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Relationships between selected sperm characteristics and fertilisation success in the beluga sturgeon, *Huso huso* (Linnaeus, 1758)

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ABSTRACT: We examined the relationships between a number of sperm characteristics (motility, concentration and pH) and sperm fertility (egg fertilisation and hatching rate) in 10 mature females and four mature males of the beluga sturgeon, *Huso huso*. The fish were treated with luteinising hormone-releasing hormone agonists (LHRH-A2), at a dose of 5–10 µg/kg of body weight (BW) by injection into the muscle between the dorsal and lateral scutes. Significant linear relationships were found between sperm pH and fertilisation rate (R^2 = 0.82, P < 0.01) and sperm pH and hatching rate (R^2 = 0.75, P < 0.05). Highly negative relationships were also observed between sperm concentration and fertilisation rate (R^2 =–0.71, P < 0.05) and sperm concentration and hatching rate (R^2 = –0.83, P < 0.01). We suggest that understanding these correlations is critical in the evaluation of sperm quality and for the preparation of media (extender) for use in sperm dilution to improve sperm motility/fertility and therefore increase fertilisation success in beluga sturgeon production.

Keywords: sperm quality parameters; fertilisation; hatching; *Huso huso*

Maximising reproductive success is a key issue in aquaculture and limiting factors are the quality of male and female gametes. Gamete quality in the wild, or in captivity, is influenced by many factors and can be highly variable (Coward et al. 2002; Rurangwa et al. 2004; Alavi et al. 2008). Factors influencing the quality of sperm, and the methods for determining them, have been extensively reviewed. These factors include sperm production indices (sperm concentration); chemical and biochemical composition of the seminal plasma; sperm motility; and fertilisation rate, and these have frequently been used as indicators of sperm quality in different fish species (Alavi et al. 2012). Fertilisation success (fertility) was one of the earliest estimators used to accurately estimate egg quality and is also the most integrative estimator of sperm quality. Ultimately, the ability to fertilise or be fertilised is the key indicator of gamete quality (Bobe and Labbe 2010).

Because of their life history characteristics, including their slow growth and late age at maturity, sturgeons are particularly sensitive to even low levels of exploitation and habitat destruction. Beluga, *Huso huso*, as the largest and most long-lived species of sturgeon, is now one of the vulnerable species. It is listed in the Red book of the International Union for Conservation of Nature (IUCN); the Azov subspecies of beluga is listed in the Red book of the Russian Federation (2001) (Khodorevskaya et al. 2009). Therefore, the conservation of this species of sturgeon is a priority task. Understanding the reproduction of wild populations of sturgeon is thus essential to ensure the preservation of this ancient group of animals (Haxton 2006).

Several published studies have analysed sperm quality and its relationship to reproductive success in a variety of sturgeon species, such as paddlefish, *Polyodon spathula* (Linhart et al. 1995; Cosson and Linhart 1996); white sturgeon, *Acipenser transmontanus* (Ingermann et al. 2002); Siberian sturgeon, *A. baeri* (Gallis et al. 1991); and Persian sturgeon, *A. persicus* (Alavi et al. 2004; Aramli et al. 2013a; Dadras et al. 2014). Unfortunately, data concerning the biological aspects of sperm and their fertility in

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the beluga sturgeon are rare (Aramli et al. 2013b). The aim of this research was to determine the physiological relationships between some sperm quality parameters (sperm motility, pH and concentration) and fertility (fertilisation and hatching rate) during the spawning season in beluga sturgeon.

MATERIAL AND METHODS

Fish and sampling. Ten females (30–45 kg, 1–2 m in length) and four males (20–25 kg, ~ 1.5 m in length) beluga were captured in the south-western Caspian Sea during the artificial propagation season in spring 2011 and transported to the Rajaee Sturgeon Hatchery Centre (Sari, Mazandaran, Iran; 36°37′ N, 53°05′ E). Female and male broodstocks were injected with an analogue of the releasing hormone LHRH-A_2 (D-Ala^6 GnRH Pro^9-NEt), as described by Nazari et al. (2010) at a dose of 5–10 μg/kg of body weight (BW). The hormone was administered to each female with an initial dose of 10% of the total dose. The final dose of hormone was administered 12–14 h after the initial dose. Water temperature, oxygen content and pH were 12.5 and 14.0 °C, >5 mg/l and 7.1–7.3, respectively, during the experiments. Ovulation occurred within 18–22 h after the second injection. Simultaneously, four sexually mature males, which had been already caught and held in the tank, were injected with LHRH-A_2 for later use in propagation. The males were given one dose of hormone at the same time as the females were administered the final dose. Spermiation occurred within 18–20 h. Females were anesthetised (with 30 mg/l clove oil) and placed in lateral recumbency on a table. A finger was inserted into the gonopore to stretch the opening slightly. A scalpel (with a straight blade narrower than the gonopore) was inserted carefully into the gonopore opening, and a 1.5–3 cm incision was made through the ventral area of the oviductal (Mullerian duct) wall. The scalpel was withdrawn and the incision probed with one finger to ensure that the opening was not obstructed. The fish was inverted and slight pressure was applied to the abdominal region by two individuals: the ova flowed through the incised opening in the oviduct and out of the gonopore (Stech et al. 1999; Aramli et al. 2014). Coelomic fluid was removed by pouring the eggs onto a screen suspended over a beaker and the eggs were immediately used for propagation.

Sperm quality analysis. Tris-HCl buffer (10 mM, pH 8.0) containing 0.25% pluronic (a substance that prevents spermatozoa from sticking to slides) was used as activating medium (AM).To trigger motility; the fresh sperm were diluted in AM with at a dilution of 1 : 1000 (Osipova et al. 2014). Spermatozoa motility was recorded with a dark-filed microscopy (400×, Olympus CK2, Tokyo, Japan). The duration of motility was determined by recording the time taken from activation to the complete cessation of activity by the last spermatozoa in a field. One person conducted all of the sperm motility observations to reduce the degree of variation. Sperm density was estimated using a Burker cell haemocytometer (Meopta, Czech Republic) at 200×magnification on an Olympus BX 50 phase contrast microscope (Olympus). The pH was measured directly using a tonometer with a combination of a glass electrode and pH metre (Philips PW9145) calibrated at 15 °C (BDS Colorkey buffers).

Fertilisation and hatching rate assessment. Fertilisation was carried out with a pool of semen from two to three males at a rate of 10 ml semen/kgof ovulated eggs. The pool of semen from four males was water-diluted (1 : 200; volume of semen:volume of water) (Dettlaff et al. 1993). Fungal infection was isolated from fertilisation during the embryonic development or alternatively, malachite green was used (5 mg/l; 5 ppm solution of malachite green for 10 min) (Williot 1990). Fertilised eggs were washed with silt solution to prevent adhesion and placed in upwelling incubation units (Youshchenko, an incubator with a moving frame, which received wide recognition, developed by Youshchenko in 1953) with 400 g in each packet, receiving filtered flowing river water. The temperature of the water was 16–18 °C during the incubation period. To measure fertilisation rate, 3 h after fertilisation, 100 eggs were randomly preserved in 10% formalin as described by Kohnehshahri and Azari Takami (1974). The following formula was used to calculate the fertilisation rate:

$$\text{Fertilisation rate (\%)} = \text{number of fertilised eggs} - \text{number of total eggs} \times 100$$

To measure the hatching rate, 200 larvae were randomly sampled from each female (three replicates per fish) separately in a small plastic tank (of 30 l) that received running water (at a flow rate of 0.5 l/min). Yolk sac larvae were counted on Day 8 of incubation and hatching rate was expressed as the

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percentage of yolk sac larvae. Excreted materials were siphoned from tanks and the mortality rate for every tank was recorded each morning.

Data analysis. Results were analysed using SPSS statistical software (Version 11.5, Chicago, IL, USA). Correlations between parameters were estimated using the Pearson Correlation Test. All data were expressed as the means ± standard error (n = 3).

RESULTS

Sperm characteristics (motility, concentration and sperm pH) and sperm fertility (egg fertilisation rate and hatching rate) are shown in Table 1. The correlations between sperm characteristics and fertility are shown in Figure 1A–1D. Significant positive relationships were found between sperm pH and egg hatching rate (R^2 = 0.75, P < 0.05) and egg fertilisation rate (R^2 = 0.82, P < 0.01). In addition, highly negative relationships were found between sperm concentration and egg fertilisation rate (R^2 = –0.71, P < 0.05) and egg hatching rate (R^2 = –0.83, P < 0.01). No significant relationships were observed between the other parameters measured in the present study.

Table 1. Sperm characteristics, and egg fertilisation and hatching rate of beluga sturgeon (*Huso huso*)

Parameters	Mean	SD
Motility (s)	261	38
Concentration (spz/ml)[9]	2.4	0.74
pH	7.9	0.65
Egg fertilisation rate (%)	81	10.21
Egg hatching rate (%)	75	12.79

DISCUSSION

The fertility of spermatozoa depends on several sperm parameters, including the concentration of spermatozoa (Ginsburg 1968); the velocity of the spermatozoa (Schlenk and Kahmann 1938); the duration of sperm motility (Billard 1983); the percentage of motile sperm (Billard 1983); the motility pattern of the sperm during active movement (Tsvetkova et al.1996); and the biochemical composition of both the seminal plasma and the spermatozoa (Ciereszko et al. 2000). Our findings indicate that both sperm pH and concentration influence egg fertilisation rate in *H. huso*. Our results agree

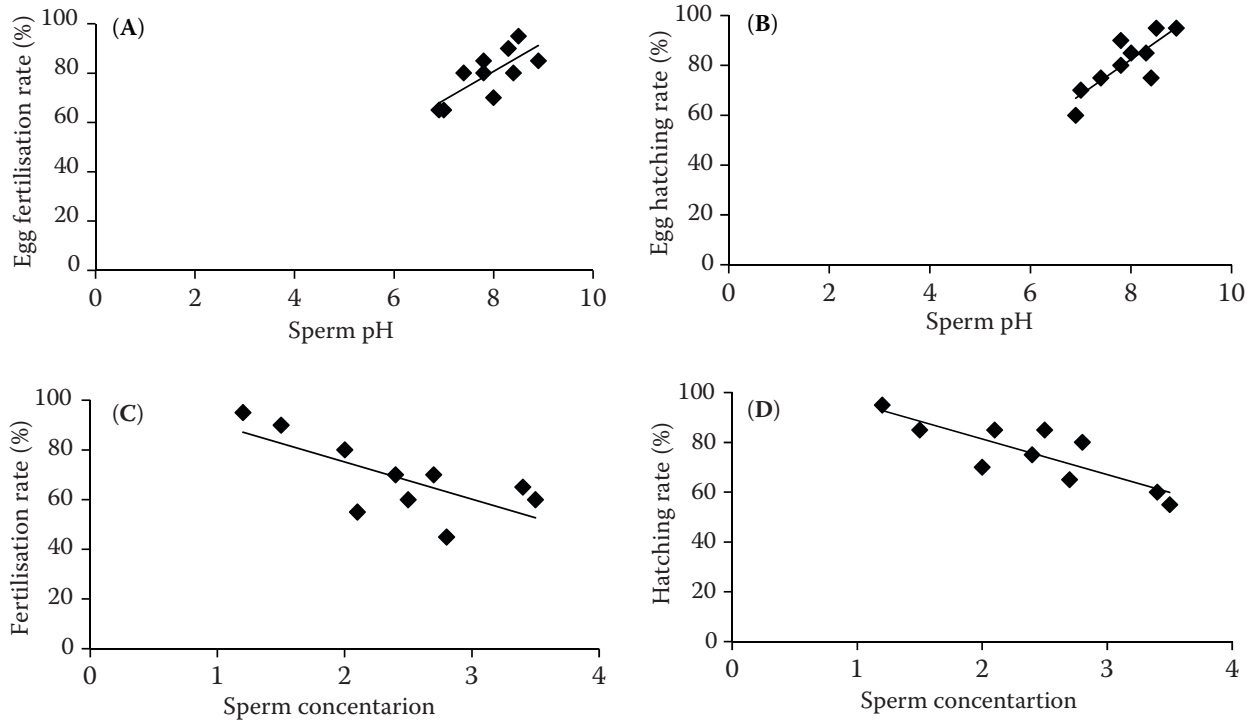

Figure 1. Significant correlations exist between (**A**) sperm pH and egg fertilisation rate (y = 11.65x–12.59, R^2 = 0.75, P < 0.05), (**B**) sperm pH and egg hatching rate (y = 14.11x–30.54, R^2 = 0.82, P < 0.01), (**C**) sperm concentration and egg fertilisation rate (y = –14.97x+105.0, R^2 = –0.71, P < 0.05), and (**D**) sperm concentration and egg hatching rate (y = –14.29x–109.9, R^2 = –0.83, P < 0.01) in beluga sturgeon (n = 10)

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with the findings of Dadras et al. (2014) on Persian sturgeon, *A. persicus*. The effects of pH on the fertility of sperm have also been described by Saad and Billard (1987) and Chao et al. (1992), who found a positive relationship between sperm pH and sperm fertility in *Cyprinus carpio* and *Epinephelus malabaricus*, respectively. In accord with our results, another study by Tuset et al. (2008) on Rainbow trout (*Oncorhynchus mykiss*), found that sperm fertility was inversely related both to the duration of sperm movement and sperm concentration. In contrast, Casselman et al. (2006) found a significant positive relationship between the number of sperm and their fertility in Walleye, *Sander vitreus.* The pH of the activation medium has been identified as a major factor affecting the motility and velocity of fish sperm (Stoss 1983; Alavi and Cosson 2005), which subsequently influences sperm fertility (Billard et al. 1995; Linhart et al. 2006). In sturgeon, the optimal pH for sperm motility has been reported as pH 8–9 (Gallis et al. 1991; Cosson and Linhart 1996; Ingermann et al. 2002; Alavi et al. 2004). In white sturgeon, *A. transmontanus*, sperm pre-incubated at pH 8.2 or higher showed higher motility following transfer into water as a swimming medium, but motility decreased or ceased when sperm were pre-incubated at pH 7.5 or lower (Ingermann et al. 2002). These results suggest that the high sensitivity of sperm motility to pH might be related to the low buffering capacity of the seminal plasma. Moreover, Ingermann et al. (2002) demonstrated that within the pH range 7.5–8.5, sperm contribute perhaps 5%–10% of the sperm buffering capacity, whereas they appear to make no contribution above a pH range of 6.0–7.0. The relatively minor contribution of sperm activation at the higher pH values seems unlikely to be physiologically significant and may simply be a consequence of the presence of sperm proteins. Low sperm buffering capacity represents a physiological adaptation allowing the epithelial cells of the reproductive tract to exert control over the degree of sperm motility by regulating sperm pH via bicarbonate secretion (Ingermann et al. 2002; Dadras et al. 2014). The concentration of sperm has traditionally been used to assess sperm quality. It is an important parameter, which has an impact on fertilisation success and is characteristic for a species (Agarwal et al. 2004; Agarwal 2005). The negative relationship between sperm fertility and sperm concentration reported in this study is difficult to interpret. However, considering the polyspermy phenomenon in sturgeon species, it is necessary to adjust the amount of sperm used to fertilise eggs (Ginsburg 1968; Azari Takami 1999). Although several studies have focused on sperm quality indices vs. fertility in sturgeons, the high variability of results indicates that this issue remains to be fully understood. Our findings are not conclusive because of the low number of individuals analysed, but they do point to a need for detailed study of these parameters as a prerequisite for understanding sperm competition and sperm fertility. However, this introductory study of the effects of these factors is helpful to the aquaculture industry as it allows the development of optimal methods for artificial reproduction and contributes to our knowledge of better short- and long-term fish sperm preservation conditions.

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