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RESEARCH PAPER

The Effects of Different Sperm Activator Medias on the Velocity and Movement Style Brown Trout (*Salmo trutta* Linnaeus, 1792) Sperm Cells

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Abstract

This study was aimed to determine the effects of Na^+, K^+ and Ca^{2+} ion-based different sperm activator medias (SAM) on the velocity and movement style of Brown trout (*Salmo trutta*) sperm cells. SAMs were prepared such as SAM1 (9 mM NaCl, 50 mM Glycine and 20 mM Tris), SAM2 (103 mM NaCl, 40 mM KCl, 20 mM HEPES), SAM3 (125 mM NaCl, 30 mM Tris), SAM4 (60 mM NaHCO3, 50 mM Tris) and SAM5 (125 mM NaCl, 1 mM CaCl2, 30 mM Glycine, 20 mM Tris), respectively. It was kept at the same pH: 9.0 for all activators. According to the study data, the statistically significant increases ($P<0.05$) were found in the velocities of sperm cells in SAM5 activator compared to other activators. All activators showed an effect of increasing and decreasing on the values of linearity and amplitude of lateral displacement of the spermatozoa head in movement style of sperm cells but these were found to be statistically insignificant ($P>0.05$). As a result, unlike other activators, the presence of the Ca^{2+} (1 mM) ion in the SAM5 activator has resulted in an increase in the velocities of sperm cells in brown trout sperm cells.

Introduction

The brown trout (*Salmo trutta* Linnaeus, 1758) belongs to the Salmonidae family. Species have a wide distribution range and naturally live in Western Asia, including Europe, North Africa and Anatolia. Brown trouts have been moved beyond natural habitats such as North and South America, New Zealand, Australia, and Africa, although it has naturally existed for over a hundred years. This species is important for sportive fishing and aquaculture (Şahin, Akbulut, Çakmak & Çavdar, 2010). *Salmo trutta* has increased with the way aquaculture production, including marine and inland waters and produced about 2924 tons as of 2017 in Turkey (TUIK, 2017).

In fish with external fertilization, the sperm cells are immobilized in the testis and seminal fluid, and the initial of motility depends on the conditions of fertilization environment. While many factors are known to regulate motility of sperm cells, ion concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^- etc.), osmolality and pH in activator media are among the most important of these. In trouts, when the sperm cells are released into freshwater, start to activate with reduced the concentration of K^+ ions (Alavi & Cosson, 2006; Browne *et al*., 2015; Dzyuba & Cosson, 2014). It was also found that rainbow trout sperm cells were immobilized after diluted with solutions containing high concentrations of K^+ ion. It was determined similar situation in sperm cells in chum salmon, *Oncorhynchus keta*. However, dilution in isotonic NaCl has been found to trigger immediate activation of sperm cell motility (Alavi & Cosson, 2006). Also in the motility of rainbow trout sperm cells, Na^+ / H^+ exchange may be responsible for the participation of Na^+ ions (Dzyuba & Cosson, 2014). Thus, investigators have found that sperm cell motility begins by decreasing the K^+ ion concentration and increasing Ca^{2+} ion concentrations. Particularly obtained data show that when the motility begins, intracellular Ca^{2+} ion concentrations increase. Therefore, Ca ion concentration is a necessity for the motility of sperm cells (S. M H Alavi, Gela, Rodina, & Linhart, 2011; Alavi & Cosson, 2006; Cosson, Billard, & Letellier, 1989; Tanimoto & Morisawa, 1988).

After activation of the fish sperm cells, their movements begin on a very low curvilinear path. But

they path more curved forms and consequently concentric circles in at the last stages of motility or at the risk of sperm contamination or when activated with inappropriate activators. In this context, linearity and percentage of motile cells are evaluated as fertility indicator (Rurangwa, Kime, Ollevier, & Nash, 2004). Computer assisted sperm analysis systems are very popular for the practical analysis of sperm cell motility in more species and have taken the methods of predicting the analyzer's individual emotions with classical methods used in the past (Fauvel, Suquet, & Cosson, 2010). Among all these literature, it was thought that this study is important in terms of not having a detailed study on sperm activation of *Salmo trutta* fish species.

Materials and Methods

Sperm Collection and Experiments

Brown trout (*Salmo trutta*) males (1657±120 g) maintained in the hatchery station at a commercial farm, Malatya, Turkey in December, 2017. Fresh sperm samples from 6 individuals were performed by massage from front to back of the fish abdomen without an anesthesia. Then, sperm samples was pooled and diluted at ratio 1:100 with an immotile solution (IMS) (103 mM NaCl, 40 mM KCl, 1 mM $CaCl_2$, 0.8 mM $MgSO_4$, 20 mM Hepes, pH 7.8) (Lahnsteiner, Mansour, & Kunz, 2011) with prepared a stock solution of immobilized sperm. All immobilized sperm samples were kept on ice during the procedure. The sperm samples were activated under the microscope with sperm activation media (SAM) at ratio 1:20 and finally the dilution rate was 2000 times.

Sperm activator media (SAM):

- **SAM1** (9 mM NaCl, 50 mM Glycine and 20 mM Tris, pH: 9.0) (Aguilar-Juárez, Ruiz-Campos, & Paniagua-Chávez, 2014)
- **SAM2** (103 mM NaCl, 40 mM KCL, 20 mM HEPES, pH: 9.0), (Eric and Randall, 2016)
- **SAM3** (125 mM NaCl, 30 mM Tris, pH 9.0) (Cosson, Billard, Gatti, & Christen, 1985)
- **SAM4** (60 mM $NaHCO_3$, 50 mM Tris, pH 9) (Lahnsteiner *et al*., 2011)
- **SAM5** (125 mM NaCl, 1 mM $CaCl_2$, 30 mM Glycine, 20 mM Tris, pH 9.0) (Billard, 1978; Nynca, Dietrich, Dobosz, Grudniewska, & Ciereszko, 2014)

In the activation time, the sperm samples were examined under Olympus BX53 microscope with 200x magnification lens and Sony CCD camera with 30 fbs were used to video of sperm samples. Sperm cell velocity parameters such as VSL: straight line velocity (µm/s), VCL: curvilinear velocity (µm/s), VAP: angular path velocity (µm/s) and movement style parameters such as LIN, linearity (%), the ratio of net distance moved to total path distance and ALH: amplitude of lateral displacement of the spermatozoa head (µm) (Fauvel *et al*., 2010) were carried out by the computer assisted sperm analysis systems, BASA-Sperm Aqua, produced by Merk Biotechnology Ltd. Co. in Turkey. Chemicals such as NaCl, KCl, $CaCl_2$, $NaHCO_3$, $MgSO_4$, Glycine, Tris and HEPES were purchased from distributor of Sigma-Aldrich Co. in Turkey.

Statistics Analysis

Descriptive analysis was done in Mean±SE, $P<0.05$. One-way ANOVA test-Tukey HSD was used between all groups after homogeneity of each group was tested through Test of Homogeneity of Variance. Normality test was performed between the data in the SPSS 17 program. Graph Pad Prism 5 was used to create graphics.

Results and Discussion

In comparison to other groups, sperm samples in SAM5 had increased in VSL value, 64.33±1.29 µm/s and it was the highest in all groups. The changes in the mean VSL value with SAM5 were found to be statistically significant ($P<0.05$). A similar case was obtained at the VAP value of the sperm samples in SAM5. While the mean of VAP value in SAM5 was 76.64±3.75 µm/s, the lowest value was found in SAM3 with 39.56±1.79 µm/s.

While the highest of the VCL value was found in SAM5 and SAM2 as 123.77±5.88 µm/s and 129.40±3.27 µm/s, respectively, the lowest VCL value was seen in SAM4 with 108.19±7.79 µm/s. The differences between VCL mean values were statistically significant ($P<0.05$) in SAM2. When it was found that the differences were insignificant ($P>0.05$) regarding LIN and ALH, the highest values of LIN and ALH were determined in SAM5 (Figure 1).

In this study, it is thought that the increase of VSL, VCL and VAP values *Salmo trutta* sperm cells in SAM5 activator depends on the presence and the amount of Ca^{2+} ions in the activator. However, the obtained the VSL and VCL values of sperm cells are similar or parallel to the VSL and VCL values of the control group in the study of researchers (Nynca *et al*., 2014) who has used the same type and same content (1 mM $CaCl_2$) of sperm activator media. A similar situation in terms of the contribution of Ca^{2+} ions to the velocities of sperm cells was found in the velocities of *Salmo trutta trutta* sperm cells by the study of Dziewulska and Domaga, (2013). Investigators observed that sperm cell velocities were negatively affected in activator with higher concentrations than 4 mM Ca^{2+}, while the highest velocity was observed in 1 mM Ca^{2+} containing activator (Dziewulska & Domagała, 2013). Activation study on sperm cells of *Acipenser ruthenus* also found that activator with 1 mM Ca^{2+} ion gave the highest cell

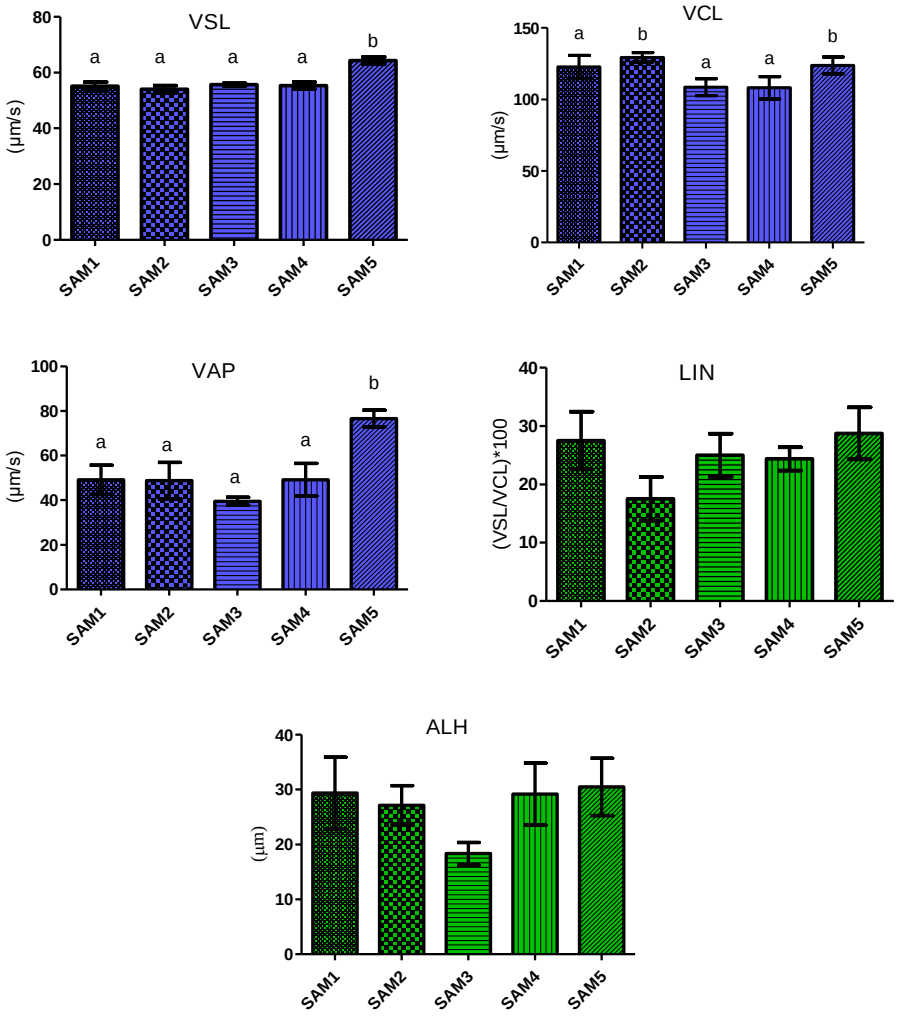

Figure 1. The effects of different sperm activator media (SAM) on straight line velocity (VSL), curvilinear velocity (VCL), average path velocity (VAP), the linearity (LIN), beat cross frequency (BCF) and amplitude of lateral displacement of the sperm head (ALH) on *Salmo trutta* sperm cells. The different letters[a,b] reflect statistically different values (P<0.05*;* Mean±S.E.).

velocity (S. M H Alavi *et al*., 2011). While it was found that the rainbow trout sperm cells were activated after 2.5 mM Ca^{2+} addition (Tanimoto & Morisawa, 1988), the sperm cells of *Barbus grypus* species were found to decrease after 10 mM but the fastest velocity in 5 mM Ca^{2+} addition (Öğretmen, Gölbaşi, & Inanan, 2014). Cosson *et al*. (1989) determined that the antagonist effect of Ca^{2+} (1 µ*M*-10 m*M*) can start when the concentration of K^+ in sperm activator media was decreased such as 2 m*M*-10 m*M* (Cosson *et al*., 1989).

In this study, it is tought that the presence and amount of Ca^{2+} in the SAM5 activator is positively effected on the velocity and the activation of *Salmon trutta* sperm cells, and this result is supported by the results of other researchers (Alavi *et al*., 2011; Alavi & Cosson, 2006; Cosson *et al*., 1989; Dziewulska & Domagała, 2013; Nynca *et al*., 2014; Öğretmen *et al*., 2014; Tanimoto & Morisawa, 1988).

Dziewulska and Domagala (2013) determined that the sperm cell velocities of *Salmo trutta trutta* sperm cells reduced when KCl concentration incresead in activator. They also showed that a significant decrease at 2 mM KCl in 10th second after activation of sperm cells. At the end of their study, 4 mM KCl and more concentrations were inhibited totally of activation *Salmo trutta trutta* sperm cells. When they examined the NaCl effects, they found that the sperm cells had the highest velocity in 60-90 mM NaCl but decreased their velocity after 120 mM NaCl (Dziewulska & Domagała, 2013).

In a study which research about the activation and the velocity of *Acipenser ruthenus* sperm cells, researchers found that 0.35 mM concentration of K^+ ions completely inhibited, 0.25 mM K^+ activated but lower the velocity and the concentration of 0.1 mM K^+ activated to the sperm cells in rate of 100% (Alavi *et al*., 2011). In other study, the sperm cells of *Barbus grypus* species have inhibited completely at 100 mM K^+ and the highest velocity at 5 mM K^+ but rapidly decreased after 20 mM K^+ (Öğretmen *et al*., 2014).

In this study, while the VCL value of *Salmo trutta*

sperm cells was high in SAM2 (103 mM NaCl) media, it decreased in SAM3 (125 mM NaCl) media. Our results have supported by some researchers who determined that the more concentration of 120 mM NaCl decrease the velocity of the sperm cell (Alavi *et al*., 2011; Dziewulska & Domagała, 2013; Dzyuba & Cosson, 2014; Öğretmen *et al*., 2014).

This study was aimed to determine the effects of Na^+, K^+ and Ca^{2+} ion-based different sperm activator medias (SAM) on the velocity and movement style of Brown trout (*Salmo trutta*) sperm cells. In conclusion, it has been determined that the amounts and the presence of 1 mM $CaCl_2$ and 103 mM NaCl in the sperm activator media are suitable and advisable for the activation and the velocity enhancer of *Salmo trutta* sperm cells. However, it should research about the effects of different concentrations of Ca^{2+}, Na^+ and K^+ ions or etc. on the velocity of sperm cells and skills of the egg fertilization.

References

Aguilar-Juárez, M., Ruiz-Campos, G., & Paniagua-Chávez, C. G. (2014). Cold storage of the sperm of the endemic trout Oncorhynchus mykiss nelsoni: A strategy for short-term germplasm conservation of endemic species. *Revista Mexicana de Biodiversidad*, *85*(1), 294–300. https://dx.doi.org/10.7550/rmb.36352

Alavi, S. M. H., & Cosson, J. (2006). Sperm motility in fishes. (II) Effects of ions and osmolality: A review. *Cell Biology International*. https://dx.doi.org/10.1016/j.cellbi.2005.06.004

Alavi, S. M. H., Gela, D., Rodina, M., & Linhart, O. (2011). Roles of osmolality, calcium - Potassium antagonist and calcium in activation and flagellar beating pattern of sturgeon sperm. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *160*(2), 166–174. https://dx.doi.org/10.1016/j.cbpa.2011.05.026

Billard, R. (1978). Some data on gametes preservation and artificial insemination in teleost fish. *Actes de Colloques Du C.N.E.X.O.*, *8*, 59–73. http://archimer.ifremer.fr/doc/00000/6021/

Browne, R. K., Kaurova, S. A., Uteshev, V. K., Shishova, N. V., McGinnity, D., Figiel, C. R., & Cosson, J. (2015). Sperm motility of externally fertilizing fish and amphibians. *Theriogenology*. https://dx.doi.org/10.1016/j.theriogenology.2014.09.018

Cosson, M. P., Billard, R., Gatti, J. L., & Christen, R. (1985). Rapid and quantitative assessment of trout spermatozoa motility using stroboscopy. *Aquaculture*, *46*(1), 71–75. https://dx.doi.org/10.1016/0044-8486(85)90178-4

Cosson, M. P., Billard, R., & Letellier, L. (1989). Rise of internal Ca^{2+} accompanies the initiation of trout sperm motility. *Cell Motility and the Cytoskeleton*, *14*(3), 424–434. https://dx.doi.org/10.1002/cm.970140312

Dziewulska, K., & Domagała, J. (2013). Effect of pH and cation concentrations on spermatozoan motility of sea trout (*Salmo trutta* m. trutta L.). *Theriogenology*, *79*(1), 48–58. https://dx.doi.org/10.1016/j.theriogenology.2012.09.008

Dzyuba, V., & Cosson, J. (2014). Motility of fish spermatozoa: From external signaling to flagella response. *Reproductive Biology*. https://dx.doi.org/10.1016/j.repbio.2013.12.005

Fauvel, C., Suquet, M., & Cosson, J. (2010). Evaluation of fish sperm quality. *Journal of Applied Ichthyology*, *26*(5), 636–643. https://dx.doi.org/10.1111/j.1439-0426.2010.01529.x

Lahnsteiner, F., Mansour, N., & Kunz, F. A. (2011). The effect of antioxidants on the quality of cryopreserved semen in two salmonid fish, the brook trout (Salvelinus fontinalis) and the rainbow trout (Oncorhynchus mykiss). *Theriogenology*, *76*(5), 882–890. https://dx.doi.org/10.1016/j.theriogenology.2011.04.019

Nynca, J., Dietrich, G. J., Dobosz, S., Grudniewska, J., & Ciereszko, A. (2014). Effect of cryopreservation on sperm motility parameters and fertilizing ability of brown trout semen. *Aquaculture*, *433*, 62–65. https://dx.doi.org/10.1016/j.aquaculture.2014.05.037

Öğretmen, F., Gölbaşi, S., & Inanan, B. E. (2014). Inhibitory effect of K^+ and Ca^{2+} concentrations, pH, and osmolality of activation solution on motility of shabut (Barbus grypus Heckel 1843) spermatozoa. *Turkish Journal of Veterinary and Animal Sciences*, *38*(3), 245–252. https://dx.doi.org/10.3906/vet-1308-9

Rurangwa, E., Kime, D. E., Ollevier, F., & Nash, J. P. (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*, *234*(1–4), 1–28. https://dx.doi.org/10.1016/j.aquaculture.2003.12.006

Tanimoto, S., & Morisawa, M. (1988). Roles for Potassium and Calcium Channels in the Initiation of Sperm Motility in Rainbow Trout. (K^+/Ca^{2+}/channel/sperm motility/rainbow trout). *Development, Growth & Differentiation*, *30*(2), 117–124. https://dx.doi.org/10.1111/j.1440-169X.1988.00117.x

TUIK, (2017). Turkish Statistical Institute, Fishery Products. http://www.tuik.gov.tr/PreTablo.do?alt_id=1005.