ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

# Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 8 (3): 375-379, 2009
ISSN 1682-296X


# Determination of Sperms' ATP Content of Golden Grey Mullet (*Mugil auratus*) at Different Conditions

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**Abstract:** Aim of this study was determination of sperms' ATP content of golden grey mullet in different time, temperature and extenders. Caspian Sea Mugilidea is one of the most important fish of the sea fishery which nowadays is the predominant catches fish of the mentioned sea. The ATP content of mentioned fish sperm is an important index of fish fertility determination of the sample's ATP concentration was done by ultra sensitive bioluminescence method. ATP content of sperms were determined at two different sampling temperature (10-12 and 18-20°C) and two different keeping temperature (4°C and room temperature) for 6 h and also ATP content assayed until 10 days storage in the three extender types (glycerol, 0.7 and 0.65% salt solution). Results of the present study showed that, ATP content of sperms, collected at 10-12°C was 74.04±7.22%, in comparison to 18-20°C. The ATP content of sperms during 6 h keeping at 4°C and room temperature were 90.26±0.91% and 17.17±1.49%, respectively. Determination of sperms' ATP content after 5 days keeping in glycerol, 0.7 and 0.65% salt solution revealed that glycerol or 0.65% salt solution is better extender than 0.7% salt solution. But sperms which were kept in mentioned extenders for 10 days showed that glycerol was better than salt solutions based on sperm ATP content saving. Results revealed that, in order to save sperms' ATP content of golden grey mullet, sampling at 18-20°C and keeping in glycerol as extender is recommended.

**Key words:** Extender, *Mugil auratus*, bioluminescence

## INTRODUCTION

Sperm is an important factor of reproduction, survival and stock duration. Environmental variables, particularly nutrition, are ultimately important in affecting sperm quality and reproductive timing (Carl *et al*., 2001). Study of sperm by parameters like fertility, motility, rate of motile sperms, motility speed, flagella beating frequency, ATP content, chemical and biochemical factors, help us for distinction of sperm quality and control the reproduction procedure of animals such as aquatic creatures. As a result expanded investigations were done by different researchers on this cell. The studies in relation to sperm ATP content of fish are slight, so any research has been done on this subject and this matter could be a cause for an unsuccessful artificially bred process of some fishes. On this issue more studies should be done on the basis of determination of sperm's ATP content of fishes.

Sperms swim using flagella beating, the energy for which is provided by the hydrolysis of ATP (Cosson *et al*., 2000; Kim *et al*., 2009). Motility is dependent both on ATP which is stored prior and that synthesized during the motility phase (Burness *et al*., 2004). In most externally fertilizing fish species, ATP synthesis is unable to sustain the high rates of ATP hydrolysis which is required during motility and as a result ATP levels rapidly decline (Yeates *et al*., 2007). This decline in ATP is paralleled by decline in flagella beat frequency and sperm swimming speed (Christen *et al*., 1987; Perchec *et al*., 1998). There is considerable variation among species of fish in the content of ATP used to support motility of sperms (Mansour *et al*., 2003; Casselman, 2006). In this study, sperm's ATP content of golden grey mullet was determined in different condition, time and temperature.

## MATERIALS AND METHODS

In this study, in 30th Oct 2005 some golden grey mullet were caught from Caspian sea with water temperature 18-20°C and salinity 12-13 ppm. The fishes were caught for repeating in storm and reduced weather temperature (in 6th Nov 2005) which water temperature was 10-12°C and salinity 12-13 ppm. Ten males (didn't spawning) were selected and made ready sample of them. They were approximately 25-30 cm length and

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900-1000 g weight. After sampling, sperms were immediately transferred to laboratory in a closed test tube, in ice box. In order to determine ATP content of stored sperms in room temperature and 4°C for 6 h, amount of sperm sample of each fish were sampling in 10-12°C, stored in 4°C and room temperature. Also, for determination of ATP content of stored sperms at cold in different extenders (glycerol 10% and glucose 0.3 M, 0.7 and 0.65% salt solution) after 5 and 10 days, 1 volume of sperm with 3 volume of extender were slowly mixed. Sperm samples mixing with glycerol and 0.65% salt solution were stored at -15°C and samples mixing with 0.7% salt solution stored at 1°C. In order to determination of ATP content of fresh sampled sperms (in 18-20°C and 10-12°C sea water) and the kept one (in 4°C and room temperature), firstly sperms were diluted 1:100 in HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, Molecular Formula: $C_8H_{18}N_2O_4S$) buffer solution (25 mM HEPES, 10 mM magnesium chloride, 2 mM EDTA, pH = 7.7). Then for membrane destruction and ATP exiting (Billard *et al*., 1999), micro vials were boiled in water bath at 100°C for 1 min. After mixing 50 μL of kit (Biaffin Co, GmbH, Germany) with 50 μL micro vial contain in special plate of luminescence measurement, mentioned signals measured by Luminometer in room temperature after 10 min. Taking time more than 30 caused to fall signal and standard curve invalidation. In later stage, standard curve drew on luminescence signals and ATP concentration and at the end ATP content of each fish sperm sample expressed as nmol ATP/108 sperm. In order to determination of ATP content of keep sperms in cold and different extenders after 5 and 10 days, firstly, samples which were frozen in -15°C (samples keep in glycerol and 0.65% salt solution) exited from freezer and thawed in 37°C for 30 sec (Long *et al*., 1993). The stages and processes in this experiment were repeated again on the kept samples which were in cold and different extenders, after 5 and 10 days (Yeates *et al*., 2007).

## RESULTS

In order to express the ATP content according to cell number, the number of sperms in1 ml milt sampled from 10 golden grey mullet, counted by Neobar lam and microscope (Table 1). The Result of the mean of counting the number of mentioned sperms was $239\times10^8\pm15\times10^8$. IN this study in order to determine the amount of effect of sampling temperature on ATP content of fish sperm, sampling was done in 2 temperature 10-12 and 18-20°C (Fig. 1). ATP content of the sampled sperms in mentioned temperatures measured by bioluminescence sensitive procedure. The Results of this determination showed that, ATP content of sperms, collecting at 10-12°C was 74.04±7.22%, in comparison to 18-20°C. The temperature in which the sperms were kept had an effect on ATP content of the mentioned cells. Finding the suitable temperature in keeping the sperms, they were kept in 4°C and room temperature for 6 h and then the amount of ATP content of them was measured. Result of this determination showed that ATP content of sperms during 6 h keeping at 4°C and room temperature were 90.26±0.91 and 17.17±1.49%, respectively. Usually for keeping the sperm extenders is used (Fig. 2). The extenders cause to

Table 1: Results related to number of the sperms exiting in 1cc milt

| Male | No. of sperms×108 |
|---|---|
| A | 220 |
| B | 258 |
| C | 245 |
| D | 200 |
| E | 290 |
| F | 275 |
| G | 315 |
| H | 235 |
| I | 180 |
| J | 170 |

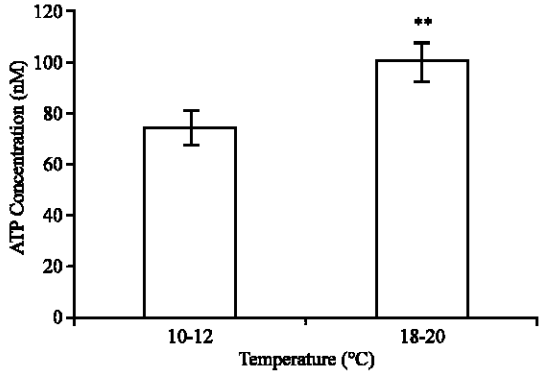


Fig. 1: Comparison of the effect of temperature on ATP content in sperm of golden grey mullet **Significant difference ($p<0.01$)

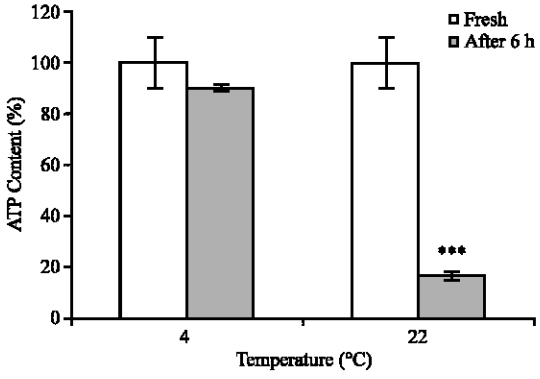


Fig. 2: Effect of temperature on keeping sperm of golden grey mullet for 6 h. ***Significant difference ($p<0.001$)

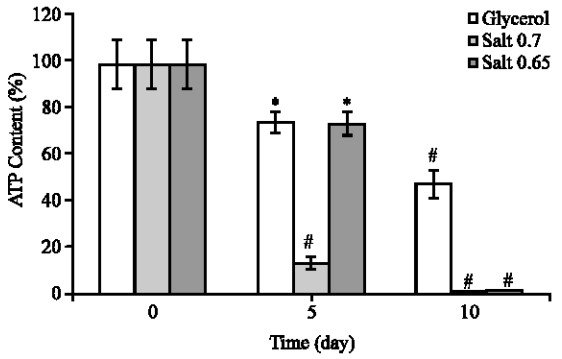


Fig. 3: TP's percentage of sperms in different days in glycerol, 0.65 and 0.7% salt solutions. *Significant difference from zero day ($p<0.05$) #Significant difference from zero day ($p<0.001$)

duration and protection of sperms activity in the keeping period. For determining the best extender, glycerol 10% and glucose 0.3 M, 0.7 and 0/65% salt solution were investigated. ATP content of sperms in mentioned solutions were measured after which sperm was kept in 1 and -15°C for 5 and 10 days. Result of this measurement revealed that glycerol or 0.65% salt solution is better extender than 0.7% salt solution. Trend of ATP content changes in different extenders during 10 days showed that glycerol was better than salt solutions for sperm ATP survival (Fig. 3).

## DISCUSSION

More researches in related to fish sperm have done in world which any one view pointed special side. A lot of research have done all around the world in related to fish sperm but each of them survey the subject from their own point of view (and look at it from the special angle) (Casselman, 2006). However, there is limited information in the case of the sperm ATP content of golden grey mullet and effects of environmental, chemical and biochemical factors on it. Up to now, there is no investigation in related to the sperm ATP content of golden grey mullet (until hasn't done), or (no investigation had been done yet on…) golden grey mullet is one of important species of fish fishery which until now (to be unsuccessful) reproduction and nourishment of it remains unsuccessful. Maybe can use from this way for induced the favorite conditions in order to reproduction and nourishment this fish species and or in special conditions for sperm keeping. May be this experiment and its findings could smooth the path to induce the favorite conditions in order to reproduction and nourishment this fish species. It might be useful for finding the best condition in which the sperm must be keeping. Perchec *et al.* (1998) showed in the absence of urine contamination, spermatozoa had ATP content in the range of 8-9 nmol/108 sperm. In contrast, when milt was contaminated with urine, the ATP content was 4-5 nmol/108 sperm. Indeed the low osmolality of urine by an early activation cause to early degradation in sperm ATP content and decrease in the quality of carp spermatozoa. So, in this study for prevention of sperm quality decreasing, during of sampling, sperm contamination by urine or any other pollutant be prevented. Burness *et al.* (2004), for determination of ATP content, add the luciferin-luciferase (dissolved in 100 mM glycine, 20 mM Mgso4, pH 7.4) to sample and measured bioluminescence signals by using a 96-well Lmax Luminometer (Molecular Devices, Sunnyvale, CA, USA). The ATP content of each sample calculated by using ATP standards and expressed as pmol/108 sperm. De Baulny *et al.* (1999) measured the ATP content by using bioluminescence (ATP bioluminescence assay, kit cls; Boehringer-Mannheim) after extraction with a trichloroacetic acid 2%, EDTA 2mM medium and expressed as nmol/108 sperm. Perchec *et al.* (1998) measured the ATP content by bioluminescence. First the sperm lyses of common carp by 1/100 dilution into boiling HEPES buffer and then with addition luciferin-luciferase to sperm, luminescence signals measured using a Biocounter M2010A Lumac/3M. Then the ATP content calculated using ATP standards and expressed as nmol/108 sperm (Kim *et al.*, 2009). In this study for measurement the ATP content used bioluminescence procedure. First the sperm sample mixed 1/100 into boiling HEPES buffer then 50 µL of it with 50 µL of bioluminescence sensitive kit (Biaffin Co, GmbH, Germany) mixed into special plate of luminescence measurement and luminescence signals measured using Luminometer (Lumistar, BMG Labtech GmbH, 5|10-19 mol/well, Germany). Then the ATP content of each sample calculated using ATP standards and expressed as nmol/108 sperm. Results due to this study defined the appendix objects.

The ATP content of sperms fresh sampled in 10-12°C, was 74.04±7.22%, ATP content of sperms fresh sampled in 18-20°C (sperm sampled in 18-20°C, 17.04±1.09 nmol/108 sperm, N=10, sperm sampled in 10-12°C, 12.21±0.9 nmol/108 sperm, N = 10, $p>0.05$).

The most sperm motility and its ability for insemination of egg is seen after sampling. Sometimes in case of artificial insemination the sperm keeping is necessary. Period of sperm motility is also affected by the keeping temperature so that according to reports of Slominska (Slominska and Jezierska, 2000), short term (15 min) keeping of sperm at 20°C shortened their motility

time and they also observed that the sperm motility of common carp reduced by about 50% after 2 h keeping in 20°C. The results of research of these 2 coworkers indicated the possibility of milt storage in refrigerator (5°C) up to 24 h for common carp and 8 hours for grass carp and *S. salar*, without considerable reduction of sperm quality (Yeates *et al*., 2007). The number of sperm (*Sander vitreus*) used in the experimental trials was kept constant, variation in sperm swimming speed (at 10 s after activation) explained approximately 90% of the variation in a male's fertilization success. These findings demonstrate that the variation in sperm quality found in wild spawning populations has the potential to dramatically influence male reproductive success (Casselman *et al*., 2006).

The obtained results from this study indicated that ATP content of stored sperm in 4°C for 6 h was 90.26±0.91% ATP content of fresh sampled sperm in 10-12°C, that this difference was not significant (n = 10, p>0.05). The ATP content of sperm keep in laboratory for 6 h was 17.17±1.49% the ATP content of fresh sampled sperm in 10-12°C. Mentioned difference was significant (n = 10, p<0.001).

The ATP content of the kept sperms in glycerol at -15°C after 5 days, the ATP content of the kept sperms in salt solution 0.7% at 1°C after 5 days and The ATP content of the kept sperms in salt solution 0.65% at -15°C after 5 days respectively was 74.19±4.5, 13.65±2.81, 73.58±6.68% the ATP content of fresh sampled sperms in 10-12°C, that this difference was significant (n = 10, p<0.001), glycerol and salt solution 0.65% as compared with salt solution 0.7%, preserve the upper percent of ATP.

The ATP content of the kept sperms in glycerol at -15°C after 10 days, ATP content of the kept sperms in salt solution 0.7% at 1°C after 10 days and ATP content of the kept sperms in salt solution 0.65% at -15°C after 10 days respectively was 47.67±6.2, 0.69±0.06 and 1.05±0.12% ATP content of fresh sampled sperm in 10-12°C, that this difference was significant (n = 10, p<0.001) and glycerol as compared with salt solution 0.7 and 0.65% which preserve the upper percent of ATP.

Keeping the sperm of European catfish Silurus glanis by cryo preservation with different cryo protect ants ($Me_2SO$, DMA, glycerol, methanol and propylene glycol) indicated that the best protection was given by DMA (10,15%), $Me_2SO$ (15%). Calvi (Calvi and Maisse, 1998) in case of trout *Oncorhynchus mykiss* reported that ATP level and sperm motility of this fish considerably decreased after thawing. But De Baulny *et al*. (1999) reported that European catfish sperm behave very differently without decrease of ATP level (Stoltz and Neff, 2006). European catfish frozen sperm with DMA result in a considerable increase of ATP level of sperm. Because DMA causing to direct stimulation of ATP synthesis that this increase of the ATP level during the process is probably a decisive factor in the freezing resistance of European catfish sperm in the presence of DMA. But obtained results from experiments of this study showed that the best extender is glycerol 10% and glucose 0.3 M.

The ATP content of the kept sperms in glycerol in -15°C after 5 days and ATP content of the kept sperms in glycerol in -15°C after 10 days respectively was 74.19±4.5 and 47.67±6.2% ATP content of fresh sampled sperms in 10-12°C, that this difference was significant (n = 10, p<0.001).

The ATP content of the kept sperms in salt solution 0.7% in 1°C after 5 days and the ATP content of the kept sperms in salt solution 0.7% in 1°C after 10 days respectively was 13.65±2.81 and 0.69±0.06% the ATP content of fresh sampled sperms in 10-12°C, that this difference was significant (n = 10, p<0.001).

The ATP content of the kept sperms in salt solution 0.65% in -15°C after 5 days and the ATP content of the kept sperms in salt solution 0.65% in -15°C after 10 days, respectively was 73.58±6.68 and 1.05±0.12% the ATP content of fresh sampled sperms in 10-12°C, that this difference was significant (n = 10, p<0.001).

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