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Immune responses and hematological variables of cultured great sturgeon (*Huso huso*) subjected to 11-ketotestosterone implantation

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Abstract

Despite studies on the effect of androgens in female teleost fish, many aspects of androgen supplementation in female sturgeon gametogenesis remain elusive. The aim of the study was to investigate the immunological and physiological responses of 4-year-old previtellogenic great sturgeon (*Huso huso*) (body weight 5580±165 g) to 11-ketotestosterone (11-KT) implants. The fish were divided into two groups of 6 fish: 11-KT group, which received a single intraperitoneal implant of 2.5 mg 11-KT and a placebo group, which was implanted without 11-KT for 56 days. Just before implantation and on days 21 and 56 post-implantation, serum samples were taken to measure immune parameters (IgM, C3 and C4) and some related biochemical and hematological indices. Results showed a significant reduction in IgM concentrations after implantation in both the 11-KT and placebo groups ($p<0.05$). The concentration of C4 showed an opposite trend and significantly increased in both the placebo and 11-KT groups ($p<0.05$). The concentration of C3 and IgM to protein (IgM/p) ratio showed no significant differences during the experiment ($p>0.05$). Serum testosterone (T) levels in the 11-KT implanted group increased significantly on days 21 and 56 post-implantation ($p<0.01$). The albumin to globulin ratio showed no significant change in the 11-KT group ($p>0.05$), but a significant difference was observed in the placebo group during the study period ($p<0.05$). In hematological parameters, the number of red blood cells significantly increased after 56 days compared with that after 21 days in both experimental groups ($p<0.05$), while white blood cells significantly increased in the 11-KT group after 56 days ($p<0.05$). The results indicated that implantation with or without 11-KT could suppress some immunity parameters of great sturgeon.

Keywords: 11-ketotestosterone, Immunity, Beluga sturgeon.

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Introduction

Sturgeons are among the most precious commercial fish because of their caviar, but also for their meat and as ornamental fish. These valuable species are threatened with extinction and are listed in Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Ludwig, 2008; Keyvanshokooh and Gharaei, 2010). For this reason, aquaculture is considered an alternative for the production of sturgeon caviar and meat. A major constraint to the development of great sturgeon (*Huso huso*) aquaculture is that sexual maturation in this species takes very long, but it is possible to overcome some difficulties that sturgeons face in the wild by reducing the age of puberty under aquaculture conditions (Carmona *et al*., 2009). In recent years, some hormonal treatments have been studied to decrease the long duration of the previtellogenic stage (Poursaeid *et al*., 2012; Akhavan *et al*., 2015). 11-ketotestosterone (11-KT) is one of the most potent androgens in male teleost fish. However, several studies have documented the ability of 11-KT to stimulate female fish maturation in short finned eel *Anguilla australis* (Lokman *et al*., 2007), Atlantic cod *Gadus morhua* (Kortner *et al*., 2009) and Coho salmon *Oncorhynchus kisutch* (Forsgren and Young, 2010). Such studies have focused on the effects of 11-KT on the reproductive physiology of females. Regarding the effect of 11-KT on fish immunity, the scarce literature available shows that 11-KT can also modulate the responses of the immune system. In common carp *Cyprinus carpio*, intraperitoneal injections of 11-KT suppressed phagocytosis, superoxide anion and nitric oxide production by head-kidney macrophages in a dose-dependent manner. However, *in vitro* incubation of carp macrophages with 11-KT did not affect the production of superoxide anion in comparison with the control group (Yamaguchi *et al*., 2001; Watanuki *et al*. 2002). In general, these results suggest that 11-KT affects the immune system of fish by modulating the function of phagocytic cells.

Immunoglobulins such as IgM and complement components C3 and C4 are common serum biochemical and hematological parameters used for the evaluation of immunity condition. Immunoglobulins play a crucial role in adaptive immune responses (Uribe *et al*., 2011). They have several functions such as complement activation of lyses and opsonize pathogens (Boshra *et al*., 2004), and agglutinate phagocytosis and cellular cytotoxicity (Ye *et al*., 2013). C3 and C4 are the best-known complement components and interact with many proteins to control cell adhesion or cell-to-cell communication (Lange *et al*., 2004a). Hematological parameters such as erythrocyte count, hematocrit, hemoglobin concentration and differential blood smears are widely used as indicators of fish health (Houston, 1997).

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The use of exogenous hormone treatments in fish to modulate gonad and gamete development (Verslycke *et al*., 2002), can also produce additional endocrine disorders (Zhang *et al*., 2014). Previous studies in several fish species have suggested that androgens can modify fish immune responses (Hou *et al*., 1999; Chaves-Pozo *et al*., 2003; Kurtz *et al*., 2007; Kortner *et al*., 2009). This is probably due to the presence of androgen receptors in different immune cell types. Steroid hormones can act on most immune cells through steroid receptors or receptor-independent mechanisms (Khan and Ansar Ahmed, 2015). As mentioned above, a number of studies have clearly demonstrated that 11-KT is the most potent androgen in stimulating gonadal development in fish. On the other hand, 11-KT may be a good candidate for accelerating sexual maturation in great sturgeon during the previtellogenic stage. In contrast, 11-KT implantation may suppress fish immunity. In this case, evaluating the effects of 11-KT on the fish immune system is inevitable.

The objective of the present study was to figure out the effects of 11-KT implantation on immune responses in female great sturgeon. Because *in vivo* studies are limited, the present study will help to understand the immune system responses after 11-KT administration in sturgeon. For this purpose, serum immune responses (C3, C4 and IgM), biochemical parameters (total protein, albumin, globulin and triglyceride) and hematological features red blood cell (RBC), hematocrit (HCT), hemoglobin (HB), white blood cell (WBC), lymphocytes, neutrophils, monocytes and eosinophils) were measured before and after (21 and 56 days) hormone implantation.

Materials and methods

Fish maintenance and implanting

The experiment was conducted at the Dr. Yousefpour Fish Hatchery Center (Siahkal, Guilan, Iran) over 56 days. Twelve previtellogenic (4 year old fish, average weight 5580±165 g) great sturgeon from a stock of 50 cultured fish were selected following gonad observation and histological examination of ovary biopsy (Mojazi Amiri *et al*., 1996; Falahatkar *et al*., 2011). The fish were anesthetized using 400 mg/L clove powder extract (Poursaeid *et al*., 2015) on the first day. The abdomen wall was opened with a sterile scalpel and a small portion of ovary was collected for histology. The selected fish were tagged through the dorsal fin and were randomly divided into two groups (six fish per each group; Table 1). One pellet containing 2.5 mg 11-KT was implanted intraperitoneally in one group and a pellet without 11-KT was implanted in the other (placebo group). The surgery site was carefully sutured with a non-absorbable silk thread (0.9 mm diameter) and a surgical needle (No. G 414/4, ACUFIRM, Dreieich, Germany).

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Table 1: Weight, length and condition factor of great sturgeon (mean ± SE, n=6).

	Placebo group	11-KT group
Body weight (g)	5530 ± 200	5800 ± 170
Total length (cm)	105.3 ± 1.5	105 ± 0.9
Condition factor	0.47 ± 0.01	0.50 ± 0.01

Fish were injected with 1 ml Oxytetracycline 10% (Razak, Tehran, Iran) and then, the site of incision was disinfected with povidone Iodine-Najo 10% (Iran Najo Pharmaceutical Co., Tehran, Iran) (Poursaeid *et al*., 2015). All fish handling and experimental procedures were authorized by the Animal Ethics Committee, University of Guilan.

The fish were held under the same temperature and photoperiod in circular fiberglass tanks (3 m diameter, 50 cm depth and 4 m^3 volume). The tanks were supplied with flow-through river water at 33.2±3.3 L min^{-1} throughout the experiment. The mean water temperature and dissolved oxygen were 12.9±1.1°C and 6.9±0.3 mg L^{-1}, respectively. During the study, fish were fed (1% of body weight) two times a day at 8:00 and 17:00 h (except 2 days prior to each sampling) with a sturgeon commercial diet (Faradaneh, Shahrekord, Iran) containing 42% crude protein, 14% crude fat, 3% crude fiber, 10% ash, 10% moisture and 1% phosphorus.

Implants manufacturing

Sustained-release androgen implants were prepared using a matrix of cholesterol and cellulose (Crim *et al.*, 1988). 11-KT implants were prepared according to the method described by Lokman *et al* (2015). Briefly, dry 11-KT was mixed with the matrix (cholesterol: cellulose=95:5). Ethanol (50%) was added (1:4 v/w) and mixed. Then, the paste was dried at 37°C for 45-90 min. Free-flowing powder was obtained by sieving dried residue via 550 µm mesh for subsequent compounding in a pellet press. The 11-KT implants (8 mm long, 2 mm diameter, 28-31 mg weight) contained 2.5 mg of 11-KT, while placebo implants contained only the compressed matrix.

Blood sampling

Blood collection was done prior to implantation (day 0), as well as on days 21 and 56 of the experiment. Blood samples were obtained from the caudal vein using 5 ml non-heparinized syringes. A total of 2 mL blood was transferred into the tubes containing 50 IU ml sodium heparin (Alborz Darou Co., Tehran, Iran) for hematological assays. The remnant blood from each fish was transferred into tubes without heparin, for the determination of serum biochemical and immunological parameters. Blood samples were kept on ice until centrifugation at 1500 g for 10 minutes at 4^0C. Obtained serum was removed and aliquoted into individual 0.2 mL Eppendorf tubes and stored at -70^0C until analysis (Kohn *et al.*, 2013).

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Measurements of 11-KT and T levels in blood samples

Additional 5 ml blood samples were obtained with non-heparinized syringes and allowed to clot for 5-6 hours at 4^0C. Serum was collected after centrifugation (10 min at 1,500 g) and stored frozen (-70^0C). The 11-KT and testosterone levels were measured through radioimmunoassay using the Immunotech kit (Marseille, France), according to the method described by Pankhurst and Carragher (1992).

Measurement of immune parameters

The total serum immunoglobulin (IgM) content (Khoshbavar-Rostami *et al*., 2006) and levels of complement components (C3 and C4) (Ortuno *et al*., 1998) were measured using the immunoturbidimetric method with a spectrophotometer (Unico UV-2100, New Jersey, USA) and commercial kits (Pars Azmun, Karaj, Iran) at a wavelength of 340 nm. The ratio of IgM to protein was calculated after total protein level quantification.

Biochemical and hematological assays

Total protein, albumin and triglycerides were measured using the Biuret method (Rehulka *et al*., 2003) using commercial kits (Pars Azmun, Karaj, Iran) and a spectrophotometer (Unico UV-2100, New Jersey, USA) following the manufacturer's instructions. Globulin levels were obtained by subtracting the amount of albumin from total protein (Kumar *et al*., 2005), and the albumin to globulin ratio (A/G ratio) was calculated.

The number of RBC and WBC were counted by an improved Neubaeur hemocytometer using Lewis diluent (Houston, 1990). Hematocrit levels (HCT) were measured using microhematocrit heparinized capillary tubes and a microhematocrit centrifuge. Hemoglobin concentrations (HB) were measured using the cyanomethemoglobin method (Rehulka *et al*., 2003). Furthermore, two blood smears for each fish were made with drops of blood, allowed to air dry and fixed in ethanol. The prepared slides were stained using 10% Giemsa solution for 20 min. Slides were examined using a light microscope (Olympus DP 12, Tokyo, Japan). One random field in each slide was selected and the number of lymphocytes, monocytes, neutrophils and eosinophils counted.

Data analysis

Data normality and variance homogeneity were checked by Kolmogorov-Smirnov and Levene's tests, respectively. Mann-Whitney tests were used to compare the differences between 11-KT and placebo groups with regard to serum levels of T and 11-KT. Other data were analyzed by two-way ANOVA and a Tukey post hoc multiple comparison test using SPSS software v. 19.0 (SPSS, Inc., Chicago, IL, USA). Interaction effects of treatment and time were analyzed using general linear model (GLM)/univariate.

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Data differences were considered statistically significant at $p<0.05$.

Results

Immunological parameters

Serum IgM levels in fish implanted with 11-KT did not differ to the levels quantified in the placebo group at each sampling. The serum IgM concentrations decreased significantly, in both experimental groups, from day 0 (41.15±2.95 and 42.95±2.69 mg dl^{-1} in the placebo and 11-KT groups, respectively) to day 21 (32.88±2.95 and 33.48±2.69 mg dl^{-1} in the placebo and 11-KT groups, respectively) and remained low on day 56 (33.14±2.95 and 33.40±2.95 mg dl^{-1} in the placebo and 11-KT groups, respectively) ($p<0.05$; Fig. 1). However, the IgM/total protein ratio did not vary significantly during the experiment. The C4 levels increased significantly in both experimental groups on day 21 and 56 after implantation (Fig.1; in the placebo group from 15.33±1.10 on day 0 to 19.52±1.24 on day 21 and 23.64±1.11 on day 56; in the 11-KT group from 16.57±1.01 on day 0 to 19.52±0.93 on day 21 and 24.49±1.01 mg dl^{-1} on day 56. There were no significant changes in C3 serum levels in both experimental groups (Fig. 1).

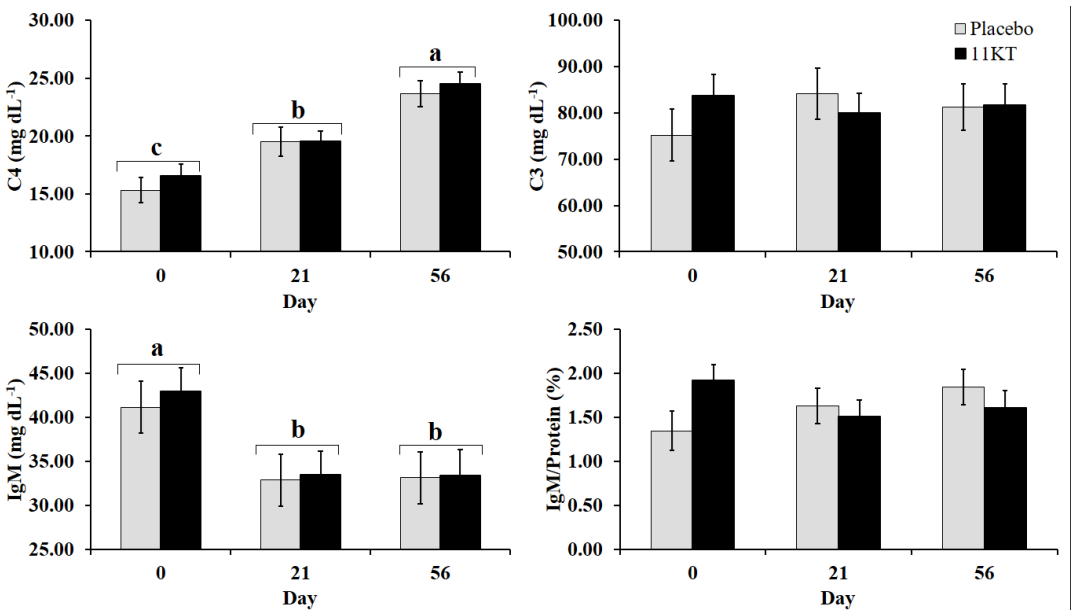


Figure 1: Effects of 11-KT implantation on innate immunity of blood serum in great sturgeon (*Huso huso*) on days 0 (before implantation), 21 and 56 of the experimental period (mean±SE, n=6). Letters denote statistically significant differences (Two-way ANOVA, $p<0.05$) with respect to the time effect (no significant difference between the placebo and 11-KT groups in each stage).

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11-KT and T levels

The 11-KT-implant resulted in marked effects on the concentration of circulating 11-KT and T. Serum T levels in the 11-KT implanted group increased significantly from 0.52±0.03 mg ml^{-1} (on day 0) to 1.33±0.02 and 3.17±0.36 ng ml^{-1} on days 21 and 56, respectively (Fig. 2A; $p<0.01$). Serum 11-KT levels in 11-KT group (76.06±14.12 ng ml^{-1}) on day 56 was 40-fold higher than the levels measured in placebo group (Fig. 2B; $p<0.01$).

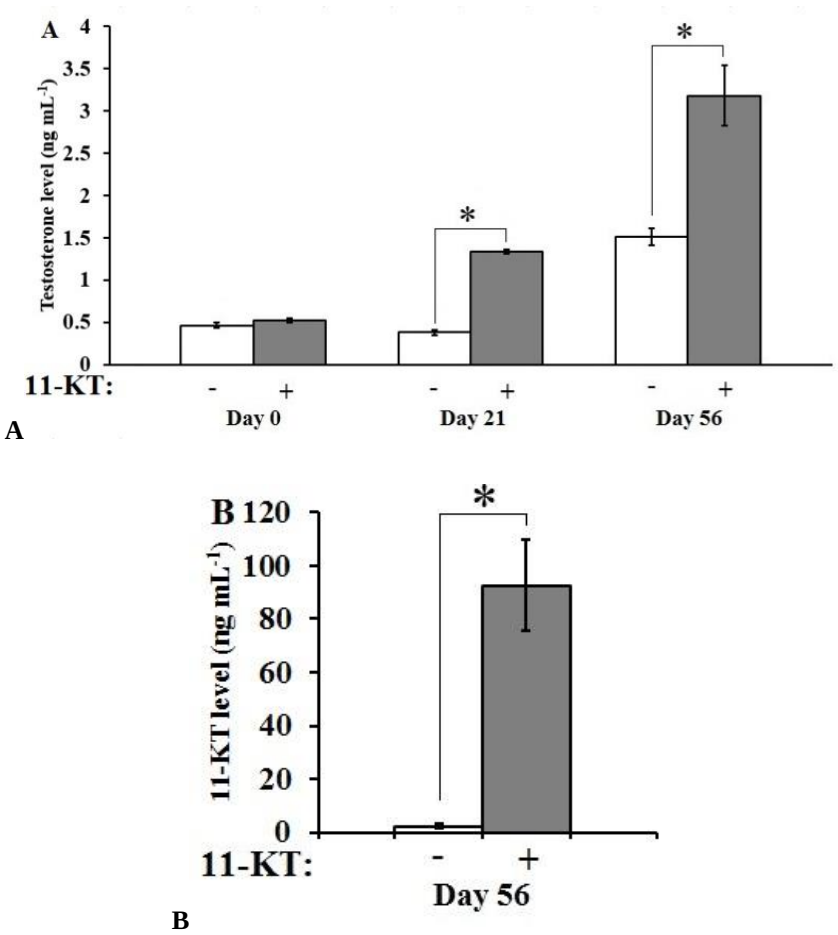


Figure 2: Serum testosterone (A) and 11-KT (B) levels following treatments with 2.5 mg 11-KT for 56 days. Each bar represents the mean ± SEM (n = 6) in great sturgeon (*Huso huso*). Asterisks (*) show significant differences between placebo (-) and 11-KT (+) groups ($p<0.01$).

Biochemical parameters

Differences between placebo and 11-KT groups at each sampling were not detected for any of the determined serum biochemical parameters. Total protein, albumin and globulin levels were found to be slightly lower in serum on day 56 compared to day 0, although the difference was not significant ($p>0.05$; Table 2). Similarly, the levels of triglycerides in both the placebo and 11-KT groups gradually decreased non-significantly on days 21 and 56 of the experiment. The A/G ratio

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did not vary during the experiment, except in the placebo group, which showed significantly higher levels on day 21 of the experiment in comparison to days 0 and 56 (Table 2).

Table 2: Biochemical parameters in blood serum of great sturgeon (*Huso huso*) implanted with or without 11-KT for 56 days.

	Placebo-day 0	11KT-day 0	Placebo-day 21	11KT-day 21	Placebo-day 56	11KT-day 56	*p*-value
Total protein (g dl^{-1})	2.26 ± 0.26	2.52 ± 0.34	2.29 ± 0.25	2.31 ± 0.21	1.90 ± 0.20	2.17 ± 0.09	0.965
Albumin (g dl^{-1})	0.65 ± 0.07	0.73 ± 0.14	0.75 ± 0.07	0.56 ± 0.06	0.47 ± 0.07	0.62 ± 0.03	0.509
Globulin (g dl^{-1})	1.61 ± 0.21	1.79 ± 0.24	1.67 ± 0.21	1.76 ± 0.19	1.43 ± 0.13	1.55 ± 0.06	0.369
A/G ratio	0.42 ± 0.05	0.43 ± 0.07	0.49 ± 0.06*	0.33 ± 0.44*	0.32 ± 0.02	0.40 ± 0.01	0.482
Triglyceride (mg dl^{-1})	191.49 ± 33.92	177.26 ± 33.30	146.24 ± 26.02	174.64 ± 10.44	116.80 ± 12.65	168.36 ± 23.44	0.328

Values are presented as mean ± S.E (n=6). Asterisk (*) shows significant effect of treatment on A/G ratio (for day 21, F=6.586 and *p*-value=0.016).

Hematological parameters

Differences between the placebo and 11-KT groups on days 21 and 56 after implantation were not detected. The number of RBC in both the placebo and 11-KT groups and the number of WBC in the 11-KT group significantly increased on day 56 in comparison with the numbers on day 21 ($p<0.05$; Table 3). Other hematological parameters did not show significant changes during the experiment and between the two experimental groups ($p>0.05$; Table 3).

Table 3: Hematological indices in great sturgeon (*Huso huso*) implanted with or without 11-KT for 56 days.

Index	Treatments				*p*-value
	Placebo-21 days	11-KT-21 days	Placebo-56 days	11-KT-56 days	
RBC (× 10^3 cell mm^{-3})	609.43 ± 16.17^c	587.14 ± 16.12^c	701.50 ± 33.98^b	773.25 ± 20.91^a	0.000
HCT (%)	27.00 ± 1.02	26.57 ± 0.78	24.67 ± 1.33	27.13 ± 0.77	0.304
HB (g dl^{-1})	6.17 ± 0.21	6.09 ± 0.19	5.60 ± 0.27	6.21 ± 0.17	0.195
WBC (cell mm^{-3})	4528.57 ± 533.95^b	4528.57 ± 548.90^b	5866.67 ± 533.95^{ab}	8225.00 ± 1337.88^a	0.017
Lymphocytes (%)	75.43 ± 1.09	72.86 ± 1.12	73.83 ± 1.83	71.88 ± 2.14	0.466
Neutrophils (%)	19.86 ± 0.91	21.85 ± 0.83	20.67 ± 1.61	23.00 ± 1.54	0.322
Monocytes (%)	3.85 ± 0.34	4.86 ± 0.46	4.50 ± 0.43	4.62 ± 0.60	0.194
Eosinophils (%)	0.86 ± 0.26	0.43 ± 0.20	1.00 ± 0.37	0.50 ± 0.27	0.420

Values are presented as mean±S.E (n=6). In each row, different superscripts indicate significant differences ($p<0.05$). RBC, red blood cells; WBC, white blood cells; HCT, hematocrit; HB, hemoglobin.

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Discussion

This study was carried out to evaluate whether immunological and hematological parameters were affected by implantation of an androgenic hormone, 11-KT, in previtellogenic great sturgeon. In this species, the effect of 11-KT on the studied parameters, were not previously reported. In fish species, the effects of androgens on immune related-parameters are poorly studied, showing contradictory results. While testosterone increases innate immune parameters (Cuesta *et al*., 2007), it seems that 11-KT induces a decrease in immune response (Yamaguchi *et al*., 2001; Watanuki *et al*., 2002; Kurtz *et al*., 2007). Androgens can be metabolized and transformed in several fish tissues, thus, their effects could be due to the administered androgen form or produced metabolites (Chaves-Pozo *et al*., 2018).

In this study, implantation was conducted by intraperitoneal injection of hormone pellets containing 2.5 mg 11-KT, previously tested by our group to stimulate ovarian development (unpublished data). Our results indicated that the implants (both placebo and 11-KT) provoked immunosuppressive reaction in previtellogenic great sturgeon. Serum IgM levels were significantly lower at the end of the study than at pre-implantation stage. A similar response was reported in juvenile rainbow trout *Oncorhynchus mykiss* (Hou *et al*., 1999). It is proven that low levels of IgM promote immunodeficiency in fish, while high IgM values are related to inflammatory and pathological conditions (Buckley, 1986). According to immunocompetence-handicap hypothesis, testosterone (T) inhibits the immune response handicapping animal health, thus increasing T serum levels causing activation of the immune response (Folstad and Karter, 1992). The results observed in rainbow trout (Hou *et al*., 1999), common carp (Saha *et al*., 2004) and three-spined stickleback *Gasterosteus aculeatus* (Kurtz *et al*., 2007) confirm this hypothesis. In the present study, higher 11-KT and T serum levels were observed on days 21 and 56 post-implantation in the 11-KT group in comparison to day 0. The IgM levels decreased simultaneously during the experiment, but this effect cannot be due to 11-KT exposure because IgM levels also decreased in the placebo group. Thus, we cannot conclude that 11-KT is immunosuppressive in great sturgeon, but decreased IgM and results of some hematological parameters show that not only pellets with 11-KT, but also pellets without 11-KT had immunosuppressive effects. In sunfish, *Lepomis macrochirus* no relationship was found between circulating 11-KT concentrations and immunity parameters (Loggie, 2016). Other studies showed that androgens modulate the fish immune response (Chaves-Pozo *et al*., 2003). Molecular studies may show how 11-KT affects sturgeon immunity.

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Implantation could be an extrinsic stressor that provoked fish responses. Cortisol as a hormone involved in immunity and reproduction, is one of the main indices of stress in fish (Wendelaar Bonga, 1997; Goikoetxea *et al*., 2017) that induces immunosuppression. Unfortunately, we do not have data on cortisol, however, the role of this hormone in decreasing IgM levels in this study cannot be ignored. The unchanged ratio of IgM to protein during the experiment shows implantation does not affect the amount of IgM protein compared to other proteins.

There are about 35 soluble complement proteins that are an important part of the defense response in vertebrates. These complements have vital roles in phagocytosis, inflammatory response, clearing immune complexes, as well as induction and enhancement of antibody responses in the innate and adaptive immunity (Holland and Lambris, 2002; Boshra and Sunyer 2006; Mauri *et al*., 2011). In teleost fish, the complement system (including C3 and C4) may not only perform a defense function in the early developmental stages of fish, but may also display a role in other development processes (Lange *et al*., 2004a, 2004b). The role of complement proteins in the generation of different organs and not only in defense against invading pathogens has been demonstrated (Dalmo, 2005). The role of complement opsonization has been documented in several species, such as common carp, catfish and salmonids (Uribe *et al*., 2011). In the present study, there was no significant difference in the C3 levels, while C4 levels increased significantly after implantation and reached a maximum at the end of the experiment. Increasing complement protein activities in fish is very important for mediating and enhancing humoral immunity. High levels of C4 and unchanged C3 levels could be explained on the basis that implantation does not affect the health of fish. Some complement proteins are produced in the leucocytes. Our results do not show the trend of changes in leucocytes due to the lack of data on day 0, but there is a significant increase between days 21 and 56. Hence, we can conclude that complement activity (C4) is modulated by increasing the number of leucocytes.

Total proteins in the plasma have been used as an indirect indicator of immune status in fish (Yang and Chen, 2003; Patriche *et al*., 2011). It has been shown that in some teleosts, the concentration of serum proteins decreases under long-term exposure to stressful conditions (Yin *et al*., 1995; Sala-Rabanal *et al*., 2003). The mechanisms are not elucidated yet, but under stressful conditions tissues and organs, including the immune system do not function properly (Tort, 2011). Stronger innate immune response is thought to be associated with increased serum protein, albumin and globulin levels in fish (Wiegertjes *et al*., 1996). In our study, protein indices including

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total protein, albumin and globulin did not show significant changes throughout the experiment, suggesting that 11-KT implantation has no effect on the synthesis and depletion of protein or on the stimulation of immune response in previtellogenic great sturgeon. On the other hand, concentration of protein alone would not certainly indicate the health of the fish. The A/G ratio is an appropriate index used to follow relative alteration in the serum or plasma protein composition and gives a clearer status of the fish. In our study, the increase in A/G ratio in the placebo group on day 21 shows globulin decreasing that means an immune response (Aydin *et al*., 2001). However, a low A/G ratio may display abnormalities in the liver or a shift from albumin to globulin production (Scott Foott *et al*., 1996). In fish, triglyceride levels are often considered as an endpoint of the health status. Our results showed a relatively slight decrease in serum triglyceride concentrations during the experiment although no significant differences among groups were observed.

Many factors such as diet, strain, age, sex, season, capture method, stressors and sexual maturity significantly fluctuate hematological parameters in fish (Langston *et al*., 2002; Svetina *et al*., 2002; Kori-Siakpere *et al*., 2005; Nikoo *et al*., 2010). Our results revealed that the values of RBC and WBC in both the placebo and 11-KT groups significantly increased 56 days after implantation. The higher levels of RBC could be explained to the increased metabolic activity of the animal. Increasing WBC values could be reflecting immunostimulatory effects in the 11-KT group. On the other hand, lymphocytes (T and B) mediate effective adaptive immunity (Pancer and Cooper, 2006). In salmonid, 11-KT inhibits lymphocyte proliferation (Slater and Schreck, 1993; Cook *et al*., 1994). Our result showed that lymphocytes were not influenced by 11-KT.

The results showed the *in vivo* effects of implantation on the circulating IgM levels and complement protein activity, as well as some biochemical and hematological parameters of previtellogenic great sturgeon as major immune status indicators. The C4 concentration, as well as RBC and WBC counts increased, whilst IgM decreased. However there was no change in C3 and biochemical indices. Although these results partly suggest that implantation may have had immunosuppressive effects in previtellogenic stage sturgeons, more studies at transcriptional and functional levels are needed to elucidate mechanisms associated with immune responses after hormone implantation.

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