



Roles of Ionic and Non-Ionic Osmolarities and Different pH Levels on Triggering Sperm Motility in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Since advantageous properties such as fast growth rate, tolerance to severe water conditions, flesh quality, variations in feeding and high reproductive performances in different habitats, Nile tilapia (*Oreochromis niloticus*) become a popular cultured fish species all over the world. Even though its worldwide popularity in aquaculture, some ecological conflicts on distribution of Nile tilapia in nature has been debated in many countries like Türkiye, describing as an invasive species. The present study aims to present how osmolality and pH affect sperm activation in Nile tilapia in terms of sperm motility and velocity as well as duration of motility at 12th and 300th seconds post-activation. For this purpose, two successive experiments have been carried out. Firstly, spermatozoa motility was activated both ionic (NaCl, KCl, CaCl₂, MgCl₂ and NaHCO₃) and non-ionic (glucose, urea, and glycine) solutions at 300, 250, 150, 100, and 50 mOsmol/kg. Additionally, motility was also activated by distilled water. Secondly, the role of pH on triggering sperm motility was determined by diluting spermatozoa in NaCl-based activating medium at pH levels ranged from 6 to 10. Spermatozoa showed the highest motility parameters when they were activated in NaCl and NaHCO₃ as ionic solutions and glucose as a non-ionic medium at 100 mOsmol/kg. Sperm motility and velocity were found 96±2% and 91±5 µm/s at 12th s and 64±4 % and 52±4 µm/s at 300th s respectively while duration of motility was determined as 1117±53 s (18'37''±53'') in NaCl at 100 mOsmol/kg. The results represented that initiation of Nile tilapia motility is directly activated by a hypo-osmotic shock induced by both ionic and non-ionic media, however, contents of the media induced significant differences in motility parameters. Moreover, it has also demonstrated that Nile tilapia spermatozoa can be activated in a wide range of pH including acidic pH values. Consequently, these findings on sperm motility properties of Nile tilapia contribute essential information not only for aquaculture practice, but also for researchers interested in ecological distribution of the species.

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Introduction

Immotile spermatozoa are activated after contact with an aquatic environment and then acquire a motility ability to fertilize egg, which they lose within minutes in most fish. During this period of time, the quality of spermatozoa motility is one of the main reasons that improves fertilization success (Alavi & Cosson, 2006). This quality is generally defined by kinetic characteristics of motility including the percentage of motile cells, sperm velocity and the duration of motility. When spermatozoa contact with an activating medium that is capable of initiating motility, these motility characteristics are highly affected by the concentrations of ions (such as Na⁺, K⁺, Ca²⁺, Mg²⁺), osmolality and pH values of this medium (Krasznai et al., 1995; Cosson, 2004). As a function of these characteristics in an activation solution, the depolarization of sperm

membrane is promoted; thereafter, flagellar motility commences, and this ensures the movement of the sperm cell (Morisawa & Suzuki, 1980). In general, seminal plasma osmolality which keeps spermatozoa quiescent is around 300 mOsm/kg in fish. Apart from ionic composition and other factors, the osmolality value of the activation medium should be below 300 mOsm/kg in freshwater fish species to initiate motility (Dreanno et al., 1999). In other words, the triggering of sperm motility in freshwater fish species is required for a hypo-osmotic shock. On the other hand, although it has less affect particularly on initiation of motility compared to osmolality, pH level of the activation medium could influence these motility characteristics (Alavi and Cosson, 2005).

Nile tilapia (*Oreochromis niloticus*) belongs to the family Cichlidae and is naturally distributed in the tropical and subtropical regions of Africa (El-Sayed & Fitzsimmons, 2023). Nile tilapia has some advantageous properties such as fast growth rate, tolerance to severe water conditions, flesh quality, and variations in feeding. Moreover, it could show a high reproductive performance in different habitats (Dikel et al., 2003; Bwanika et al., 2004). These characteristics have allowed the fish to become a popular cultured fish species all over the world. Starting from 1960s, this species has been introduced from Africa to different continents to use in aquaculture and enhance fisheries (Dadzie, 1992). Up to now, it has spread throughout from U.S.A to the Far East. The global farmed Nile tilapia production has exceeded over 5 million tons in 2020s. Although it has a fluctuant trend, the farmed production of Nile tilapia in Türkiye is reported. According to Republic of Türkiye Ministry of Agriculture and Forestry, the aquaculture production of Nile tilapia in Türkiye was varied from 6 to 58 tons between the years of 2014 and 2021 while there has been no production record in 2022 and 2023. Even though its worldwide popularity in aquaculture, some ecological conflicts on distribution of Nile tilapia in nature has been debated in many countries like Türkiye. Nile tilapia is described as an invasive species in the inland water of Türkiye (Çiçek, 2021). The presence of this species has been documented in the different provinces since 2005, and these reports have been increasing with new ones such as Eskişehir, Burdur and Mersin being added in recent years (Arslan et al., 2021; Ergüden et al., 2022). Obviously, any findings about reproductive physiology, like sperm motility properties, of Nile tilapia can provide valuable information not only for aquaculture practice, but also for researchers interested in ecological and geographical distribution status of this species.

In freshwater fishes, studies on sperm motility activation have been considerably focused on species belonging to the Acipenseridae, Cyprinidae and Salmonidae families, probably since they have included commercially important cultured species for quite some time (Rurangwa et al., 2004; Alavi & Cosson, 2005;2006). However, there is limited information available on sperm motility properties of Cichlidae species, specifically interaction between alterations in motility parameters and activation media at different osmolarities and pH levels. The aims of the present study were to investigate how osmolality and pH effect on sperm activation in Nile tilapia in terms of motility parameters. For this purpose, firstly spermatozoa motility was activated both ionic (NaCl, KCl, CaCl₂, MgCl₂ and NaHCO₃) and non-ionic (glucose, urea, and glycine) solutions at 300, 250, 150, 100, and 50 mOsmol/kg. Furthermore, the role of pH on triggering sperm motility was determined by diluting sperm in activation media that contain NaCl at pH levels ranged from 6 to 10.

Materials and Methods

Fish and sperm collection

Spermatozoa samples from five mature males (body mass 108–121 g) of Nile tilapia were used for the experiments. The water temperature was maintained at

28°C. Before the sampling, the males were anaesthetized by immersion in clove oil (50 µl/L, Öğretmen et al., 2014). Firstly, the urinary bladder was emptied by hand pressure at the abdomen, then the males were stripped after the genital papilla and the abdomen were thoroughly dried. Semen samples were collected with a 20 µL micropipette using as a catheter and placed immediately on ice. Samples were immediately controlled under the microscope and discarded if any movement of spermatozoa observed due to contamination with urine. The samples of individual males were not pooled.

Sperm Motility Assessment and Activating Solutions

To examine the effect of osmolality, sperm motility was triggered by both ionic and non-ionic solutions at 300, 250, 150, 100, and 50 mOsmol/kg. In the experiments, NaCl, KCl, CaCl₂, MgCl₂ and NaHCO₃ for ionic solutions and glucose, urea and glycine for non-ionic solutions were used and adjusted to pH 8.0. All chemicals were purchased from Sigma Aldrich Chemical Company (Steinheim, Germany). Osmolalities were calculated based on the concentrations (Vujovic et al., 2018). In addition, sperm motility was also activated by distilled water. After the activation, motility (percentage of motility, %) and velocity (curvilinear velocity-VCL, µm/s) were determined at 12th and 300th seconds post-activation as well as total duration of motility. For trigger motility, samples were mixed with the activation solutions in Eppendorf tubes at 1:300 (v:v) ratio which place in in a temperature-controlled water bath at 25°C. Sperm motility was recorded using a Leica DM750 microscope with phase contrast attachment mounted a Leica MC190 HD camera (Leica Microsystems, Wetzlar, Germany). Spermatozoa were evaluated in each recording by ImageJ with a specific CASA plug-in (Wilson-Leedy and Ingermann, 2007; Schneider et al., 2012). Velocity values higher than 20 µm/s were considered as motil (Dzyuba et al., 2019), according to this velocity, motility levels were determined. The duration of motility was determined as a time period until forward movement stopped and circular movement began (Billard & Cosson, 1992; Dietrich et al., 2005). Determination of motility parameters was done in quintuplicate for each dilution.

Statistical Analysis

All values were expressed as mean ± SD. Percentage and velocity data were performed arc-sin and log₁₀ transformations, respectively before statistical analysis. One-way ANOVA followed by Tukey's test was used to reveal the results of treatments were significantly different from each other including the control group (P < 0.05).

Results

Effects of Ionic and Non-Ionic Osmolarities on Motility

When Nile tilapia spermatozoa were activated by distilled water, motility values were found as 81±5% at 12th s and 34±2 at 300th s while velocity values were 76±5 µm/s and 28±4 µm/s. Duration of motility was determined as 00:57 573 ±57s (9'33''±57'') in distilled water.

Spermatozoa motility was activated both ionic and non-ionic solutions at 250, 150, 100, and 50 mOsmol/kg, and percentages of motility values were depicted in Figure 1.

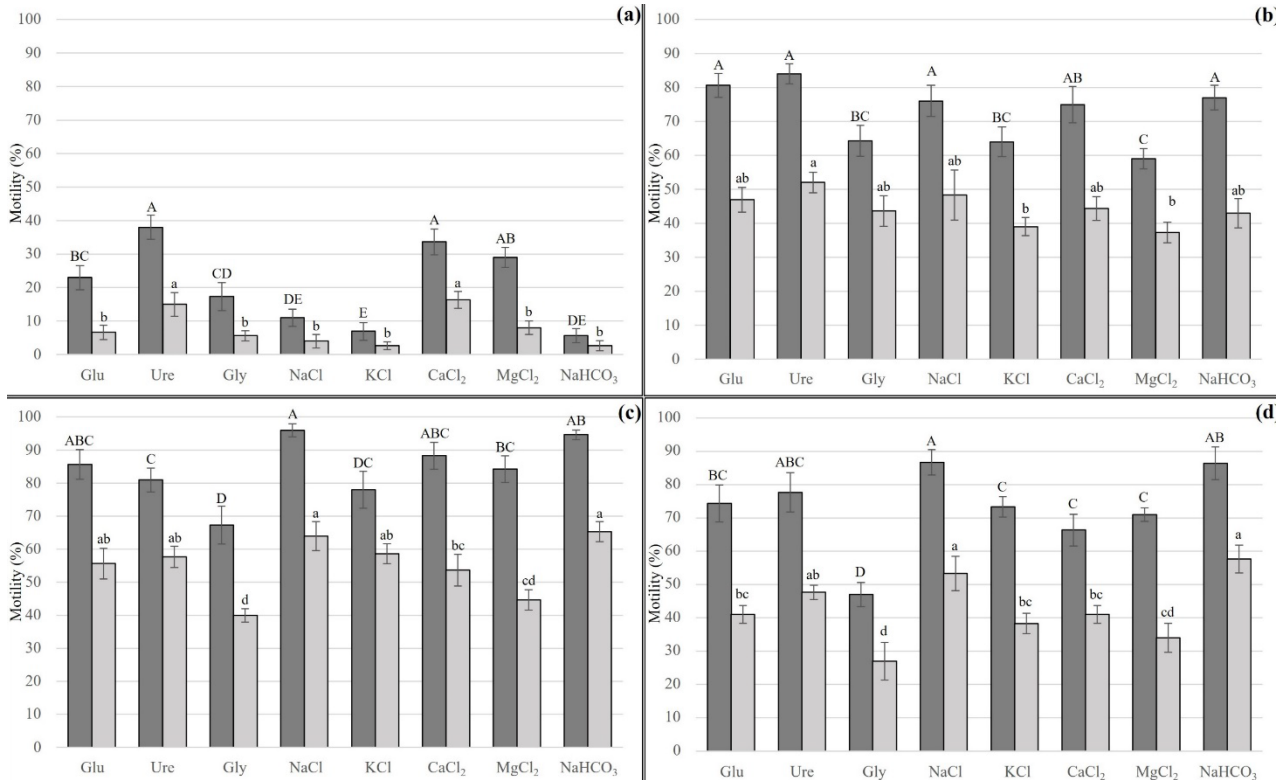


Figure 1. Percentage of motile spermatozoa (%) of Nile tilapia spermatozoa triggered by glucose (Glu), urea (Ure), glycine (Gly), NaCl, KCl, CaCl₂, MgCl₂ and NaHCO₃ at 12th (■) and 300th (□) seconds post-activation at 250 (a), 150 (b), 100 (c), and 50 (d) mOsmol/kg.

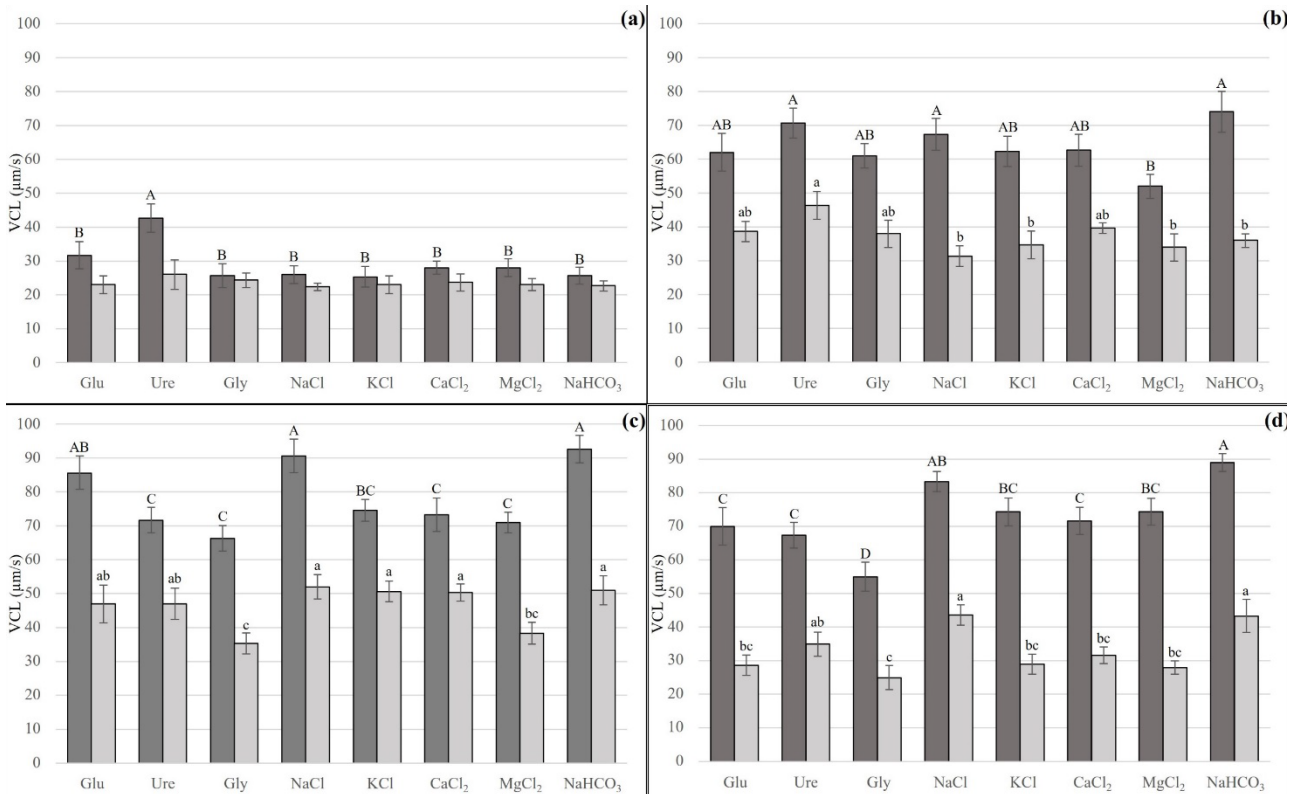


Figure 2. Curvilinear velocity (VCL, $\mu\text{m/s}$) of motile spermatozoa of Nile tilapia spermatozoa triggered by glucose (Glu), urea (Ure), glycine (Gly), NaCl, KCl, CaCl₂, MgCl₂ and NaHCO₃ at 12th (■) and 300th (□) seconds post-activation at 250 (a), 150 (b), 100 (c), and 50 (d) mOsmol/kg.

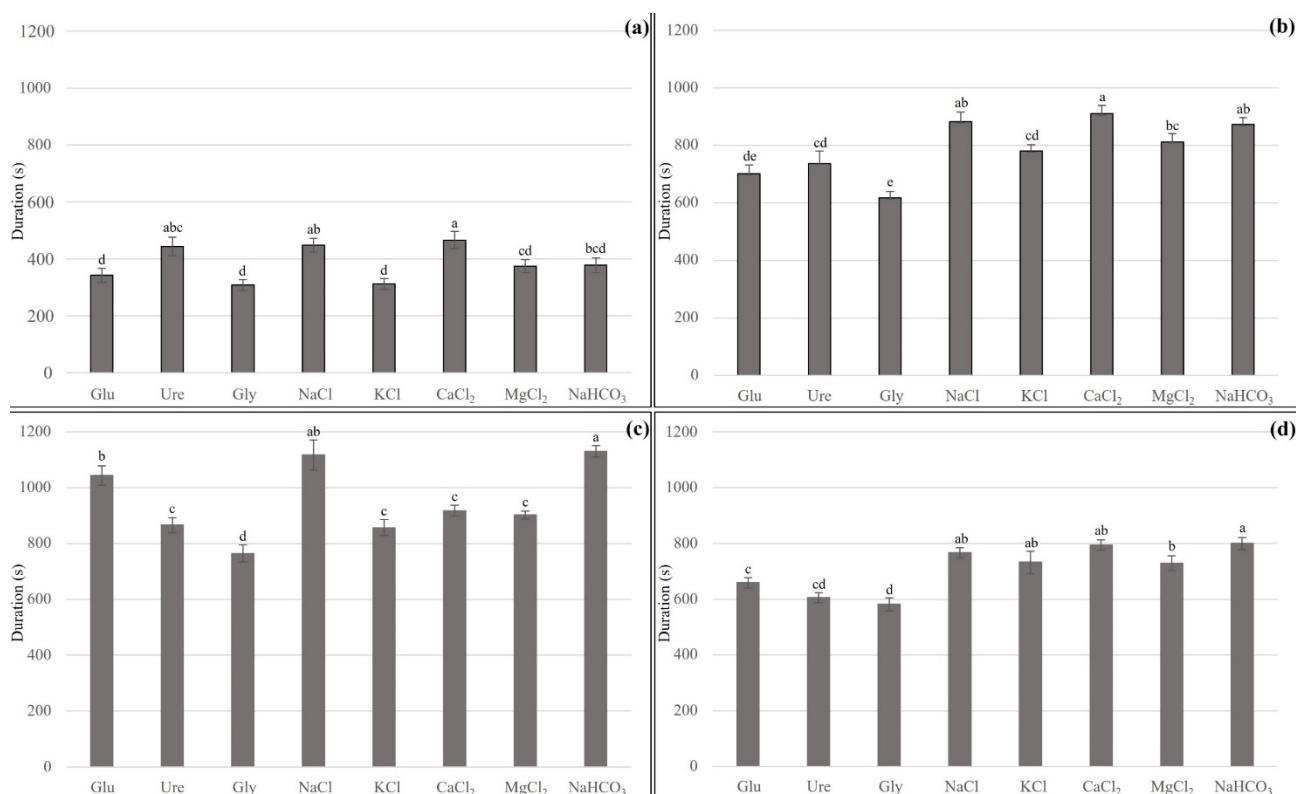


Figure 3. Durations (s) of sperm motility of Nile tilapia spermatozoa triggered by glucose (Glu), urea (Ure), glycine (Gly), NaCl, KCl, CaCl₂, MgCl₂ and NaHCO₃ at 250 (a), 150 (b), 100 (c), and 50 (d) mOsmol/kg.

The triggering of spermatozoa motility was completely suppressed at 300 mOsmol/kg for all solutions. The motile spermatozoa were observed at ≤ 250 mOsmol/kg of all used solutions at 12th and 300th s. The lowest motility values (ranged from 6 to 38 % at 12th s and ranged from 3 to 6 % at 300th s) were found at 250 mOsmol/kg while the highest ones (ranged from 67 to 96 % at 12th s and ranged from 40 to 65 % at 300th s) were found at 100 mOsmol/kg. Regarding all data set on the motility, the highest motility values at 12th s were recorded as $96 \pm 2\%$ and $95 \pm 2\%$ when spermatozoa were diluted in NaCl and NaHCO₃ as ionic solutions at 100 mOsmol/kg. On the other hand, the lowest motility value at the same time period were found as $67 \pm 6\%$ when spermatozoa were activated in glycine at 100 mOsmol/kg. Similarly, approximately 60% of motility as the highest motility values at 300th s was observed after the NaCl and NaHCO₃ dilutions at 100 mOsmol/kg.

VCL values of motile spermatozoa activated in the different osmolality of both ionic and non-ionic solutions were represented in Figure 2. Except for velocity of spermatozoa (43 ± 4 $\mu\text{m/s}$) diluted in urea at 250 mOsmol/kg, VCL values showed no significant difference in the other dilutions. Moreover, non-significant velocity values found in all used solutions at 300th s ($P < 0.05$) at 250 mOsmol/kg. Velocity of the motile spermatozoa increased at ≤ 150 mOsmol/kg of all used solutions. The highest velocity average values were found at 100 mOsmol/kg, as ranging from 66 to 93 $\mu\text{m/s}$ at 12th s while 35 to 52 $\mu\text{m/s}$ at 300th. At this osmolality, VCL values found by NaCl and NaHCO₃ dilutions were significantly higher than the other dilutions (91 ± 5 and 93 ± 4 $\mu\text{m/s}$ respectively) while no significant difference in VCL values was detected in KCl, CaCl₂ and MgCl₂ dilutions. Among the non-ionic solutions, glucose dilution has induced the higher velocity values (86 ± 5 $\mu\text{m/s}$) than those obtained by glycine and urea at 100 mOsmol/kg.

Durations of spermatozoa motility activated both ionic and non-ionic solutions at 250, 150, 100, and 50 mOsmol/kg were shown in Figure 3. In general, spermatozoa durations recorded at 100 mOsmol/kg could be roughly classified as 300 to 450 s at 250 mOsmol/kg, 600 to 900 s at 150 mOsmol/kg, 750 to 1150 s at 100 mOsmol/kg, 600 to 800 s at 50 mOsmol/kg in terms of osmolality for all used dilutions. The highest durations of spermatozoa motility were observed as 1117 ± 53 s ($18^{\circ}37' \pm 53''$) for NaCl and 1131 ± 20 s ($18^{\circ}51' \pm 20''$) for NaHCO₃ among ionic solutions while 1044 ± 35 s ($17^{\circ}24' \pm 35''$) for glucose among non-ionic solutions at 100 mOsmol/kg.

Effects of pH on Motility

After spermatozoa motility was activated in ionic and non-ionic solutions, the effect of pH on motility was tested using with 100 mOsm/kg NaCl. Percentage of motile spermatozoa and velocity recorded at 12th and 300th seconds post-activation, and duration of motility were observed at pH ranged from 6 to 10 (Figure 4). The percentage of motile spermatozoa ($>90\%$) and velocity (around 90 $\mu\text{m/s}$) values showed no significant differences at 12th s ($P > 0.05$), while those values at 300th s showed significant decreases especially spermatozoa activated with pH 6, 6.5, and 10 solutions. The lowest motility parameters found at pH 6 as $39 \pm 2\%$, 33 ± 2 $\mu\text{m/s}$ at 300th s. No significant difference in spermatozoa motility was detected in pH 7, 7.5, 8, 8.5, 9, and 9.5 solutions. Even this time period, similar velocity values noticed in spermatozoa activated in pH 7.5, 8, 8.5, and 9 solutions. Moreover, these significant differences found at 300th s were reflected to motility durations.

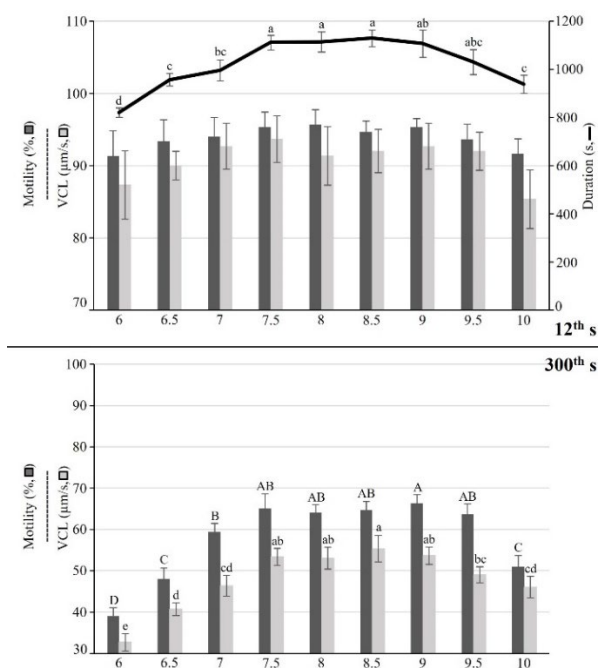


Figure 4. Percentage of motile spermatozoa (%), velocity (VCL, $\mu\text{m/s}$), duration (s) of sperm motility of Nile tilapia spermatozoa triggered by 100 mOsm/kg NaCl at different pH levels. at 12th (a) and 300th (b) seconds post-activation

The significantly lowest duration was recorded in pH 6 solution as 820 ± 20 s ($13'40'' \pm 20''$) while durations in pH 7.5, 8, 8.5, 9, and 9.5 solutions were determined as >1000 s ($>16'40''$).

Discussion

Fish spermatozoa are uniquely adapted to succeed in challenging external environments such as sea, brackish or fresh water after being released from spermatid ducts (Morisawa & Suzuki, 1980). Although sperm cells have an ability to deal with adverse conditions originating from external media, their motility features could be negatively affected by those conditions (Cosson, 2010). In laboratory conditions, relationship and interaction between motility characteristics and ions, osmolality and pH can be revealed using artificial fertilization media instead of natural ones (Inanan, 2020). The initiation of motility and motility features are species-specific and well documented for some cultured species such as rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) compared to other species (Alavi & Cosson, 2005; 2006). Nevertheless, it is assessed that there is a need for such studies on Nile tilapia spermatozoa.

In this study, it has been shown that both ionic (NaCl, KCl, CaCl_2 , MgCl_2 and NaHCO_3) and non-ionic (glucose, urea, glycine) solutions at osmolalities ≤ 250 mOsmol/kg initiate sperm motility in Nile tilapia. This demonstrates that the hypo-osmotic shock for triggering sperm motility could be produced by not only ions but also non-ionic agents. Osmolality is one of the dominant factors which controls initiation of motility and quality of its characteristics (Morisawa, 2008). In general, osmolality is the key factor in triggering sperm motility of cyprinids and marine fish species while osmolality in combination with K^+ concentration is

controlling motility in salmonids and sturgeons (Alavi & Cosson, 2006). Within this context, initiation of sperm motility in Nile tilapia has a similar pattern with cyprinids such as common carp, common barbell (*Barbus barbus*) and redbreil dace (*Clinostomus elongatus*) (Morisawa et al., 1983; Alavi et al., 2009; Butts et al., 2013).

It was revealed that different osmolality levels affect motility and sperm velocity, and hence the duration of Nile tilapia spermatozoa. These findings are consistent with other freshwater species. The highest motility and velocity values were found at osmolality of 100 mOsmol/kg. The lower values of these parameters determined above and below 100 mOsmol/kg could be mainly due to an inadequate impulse of hypo-osmotic shock and flagellar damage, respectively. In the hypo-osmolality dependent mechanism, intracellular signaling is required for ongoing hypo-osmotic shock after initiation of motility to transfer intracellular signaling throughout spermatozoon for maintain motility (Billard & Cosson, 1992). In this regard, motility parameters can be confined by the higher osmolalities which are unable to suppress motility. On the other hand, some cytoplasmic irregular bulges and curling could be form in sperm flagella when osmolality is ≤ 50 mOsmol/kg. Flagellar damages cause lessening flagellar movement and results in decreases in velocity. This phenomenon is very well established in Northern pike (*Esox lucius*) spermatozoa (Alavi et al., 2009). That also might be the main reason for the lower motility parameters obtained by distilled water in the current study.

The limited knowledge has been found in recent previous studies on sperm characteristics in activation of Nile tilapia spermatozoa. Dzyuba et al., (2019) reported that changes in temperature of activating medium directly and markedly fluctuate sperm motility characteristics including duration. They obtained $> 90\%$ of motility, around $45 \mu\text{m/s}$ velocity, and approximately 800 s ($13'20''$) duration at 30th s post-activation and 25°C in an activating solution containing 45 NaCl, 40 mM; Tris-HCl, 10 mM; pH 8.2. Piamsomboon et al., (2019) also suggested that $> 90\%$ motility were observed when Nile tilapia spermatozoa were activated by pond water. In the *Oreochromis* genus, sperm studies have mostly focused on Mozambique tilapia (*Oreochromis mossambicus*). Like Nile tilapia sperm characteristics, Mozambique tilapia spermatozoa have a long motility duration and low velocity compared to spermatozoa commonly farmed fish species like common carp and rainbow trout. It was reported that Mozambique tilapia spermatozoa shown a prolonged motility as >30 min duration and $70\text{--}80 \mu\text{m/s}$ initial velocity at room temperature (Morita et al., 2004). Moreover, this species, unlike Nile tilapia, can be acclimatized to euryhaline waters, and therefore, scientific awareness of it has been promoted compared to Nile tilapia. In Mozambique tilapia spermatozoa, it has been shown that spermatozoa activation from freshwater-acclimated and seawater-acclimated broodstocks need different osmolalities (Linhart et al., 1999). Based on the available knowledge so far, Nile tilapia could not be fully acclimatized to saline waters, especially seawater, even though there are some efforts on it (Mirera & Okemwa, 2023). It might be necessary to consider the occurrence of salinity-based acclimation for spermatozoa for future acclimatization studies.

In general, compared to osmolality, pH levels have a more limited effect on fish sperm motility. In this study, it has shown that Nile tilapia spermatozoa could be activated in a wide range of pH. However, decreases in motility duration were observed at low pH levels. These durations were parallel to motility and velocity recorded at 300th s. During the course of motility, the extreme pH levels didn't influence motility parameters in the first place but later did. The long period of mobility may have played a contributory role in this influence. Although the effects of pH levels on activation of fish sperm vary among species, motility can be triggered in activating solutions with a range from 7 – 9 pH in general (Redondo-Muller et al., 1991; Billard & Cosson, 1992; Linhart et al., 2002). It should be emphasized that the ingredients of activating solution might have effects on motility activation in terms of pH levels (Cosson & Linhart, 1996). On the other hand, initiations of motility in different species, even in the same genus, react differently to the low pH. Ciereszk et al. (2010) showed that at a pH of 6, motility was found around %60 for brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*) and around 10% for Atlantic salmon (*Salmo salar*). The pH level within spermatozoon is lower than external pH (Gatti et al. 1990). This could be related to alterations in membrane potential which is a requirement for motility activation (Boitano & Omoto, 1991). The extreme external pH levels could disrupt the formation of membrane potential which could have motility inhibiting effects. Moreover, internal pH is a possible cellular secondary messenger for initiation of motility (Krasznai et al., 1995). The transmission impairment in this pH message throughout the sperm cell might prevent the exchange through ion channels in the membrane (Krasznai et al., 2003). Like the possible disruption in membrane potential, any malfunction in ion channels can cause decreases in motility parameters. Additionally, external pH levels can substantively improve or decline the flagellar beat frequency of spermatozoa (Alavi & Cosson, 2006). Changes in the flagellar beat frequency alter duration of sperm motility. For instance, total duration of motility of sperm was recorded as around 60 s at 6.0 pH while approximately 200 s at 8.0 pH in *Acipenser persicus* (Alavi et al., 2004).

Besides its aquaculture, Nile tilapia is also an ecologically important species. This fish has been identified as an invasive species in the water bodies of different countries like Türkiye after it was introduced from African inland waters where it naturally occurs. It can be evaluated that sperm motility features of Nile tilapia listed as motility obtained in a wide range of temperature and pH, and long motility time might contribute to its abundance in the various water bodies of different regions. Dzyuba et al. (2019) shown that motility of Nile tilapia spermatozoa could be highly ($\geq 80\%$) activated at 15, 25 35, and 45 °C, and indeed they observed motility at the extreme temperatures like 5 and 50 °C. Moreover, the current study has revealed that sperm activation could be triggered in a wide range of pH values, including the acidic range. pH levels in the natural water bodies are altered by many impacts like industrial pollution and algal blooms (Pinheiro et al., 2021). These features of Nile tilapia spermatozoa are reflective and compatible with this species' ability to survive in diverse water bodies.

Overall, it is useful to remark on some critical issues about the determination of the characteristics of Nile tilapia spermatozoa for further studies. As is often the case, urine contamination could occur in fish during semen sampling by the stripping. In that point, urine contamination could considerably impair pH-dependence of sperm motility activation as well as the osmolarity condition (Ciereszko et al., 2010). Semen contamination with urine can be easily avoided in some fish while it is more difficult to manage in other fish. In Nile tilapia, this is a challenging problem that needs to be overcome. Anatomically, Nile tilapia has a large urinary bladder which must be drained before the stripping. Long motility duration allows sperm activation by urine to be readily recognized during the microscopic examination. Nevertheless, the immobility of Nile tilapia spermatozoa should be immediately checked under the microscope after stripping if they are used in further applications or experiments. Moreover, another critical issue for Nile tilapia spermatozoa is the temperature of activating media. Regarding their sensitivity to temperature, a lesser or a greater duration of motility for the same spermatozoa sample could be recorded. For this reason, determination of sperm motility parameters in Nile tilapia is required to be conducted under constant temperature conditions.

The determination of the optimum motility conditions provides information that can be used in long-term and short-term preservation of spermatozoa (Kanyılmaz & İnanan, 2020). It is also essential to obtain high percentage embryos which is necessary for studies on embryos and larvae (İnanan, 2024). More importantly, such studies are especially useful as a basis for new candidate finfish species in aquaculture. For instance, the Turkish production of aquaculture is dominated by rainbow trout, the European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) while there is a need to boost production capacity for new candidate fish (Yigit et al., 2023). Sperm management is essential for effective reproduction of fish species (Linhart et al., 2020).

Conclusion

The results of the current study show that initiation of Nile tilapia motility is directly activated by a hypo-osmotic shock induced by both ionic and non-ionic media. However, the agents in the media caused significant differences in sperm motility, velocity and duration. Spermatozoa showed the highest motility parameters when they were activated in NaCl and NaHCO₃ as ionic solutions and glucose as a non-ionic medium at 100 mOsmol/kg. Moreover, it has also demonstrated that Nile tilapia spermatozoa can be activated in a wide range of pH including acidic pH values. Since some favorable features such as long duration of motility, relatively less affected by acidity, and motility activation based on ion-free hypo-osmotic shock, Nile tilapia spermatozoa could be a good model for fish sperm motility studies focused on understanding of motility mechanism, the energetics of sperm motility. Further studies should test Nile tilapia spermatozoa to show how motility parameters are affected by substances such as antioxidants, vitamins or even toxicants like pesticides and drugs.

Declarations

Ethical Approval Certificate

The experimental procedures of this study were approved by the Experimental Animal Application and Research Center (ASÜ-DEHAM) of Aksaray University (2021/1-3).

Author Contribution Statement

Burak Evren İnanan: Designed the study, data collection, conceptualization, interpreted data, and writing the original draft

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Conflict of Interest

The author declares no conflict of interest.

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