations. One  $\mu\text{l}$  of pre-diluted sperm with ovarian fluid was activated directly in activation medium of 0.3% NaCl at ratio 1: 1000 and immediately recorded with a 3 CCD video camera (Panasonic wv.cp 240 Japan) mounted on a dark-field microscope (Leica USA). The duration of motility was measured immediately after initiation of sperm activation until 100 % spermatozoa were immotile and expressed as duration of motility (Dadras *et al.*, 2014). The percentage of motility was defined as the percentage of progressively motile spermatozoa within each activated sample. Progressively motile spermatozoa were

defined as actively swimming in a forward motion. Only forward moving sperm was judged motile and sperm cells that vibrated in place were not considered motile. Observations were made within two hours of semen and ovarian fluid collection. Each analysis was taken in triplicate for each sample. Average of the three measurements was used for the results. All experiments were performed at room temperature ( $20-22^{\circ}\text{C}$ ). Before data analysis by ANOVA, Duncan test was used for normality of data distribution and homogeneity of variance. Sperm motility parameters were compared using repeated measures one-way ANOVA followed by Duncan post hoc. All analyses were performed on samples from ten males and ten females in replicates. Statistical data were analyzed using SPSS 16.

## Results

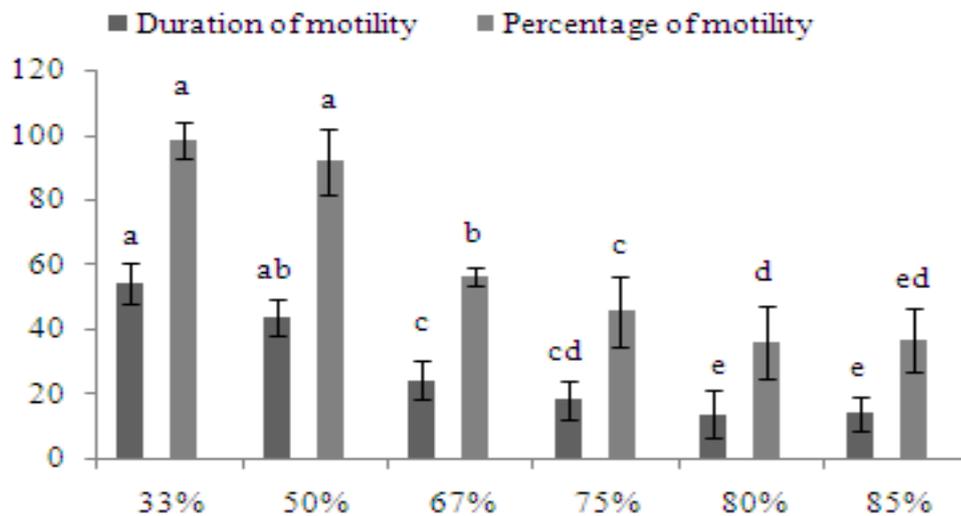
Ionic and biochemical compositions of the ovarian fluid are presented in Table 1. Sodium was the predominant ion measured in ovarian fluid and the potassium concentration was measured to be  $8.7\pm 0.1$  mM. High concentrations of ovarian fluid (67, 75, 80 and 85%) significantly decreased the duration of sperm motility compared to low concentrations (33 and 50%). The highest values were observed with 33 and 50% of ovarian fluid ( $54\pm 6.4$  and  $43.6\pm 5.6$ , respectively) and the lowest in other treatments ( $24.2\pm 6.2$ ,  $18\pm 5.9$ ,  $13.6\pm 7.5$  and  $13.9\pm 5.4$ , respectively) (Fig. 1). The percentage of sperm motility was higher with 33 and 50% of

ovarian fluid ( $98.3 \pm 5.7$  and  $91.6 \pm 10.2$ , respectively) compared to other treatments ( $56.3 \pm 2.9$ ,  $45.3 \pm 11.1$ ,  $36 \pm 13.5$  and  $36.7 \pm 9.7$ , respectively) (Fig. 1). In the individual responses of sperm quality parameters, four males (1, 3, 4 and 9) had significantly higher

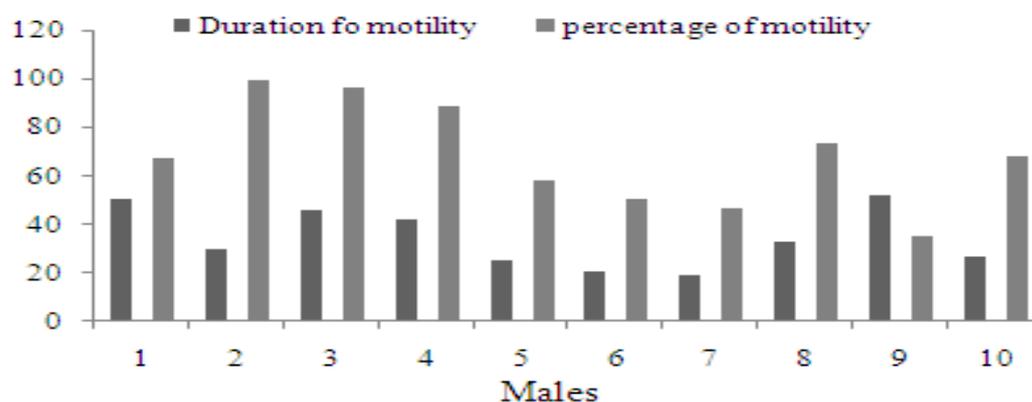
duration of motility when activated with ovarian fluid than those recorded for other males (Fig. 2). Male numbers of 2, 3, 8 and 10 had significantly higher percentage of motility with ovarian fluid (Fig. 2).

**Table 1: The ionic and biochemical compositions of the ovarian fluid of *Rutilus kutum*.**

Ovarian fluid characteristics	Minimum	Maximum	Mean $\pm$ SE
Na <sup>+</sup> (mML <sup>-1</sup> )	80.1	120.5	98.3 $\pm$ 14.3
K <sup>+</sup> ( mML <sup>-1</sup> )	4.2	11.9	8.7 $\pm$ 5.1
Ca <sup>+2</sup> (mML <sup>-1</sup> )	7.1	14.3	9.7 $\pm$ 2.6
Mg <sup>+2</sup> (mML <sup>-1</sup> )	2.9	8.9	8.08 $\pm$ 2.2
pH	7.56	8.87	7.7 $\pm$ 0.4
Osmolality (mOsmol kg <sup>-1</sup> )	202.5	285	237.5 $\pm$ 22.1
Glucose (mML <sup>-1</sup> )	188.6	467.8	342.3 $\pm$ 85.4
Total protein (mg 100 ml <sup>-1</sup> )	7.4	22.4	12.9 $\pm$ 4.4
Cholesterol (mML <sup>-1</sup> )	281.9	868.2	585.9 $\pm$ 193.8



**Figure 1: Effect of different percents of ovarian fluid on sperm motility traits parameters represented by data from ten males. Means with the same letters are not significantly different ( $p < 0.05$ ).**



**Figure 2:** Effect of *Rutilus kutum* ovarian fluid on individual male motility activation registered in the ten males.

### Discussion

In teleost fish, sperm motility is one of the biomarkers used for assessment of sperm quality (Lahnsteiner *et al.*, 1998; Golpour *et al.*, 2013). Our data showed a clear sperm motility improvement with a certain level of ovarian fluid. The highest motility parameters were recorded mainly with 33 and 50 % ovarian fluid (Fig. 1). The most evident effect of the presence of ovarian fluid was the prolongation time of all motility parameters. Billard, (1992) claimed that some substances in ovarian fluid or seminal plasma protect sperm. The protein and carbohydrate fractions of ovarian fluid (Lahnsteiner *et al.*, 2001) or pH enhance the motility (Wojtczak *et al.*, 2007). In agreement with our results, Diogo *et al.* (2010) reported sperm motility improved with a certain level of ovarian fluid (25%) in the activation medium. Some chemical constituents of ovarian fluid influence ATP metabolism such that the rate and duration of energy production are increased (Turner and Montgomerie, 2002). Ovarian fluid constituents may

protect cells during exposure to medium, as does freshwater and seawater during sperm activation, reducing the percentage of destroyed cells. Also, ovarian fluid lowers the osmolarity of the medium diminishing the harmful effect of medium. Although the presence of ovarian fluid generally improves sperm motility in several species, its individual effect has been reported to be scattered. In our study, ten males with almost the same sperm quality enhanced their sperm characteristics in the presence of ovarian fluid. We found that duration of motility in males (1, 3, 4 and 9) and percentage of motility in males (2, 3, 4, 8 and 10) were significantly higher. Like the study by Urbach *et al.* (2005) sperm ability to swim in ovarian fluid solution depended on the male's identity and its sperm traits. Also, some studies with freshwater species proposed that ovarian fluid may benefit dominant males, since they release milt in closer contact with eggs than subordinate males (Liley *et al.*, 2002). The mechanisms explaining the

differences in motility characteristics of spermatozoa are unknown. Biochemical characteristics of the semen can be modulated by numerous external and internal factors such as age, reproductive behavior, season, temperature, water composition, stress, hormonal changes, nutrition, etc. (Ciereszko *et al.*, 1996; Ciereszko, 2008; Seifi, 2011). With regard to these factors, it is difficult to determine primary factors causing differences in sperm motility characteristics among individual males. Therefore, it can be concluded that males and females either differ in terms of motility characteristics or ability of their ovarian fluid to enhance sperm movement. In other words, some females could possibly increase their chances of eggs fertilization by producing ovarian fluid with superior ability to enhance sperm movement. On the other hand, some males produce spermatozoa with superior motility characteristics despite variability in motility-modulating activity of particular ovarian fluid. The positive influence of ovarian fluid on sperm motility may not only be due to its physico-chemical characteristics such as pH, osmolarity and viscosity, but also inorganic composition (Lahnsteiner *et al.*, 1995) and sperm activating factors (Ohtake, 2003). Altogether, these facts may be the origin of the effect of ovarian fluid on sperm motility, though the full mechanism is still unknown. Further investigation is necessary to determine the physiological bases behind this phenomenon.

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