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Original Article

Penicillin improves the milt quality of Persian sturgeon, *Acipenser persicus* during short-term storage

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Abstract: This study was conducted to examine the effects of antibiotic (5000 units of penicillin) on sperm quality of Persian sturgeon, *Acipenser persicus* during 9 days in vitro storage of milt. For this purpose, the milt samples were stored in the presence and absence of 5000 units of penicillin. Freshwater was used as sperm activator. The milt samples were stored at 4°C and the motility indices were measured 0, 3, 6 and 9 days after storage. The sperm duration and percentage of sperm motility decreased after 6 days of storage both in the presence and absence of antibiotic, although this decrease was more significant in the absence of antibiotic. After 9 days of storage, the lowest values of sperm motility indices was recorded for antibiotic receiving milt samples while no motile spermatozoa observed for antibiotic-free milt samples. In conclusion, our results demonstrated that 5000 units of penicillin improve the Persian sturgeon milt quality during short-term storage.

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Introduction

The short and long term storage of fish spermatozoa are widely used to enhance the efficiency of artificial propagation of fish especially endangered species. Short-term storage of fish milt is useful to reduce the risk of disease transmission, to decrease the costs of brood stock holding in the hatchery, to synchronize male and female gamete availability and to transport the milt samples between different regions (Cloud et al., 1990; Degraaf and Berlincky, 2004). The milt quality during short-term storage is affected by temperature, oxygen supply, sterility, addition of antibiotics, and proper gas exchange (Scott and Baynes, 1980; Babiak and Dabrowsky, 2003; Jensen and Alderdice, 1984). Some studies showed that the anaerobic conditions and associated microbial contaminations may reduce sperm motility and viability during short-term storage of fish milt. For example in Channel catfish, *Ictalurus punctatus*, the milt quality decreased during 10 days storage at 4°C due to the increasing load of bacteria and subsequently the production of extracellular enzymes and consumption of oxygen (Jenkins and Tiersch, 1997). Few studies have used antibiotics in order to the inhibition of bacterial activity during short-term storage of fish milt. In this respect, data is very rare about sturgeon fishes especially Persian sturgeon, *Acipenser persicus*. For instance, the milt quality of Paddlefish, *Polyodon spathula* in terms of sperm motility duration and percentage improved in the presence of 5000 units of penicillin + 5 mg streptomycin/ml (Brown and Mims, 1995).

The sturgeons are ecologically and economically valuable fish species that have been considered in IUCN red list due to significant decreases in their stocks in the nature. At now, the short-term storage of sturgeon milt is used widely in the hatcheries. The

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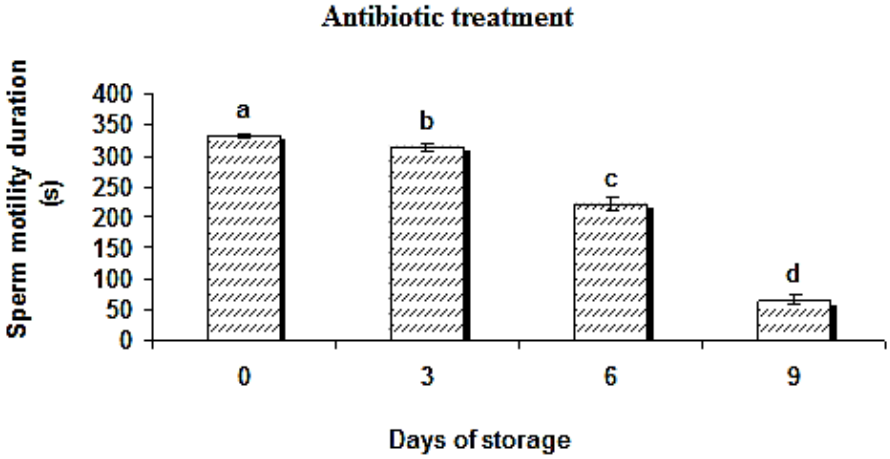

Figure 1. Changes of duration of Persian sturgeon (*Acipenser persicus*) sperm motility during short-term storage of antibiotic receiving milt samples. Different letters indicate significant differences among samples (P<0.05).

increase of short-term storage efficiency can enhance the management of sturgeon propagation and aquaculture in hatchery. Therefore, this study was aimed to examine the effects of antibiotic (5000 units of penicillin) on viability of Persian sturgeon spermatozoa during short-term storage of milt.

Material and Methods

Adult Persian sturgeon were obtained from Shahid Beheshti Artificial Sturgeon Propagation and Rearing Center (Rasht, Iran) during spawning season. After spermiation, the milt samples were collected by 50 ml syringe to prevent contamination with feces, urine, blood, or water, poured into separate sterilized plastic petri dishes. Immediately after milt collection, 10 milt samples with good quality obtained from separate males were considered for the experiment. 5000 units of penicillin were added to 5 milt samples and 5 antibiotic-free milt samples were considered as control group. After addition of penicillin, the milt samples were stored in refrigerator at 4°C. The sperm motility indices were measured according to Alavi et al. (2006) four times including 0, 3, 6 and 9 days after storage. To induce the initiation of sperm motility, a 50 µl drop of freshwater placed on a glass slide and then a drop of 1 µl fresh sperm was diluted using a microsampler. Then, sperm motility was measured according to Rurangwa et al. (2004) by a semi-quantitative method. In this regard, the motility was recorded by a video camera coupled with the optical lens of microscope. At the end, the video recordings were reviewed and the motility was presented as the percentage and duration of motility after the onset of motility until 100% of spermatozoa were immotile. Only forward-moving sperm were considered motile, those simply vibrating or turning on their axes was considered immotile (Aas et al., 1991).

The SPSS software (version 16) was used for data analysis. The normality of data was investigated by Kolmogorov-Smirnov test but because of percentage data (percentage of motile spermatozoa) did not have a normal distribution, proportional data were converted by angular transformation ($\arcsin\sqrt{p}$). One-way analysis of variance (ANOVA) was employed to compare the means of sperm motility indices in different times of storage. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which groups were different. Also, the independent samples t-test was used for the comparison of the means of sperm motility indices between antibiotic receiving and antibiotic-free milt samples.

Results

In antibiotic receiving milt samples of Persian sturgeon, the duration and percentage of sperm

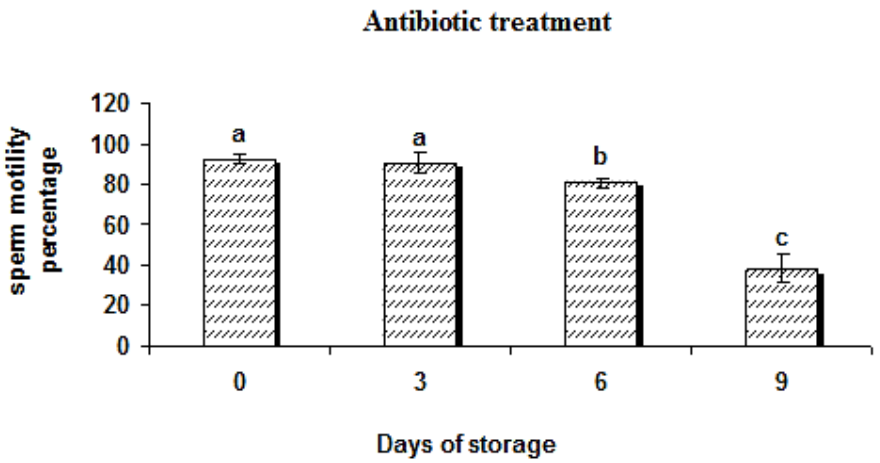

Figure 2. Changes of percentage of Persian sturgeon (*Acipenser persicus*) sperm motility during short-term storage of antibiotic receiving milt samples. Different letters indicate significant differences among samples (P<0.05).

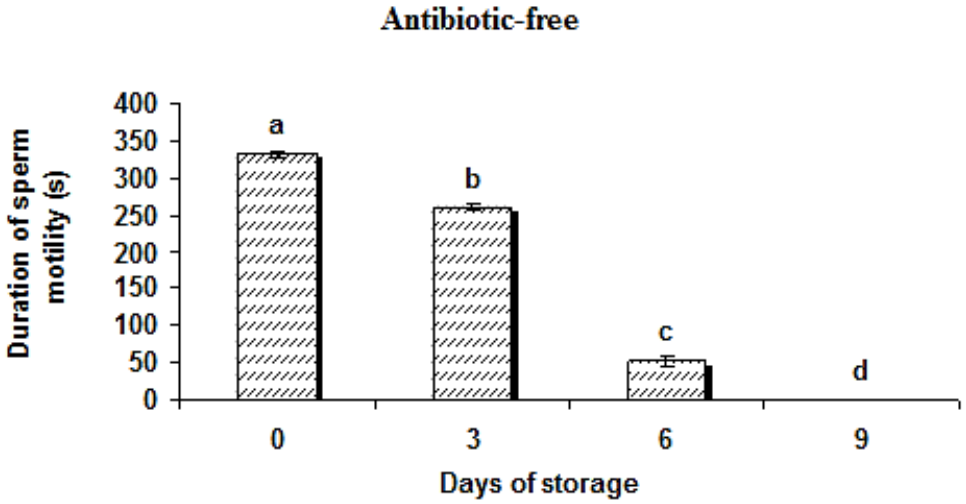

Figure 3. Changes of duration of Persian sturgeon (*Acipenser persicus*) sperm motility during short-term storage of antibiotic-free milt samples. Different letters indicate significant differences among samples (P<0.05)

motility were stable in 0 and 3 days (P>0.05) and then decreased after 6 and 9 days of storage (Fig. 1, Fig. 2) (P<0.05). In antibiotic-free group, the duration and percentage of sperm motility decreased significantly after 3 days of storage so that no motile spermatozoa was observed after 9 days of storage (Fig. 3, Fig. 4) (P<0.05). Decreases in sperm motility was more significant during the storage days in the absence of antibiotic. The overall values of sperm motility duration and percentage in antibiotic receiving milt samples was significantly higher than that of antibiotic-free one (P<0.05) (Table 1).

Discussion

Our results demonstrated that 5000 units of penicillin improve the milt quality of Persian sturgeon in terms of sperm motility and percentage of motile spermatozoa during 9 days short-term storage at 4°C. Studies on other fish species have confirmed that antibiotics such as penicillin, streptomycin and erythromycin can maintain the viability of spermatozoa during milt storage (Stoss et al., 1978; Stoss and Refstie 1983; Brown and Mims, 1995; Jenkins and Tiersch, 1997; Segovia et al., 2000). In rainbow trout, *Oncorhynchus mykiss* decreasing of fertilization capacity during milt

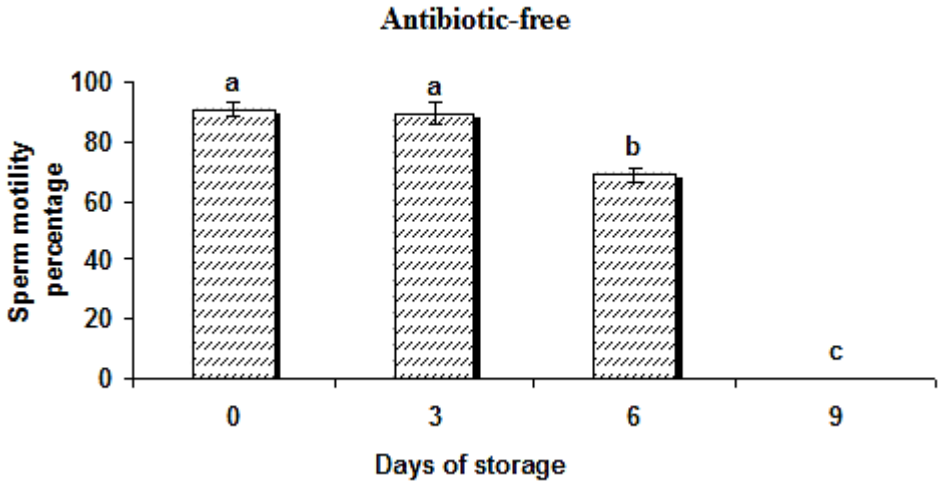

Figure 4. Changes of percentage of Persian sturgeon sperm motility during short-term storage of antibiotic-free milt samples. Different letters indicate significant differences among samples (P<0.05).

storage was significant in absence of 125 units of penicillin and 125 µg streptomycin per ml sperm than antibiotic receiving milt samples. In Paddlefish, an extender containing antibiotic provided better fertilization rate after 14 and 25 days of storage. Anaerobic conditions and associated microbial contamination may reduce sperm motility and viability. Channel catfish sperm maintains its motility at 4°C for 10 days, until the bacterial infection especially by genus *Pseudomonas* (65%), decreased the percentage and duration of sperm motility by production of extracellular enzymes and consumption of oxygen (Jenkins and Tiersch, 1997). The same authors demonstrated that, in non-sterile solutions, motility was completely lost after three days. However, a higher concentrations of antibiotics (gentamicin, 750 µg ml^{-1}; ampicillin, >250 µg ml^{-1}) reduced sperm viability and mitochondrial function in tilapia, *Oreochromis niloticus* (Segovia et al., 2000).

Our result from antibiotic-free milt samples showed that Persian sturgeon spermatozoa maintain their motility in appropriate level only during 3 days of storage. But in antibiotic receiving milt samples, the appropriate sperm motility was maintained for 6 days. Therefore, the use of penicillin can enhances the viability of Persian sturgeon spermatozoa in terms of motility percent and duration during in vitro short-term storage.

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