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# Maturation, Artificial Propagation and Some Physiological Indices in Farmed Ship Sturgeon, *Acipenser nudiventris*

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**Abstract:** This study was carried out with the aim at brood stocking of ship sturgeon (*Acipenser nudiventris*) as an endangered species at farmed condition. A total of 12 farmed fish (6 males and 6 females) at ages 5-7 years, were stocked in 6 fiberglass tanks with volume 4000 $m^3$ water, separately (including 2 fish per each tank). Males were fed diet without soybean but containing vitamins. Females were fed with diet containing both soybean and vitamins. GnRH hormone used for artificial propagation inducing of breeders at two step of injection for females (dose 10 µg/kg BW with 20:80 ration) and one step of injection for males at concentration 20 µg/kg BW. Significant differences ($P<0.05$) were observed at testosterone levels (T) between mature and immature males and females. 17α-hydroxyprogesterone level was 0.10±0.054 ng/ml and 0.03±0.005 ng/ml in propagated and immature females, respectively. 17β- estradiol ($E_2$) was 47.5±1.91ng/ml and 21.6±3.02 ng/ml in propagated and immature females, respectively. Significant difference ($P<0.05$) was also detected in albumin level in immature and propagated females. Glucose, cortisol, cholesterol and triglyceride levels showed no significant differences in males and females. This study emphasis on applied research and commercialization of the results towards the development of artificial breeding and rearing in ship sturgeon to produce caviar and enhance sturgeon aquaculture.

**Key words:** Brood stocking · Artificial Propagation · Farmed *Acipenser nudiventris* · Reproductive Physiology

## INTRUDUCTION

Sturgeons are the most important fishes that their wild population has been decreased sharply in major parts of the northern hemisphere especially in the Caspian Sea in recent decades. Scientists believe that one of the practical ways for preventing of sturgeon extinction is captive breeding. Ship sturgeon, *Acipenser nudiventris* is one of the most important endangered species. The choice methods for rescues of sturgeon stocks is study on their reproductive system performance and detection of all effective parameters on gonad, but the long-term maturation time is the major problem for study on their sexual maturation. Totally, effective factors on sturgeon reproductive system could be divided into internal factors including physiological, genetical and all process related to endocrine glands and external factors such as light, temperature, salinity, pH, nutrition and also some other physicochemical properties of water [1]. Beside one of the most important external factors, effective on reproductive system of aquatic animals is diet ingredients [2]. Based on Hoar *et al.* [1], the most fluctuations of sex hormones in fish are depended on diet ingredient compositions and environmental conditions that caused to reproductive behaviors. Vitamins C and E affected steroid endogenesis and vitellogenesis process [1]. Bahmani *et al.* [ 3], Bahmani *et al.* [4] and Bahmani *et al.* [5], investigated the effect of diets including both soybean and vitamins C and E on females and diet without soybean for males in sturgeons, *Acipenser stellatus*, *Acipenser persicus*, *Acipenser nudiventris* and *Huso huso* that lead to production of broodstocks and fingerlings in these fishes at farmed conditions.

Barannikova *et al.* [6] studied the fluctuations of 17β-estradiol, testosterone and 11 keto-testosterone levels in *Acipenser stellatus*, *Acipenser nudiventris* and *Huso*

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*huso* via growth and final maturation stages of gonads by hormonal treatments and emphasized on their correlation with teleost fish.

The aim of the present research was to brood stocking of ship sturgeon, *Acipenser nudiventris* at farmed conditions and measuring some related parameters such as sexing, rearing conditions and bioecological parameters by evaluating the relationship between blood indices and sexual hormones level changes in males and females at different sexual maturation stages. So reaching to the biotechnique of broodstock and artificial reproduction in farmed sturgeon especially *A. nudiventris* is the main new objects that which provide changes in completing development of this strategic industry.

## MATERIALS AND METHODS

**Fishes and Rearing Condition:** 12 farmed ship sturgeons, *A. nudiventris* (6 males and 6 females at ages 5-7 years) were selected using histological patterns and biopsy and stocked in fiberglass tanks in the aquaculture department of International Sturgeon Research Institute (ISRI) during 2009-2012. Fish were fed with special food diets (Including soybean for females and without soybean for males containing 38-40 % protein, 13-15% fat, 19.5-20 Mj/kg energy and 1% vitamins and minerals) with amount of 3-5% of body weight considering to water temperature. Fish were biometered and their total weight and length were measured seasonally at controlled conditions during rearing, propagation and hatching periods, after anesthetizing by clove powder at concentration 100-150 ppm.

**Larvae Rearing Condition:** The larvae were held in circular concrete ponds installed in an isolated building. Tanks composed of an aeration pump equipped with air stones for oxygen enrichment. Freshwater was obtained from Sepidroud River in appropriate proportions. The ponds were located in an isolated building supplied with a natural photoperiod provided. The fresh water was bacteria-free well water supplied at a constant temperature of 20°C, which was de-gassed and further oxygen-enriched via aeration. The only available food item that was accepted by larvae on a regular basis (Over the long - term) was live food (*Artemia nauplii* and daphnia). The larvae were fed five times a day and tanks were cleaned daily.

**Water Physicochemical Parameters:** Temperature, pH and dissolved oxygen were checked daily with WTW™ devices (Germany).

**Sex Determination:** Fish were sexed both by biopsy and laparoscopy methods (Using a Stema Company; model M-CAM1700, 30 degree telescope with 4 mm in size and with length 17.5 cm and with the cold light source a halogen 250 W, Germany) seasonally [7].

**Histological Study:** For histological studies, gonad samples were collected by biopsy and tissues of gonad fixed in Bouin's fluid during 48 hours and were dehydrated, paraffinated, mounted, sectioned (5-7 µm) by Microtome set (Leitz 1512, Germany), stained by Hematoxylin and Eosin (H & E) and were studied by light microscope [8-10].

**Hematological and Biochemical Analysis:** Blood sampling and biochemical studies were carried out seasonally. F or hematological study, blood was collected from each fish and transferred to the hematology Lab (Physiology and biochemistry department of ISRI) for further analysis. Plasma samples were separated using a centrifuge (Labofuge 200, Heraeus Sepatech, Germany) at 3000 rpm for 10 min. Samples were stored at -20°C [11, 12]. Hemoglobin (Hb) and albumin levels were measured using a photometric and spectrophotometer methods (UV/VIS- model 6505, Jenway Company, England) and Pars Azmon kit (Iran) with related wave at the Hematology Lab.

Blood indices such as red blood cells (RBC), white blood cells (WBC) and haematocrit (Hct) were measured based on regular methods [13].

**Hormones Analysis:** Cortisol and sex steroid hormone levels (Including testosterone, 17α-hydroxy progesterone and 17β-estradiol) were measured using a radioimmunoassay (RIA) and Immunotech kit, $I_{125}$ (France) and gamma counter LKB (Finland) based on ng/ml in the Dr. Fadaie laboratory [14-16].

**GnRH Protocol:** Injection of GnRH was done based on two step of injection in females (At concentration 10 µg/kg during 6-12 hours, 20:80 ratio) and one step of injection in males (20 µg/kg) based on temperature and physiological condition of broodstock via muscular injection [ 9].

Fig. 1: Sperm obtaining from males

Fig. 2: Microincision of oviduct in female

**Polarization Index (PI):** Polarization Index determined by Germinal Vesicle (GV) index was measured for accurate time detection of reproduction and complete ovulation after injection [9].

**Sperm and Egg Obtaining:** Egg obtained by microincision and cutting 1.5-3 cm of oviduct and pressuring of fish body from head to tail without killing the breeders [ 16]. Sperm obtained by syringe with volume 50 cc (Figures 1, 2).

**Statistical Method:** \Minimum, maximum, variance and standard error used to describe biochemical indices. One-way ANOVA used for comparison between 2 groups and Duncan's test used for differential studies. Levene's test was used for testing equality of variance. An independent-sampled t-test used to detect differences between hormonal and biochemical indices. Data analysis and drawing diagrams was done using Excel and SPSS17 software. Data are presented as Mean ± SE.

## RESULTS

**Fluctuations of Water Parameters During Rearing Period:** Minimum and maximum of temperature were 8±1.5 and 27.4±1.4 in January (winter) and July (summer), respectively. There was an adverse relationship between temperature and dissolved oxygen (Table 1).

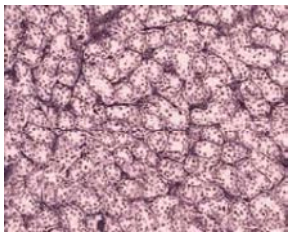

Fig. 3: Spermatocytes in males at stage II (20X)

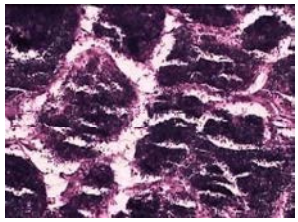

Fig. 4: Sperms at stage IV

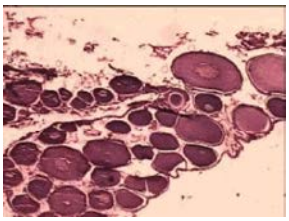

Fig. 5: Oocytes at stage II females (20X)

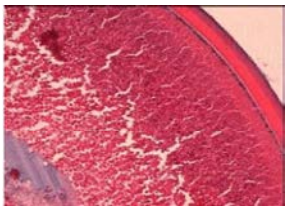

Fig. 6: Egg at stage IV in females (20X)

Table 1: Mean of water physicochemical parameters during different months

| Months | Temperature (°C) | $O_2$ (mg/l) | pH |
|---|---|---|---|
| March | 15.5±1.5 | 8.4±0.6 | 7.8±0.1 |
| April | 16.5±1.4 | 8.2±0.5 | 7.8±0.4 |
| May | 20.1±1.3 | 8.1±0.5 | 7.9±0.2 |
| Jun | 24.2±1.1 | 7.2±0.6 | 7.8±0.1 |
| July | 27.4±1.4 | 7.4±1.1 | 7.8±0.3 |
| August | 24±1.1 | 7.9±0.8 | 7.8±0.3 |
| September | 23±1.5 | 7.8±0.4 | 7.9±0.5 |
| October | 18.2±1.1 | 8.2±0.3 | 7.5±0.5 |
| November | 12.6±1.2 | 8.1±0.5 | 7.8±0.3 |
| December | 8.7±1 | 8.5±0.2 | 7.9±0.6 |
| January | 8±1.5 | 8.9±0.6 | 7.7±0.2 |
| February | 11.5±1.3 | 8.7±0.4 | 8.2±0.5 |

**Sex Determination:** Results of sexing showed that, 6 fish were males and 6 were females at the beginning of experiment. Gonadal development studies showed that 3 of females and 3 of males were at stage IV of sexual maturation. In males, secondary spermatocytes appeared

at stage II and sperm observed in testes of fish at stage IV. Germinal vesicle (GV) status or polarization index (PI) ranged at 8-12 at stage IV (Figures 3-6).

**Biometric Parameters:** Based on the obtained results, total length and total weight changed during experiment and reached a 8.5±0.7 kg and 115.6±3 cm in males; and 11.5±0.5 kg and 123 cm in females, respectively without significant differences (P>0.05).

**Blood Indices:** Blood indices results showed no significant difference in white blood cells (WBCs), red blood cells (RBCs), hematocrit (Hct) and hemoglobin (Hb) indices between males and females at different stages (P>0.05).

**Hormonal Indices in Males:** The mean levels of testosterone in spermiated and none-spermiated fish were 46.17±9.22 ng/ml and 24.42±9.88 ng/ml, respectively and showed significant differences (P<0.05) (Figure 7). The mean levels of 17α-hydroxyprogesterone in spermiated breeders and none-spermiated fish were 0.06±0.006 ng/ml and 0.05±0.009 ng/ml, respectively and showed no significant difference (P>0.05) (Figure 8).

The mean levels of 17β-estradiol ($E_2$) in spermiated and none-spermiated fish were 23.21±5.75 ng/ml and 17.58±3.51 ng/ml, respectively and showed no significant differences (P>0.05) (Figure 9).

**Hormonal Indices in Females:** The mean levels of testosterone in propagated and immature fish were 49.98±2.68 and 32±569 ng/ml, respectively with significant differences (P<0.05) (Figure 10). The mean levels of 17α-hydroxyprogesterone in propagated broodstock and immature fish were 0.10±0.054 ng/ml and 0.03±0.005 ng/ml, respectively (P<0.05) (Figure 11). The mean levels of 17β-estradiol ($E_2$) in propagated breeders and immature fish were 47.5±1.91 and 21.6±3.02 ng/ml, respectively (P<0.05) (Figure 12).

**The Comparison of Biochemical Indices Between Females and Males:** The mean level of albumin in propagated broodstock and immature females were 1.54±0.07 and 1.35±0.06 mg/dl, respectively (P<0.05) (Figure 13).

The mean level of albumin in spermiated breeders and none-spermiated fish were 1.49±0.01 mg/dl and 1.43±0.02 mg/dl, respectively (P>0.05) (Figure 14).

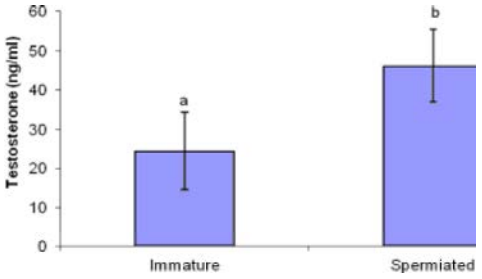


Fig. 7: Mean testosterone changes in males

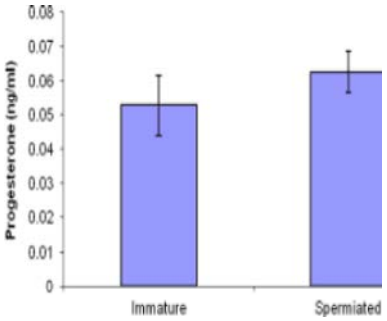


Fig. 8: Mean 17a-hydroxyprogesterone changes in males

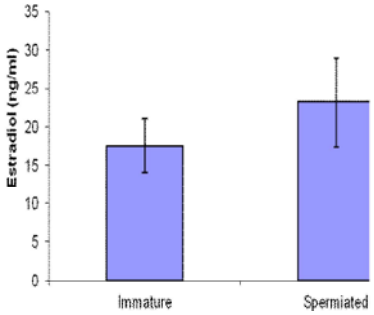


Fig. 9: Mean 17ß- estradiol (E2) changes in males

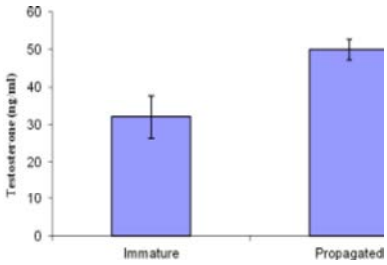


Fig. 10: Mean testosterone level in females

Some other biochemical indices results such as cortisol, glucose, cholesterol and triglyceride showed no significant differences in males and females (P>0.05).

**Artificial Propagation and Fingerlings Production:** During 20-28 hours after hormone injection, 3 females of *A. nudiventris* with mean weight 8.8, 10 and 14.5 kg were

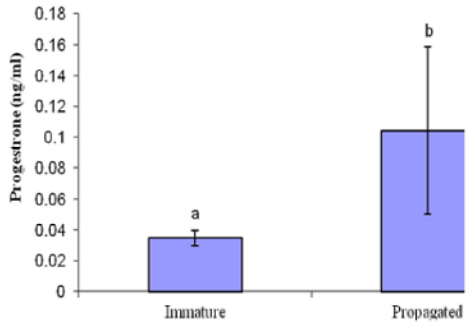


Fig. 11: Mean 17a-hydroxyprogesterone level in females

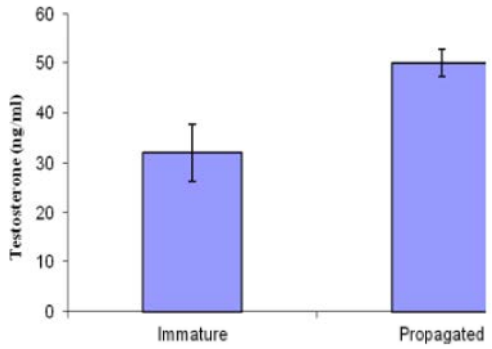


Fig. 12: Mean 17ß-estradiol level in females

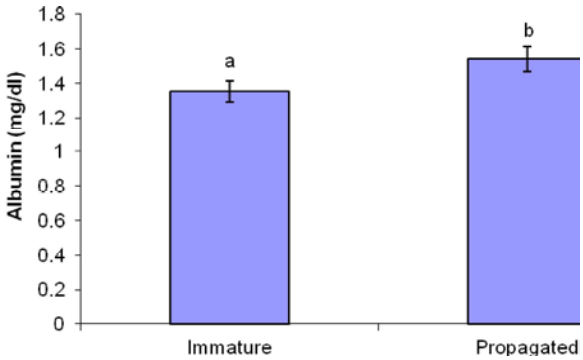


Fig. 13: Mean levels of albumin in females

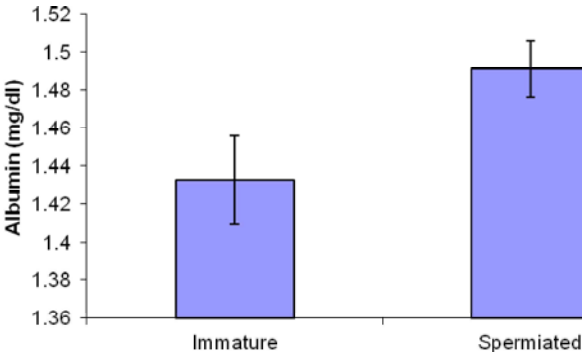


Fig. 14: Mean levels of albumin in males

ovulated and 1000, 1100 and 1900 g of eggs were obtained, respectively. Mean number of eggs per gram were 61.

It was obtained 180 ml sperm from 4 breeders at 10-20 hours after injection. Sperm having health motivation were fertilized with eggs. Fertilization rate were 96, 94 and 76 %, respectively. After incubation, about 115000 larvae were produced. Larvae were handled to Veniro concrete ponds and released with weight 3-5 g after feeding with live food (Daphnia and nauplii of Artemia).

## DISCUSION

Intracellular morphology signs were reported in *Acipenser transmontanus* [17] and *Huso huso* [4, 8, 18] indicating start of yolk absorption in egg. Vitelline development and increase in oocyte diameter showed the high quality of eggs, while fat granule deposition in eggs indicating low quality of eggs at stage IV [9]. Results of studies showed that stress will be led to apoptosis [16] in eggs at stage IV, jelly layer, internal and external *zona radiata*, fat layer, pigments, nucleus, nucleolus and micropile will be appeared. These results were according to Dettlaff *et al.* [19] in *Acipenser nudiventris*, Bahmani *et al.* [18] in *Acipenser persicus* and [7] in wild population of *Acipenser stellatus*. The obtained results showed that morphology of gonad in *A. nudiventris* were various at different body weights including increase of vesicle, increase in volume and gonad color changes. Reproductive development in some female broodstock of *A. nudiventris* was studied by measuring nucleus location in eggs. Fish that had polarization index (PI) lower than 0.07 were at stage IV of sexual maturation. Bahmani *et al.* [3] and Bahmani *et al.* [5] reported the same results in *Acipenser stellatus*. It was detected at present study that breeding of farmed *A. nudiventris* is possible and they matured sooner (At age 7 year old) than wild broodstocks (At age 10, 12 year old) sexually. It appears that we can decrease sexual maturation age in this species by using suitable management of temperature, feed diets and better selection of fish for broodstock. Bahmani and Kazemi [8], reported that sex maturation age in male *Huso huso* and *Acipenser stellatus* [16] was 6-year-old. Other farmed conditions [4, 7, 8, 17 and 18] indicated asynchronous at sex development stages of gonad histologically depended on endemic and climatic status, rearing conditions and other related factors.

Based on the obtained results, testosterone level was more in spermiated males *A. nudiventris* than immature fish. In females, testosterone level increased according to

sex development and was more in propagated breeders than immature fish. Totally, the level of testosterone was more in males than females, but progesterone level was lower in males. Testosterone level was increased due to decreasing in aromatize enzyme activity and reducing in requirement to estradiol and misusing of testosterone. Increase in progesterone production at final stages of sexual maturation led to increase in use of 17α-hydroxyprogesterone and decrease in blood serum [20]. The mean of 17α-hydroxyprogesterone and 17β-estradiol was lower in spermiated males than immature of *Acipenser nudiventris* with no significant difference ($P>0.05$), while the mean level of these hormones in propagated female broodstocks was lower than immature fishes ($P<0.05$) due to the role of these hormones in vitellogenesis and final maturation in females and their capability for changing to each other by aromatizing based on requirement. Generally, the mean level of these hormones in females was more than in males. These results were according to many some other study results. Similar results in *A. stellatus*, *Huso huso*, *A. persicus* and *A. nudiventris* were previously reported [3-5].

Bahmani *et al.* [3] indicated that testosterone (T), 17α-hydroxyprogesterone and 17β-estradiol ($E_2$) are the most important indices for sex maturity detection in *A. stellatus*. Therefore, with measuring their levels, detecting of sex maturation time in farmed sturgeon broodstock and management of their propagation is possible. Bahmani *et al.* [4] and Bahmani *et al.* [21] found that testosterone levels of farmed *Huso huso* was greater in females than males. Barannikova *et al*. [22] reported that testosterone level was greater in males than females in *H. huso* but progesterone level was more in males in Volga River in spring (April). In fish captured in autumn, serum testosterone and 17α-hydroxypro gesterone levels were lower than fish captured in spring. Males in winter had lower level of testosterone. Sex hormone levels were different between males and females in July (summer). $E_2$ and testosterone levels in female breeders were 4.22 and 359.6 ng/ml in Danube River, respectively. Barannikova *et al.* and Ceapa *et al.* [22, 23] reported that testosterone level increased in both sex in spring and reached a maximum (184.8±11.8 ng/ml in males and 105.2±30.35 ng/ml in females), while decreased and reached to 40.9±11.8 in females and 69±26.71 ng/ml in males (At stage III) in fish captured in winter males and females. In fact, seasonal changes in steroid hormone levels depended on reproductive cycle affected sexual behaviors and were necessary in vertebrate reproduction [24]. 17α- hydroxylprogesterone formation occurred in techa cells at all stages. This steroid hormone spread in granulosa cells and named adult simulative hormone in many species of fish. Nagahama and Molly *et al.* [25, 26] indicated that testosterone and 17α-hydroxyprogesterone play the main role during oocyte maturation in *Acipenser transmontanus*. Nagahama *et al*. [27] confirmed that testosterone and estradiol levels decreased in females during gonad development because of decreasing in aromatize enzyme activity in follicle layer that controlled by gonadotropins. Thomas [28] found that cortisol hormone levels used as a suitable factors for biochemical indices resulting abnormal functions in reproductive system at stress conditions. Luteus [29] measured 17α-hydroxyprogesterone level as an index for sexing in female *A. transmontanus* breeders [30]. Studies of other authors showed that the levels of $E_2$, 17α-hydroxyprogesterone and cortisol were increased via final maturation [27, 31 and 32] and they indicated that testosterone and 17α-hydroxyprogesterone levels in males and females increased at final sex maturation stage depended on gonad development in *Epinephelus morio* and reached to maximum in spring. Bahmani *et al*. [11] and Bahmani *et al*. [14] confirmed cortisol hormone role as an stress index in decreasing of sex steroid hormones in *Acipenser persicus* on migration time in Sepidroud and Gorganrood Rivers located in southern part of Caspian sea.

Cholesterol acts as a precursor for steroid hormones and the level of cholesterol and triglyceride level decrease with beginning of reproductive activities so that stocked energy used for reproductive functions [33-35]. Internal level and normal limit of cholesterol indicated a good status of nutrition in fish. It is confirmed that diet ingredients can be affected on serum biochemical indices in fish [36].

The present study showed the possibility of broodstock production and artificial propagation in farmed ship sturgeon, *A. nudiventris* as endangered species without killing them using microincision of oviduct method and measuring sex-steroid hormone levels. This is good news for commercialization of this operational study in development of sturgeon rearing and propagation industry in order to caviar and fingerling production at farmed conditions.

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