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Temperature response to sperm motility of Russian sturgeon semen during the post-activation period

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Özet

Sıcaklığın Karaca Mersini Spermlerinin Sulandırmadan Sonraki Hareketliliğine Etkisi

Bu çalışmada karaca mersininin (*Acipenser gueldenstaedtii* Brandt, 1883) sperm kalitesi ve sulandırmadan sonra sıcaklığın sperm hareketliliği ve yaşama süresine etkisi çalışılmıştır. Spermler, 0.5 µg/kg sulfagon enjeksiyonu yapılan dört balıktan alınmıştır. Sperm kalitesi belirlendikten sonra, tatlı su ile sulandırılarak farklı sıcaklıklarda (4-24°C) muhafaza edilmiştir. Hareketliliğin, karaca mersin balığının yumurta inkübasyonu sıcaklığına en yakın olan 14°C'de 28. dakikaya kadar devam ettiği ve canlılığın %5 olduğu görülmüştür. Sperm canlılığının 14°C'de 8. dakikaya kadar %50'den fazla olduğu belirlenmiştir. Bu nedenle karaca mersini spermi sulandırıldıktan sonra 8. dakikaya kadar döllemede kullanılabilir.

Anahtar Kelimeler: Sperm hareketliliği, Karaca mersini, sıcaklık, üreme

Abstract

In this study, sperm quality of Russian sturgeon (*Acipenser gueldenstaedtii* Brandt, 1883) and effect of temperature on sperm motility and viability after diluting with fresh water studied. The sperm were collected from four males after 0.5 µg/kg surfagone injection. After sperm quality determined, diluted with fresh water and kept various temperatures from 4 to 24C°. The spermatozoa motility and viability were examined five minutes intervals until motionless stage. The motility and viability of Russian sturgeon spermatozoa decreases when the temperature is increased from 4 to 24°C. Motility was seen to in progress at the 14°C which is close to incubation degree of Russian sturgeon eggs until 28th minute and viability recorded as 5% percent at that time. The viability of spermatozoa was determined more than 50 percent at the 14°C, therefore Russian sturgeon sperm can be use fertilization until 8 min after activated with fresh water.

***Keywords*:** Sperm motility, Russian sturgeon, temperature, reproduction

This study was presented in the International Scientific Conferance on Innovation Tecnologies in Aquqculture, 21-22 September 2009, Rostov on Don, Russia

Introduction

The origin of culture of sturgeons in Russia began in 1869 and research program on developmental biology of sturgeons in the former USSR were motivated by the need to establish hatcheries for stocking. This need had arisen during 19301940 due to construction of river dams artificial reproduction of sturgeon species was initiated by means of hormone injection by Russian scientist after 1950s (Chebanov and Billard, 2001; Dettlaff and Goncharov, 2002). In spite of commercial culture of sturgeons was initiated in early 1970s (Kozlov, 1993; Chebanov *et al*., 1998), sturgeon culture studies in Turkey, have launched in 2001 (Çelikkale *et al*. 2002; Memiş *et al*., 2006) nearly 30 years later than the other neighbour countries.

The fish farming industry has been more focused on the quality of eggs or larvae rather than that of sperm, even though the quality of both gametes may affect fertilization success and larval survival. Artificial reproduction in hatcheries as in the rocess of broodstock

selection, as well as in the management of cryo-sperm banks requires adequate, rapid and sensitive tools to assess sperm quality during the different steps of artificial insemination (Rurangwa *et al*., 2004).

In case of preservation of sturgeon sperm at room or +4°C, semen undiluted and fertilizing capacity lengths of 5-6 days (Dettlaff et al., 1993). In order to prolong the survival length of sperm, milt was usually diluted in extenders of various compositions. Supply of oxygen and pH were critical factors in the short term storage of sperm (Billard *et al*., 2004).

Poor sperm quality can be a limiting factor in their culture; however, even when fertilization success is high, differences in sperm quality between males when mixed sperm from multiple males is used may severely reduce the apparent population size and may affect the future genetic integrity of the stock.

Spermatozoa of are stored in seminal plasma fluid in the genital tract and in contrast with mammals, most externally fertilizing teleosts have sperm that are immotile on ejaculation. Spermatozoa only become motile and metabolically active after release into the water.

The gametes of chondrostean fish such as the sturgeon and paddlefish differ from the general teleost model. Sperm with an acrosome and eggs with numerous micropyles coexist in sturgeons (Linhart and Kudo, 1997; Linhart *et al*., 1995).

Sperm motility is of major importance as a measure of quality, especially in commercial fish production, it is necessary to evaluate sperm quality in order to increase the efficiency of artificial fertilization (Rurangwa *et al*., 2004). Sperm is immediately activated when transferred into swimming medium.

Less information is available on the effects of temperature on motility duration of sturgeon spermatozoa and these data are highly variable in the literature. Motility in semen is mainly controlled by KCI in salmonids, and probably also in sturgeons, and by osmotic pressure in other freshwater and seawater fish species, but other factors, such as concentration of surrounding metabolites and ions (Ca_2C, Mg_2C, etc.), pH and temperature also influence motility characteristics (Alavi and Cosson, 2005). In this research, effects of temperature on motility of Russian sturgeon (*Acipenser gueldenstaedtii* Brandt, 1883) spermatozoa were studied and presented.

Materials and Methods

In this study, nine years old four males from the broodstock, which have kept in fresh water ponds in the facilities of SUMAE (Central Fisheries Research Institute). Fish were feed with row fish under the natural photoperiod regime of facility. Fish were not fed 30 days before semen collection. Sturgeon weights were 10.5±0.96 kg and 0.5 µg/kg surfagone injected to each male into the muscle near the 3th dorsal plate as single dose on 02 June 2009. After hormone ejections, sperm collected from each male at 28th hours.

To avoid any sperm contamination by urine, the urinary bladder emptied by gently squeezing the fish belly. The genital region cleaned and dried and then sperm carefully sucked into a syringe applying abdominal pressure and stored at 4°C until use. The fresh sperm were placed in twelve 50 ml containers in which three containers for one fish in the ice box were transferred to the SUMAE lab.

In the laboratory, the motility of the each batch semen was assessed immediately under a microscopy (magnification 40x10; Nikon E 400), by putting on the lam 1 µl sperm and 50 µl fresh water. The pH of sperm solution was measured by pH meter (Mettler-Toledo AG, Analytical, Switzerland). After semen qualities determination, all batch of semen from fish were pooled. Further studies were conduct with pooled sperm.

During the entire experiment, the sperm motility was evaluated by using five categories, as traditional (1-5) and new (+) as described by Liu *et al.,* (2006) scoring methods. The viability of sperm described as percent (%). The new scoring method described by Liu *et al.,* (2006) follows below.

1- Drastic and extreme rapid movement (++++): the path of sperm motion was so fast that it was impossible to clearly follow individual sperm. Estimation was made as percent (%).
2- Fast movement (+++): the speed of moving sperm is very fast.
3- Slow movement (++): the speed of moving sperm is very slow.
4- Vibration (+): sperm does not move forward but its tail shows right and left vibrations.
5- Motionless (-): most sperm do not move and shows no movement.

In this study, four trails were carried at the same time by four people. After sperm quality determined, sperm were diluted 1:50 ratio with fresh water with pre-set temperature and kept various temperature as 4°C (fridge), 9°C (incubator, sanyo, Japan), 14°C (incubator, sanyo, Japan),, 19°C (incubator, Japan), and 24°C (water bath) due to maintain stable of their temperature. After dilution, motility and viability of spermatozoa were examined within first at 3 minute and following 5 minute intervals until motionless stage which most sperm do not move and shows no movement.

Results

Motility and viability ratio of sperm activated with fresh water at the different temperature were given in table 1 and figure 1 respectively. It was seen that the motility and viability ratio of sperm decreased depending on increasing temperature. Motility was seen to in progress at the 14°C which is close to incubation degree of sturgeon eggs until 28th minute and viability ratio recorded as 1-5% at that time. Sperm were kept at +4°C maintained their viability well into the 40-50th minute. After 28th minute, motility slowed and forward movements were not observed between 33 and 43 minute, but shake of tail.

Table 1. Motility of sperm after activated with fresh water at different temperatures

Time after activation (min.)	4°C	9°C	14°C	19°C	24°C
0	+++	++++	++++	++++	++++
3	+++	++++	+++	+++	+++
8	+++	++	++	++	++
13	+++	+++	++	++	++
18	+++	++	++	++	+
23	++	++	++	+	-
28	++	+	+	-	
33	+	-	-		
38	+				
43	+				

Discussion

Sturgeon can be propagated when water temperature ranges from 10 to 20 °C (Mims *et al*., 2002) and Kozlov (1993) reported that the spawning temperature of Russian sturgeon is 11-23 °C.

Ovulation and spermiation are stimulated by injecting either fresh or dried common carp (CCP) (Mims *et al*., 2002; Urbányi *et al*., 2004), sturgeon (SP) pituitaries (Lahnsteiner *et al*., 2004), a synthetic Luteinizing Hormone Releasing Hormone analogue, LHRH-a (Mirzoyan *et al*., 2006), natural salmon GnRH (Glogowski *et al*., 2002),Gonadotrophin Releasing Hormone Analo-

gues (GnRHa) (Williot *et al*., 2002) and surfagon (Emel'yanova *et al*., 2006; Petrushina, 2007).

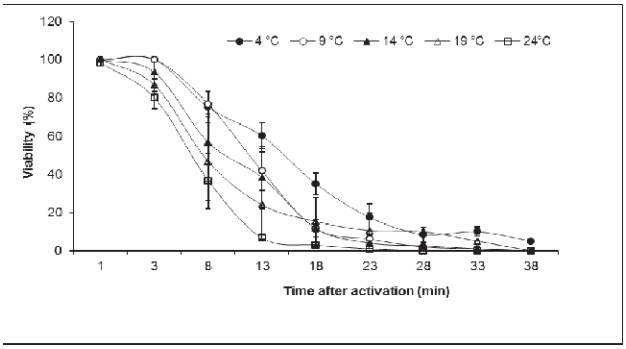

Figure 1. The viability of sperm (%) after activated with fresh water at different temperature, (Mean±SE).

Emel'yanova *et al*., (2006) reported that 2-20µg/kg surfagon may be used for ovulation of oocytes in Zebrasoma scopas (Acanthuridae) and Petrushina, (2007) recomended that Surfagon may be injected singly or fractionally in the dose of 1-2 µg/kg in traditional terms at optimal water temperatures for sturgeon. In this study, spermiation was stimulated 0.5 µg/kg surfagon at 15°C by single injection within intramusular near the 3th dorsal placa.

Sperm quality in farmed fish may be affected by different components of broodstock husbandry, during collection and storage of sperm prior to fertilization or the fertilization procedure. Although other approaches for quantification of sperm quality have been suggested, motility is most commonly used since high motility is a prerequisite for fertilization and correlates strongly with fertilization success (Rurangwa *et al*., 2004; Bozkurt *et al*., 2006a).

Spermatozoa motility varies according to the type of fish species. For example, spermatozoa motility of *salmo trutta fario* was 81% at 97.4 s (Bozkurt *et al*., 2006a), for *Cyprinus carpio* as 79% at 684 s (Bozkurt *et al*., 2006b) and of *Onchorynchus mykiss* was 81% at 38 s (Canyurt and Akhan, 2008) reported recently.

Spermatozoa of sturgeons and paddlefish are essentially immotile in the seminal plasma (Linhart *et al*. 1995; Cosson and Linhart, 1996; Cosson *et al*. 2000; Linhart *et al*. 2003; Alavi *et al*. 2004). Mims (1991) reported that the motility duration of the spermatozoa of paddlefish, (*Polyodon spathula*), can be up to 4.4 min, while Linhart *et al*. (1995) have reported that only 1-5% of spermatozoa are motile at 6 min. Williot *et al*. (2000) found that the motility of Siberian sturgeon spermatozoa decreases when the temperature is increased from 10 to 17.5°C. In this study, spermatozoa were motile at 43 minute at 4°C and in 13 min at the 24°C (Table 1).

Mims *et al.* (2002) recommended that if the motile of sperm is 75 to 100 percent, the milt can be used for fertilization in sturgeon. If less than 75 percent of the sperm are motile, use other males. In this study, more then 75 percent of the sperm was determined as motile at 3 min at 14°C. Considering recommendation of Mims *et al.* (2002) on Russian sturgeon sperm can be use fertilization until 8 min at 14°C after activated with fresh water.

Present study showed spermatozoa of Russian sturgeon were motile until 28 min and viability was 5% in the 23 min at 14 °C.

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