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EFFECT OF TWO EGG DE-ADHESION METHODS ON REPRODUCTIVE SUCCESSES OF BELUGA *Huso huso*

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ABSTRACT

This study was carried out to compare the effects of two egg de-adhesion methods including use of tannic acid and clay suspension on reproductive parameters of beluga. For this purpose, one treatment with eggs treated with tannic acid and one control group with eggs treated with clay with 4 replications was performed. According to results, the duration of adhesion removal procedure was 45 min for eggs treated with clay while this duration was 1:30 min for eggs treated with tannic acid. Also, the fertilization percent, hatching percent and survival percent of alevins were higher for eggs treated with tannic acid compared to eggs treated with clay suspension. Furthermore, the fungal pollution rate was higher for eggs treated with clay suspension than tannic acid treatment. Results of the research indicate egg de-adhesion by tannic acid as more efficient than clay suspension in terms of time saving and reproductive success of beluga.

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INTRODUCTION

Beluga is the largest and most important of the species of sturgeon in the Caspian Sea. During the last decades, due to serious declines in the wild stocks of beluga, attempts have been focused on restocking programs, especially artificial reproduction (Kiabi et al., 1999). During artificial fertilization process of sturgeons, egg adhesiveness after egg hydration is a serious problem for aquaculturists. Sturgeon eggs have three layers and the third layer secretes an adhesive jelly which is activated in water. The adhesion and coagulation of eggs affect adversely their viability and disrupt the process of artificial fertilization. To date, the use of clay suspension is a usual method for removing egg adhesion. Since the egg de-adhesion method by clay suspension is very time-consuming and needs more facilities, reliance on new methods for this purpose is necessary. By now, many materials and chemicals have been used in order to deactivate the adhesive layer of the fertilized sturgeon eggs such as eggs silk, mud, fuller's earth, chemicals (urea, NaCl, tannic acid) (Conte et al., 1988; Bouchard and Aloisi, 2002). Neitali et al. (2013) obtained a higher survival rate when trypsin was used for de-adhesion of Persian sturgeon eggs compared to the use of clay suspension. Proteolytic enzymes have been successfully applied to common carp, tench and African catfish eggs, leading to a hatching rate of over 80% (Linhart et al., 2000, 2004; Żarski et al., 2015). However, there is no information about the effects of these techniques on the reproductive success of beluga eggs. To this respect, in the present study, the efficiency of tannic acid and clay suspension on removing egg adhesion was compared and their impacts on some reproductive parameters including fertilization and hatching rates of eggs, and also the survival rate of alevins, was studied.

MATERIAL AND METHODS

This study was carried out in Shahid Marjani Artificial Sturgeon Propagation and Rearing Center, Gorgan, Iran. Three adult females (total weight: 14.5, 13.1 and 17.8 kg, respectively) and six adult males (total weight: 12.3, 13.0, 11.2, 11.8, 14.8 and 15.4 kg, respectively) were selected randomly for the experiment. To induce ovulation in females, two dosages of LHRHa (Luteinizing Hormone - Releasing Hormone analogue), including first dosage (0.01 mg/kg) and final dosage (0.09 mg/mg), were injected intramuscularly. Also,

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to induce spermiation in males, one dosage of 0.03 mg/kg LHRHa was injected coincident with the final injection of females. After hormonal treatment, the brooders were checked out once every 4 h in order to detect final maturation. When ovulation and spermiation were done, the sperm quality of males was examined according to Hajirezaee et al. (2011). In this respect, a 50 µl drop of freshwater was placed on a glass slide and then a drop of 1 µl fresh sperm was diluted using a microsampler. Sperm motility was estimated by a semi-quantitative method (Rurangwa et al., 2004). In this regard, motility was recorded by a video camera coupled with optical lens of a microscope. At the end, video recordings were reviewed and 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 judged motile, those simply vibrating or turning on their axes were considered immotile (Aas et al., 1991). At the end, the sperm samples with good quality, i.e. motility percent and duration of more than 90% and 200 s, respectively, were selected for fertilization. After egg collection, the eggs were weighted and then divided into 8 vials (each vial containing 500 g egg), including 4 vials as control group (treated with clay suspension) and 4 vials as treatment group (treated with tannic acid (0.625 g/1.25 lit freshwater)). After pooling of semen of six males, the diluted semen with freshwater was added to eggs and then mixed for a period of 4-5 min for fertilization. After fertilization, the fertilized eggs were washed by tannic acid (in treatment group) (tannic acid manufactured by TANAN Company, Iran) and clay suspension (in control group). The period of washing for treatment group was 1:30 min and 45 min for control group according to Dettlaff et al. (1993). After washing the eggs with tannic acid, the eggs were washed with freshwater and then placed in Yushchenko incubators. Two methods were used against fungal pollution during incubation period of eggs as follows: (1) chemical method: use of malachite green bath (2) mechanical method: siphoning of dead eggs
The fertilization percent was calculated in gastrula stage as follows:

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

After about 5 days, the fertilized eggs were hatched. The hatching percent was calculated as follows:

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

After weighting, 220 g of alevins from each experimental group (i.e. treatment and control) were distributed separately to Veniro tanks. After observation of yolk sac and observation of melanin plug (a sign of initiation of active feeding), the survival percent of alevins was calculated as follows:

Survival percent of alevins (%) = the number of alive alevins /total number of alevins × 100

During larviculture in veniro, the experimental tanks were siphoned daily to remove dead alevins. The dead alevins were counted each time when siphoning.

The SPSS software was used for data analysis. Because percentage data (% fertilization, % hatching and % survival) did not have a normal distribution, proportional data were converted by angular transformation (arcsin $\sqrt{p}$). The independent samples t-test was used for the comparison of the

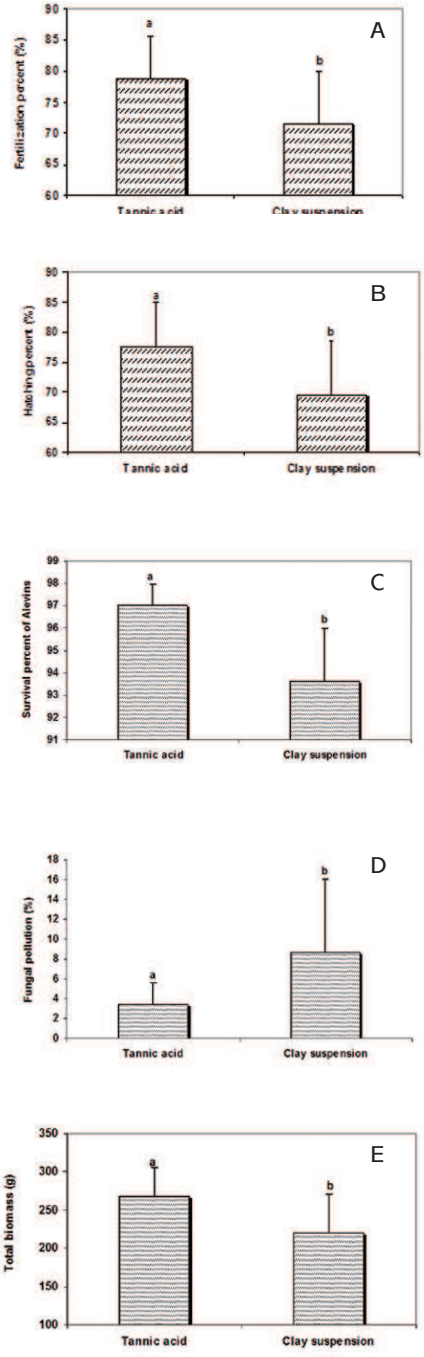

Fig 1. The comparison of egg fertilization (A), hatching, (B), alevins survival (C), fungal pollution of eggs (D) and total biomass of harvested alevins between tannic acid and clay treatments in beluga. Values in the same row with different superscript are significantly different (p<0.05)

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means between control group and the treatment group.

RESULTS

Based on sperm motility analysis, the sperm samples with good quality, i.e. motility percent and duration of more than 90% and 200 s, respectively, were selected for fertilization. The fertilization (Fig. 1A), hatching percent (Fig. 1B) and survival percent (Fig. 1C) were significantly higher in eggs treated with tannic acid compared to eggs treated with clay suspension. In contrast, the fungal pollution percent was significantly lower in eggs treated with tannic acid compared to eggs treated with clay suspension (Fig. 1D). The total biomass of harvested alevins was higher for eggs treated with tannic acid than eggs treated with clay suspension (Fig. 1E).

DISCUSSION

Adhesiveness of the eggs has always been a major problem during the hydration phase of sturgeon propagation. The eggs are so sticky that they form clumps and coatings on spawning and incubating containers so thick that abnormal development and high mortality are likely to occur (Waltemyer, 1976; Laale, 1980; Patzner and Glechner, 1996; Riehl and Patzner, 1998; Rizzo et al., 2002; Huysentruyt and Adriaens, 2005; Feledi et al., 2011). Therefore, egg adhesion must be eliminated prior to incubation (Dettlaff et al., 1993). To deactivate the jelly layer of the fertilized sturgeon eggs, silk, mud, fuller's earth, chemicals (urea-NaCl-tannic acid) and other treatments are widely used (Conte et al., 1988, Bouchard and Aloisi, 2002). However, there is no information about the effects of these techniques on the reproductive success of beluga eggs. Minimizing the duration of egg de-adhesion procedure is very important from time-saving and cost-saving aspects. In the Gulf Acipenser eggs, the use of fullers and clay decreased the duration of egg de-adhesion procedure to half (Elizabeth, 2001). In white sturgeon *Acipenser transmontanus*, a combination of urea/salt or sodium sulfite accompanying tannic acid decreased considerably the duration of egg de-adhesion procedure (Krise et al., 1986). Demska-Zake et al. (2005) observed a significant decrease in the duration of egg de-adhesion of walleye, pike perch (*Sander lucioperca*) when he used tannic acid. Also, Żarski et al. (2015) obtained the best result in de-adhesion of pike perch when eggs were submerged for 1 and 2 min in tannic acid. In our study, contrary to clay suspension, the use of tannic acid had the best result on decreasing the duration of egg de-adhesion procedure, i.e. 1:30 min *vs.* 45 min. This shows that tannic acid has faster and stronger effect on elimination of jelly layer of fertilized eggs than clay suspension. In other fish species such as pike perch, stickiness of eggs was eliminated when tannic acid was applied for 1 and 2 min (Żarski et al., 2015). Generally, the amount of jelly coat hydration varies between species, thus the length of time required for egg de-adhesion will vary (Van Eenennaam et al., 2005). Shorter duration of egg de-adhesion procedure by tannic acid is important in decreasing costs of artificial propagation of beluga. Some studies have demonstrated that de-adhesion of Tench *Tinca tinca* (Gela et al., 2003) and Lake Sturgeon (Bounchard and Aloisi, 2002) by clay suspension is time-consuming and increases the costs of artificial propagation. Fungal infections are among the most common diseases seen in tropical fish. Because fungal spores are found in all fish tanks, they can quickly colonize and create problems in stressed, injured or diseased fish and eggs. The fungus often infects damaged, diseased or infertile eggs, and the fungus can then spread to healthy eggs (Ramaiah 2006). In the present study, de-adhesive eggs by tannic acid showed lower fungal pollution and higher hatching percent and survival rate of alevins compared to de-adhesive eggs by clay suspension. This result may be due to more damaging of eggs by hard particles of clay during egg de-adhesion procedure. Neitali et al. (2013) have compared the efficiency of clay method and chemical method in de-adhesion of Persian sturgeon eggs. In their study, the lower survival rates of Persian sturgeon embryo in clay method compared to chemical method was attributed to physical damages caused by clay particles. In pike perch, use of tannic acid resulted in more hatching and survival rates of alevins (Żarski et al., 2015). As the damaging rate of eggs increases, the probability of fungal attacks increased as well. In our study, since the treatment of eggs with tannic acid and clay was after fertilization, it was unlikely that the fertilization rate was affected by the egg de-adhesion method. In conclusion, our results showed that the efficiency of tannic acid for elimination of jelly layer of beluga eggs was higher than clay suspension from cost and time-saving aspects. In this regard, the total time of egg adhesion removal procedure by tannic acid was shorter (1:30 min) than clay procedure (45 min). Also, the fungal pollution was lower in tannic acid procedure than in clay method.

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Sažetak

UČINAK DVIJU DE-ADHEZIJSKIH METODA NA REPRODUKTIVNA SVOJSTVA MORUNE, *Huso huso*

Ovo istraživanje je provedeno kako bi se usporedio efekt

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dviju de-adhezijskih metoda, uključujući korištenje tanina i suspenzije gline na reproduktivna svojstva morune. U tu svrhu provedena su 4 ponavljanja tretmana odljepljivanja ikre taninskom kiselinom i glinom koja je služila kao kontrola. Prema rezultatima, trajanje postupka odljepljivanja ikre iznosilo je 45 min za ikru tretiranu glinom, dok je za ikru tretiranu taninskom kiselinom iznosilo 1,5 minuta. Također, postotak oplodnje i postotak preživljavanja izvaljenih ličinki bio je veći za ikru tretiranu taninom u odnosu na ikru tretiranu suspenzijom gline. Također, gljivični postotak onečišćenja kod ikre tretirane suspenzijom gline je bio veći nego kod tretmana s taninskom kiselinom. Rezultati istraživanja ukazuju da je tretiranje ikre taninskom kiselinom učinkovitije nego suspenzijom gline zbog kraćeg trajanja odljepljivanja i boljeg reproduktivnog uspjeha.

Ključne riječi: de-adhezijska metoda, suspenzija gline, taninska kiselina, moruna, *Huso huso*

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