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Total Period of Motility of *Acipenser persicus* Spermatozoa in Freshwater and Saline Solution

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Abstracts: A study on motility of spermatozoa of the Persian sturgeon, *Acipenser persicus* were carried out. The time of relative cessation of sperm activity was evaluated using microscopic observation. Sperm was activated with three different swimming medium, freshwater pH 7.5, and two different buffered saline solutions, pH 7.5. Total period of the motility of *A. persicus* spermatozoa was shorter in freshwater than saline solutions. Significant correlation was found between period of sperm motility and dilution rate. Increasing dilution rate from 1:50 to 1:500 had negative effect on sperm motility and significantly decreased the duration of motility ($P<0.01$). There was also significant correlation between ionic composition of swimming medium and total period of sperm motility.

Key Words : *Acipenser persicus*, Spermatozoa, Period of motility, Dilution rate, Freshwater, Saline solution

Introduction

Sperm motility is a key parameter determining the semen fertilization capacity (Billard & Cosson, 1992; Lahnsteiner *et al.*, 1996). The spermatozoa of sturgeon, like that of other fishes, are immotile in the testis and genital tract and only are activated after releasing into external medium (Ginzburg, 1968; Scott & Baynes,

1980; Gallis *et al.*, 1991; Billard *et al.*, 1995; Cosson & Linhart, 1996). The inhibition of motility in semen is mainly due to K^+ ions (Gallis *et al.*, 1991; Cosson. *et al.*, 1999; Billard, 2000; Alavi *et al.*, 2002; Alavi, 2003). Drabkina, (1961), Linhart *et al.*, (1995) and Alavi (2003) reported the influence of other factors such as osmotic pressure in the motility duration of sturgeon spermatozoa.

The duration of spermatozoa motility varies between 3-20 min in different species of sturgeon (Ginzburg, 1968). The total period of sperm motility were reported 13 min for Grate sturgeon, *Huso huso*, (Ginzburg, 1968); 3.5 to 6 min for Russian sturgeon, *A. gueldenstaedtii*, (Drabkina, 1961); 5 to 20 min for sterlet, *A. ruthenus* (Tsvekova *et al.*, 1996), , 1.5 to 5 min for Persian sturgeon, *A. persicus* (Alavi *et al.*, 2002) and maximum 3 min in Siberian sturgeon, *A. baeri*, (Williot *et al.*, 2000) in fresh water at 15 to 20°C. Dilution rate for spermatozoa also is a key parameter for sperm motility, because the volume of diluent added determines the dynamics of activation Billard & Cosson (1992); Gallis *et al.*, (1991) and Alavi (2003) observed the period of sperm motility decreased when the dilution ratio increased in Siberian and Persian sturgeon.

The aim of present study was to determine the total period of motility of spermatozoa of *A. persicus* in freshwater in comparison with saline solutions at different dilution rates.

Material and Methods

Broodstock: The experiment carried out in April 2000 at the International Sturgeon Research Institute, Rasht, Iran. The Persian sturgeon was captured from Sefidrood River (Southern part of the Caspian Sea) and transferred to Dr. Beheshti Sturgeon Artificial Propagation and Rearing Center, near to the Sangar dam, Rasht. Five males, suitable for stripping, were selected from the ponds, few days before the experiment and kept, separated from females, in a concrete tank at 15-17°C. The Broodstock was injected with pituitary homogenized extracts of

sturgeon (a suspension of powdered, acetone dried sturgeon pituitaries) for spawning induction at does of 50mg/ total body weight (Kohnehshari & Azari Takami, 1974). Milt was collected by hand stripping, 25-30 h after injection in glass experimental tubes (Kohneshahri & Azari Takami, 1974). The tubes were stored on ice during transportation to the Laboratory of Genetic and Biotechnology. Semen of males was stored at 4°C until motility analysis.

Evaluation of Motility: According to suggested procedure by Persov (1941), sperm motility was evaluated for the total period of motility (sec), *i.e.* when the all most spermatozoa (more than 95%) were immotile. All experiments were performed at room temperature (17-20°C), using light microscopy under 40X magnification. To induce the initiation of sperm motility, milt was diluted in freshwater pH 7.5, (control) and two buffered saline solutions. The saline solution (1) containing NaCl 110mM, KCl 28.3mM, $MgSo_4$ 1.1mM, $CaCl_2$ 1.8mM, Tris-HCl 0.2 mM, pH 7.5 and saline solution (2) containing NaCl 135mM, KCl 3.1mM, $MgSo_4$ 1.3mM, $CaCl_2$ 3.4 mM, Tris-HCl 0.2 mM, pH 7.5. The pH of freshwater and saline solutions was measured with a classic laboratory pH-meter. A one-step dilution technique (Billard & Cosson, 1992) was used for inducing of motility activation. Effect of dilution rate was tested at two dilution rates (*i.e.* 1:50 and 1:500).

Data Analysis: The values are given as average ±SD. Statistical comparison were made with an independent sample test, Mann-Whiteny- u-Wilcoxon Rank Sum W Test. SPSS 9.0 was used for statistical analyses.

Results

Using the described experimental technique, highest and lowest period of sperm motility was observed in saline solution (2) at the rate of 1:50 (264.65± 32.53 sec) and freshwater at the rate of 1:500 (86.45±15.05 sec). Minimum, maximum, and average of duration of motility of the Persian sturgeon spermatozoa

at the rate of 1:50, respectively. In addition, the period of motility of spermatozoa diluted in saline solution (1) was higher than that in the freshwater (Figs. 1&2, $P<0.01$ for 1:50 and $P<0.05$ for 1:500). Also, there were significant correlation in motility duration of sperm between saline solution (1) and saline solution (2) at 1:50 and 1:500 dilution rates (Figs. 1&2, $P<0.01$). The motility duration of spermatozoa of *A. persicus* was significantly related to dilution rate after diluting in freshwater, saline solution (1) and saline solution (2) (Figs. 1&2, $P<0.01$).

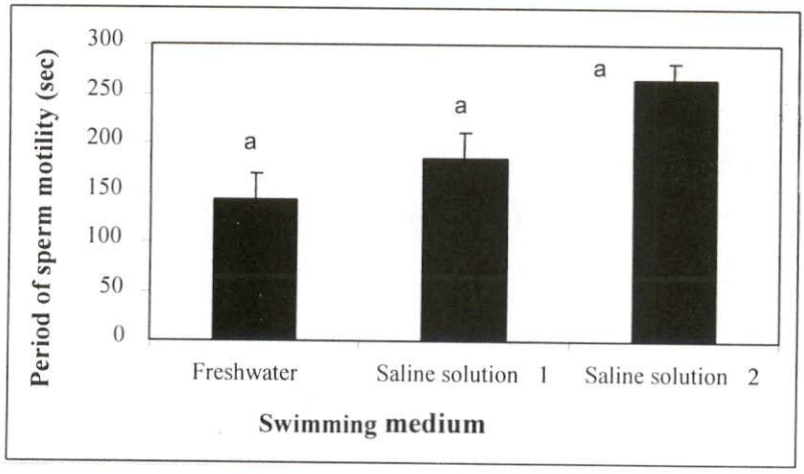

Figure 1: Period of sperm motility in *A. persicus* in freshwater and saline solutions (1) and (2) at dilution rate 1:50. Values superscripted by the same letter are not significant different (Mann-Whiteny- u-Wilcoxon Rank Sum W Test, n = 5, a: $P<0.01$)

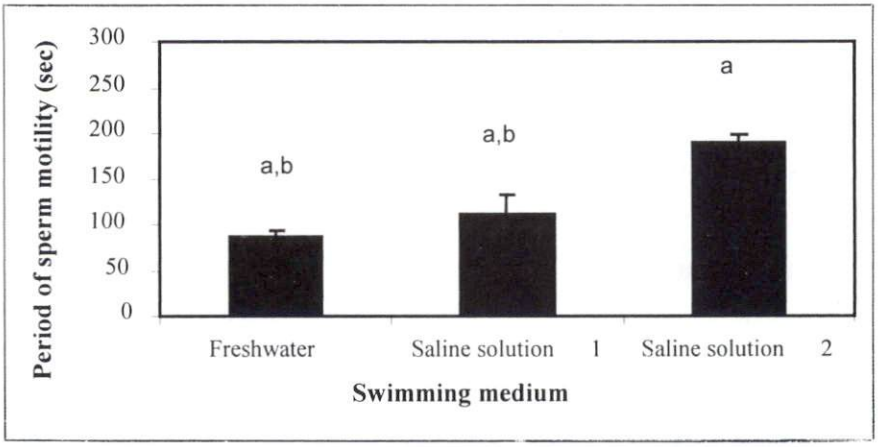

Figure 2: Period of sperm motility in *A. persicus* in freshwater and saline solutions (1) and (2) at dilution rate of 1:500. Values superscripted by the same letter are not significant different (Mann-Whiteny- u-Wilcoxon Rank Sum W Test, n = 5, a: $P<0.01$; b: $P<0.05$).

Discussion

This study showed that immotile sperm in seminal plasma of sturgeon are activated when transferred into swimming medium and the dilution rate is prerequisite factor which controls the sperm motility. Environmental factors such as temperature, ionic composition, pH and osmolarity are other factors regulating the sperm motility in sturgeon (Linhart *et al.*, 1995; Cosson & Linhart, 1996; Toth *et al.*, 1997; Linhart *et al.*, 2002; Alavi, 2003) like teleosts (Stoss, 1983; Billard & Cosson, 1992; Billard *et al.*, 1995; Lahnsteiner *et al.*, 1997; Krasznai *et al.*, 2000).

In addition, it is confirmed, again, that the motility of spermatozoa in *A. persicus*, like that of other sturgeon species (Ginzburg, 1968) and teleosts (Stoss, 1983; Billard *et al.*, 1995), is prolonged in saline solutions in comparison to freshwater. The results also show that the composition of activating solution influences the period of sperm motility in sturgeon. Synergetic effects between

ions can control motility of sperm by the membrane potential (Cosson *et al.*, 1999; Linhart *et al.*, 2002). The duration of motility increases when 1mM Ca^{2+} is added to the activating solution (Billard *et al.*, 1999). Gallis *et al.*, (1991), Linhart *et al.*, (2002) and Alavi (2003) reported that potassium at 0.1, 0.5-5 and more than 3 mM had inhibitory effects on sperm motility in the Siberian sturgeon, paddlefish and the Persian sturgeon, respectively. Toth *et al.*, (1997) and Alavi (2003) observed high biosensivity of sperm to sodium concentrations at 50mM Na^{+} in *A. fulvescence* and *A. persicus*. Alavi, (2003) reported positive effect of Mg^{2+} on motility of sturgeon spermatozoa in *A. persicus*. According to these literatures, it seems that the interaction between ions can reduce the sensivity of sperm to Na^{+} ions and the inhibitory effect of K^{+}. Probably, some differences between this study with others can be explained by physiological condition of broodstock (Billard & Cosson, 1992), method of analyzing of the sperm motility (Cosson *et al.*, 1999), technique of dilution of sperm in activating solution or freshwater (Billard *et al.*, 1995), and an important role of stress in fish spermiation process (Alavi, 2003) during transferring into hatchery, spawning induction by pituitary injection and semen sampling time. However, it is clear that spermatozoa become motile as a result of changes in the properties of the plasma membrane potential and its conductance (Cosson *et al.*, 1989; Gatti *et al.*, 1990; Boitano & Omoto, 1992; Tanimoto *et al.*, 1994; Krasznai *et al.*, 1995) and several parameters such as ionic composition modificate membrane and induce motility due to intracellular changes (Morisawa *et al.*, 1983, Cosson *et al.*, 1989; 1999).

Probably, effect of dilution rate on motility depends on spermatozoa concentration in milt (Billard, Personal Communication). So that, for stimulating of motility in fishes with high concentration of spermatozoa, needs a high dilution rate.

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