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Spermatological parameters of wild and cultured beluga (*Huso huso* Linnaeus, 1758) and their effects on fertilization rate, hatching rate, larval growth and survival rates

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ABSTRACT

To compare the reproductive performance of the wild and cultured stocks of beluga, *Huso huso* males, some spermatological parameters were measured including: sperm motility, sperm density, spermatocrit and also fertilization rate, hatching rate and larval growth and survival rates. The semen samples were sampled from 11 wild spawners and 12 cultured brooders. A half of each semen sample was allocated for spermatological analysis and remaining were used for artificial propagation. In wild beluga, sperm motility time was 339.09 ± 23.11 s; spermatozoa motility rate 83.64 ± 2.01%; sperm density 15.22 ± 3.46 $\times 10^9$ and spermatocrit 5.88 ± 1.36% while in cultured beluga, sperm motility time was 199.35 ± 29.02 s; spermatozoa motility rate 74.22 ± 2.4 %; sperm density 8.37 ± 0.24 $\times 10^9$ and spermatocrit 3.21 ± 0.29 %. The fertilization rate (%), hatching rate (%), larval growth (% g.day^{-1}), and survival rate (%) in wild beluga were 61.72 ± 14.16, 43.33 ± 11.13, 12.28 ± 0.33 and 52.66 ± 7.77, while in cultured beluga were 49.98 ± 6.55, 31.47 ± 8.49, 12.28 ± 0.62 and 57.37 ± 7.89, respectively. The spermatozoa motility (the ratio and duration of motility) were significantly different between wild and cultured brooders ($P<0.05$). The values of sperm density, spermatocrit, fertilization rate, hatching rate, larval growth and survival rate were not significantly different between wild and cultured beluga ($P> 0.05$).

Key words: Sperm density, Sperm motility, Growth rate, Survival rate, Beluga, *Huso huso*

INTRODUCTION

The great sturgeon (or beluga), *Huso huso* Linnaeus, 1758 is the most valuable and unique sturgeon fish in the Caspian Sea due to the desirability of its meat and caviar throughout the world. During the last decades, the wild stocks of beluga have declined dramatically due to various reasons such as overfishing, water pollution and construction of dams and destruction of rivers (Kiabi *et al.* 1999). Thus, this sturgeon has been considered for biological conservation and also restocking program by means of artificial propagation in the southern basin of the Caspian Sea (Kiabi *et al.* 1999). Unfortunately, the efficiency of artificial propagation of wild stocks of beluga is adversely inconstant depending on brooder and gamete availability. To eliminate this problem, the production of cultured brooders of beluga in the hatchery conditions has been considered as a strategy to overcome the shortage of needed gametes for artificial breeding. On the other hand, the very long period is required to occur first sexual maturation in sturgeons especially beluga that makes their hatchery production very expensive. Furthermore, hatchery conditions are apparently different from wild environment and may affect the process of gonadal maturation and quality of gametes. For example, under culture condition, adult fishes are exposed to various types of stressors which may affect gonad development and gamete quality (Donaldson 1981; Barton & Iwama 1991; Pankhurst & Van Der Kraak 1997; Wendelaar Bonga 1997; Iwama *et al.* 2005; Milla *et al.* 2009). Campbell *et al.* (1994) reported that chronic confinement of hatchery ponds not only

disrupt the reproductive endocrinology of trout, but also result in reduced egg size and larval survival rate in rainbow trout, *Oncorhynchus mykiss* (Walbaum). In captive condition, the fertilization rate, gamete quality and spawning behavior of spotted grouper, *Epinephelus akaara* are influenced adversely (Okumura *et al.* 2002).

Due to these reasons, the efficiency of hatchery production of brooders of beluga should be investigated. Therefore, in the present study, we investigated the reproductive performance of the wild and cultured males of beluga, by means of spermatological parameters and also fertilization, hatching, larval growth and survival rates.

MATERIALS AND METHODS

This study was carried out in Shahid Marjani Artificial Sturgeon Propagation and Rearing Center, Gorgan, Iran. Two groups of beluga males were considered for the experiment: 1) cultured fish (n = 12, total weight (TW) = 23.00 ± 3.43 kg and fork length (FL) 151 ± 8.88 cm). These brooders were reared under captivity conditions in the hatchery for a period of 4 years. 2) The wild fish (n = 11, TW = 72 ± 7.98 Kg; FL = 193 ± 9.73 cm). These brooders had been captured in the southeastern part of the Caspian Sea during their upstream reproductive migration, and then transferred to the hatchery. To induce ovulation in females, two dosages of pituitary extract including first dosage (20 mg dry pituitary/2 ml 0.7 % NaCl) followed by the final dosage (180-200 mg dry pituitary/3 ml 0.7 % NaCl) 12h later were injected intramuscularly. Also, to induce spermiation in males, 1 vial LHRH-A2 was injected coincident with final injection of females. After hormonal treatment, the brooders were checked out every 4 h one time to detect the final maturation. When the ovulation and spermiation occurred, the sperm quality of males was examined. To induce sperm motility, the semen samples was diluted in ratio of 1 (semen) : 2000 (freshwater) followed by evaluating sperm motility rate (%) immediately and sperm motility time after activation (%) under dark-field microscope coupled with a CCD video camera (SONY DXC-970MD, Japan) (Cosson *et al.* 2000). The video records were investigated by Adobe Premiere software and percentage of sperm motility was calculated in comparison with 12 captured pictures from 1s motility video. The duration of sperm motility was calculated after the onset of motility until all cells become immotile (Cosson *et al.* 2000).

Only forward-moving spermatozoa were classified as motile, while sperm cells simply vibrating or turning on their axes were considered as immotile (Aas *et al.* 1991). The sperm density was measured by haemocytometer counting chamber according to Hajirezaee *et al.* (2010a). So that, milt was diluted 1000 times in a 0.7% NaCl solution. Then, a droplet of the diluted milt was placed on a haemocytometer slide (depth 0.1 mm) with a coverslip. After 10 min (a time to allows sperm sedimentation), the spermatozoa were counted in 16 individual cells of haemocytometer under a light microscopy (Nikon, Japan). At the end, the number of spermatozoa was calculated as follows:

Sperm density ($/ml^{-1}$) = 1000 × number of counted sperm / [area (mm^2) × chamber depth (mm) × dilution ratio.

Spermatocrit was determined by centrifuging semen for 10 min at 5000 rpm in a haematocrit centrifuge (D-78532, Tuttlingen Zentrifugen, Germany) according to Piironen (1985).

After egg collection from 3 wild females, the coelomic fluid was separated, the eggs were pooled and weighted and then divided into 23 trays (each tray containing 100 g pooled egg; 11 trays for wild fish and 12 trays for cultured ones).

For fertilization, the diluted semen with freshwater in a ratio of 1 ml (semen): 200 ml (freshwater) was added to each tray and then mixed for 4-5 min.

After fertilization, to eliminate the egg adhesion, the fertilized eggs were washed by 10% clay suspension for 40 min.

After washing, the eggs fertilized by sperm obtained from wild and cultured males, were incubated separately in Yushchenko

incubators. 1 % Permanganate potassium solution was used against fungal contamination during incubation period of eggs. The fertilization rate (%) was calculated in the stage 4 blastomeres as follows:

Fertilization rate (%) = the number of fertilized eggs/total number of eggs × 100

After about 6 days, the fertilized eggs were hatched. The hatching rate was calculated as follow:

Hatching rate (%) = the number of hatched eggs/total number of fertilized eggs × 100

The specific growth rate (SGR) and survival rate (SR) of alevins until active feeding and thereafter were calculated as follow:

SGR (% day^{-1}) = 100 × (ln W_2 - ln W_1)/days, where W_1 = Mean of primary weight, W_2 = Mean of final weight; SR (%) = (total number of alive alevins / total number of alevins) × 100

Statistical analysis

The SPSS software was used for data analysis. Because the data [SR (%), SGR (%), fertilization rate (%), hatching rate (%), spermatocrit (%) and sperm motility rate (%)] did not have a normal distribution, proportional data were converted by angular transformation (arcsin √p).

The independent sample T-test was employed to compare the means between cultured and wild fish.

RESULTS

According to T-test analysis, there was significant differences between wild and cultured males of beluga in terms of the rate (%) and time of sperm motility (Fig. 1, P<0.05). The motility rate (%) and sperm motility time of wild spermatozoa were significantly higher than spermatozoa taken from cultured beluga (P<0.05). The values of sperm density, spermatocrit (%) (Fig. 2, P> 0.05), fertilization rate (%), hatching rate (%) (Fig. 3, P> 0.05), SGR (%day^{-1}) and survival rate (%) (P> 0.05, Table 1) were similar in wild and cultured males.

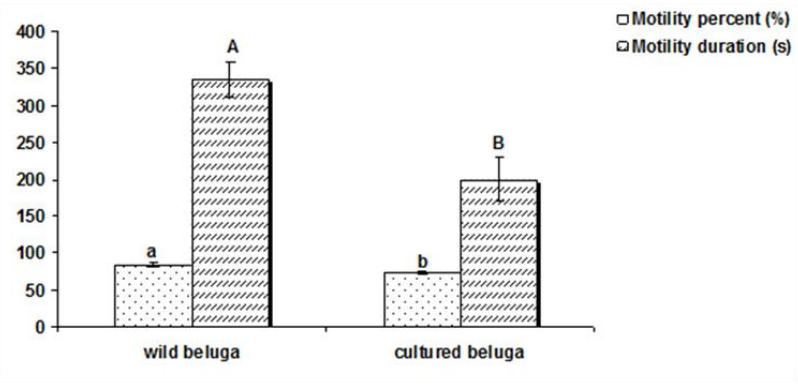

Fig 1. Comparison of rate (%) and time of sperm motility between the sperm obtained from wild and cultured male beluga. Means with same superscripts are not significantly different (P > 0.05).

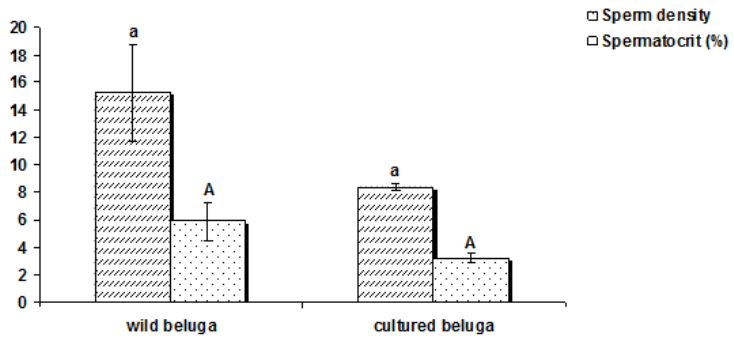

Fig 2. Comparison of sperm density and spermatocrit between the sperm obtained from wild and cultured male beluga, *Huso huso*. Means with same superscripts are not significantly different (P > 0.05).

Table 1. Comparison of larval growth and survival rates between the larvae obtained from the eggs fertilized with the sperm originating from wild and cultured male beluga, *Huso huso*. Means with same superscripts were not significantly different (P > 0.05).

parameters	Wild males of beluga	Cultured males of beluga
Survival rate $_1$ (%)	63.79 ± 8.53^a	63.58 ± 8.27^a
Survival rate $_2$	80.04 ± 3.59^a	76.87 ± 3.22^a
Survival rate $_T$	52.66 ± 7.77^a	57.37 ± 7.89^a
Specific growth rate $_1$	12.63 ± 0.45^a	12.78 ± 0.18^a
Specific growth rate $_2$	11.40 ± 0.86^a	13.03 ± 1.91^a
Specific growth rate $_T$	12.28 ± 0.33^a	12.28 ± 0.62^a

* Survival rate $_1$: survival rate of hatched alevins until active feeding; Survival rate $_2$: survival rate of hatched alevins after active feeding; Survival rate $_T$: survival rate of hatched alevins over the course of the experiment; Specific growth rate $_1$: Specific growth rate of hatched alevins until active feeding; Specific growth rate $_2$: Specific growth rate of hatched alevins after active feeding; Specific growth rate $_T$: Specific growth rate of hatched alevins over the course of the experiment.

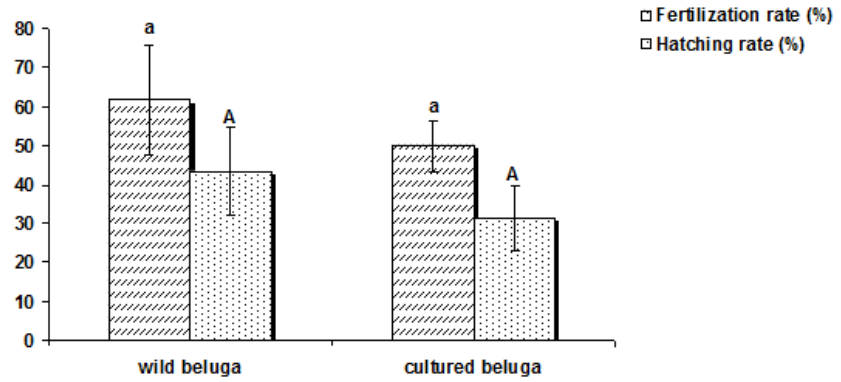

Fig 3. Comparison of fertilization and hatching rates between the sperm obtained from wild and cultured male beluga, *Huso huso*. Means with same superscripts are not significantly different (P > 0.05).

DISCUSSION

During last decades, the wild sturgeon stocks in the Caspian Sea have been faced to severe declines due to the several reasons including overfishing, water pollution, and construction of dams and destruction of rivers (Kiabi *et al.* 1999; Niksirat & Abdoli 2009). Therefore, the attempts have been focused on restocking of sturgeon species by means of artificial reproduction. Nevertheless, the output of restocking program is decreasing annually due to the decreases of wild stocks in nature. As an alternative, the productions of cultured brooders could be an appropriate way to compensate brooder shortage in wild. But, since the production of cultured brooders is very expensive and need equipment and facilities, the investigation of their reproductive performance is necessary to avoid time and cost lost. In the present study, among spermatological parameters, the rate (%) and time of sperm motility were higher in wild male beluga than in cultured individuals. In contrast, there were no differences between experimental groups in terms of fertilization rate, hatching rate, larval growth and survival rates. The sperm motility and sperm density determine the fertilization capability of spermatozoa and are often used to estimate milt quality (Suquet *et al.* 1982; Billard *et al.* 1993; Linhart *et al.* 1994; Krol *et al.* 2006; Hajirezaee *et al.* 2010b,c). Generally, many factors affect the sperm quality in fish. The factors including: age, weight and length of brooders, rearing conditions (temperature,

photoperiod, nourishment, undesirable components and animal welfare and health), artificial induction of spawning and spawning season (Hajirezaee *et al.* 2010d). The hatchery conditions are apparently different from wild environment and may affect differently the reproductive performance in the wild and cultured fishes. Therefore, the observed differences in rate and time of sperm motility between wild and cultured male beluga may be due to the differences in their living environment. For example, under cultured condition, adult fishes are exposed to various types of stressors such as confinement, crowding, handling, biopsy, transportation and hormonally-induced spawning. It is now clearly established that there is an inhibitory effect of stress on reproductive processes in teleost fishes, with effects ranging from depression of endocrine function to reduction of gamete and larval quality (Pankhurst & Van Der Kraak 1997). Morisawa *et al.* (1979) have demonstrated that the hypotonic freshwater environment establishes the hydration of testis. This may stimulate the spontaneous activation of spermatozoa in seminal fluid before stripping and make spermatozoa immotile before fertilization can occur. In sturgeons, the spermatozoa become motile when are released to environment with low osmolality such as freshwater (Alavi & Cosson 2006). In the present study, rate and time of sperm motility were higher in cultured male beluga than in wild individuals. Since the cultured male beluga have passed more time in freshwater hatchery than wild brooders, therefore the probability of testis hydration in cultured beluga is higher than in wild individuals. The testis hydration decreases the osmolality of seminal fluid and also decreases the potential of sperm motility. On the other hand, the testis hydration usually decreases spermatocrit and sperm density, while these parameters were not different between wild and cultured male beluga. This suggested likely that cultured males with employing an efficient osmoregulation, excrete the excess water of the body in response to hypotonic freshwater environment.

Our results showed that the kind of male brooder has not certain effect on reproductive performance. The larval growth, fertilization, hatching and survival rates were similar, although rate and duration of sperm motility were higher in wild beluga than in cultured individuals. There is likely a threshold level for sperm motility rate and sperm motility time so that probably the influence of these parameters on reproductive performance is evident in very low values. In conclusion, although the wild male beluga produced spermatozoa with higher motility rate, the quality of produced larvae was similar in both groups. Therefore, the production of cultured brooder beluga could be a good way to compensate the declines in wild stocks of this species in nature.

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پارامترهای اسپرم شناختی فیل ماهی (*Huso huso* Linnaeus, 1758) وحشی و پرورشی و اثرات آنها بر روی درصد لقاح، درصد تفریخ، نرخ رشد و بقای لارو

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چکیده

برای مقایسه عملکرد تولید مثلی مولدین نر فیل ماهی *Huso huso*، وحشی و پرورشی، برخی پارامترهای اسپرم شناختی شامل: تحرک اسپرم، تراکم اسپرم، اسپرماتوسیت و درصد لقاح، درصد تفریخ و نرخ رشد و بقای لارو اندازه گیری شدند. اسپرم های ۱۱ مولد وحشی و ۱۲ مولد پرورشی نمونه برداری شدند. نیمی از هر نمونه اسپرم برای تجزیه و تحلیل اسپرم شناسی اختصاص داده شد و باقی مانده برای تکثیر مصنوعی مورد استفاده قرار گرفت. در فیل ماهی وحشی، مدت زمان تحرک اسپرم ۱/۳۶ ± ۳۳۹/۰۹ ثانیه، میزان تحرک اسپرم ۲/۰۱ ± ۸۳/۶۴ درصد، تراکم اسپرم ۱۰۹ × ۳/۴۶ ± ۱۵/۲۲ و اسپرماتوکریت ۱/۳۶ ± ۵/۸۸ درصد بود، در حالیکه در فیل ماهی پرورشی، مدت زمان تحرک اسپرم ۲۹/۰۲ ± ۱۹۹/۳۵ ثانیه، میزان تحرک اسپرم ۲/۴ ± ۷۴/۲۲ درصد، تراکم اسپرم ۱۰۹ × ۰/۲۴ ± ۸/۳۷ و اسپرماتوکریت ۰/۲۹ ± ۳/۲۱ درصد بود. درصد لقاح، درصد تفریخ، نرخ رشد و بقاء لارو درفیل ماهی وحشی به ترتیب ۱۴/۱۶ ± ۶۱/۷۲ درصد، ۱۱/۱۳ ± ۴۳/۳۳ درصد، ۰/۳۳ ± ۱۲/۲۸ گرم در روز و ۷/۷۷ ± ۵۲/۶۶ درصد بودند، در حالیکه در فیل ماهی پرورشی ۶/۵۵ ± ۴۹/۹۸ درصد، ۸/۴۹ ± ۳۱/۴۷ درصد، ۰/۶۲ ± ۱۲/۲۸ گرم در روز و ۷/۸۹ ± ۵۷/۳۷ درصد بودند. تحرک اسپرم (نسبت و مدت زمان تحرک اسپرم‌ها) بین مولدین وحشی و پرورشی اختلاف ها معنی دار بودند ($P<0.05$) . مقادیر تراکم اسپرم، اسپرماتوسیت، درصد لقاح، درصد تفریخ، نرخ رشد و بقای لارو بین فیل ماهی وحشی و پرورشی تفاوت معنی‌داری نداشتند($P>0.05$).

* مولف مسئول