Int Aquat Res (2010) 2: 71-75
ISSN 2008-4935
Available online at www.intelaquares.com

International
Aquatic Research

Short communication

Germinal vesicle breakdown rates in oocytes and steroid levels in blood and ovarian fluid of the Persian sturgeon *Acipenser persicus*

Saeed Hajirezaee[1*], Gholam Reza Rafiee[1], Ramtin Hushangi[1], Ruhollah Rahimi[2], Hamid Niksirat[3], Rezvan Kazemi[4]

[1]*Department of Fisheries and Environmental Sciences, Faculty of Natural Resources, University of Tehran, Karaj, Iran*
[2]*Department of Fisheries, Faculty of Marine Sciences, Chabahar Maritime University, Chabahar, Iran*
[3]*Department of Biodiversity and Ecosystem Management, Environmental Sciences Research Institute, Shahid Beheshti University, G.C., Tehran, Iran*
[4]*International Sturgeon Research Institute, Rasht, Iran.*

Received: 3 February 2010; Accepted: 15 March 2010

Abstract

In the present study, the steroids [Cortisol (C), testosterone (T), 11-ketotestosterone (11-KT), progesterone (P), 17,20ß,21-trihydroxy-4-pregnen-3-one (20βS), 17*α*,20*β* dihydroxy-4-pregnen-3-one (DHP) and 17*α*, hydroxyprogesterone (OHP)] in both blood serum (BS) and ovarian fluid (OF) as well as the rate of Germinal Vesicle Breakdown (GVBD) were measured for the Persian sturgeon, *Acipenser persicus*. The high positive correlations were found between 20ßS and P levels in both OF and BS. Additionally, 20ßS and P exhibited the positive correlation with percentage of GVBD while a significant negative correlation was found between Polarization Index (PI) values and percentage of GVBD. The levels of 20ßS and P in both BS and OF were significantly higher in females with high GVBD rate than in females with the lowest GVBD rate. Therefore, the percentage of GVBD may be affected by 20ßS and P levels in both OF and BS as well as by developmental stage of oocytes.

Keywords: *Acipenser persicus*, Ovarian fluid, Steroids, Germinal vesicle breakdown

Introduction

It is well recognized that sex steroids play an important role in hormonal control of the reproduction in fish including sturgeons (Kime 1993; Barannikova et al. 2000, 2002).

Final oocyte maturation (FOM) in fish comprises the migration and breakdown of the germinal vesicle (GVBD) and is followed by egg release from the follicles (ovulation) (Semenkova et al. 2006). After ovulation, the eggs are plunged in ovarian fluid, a liquid media composed of organic and inorganic components which preserve eggs till spawning or stripping (Bayunova et al. 2003).

* Corresponding author. Email: S.Hajirezaee@gmail.com. Tel: +98-936-597 6145.

However, a little information about the endocrine hormones involving in final maturation of sturgeon eggs exists. Therefore, the study aimed to measure the values of steroids in blood serum (BS) and ovarian fluid (OF) as well as the percentage of GVBD in a commercially and ecologically valuable species of Caspian Sea (Persian sturgeon, *Acipenser persicus*) and to investigate their probable relationships.

Materials and methods

Twenty four Persian sturgeon females (Total length = 132-162cm: the distance between the tip of snout until the top margin of heterocercal caudal fin; Total weight = 23.5-26 Kg) were captured in the southern part of the Caspian Sea from March to June 2008 and transported to Shahid Beheshti Artificial Sturgeon Propagation and Rearing Center (SAPRC), Rasht, Iran. After delivery to SAPRC, the females were kept in pond (1m×4m×8m; water used had the following conditions: about 1.5 m^3/min in pond entrance; water temperature: 16-18 °C; dissolved oxygen: 8-8.3 mg/l and pH: 7.3-7.5).

During the experiment, the females with Polarization Index (PI: the ratio of the distance of the germinal vesicle from the animal pole over the animal-vegetal oocyte diameter × 100) comprising between 5 and 8 were selected for induction of spawning since the Persian sturgeon females show better response to pituitary preparation (PP) treatment in this PI range (Azari Takami et al. 1997).

For each female, PI values of oocytes were measured under a Stereomicroscope (Meiji EMZ-1, Meiji Techno Company, Japan) according to Billard et al. (2000). To induce the ovulation, the females were injected intramuscularly with pituitary preparation (PP) at doses of 50-70 mg. These dosages were temperature dependent as follows: 16 °C: 50 mg PP/female; 17 °C: 60 mg PP/female; 18 °C: 70 mg PP/female) (Kohneshahri and Azari Takami 1974). Within 18-24 h after PP treatment, 17 females were ovulated and the rest did not respond to injection.

BS samples were taken from the caudal vein of females using heparinized syringe (with maximum degree of 5 ml) at the time of the ovulation. Then, the blood samples were centrifuged (5000 rpm for 10 min) (Labofuge200, Heraeus Company, Germany) to separate the serum. Also, the OF samples were separated from eggs by a net with 1 mm mesh sizes. The BS and OF samples were stored at – 20 °C until hormonal analysis.

From each ovulated female, 20 g (approximately 900-1000 eggs numbers) eggs were allocated for calculation of Germinal vesicle breakdown rate (GVBD %) under a Stereomicroscope. After calculation of GVBD rate for each brooder, the brooders were divided into three groups on the basis of GVBD rate: Group I: 80-100% GVBD; Group II: 70-80% GVBD and Group III: 50-70% GVBD.

Steroids [Cortisol (C), testosterone (T), 11-ketotestosterone (11-KT), progesterone (P) and 17,20ß,21-trihydroxy-4-pregnen-3-one(20βS)] concentrations (ng/mL) were measured by Enzyme-Linked Immunosorbent Assay (ELISA) according to Bayunova et al. (2002) and Semenkova et al. (2002). Also, the concentration of 17*α*,20*β*-dihydroxy-4-pregnen-3-one (DHP) and 17*α*,hydroxyprogesterone (OHP) were measured by Radio Immuno Assay (RIA) according to Canario and Scott (1989) and Shimizu et al. (1985), respectively.

The SPSS software was used for data analysis. The values of steroids in both BS and OF were normal according to Kolmogorov Smirnov test but because of percentage data (percentage of GVBD) did not have a normal distribution, proportional data were converted by angular transformation (arcsin$\sqrt{p}$). One-way analysis of variance (ANOVA) was employed to compare the means of steroids among groups with various GVBD rates (i.e. groups I, II and III). When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which groups were different. Also, the independent samples *t*-test was used for the comparison of the means of steroids between ovarian fluid and blood serum. All correlations were tested using the bivariate correlation coefficients of Pearson. Then, linear and non-linear regression models were investigated using regression fits.

Results

The values of all steroids in OF were significantly lower than that in BS (Table 1, $P < 0.05$). There were high positive correlations between 20ßS and P levels in OF and their values in BS ($r^2= 0.59$ for 20ßS, $P < 0.001$; $r^2= 0.63$ for P, $P < 0.001$). Additionally, 20ßS and P were positively correlated with percentage of GVBD ($r^2= 0.76$ for 20ßS in BS, $P < 0.001$; $r^2= 0.47$ for 20ßS in OF; $r^2= 0.75$ for P in BS, $P < 0.001$; $r^2= 0.65$ for P in OF, $P < 0.001$). A significant negative correlation was found between PI values and percentage of GVBD (Figure1, $P < 0.001$). Also,

the levels of 20ßS and P in both BS and OF were significantly higher in females with high GVBD rate than the females with the lowest GVBD rate (Table 2, $P < 0.05$).

Table 1. Steroid concentrations (ng/mL) in blood serum (BS) and ovarian fluid (OF) of ovulated Persian sturgeon females (n= 17)

	T	11-KT	P	DHP	OHP	20βS	C
Steroids in blood serum (BS)	18.0±2.5[*1]	15.0±2.5[*]	4.4±1[*]	1.1±0.4[*]	21.9±3.1[*]	8.8±1.2[*]	205.4±17.4[*]
Steroids in ovarian fluid (OF)	3.9±1.4	2.7±1	0.5±0.3	0.4±0.2	4.4±1.2	2.2±0.6	8.0±2.2

[1] Significant differences between steroid levels of BS and OF have been indicated as *.

Table 2. The comparison of steroid levels in OF and BS of females with various GVBD rates

	Treatments[*]		
Steroids in BS	Group I (n=6)	Group II (n=6)	Group III (n=5)
T	18.7±2.1[a**]	16.2±1.4[a]	19.4±3[a]
11-KT	16.9±2.6[a]	12.8±2.1[a]	15.2±1.8[a]
P	5.6±0.6[a]	4.2±0.3[b]	3.2±0.3[c]
DHP	1.1±0.2[a]	1.1±0.6[a]	1.1±0.5[a]
OHP	25.1±3.6[a]	20.3±0.8[a]	21.4±1.3[a]
20βS	10.7±1.8[a]	8.5±0.7[b]	7±0.4[b]
C	197.5±9.4[a]	211.3±19.6[a]	207.8±21.6[a]
Steroids in OF	Group I (n=6)	Group II (n=6)	Group III (n=5)
T	4.15±1.3[a]	3.6±1.4[a]	4±1.8[a]
11-KT	2.2±0.6[a]	2.3±0.7[a]	3.7±1.2[a]
P	0.81±0.19[a]	0.5±0.1[b]	0.28±0.13[b]
DHP	0.3±0.2[a]	0.6±0.1[a]	0.5±0.2[a]
OHP	4.3±1.1[a]	4.4±1.6[a]	4.5±0.1[a]
20βS	2.8±0.5[a]	1.9±0.5[b]	1.8±0.5[b]
C	9.4±2.4[a]	6.3±1.3[a]	8.3±2.7[a]

[*] Treatments (groups): the females with various GVBD rates, Group I: 80-100% GVBD, Group II: 70-80% GVBD, Group III: 50-70% GVBD.
[**] Means with same superscripts in each row are not significantly different ($P > 0.05$).

Discussion

According to present data, the levels of all examined steroids (C, T, 11KT, P, 20βS, DHP, OHP) in OF were lower than those in BS at the time of ovulation. In agreement with our results, the T and C levels in Stellate sturgeon, *Acipenser stellatus*, ovarian fluid were also lower than those in BS at the time of ovulation, although the P levels was statistically equal between OF and BS (Barannikova et al. 2002).

Also, the T, 11-KT and E2 levels in the Russian sturgeon, *Acipenser gueldenstaedti*, ovarian fluid were lower than their values in BS (Bayunova et al. 2003). Such differences between the steroid values of BS and OF suggested that the sturgeons had mechanisms stabilizing the concentrations of a number of hormones and metabolites in the OF at the optimum level, which may be important for eggs.

For example, cortisol levels lower in the OF than in BS might be a protective mechanism for the Persian sturgeon eggs from the adverse effects of high corticosteroid concentrations in stressful conditions. Furthermore, it was likely that the higher levels of sex steroids in ovarian fluid inappropriately accelerated the overripping of eggs after ovulation.

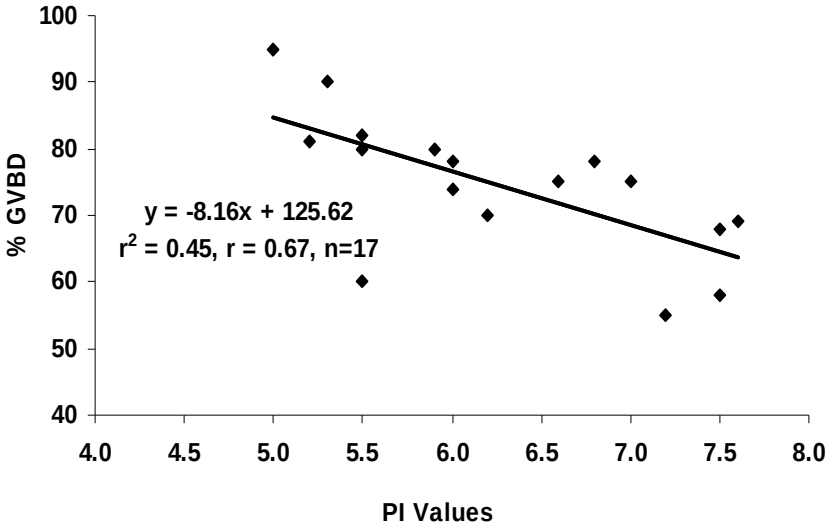

Fig. 1. The relationship between the PI (Polarization Index) values of oocytes and the percentage of GVBD (n: number of females, r^2: R square, r: Regression coefficient, y: regression equation)

In the present study, there were positive relationships between 20ßS and P levels in OF and their values in BS. These also had positive relationships with %GVBD. Also, the females with higher rate of GVBD showed the higher levels of 20ßS and P in both OF and BS than those with lower rate of GVBD. Observations in *vitro* have been demonstrated that several steroids, such as progesterone, 11-dexycortisol, 17,20β-dihydroxy-4-pregnen-3-one and 17,20β,21-trihydroxy-4-pregnen-3-one, are capable of inducing GVBD in teleost fish and in sturgeon (Semenkova et al. 2006).

On the other hand, different results were recorded about the main steroids affecting GVBD in these studies. According to an unpublished study, Semenkova et al. (2006) demonstrated that DHP was not effective in inducing GVBD in the Sterlet sturgeon, *Acipenser ruthenus*, oocytes, while 20βS and 17,20α-dihydroxy-4-pregnen-3-one induced 71.4 % and 100 % GVBD, respectively. In the sturgeon hybrid bester, OHP was the most potent steroid in inducing GVBD (Amiri, et al. 1999) whereas 11-dexycortisol induced the highest percentage of GVBD in the white sturgeon, *Acipenser transmontanus*, oocytes (Webb et al. 2000).

Such different results could be related to the different experimental designs and the physiological state of the fish and oocytes. Semenkova et al. (2006) observed that oocytes with the highest PI had higher percentage of GVBD in comparison with the the oocytes with the lowest PI as recorded for the Persian sturgeon in this study. In this regard, the females with the highest PI showed the lowest percentage of GVBD after stimulation of maturation by PP treatment. As germinal vesicle (GV) moves towards the animal pole during germinal vesicle migration (GVM), the polarization index (PI) decreases and the oocytes become more mature. In oocytes with high PI values, it is likely that the steroids are involved in GVM and GVBD. For oocytes with low PI values, the action of steroids is focused more on GVBD. Thus, the oocytes with the lowest PI (more mature) show higher levels of GVBD. This result shows an overlap between in *vitro* (Semenkova et al. 2006) and in *vivo* observations about the role of developmental stage of oocytes on maturation successes.

In the Persian sturgeon, the higher levels of 20ßS and P in OF and BS of females with high %GVBD, as well as the positive correlations these two steroids with %GVBD demonstrated that the 20ßS and P may be more effective than other steroids in stimulation of GVBD in *vivo*. It was likely that a part of maturation process was completed by steroids in OF being high positive correlations between BS and OF levels of 20ßS and P in Persian sturgeon as well as the T and 11-KT in the Russian sturgeon, *Acipenser gueldenstaedti*, (20ßS and P were not tested) (Bayunova et al. 2003).

Conclusion

The lower levels of steroids in OF than those in BS might be essential for viability of sturgeon eggs after ovulation. Also, the significant positive correlations between 20ßS and P (in both BS and OF) with GVBD rates showed more effectiveness of these two steroids in GVBD induction. Further studies are necessary to explore the roles of steroids of ovarian fluid in the egg ovulation.

Acknowledgements

The authors are sincerely grateful to the staff of International Sturgeon Research Institute and Shahid Beheshti Artificial Sturgeon Propagation and Rearing Center, Iran, Rasht for their suggestions and providing of Persian sturgeon females.

References

Amiri BM, Maebaayashi M, Hara A, Adashi S, Moberg GP, Doroshov SI, Yamauchi K. 1999. *In vitro* steroidogenesis by testicular fragments and ovarian follicles in a hybrid sturgeon, Bester. Fish Physiol Biochem 21: 1-14.

Azari Takami G, Pousti E, Ebrahimi E. 1997. Potential of reproduction in Persian sturgeon, *Acipencer persicus*. Journal of Veterinary (In Persian), Faculty of Veterinary, University of Tehran 51: 31-34.

Billard R. 2000. Biology and control of reproduction of sturgeon in fish farm. Iran J Fish Sci 2: 1-20.

Barannikova IA, Artyukhin EN, Bayunova LV, Dyubin VP, Semenkova TB. 2000. Steroids profiles in giant sturgeon females (*Huso huso* (L.)) at the beginning of anadromous migration and at induced ovulation after reservation. 1999. Proceedings of the 6th Int. Symposium on Reproduction and Physiology of Fish (ed. By B, Norberg OS, Kjesbi GL, Tarranger E, Andersson SO, Steffanson), Norway, Bergen, p. 420.

Barannikova IA, Dyubin, VP, Bayunova, LV, Semenkova, TB .2002. Steroids in the control of reproductive function of Fish. Neurosci Behavior Physiol 32: 141-148.

Bayunova LV, Barannikova IA, Dyubin VP, Gruslova AB, Semenkova TB, Trenkler IV. 2003. Sex steroids concentrations in Russian sturgeon (*Acipenser guel denstaedti* Br.) serum and coelomic fluid at final oocyte maturation. Fish Physiol Biochem 28: 325–326.

Canario AVM, Scott AP. 1989. Synthesis of 20-hydroxylated steroids by ovaries of thr dab (*Limanda limanda*). Gen Comp Endocrinol 76: 147-158.

Deshpande AK, Koide SS. 1985. *In vitro* induction of *Xenopus* oocyte maturation by 4-pregnene-17α,20β-diol-3-one. Gen Comp Endocrinol 57: 130-134.

Kime DE. 1993. “Classical” and “non-classical” reproductive steroids in fish. Rev Fish Biol Fish 3: 160-180.

Kohneshahri M, Azari Takami G .1974. Artificial propagation of sturgeons (In Persian). Tehran University Publications, Tehran, pp 46-47.

Nagahama Y. 1987. Endocrine control of oocyte maturation. In: Hormones and reproduction in Fishes Amphibians, and Reptiles (ed. By DO, Norris and RE Jones), pp171-202. Plenum Press, New York, USA.

Semenkova TB, Barannikova IA, Kime DE, McAllister BG, Bayunova LV, Dyubin VP, Kolmakov NN. 2002. Sex steroid profiles in female and male Stellate sturgeon during final maturation induced by hormonal treatment. J Appl Ichthyol 18: 375-381.

Semenkova B, Bayunova LV, Webb MAH, Kolmakov NN, Romanov AG, Barannikova IA. 2006. Effect of progestins on germinal vesicle break down in sturgeon follicles *in vitro*. J Appl Ichthyol 22: 353-357.

Shimizu A, Aida K, Hanyu I. 1985. Endocrine profiles during the short-reproductive cycle of an autumn-spawning bitterling, *Acheilognathus rhombea*. Gen Comp Endocrinol 60: 351–371.

Webb MAH, Van Eenennaam JP, Doroshov SI. 2000. Effects of steroids on *in vitro* oocyte maturation in white sturgeon (*Acipenser transmontanus*). Fish Physiol Biochem 23: 317-325.