



Assessment in Vitro Oocyte Maturation and Blood-Circulating Steroid Hormones in Persian Sturgeon (*Acipenser Persicus*) Spawning

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Abstract

The in vitro effect of 17 α , 20 β -Dihydroxyprogesterone on the maturation of Persian sturgeon (*Acipenser persicus*) oocytes for 7, 10, 12, 24 and 30th hours of incubation were investigated. Blood serum, testosterone (T), progesterone (P4) and 17- β estradiol concentrations were measured using enzyme-linked immuno-sorbent assay (ELISA). The oocytes were incubated in SIS, RM2, L-15 and PSACF media in the presence of 17 α , 20 β -Dihydroxyprogesterone at 1 μ g/ml concentration. The relationship between the fertilization rate and incubation time of the studied media during in vitro maturation of Persian sturgeon oocytes was investigated. In all four media, fertilization rate decreased with increasing duration of incubation. The highest fertilization rate was recorded in the oocytes incubated in SIS medium for 12 h. Fertilization rate decreased significantly with incubation time in SIS medium averaging 84 \pm 5.65% after 12 h and reaching 8.3 \pm 14.43% after 30 h ($P < 0.05$). No significant correlations were observed between 17- β estradiol ($R^2 = 0.0134$), progesterone ($R^2 = 0.0159$) and testosterone ($R^2 = 0.0652$) and fertilization rate ($P > 0.05$). The obtained results in the present study support the role of 17 α , 20 β -Dihydroxyprogesterone on oocyte maturation in the Persian sturgeon and also suggest that in vitro oocyte maturation assay can be used as practical tool for improving breeding management of the Persian sturgeon.

Keywords: *Acipenser persicus*, In vitro oocyte maturation, Fertilization rate, Steroid hormones, Incubation media

Introduction

The application of reproduction in vitro procedure for studying the mechanisms of hormonal regulation of oocyte maturation in sturgeons became possible after producing culture media in which inducing this process was possible by gonadotropic preparations. Vertebrate oocytes, which grow within the ovarian follicles, are arrested at the first meiotic prophase. In teleosts, as in other non-mammalian vertebrates, the principal events responsible for the enormous growth of oocytes are essentially due to the accumulation of yolk proteins within their cytoplasm (Devlin & Nagahama, 2002). After the oocyte completes its growth in size, it becomes ready for the next phase of oogenesis, that is, the resumption of meiosis (completion of the first meiotic division followed by progression to metaphase II), which is accompanied by several maturational processes in the nucleus and cytoplasm of the oocyte. This process, called oocyte maturation, occurs prior to ovulation and is a prerequisite for successful fertilization; it consists of breakdown of the germinal vesicle (GVBD), chromosome condensation, assembly of the meiotic spindle, and formation of the first polar body.

Studies using well-characterized *in vitro* systems as well as those of *in vivo* systems have revealed that oocyte maturation in fish is regulated by three mediators, gonadotropin (GTH; luteinizing hormone, LH), maturation-inducing hormone (MIH), and maturation-promoting factor (MPF).

In sturgeons, GV (germinal vesicle) requires a rather long period for complete migration during which oocyte sensitivity to the maturation-inducing hormone in vitro will change. Several in vitro studies have revealed that various steroids such as 17 α , 20 β -Dihydroxyprogesterone can stimulate oocyte maturation in teleosts (Mojazi Amiri *et al.*, 2001). Sturgeons usually do not breed naturally in captivity and must be spawned artificially using exogenous hormones. The timing of their spawning behavior (e.g., upriver migration from the ocean), presence of ripe fish in the spawning grounds, and appearance of the brood fish in the spawning season have traditionally been used to assess the stage of maturity and specific time of spawning. Selection of female fish for spawning without proper assessment of their stage of egg maturation often results in partial or no ovulation, or ovulation occurs but with low egg fertility or low embryo survival. Prediction of

successful induction of ovulation based on GV position, however, is not fully reliable because fish exhibiting intermediate stages of GV migration often result in ovulatory dysfunction. For fish with intermediate stages of GV migration, the in vitro oocyte maturation response to maturation-inducing steroids yields a higher predictive accuracy (Omoto *et al.*, 2005). Successful fertilization requires collecting properly matured gametes at the right time; otherwise, there happens a loss in fertilizing ability. Since female sturgeon mature slowly, if breeders fail to induce high quality eggs, they must wait a few years for the next breeding cycle (Mylonas and Zohar, 1998). Without determining the appropriate time for stripping, the ovulated eggs retained in the ovary or body cavity beyond a certain time span undergo over-ripening due to gradual morphological and biochemical changes that negatively affect fertility and larval development (Mohagheghi Samarin *et al.*, 2011). Thus, for avoiding over-ripening in eggs, it is important to obtain and fertilize eggs at the correct time after ovulation, especially for those fish whose eggs can only be obtained by manual stripping and fertilized artificially (Taati *et al.*, 2011). Since the culture medium composition significantly affects the sensitivity of the sturgeon follicles to gonadotropins (Talbot *et al.*, 2011), the choice of culture medium for estimating the developmental stage or physiological state of sturgeon follicles according to their reaction to gonadotropins in vitro is of great importance.

Testosterone (T) and 17- β estradiol (E2) play major roles in oocyte development and maturation (Heidari *et al.* 2010). It has been reported that plasma 17- β estradiol (E2) levels increased during the vitellogenic stage but decreased during the maturational stage in many teleost. E2 is known to induce the synthesis and release of vitellogenin protein by the liver. Progesterone may directly enhance maturation through vitellogenesis stimulation or act as a precursor to Vitellogenesis Stimulating Ovarian Hormone (VSOH) (Meunpol *et al.*, 2007).

However, little attention has been given to the relationship between maturational competence of oocyte and fertilization rate of eggs. Here, we aimed to attain knowledge about the relationship between the fertilization rate and incubation time of oocytes in vitro and determination of best time to induce ovulation in the female.

Materials and Methods

Fish and Sampling

Males (18–22 kg, 1-2 m, n = 10) and females (20–25 kg, 1.5-2 m, n = 16) Persian sturgeon, *A. persicus* (Borodin), brood fish were captured in the Caspian Sea using gillnets (length 18 m, width 5.4 m, mesh size 15 cm, water temperature 10–11.5°C) during March 2011 and transported for 6 h by boat to

the Sturgeon Hatchery Center where they were maintained for 5 days in m² holding ponds (8 m diameter, 1 m depth, 50 m³ volume) at 12.5–13°C, with a fresh water supply. Water temperature, oxygen content and pH were 14–15.5°C, >6.1 mg/L and 7.1–7.3, respectively, during the experiments. The females were surveyed for reproduction condition by oocyte diameter and degree of migration of germinal vesicle toward the animal pole. For measuring GV (germinal vesicle) position by polarization index (PI), a sample of 15-20 eggs for each female were boiled for 2 minutes and were cut along their animal-vegetal poles axis and observed under a dissection microscope with a micrometer eyepiece. The oocyte polarization index for GV position was calculated by the formula $PI = a/A \times 100$, in which a: distance between GV and cell membrane, and A: diameter of oocyte along animal-vegetal axis (Kazanskii *et al.*, 1978).

Media and Sample Preparation

The artificial media used to incubate the oocytes were commercial media, named L-15 medium (Leibovitz), RM2, SIS and PSACF. Then, Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was added to the L-15 medium as a buffering agent at a concentration of 5 mM, penicillin at 70 mg L⁻¹ and streptomycin sulfate at 100 mg L⁻¹ and pH adjusted to 7.8 with 1 M NaOH. The RM2 medium (Ringer solution modified for sturgeons) consisted of: 11.9 mM/l NaHCO₃, 111 mM/l NaCl, 3.35 mM/l KCl, 2.14 mM/l CaCl₂ (2H₂O) and pH: 8.02. SIS medium (Siberian sturgeon medium based on plasma characteristics of Siberian sturgeon) consisted of: 128 mM/l NaCl, 2.7 mM/l KCl, 1.5 mM/l CaCl₂(2H₂O), 0.84 mM/l MgCl₂(6H₂O), 0.7 mM/l Na₂SO₄, 20 mM/l Hepes buffer and pH: 7.55. The PSACF medium (Persian Sturgeon Artificial Coelomic Fluid) based on coelomic fluid characteristics of female Persian sturgeon (*Acipenser persicus*) that was obtained by sohrabnezhad *et al* (2006) and consisted of: 80 mM/l NaCl, 3.96 mM/l KCl, 0.78 mM/l MgSO₄.7H₂O, 0.26 mM/l CaCl₂, 2.42 mM/l glucose, 20 mM/l NaHCO₃, 20 mM/l Hepes, 1g BSA (bovine serum albumin) and pH: 7.5. All culture media were prepared with de-ionised water. The media were stored in a refrigerator at 4 °C and were prepared fresh every week. 17 α , 20 β -Dihydroxyprogesterone (Sigma Chemical Co., St Louis, MO) was dissolved in ethanol and added (0.2% in volume) to a final concentration of 1 μ g/ml. The average 90-100 oocytes were removed with a metal probe through a small abdominal hole and put directly in 4 plastic beakers containing 7.5 ml of the medium, which had been hormone-complemented shortly before sampling. Capped plastic beakers were incubated under normal atmosphere at 18.5 \pm 1°C for 7, 10, 12, 24 and 30 h. After incubation, the oocytes were fixed in formalin 10% solution. Then the oocytes were boiled gently for about 1-3 minutes. After boiling, the oocytes were chilled by placing the

beakers directly on ice for 15-30 minutes and then oocyte PI and GVBD in the in vitro maturation assay were determined at each sampling. Soon after the removal of the oocytes, the females were injected intramuscularly with acetone-dried sturgeon pituitary at water temperature in the range of 17-20 °C (45-50 mg for female and 35 mg for male). For establishing equal condition for fertilization and lowering the effect of male characteristics on the results we used the semen of those fish with high motility (85-92%) and based on the practical protocol of Shahid Marjani Sturgeon Fish Propagation and Rearing Center (Golestan Province, Iran), sperm of different fish were stripped separately, achieving ovum fertilized with mixed sperm. The semidry fertilization method (Azari Takami, 2009) was used and the insemination dosage was 1 percent of egg volume for each fertilization experiment. Before each insemination, the excess of coelomic fluid was removed by pouring the eggs onto a screen suspended over a beaker. After eliminating eggs adhesiveness (Azarin Takami, 2009), eggs were placed in Yushchenko incubators in a running freshwater system at 17-20 °C and in the presence of more than 6 ppm of dissolved oxygen. In order to calculate the fertilization rate, three hours after fertilization, 100 eggs were randomly removed and preserved in formalin 10 % solution where monospermic percentage was considered only for the eggs reached four-cell stage.

Blood Sampling and Steroid Analysis

Before injecting the female brood fish with acetone-dried sturgeon pituitary, blood samples (2 ml) were collected from the caudal vein using a syringe and kept in nonheparinized vials on ice until separated by centrifugation at the end of the day. After separation (5000g for 20 min), all sera were stored at -45°C while awaiting transfer to the chemistry laboratory of Gorgan University of Agricultural Sciences and Natural Resources. Serum testosterone, 17-β estradiol and progesterone levels were measured using enzyme linked immuno-sorbent assay (ELISA) as described by Matsuyama *et al* (1998).

Statistical Analysis

The obtained data were subjected to one-way analysis of variance (ANOVA) and the significant difference between the treatments was determined by Duncan's test. The differences between means were analyzed at the 5 % probability level (P value of less

than 0.05 was considered as statistically significant). All data are reported as means±standard deviation. All analyses were performed using statistical software (SPSS version 16.0, SPSS Inc, Chicago, IL). The correlation between culture media and fertilization rate were analyzed using the bivariate correlation coefficients of Pearson (SPSS version 16.0). Multiple regressions were used to determine the relationship between fertilization rate and sexual hormones and also between fertilization rate and incubation time.

Results

The obtained results showed that there was a significant difference between fertilization rate of oocytes incubated in SIS medium after 12 and 30 h of incubation ($P<0.05$). Table 1 shows the average of fertilization rate in Persian sturgeon (*Acipenser persicus*). Fertilization rate decreased significantly with incubation time in SIS medium averaging $84\pm 5.65\%$ after 12 h and reaching $8.3\pm 14.43\%$ after 30 h. Thus, samples incubated in SIS medium after 12 h showed significantly higher fertilization rate than the samples in any of the other groups ($P<0.05$).

The fertilization rate decreased significantly with increasing duration of incubation of oocytes in RM2 medium ($P<0.05$), so that it declined from $83\pm 7.07\%$ after 12 h to $23.50\pm 33.23\%$ after 24 h of incubation but this difference was not significant between 24 and 30 h of incubation ($P>0.05$).

Significant differences were observed among fertilization rates of oocytes of L-15 medium ($P<0.05$), while the difference between 24 and 30 h of incubation was not significant ($P>0.05$). In the case of RM2 medium, a significant difference was only detected between 12 and 24 h treatments, in which 12 h treatment showed significantly higher level ($P<0.05$).

The results showed that the fertilization rate of oocytes of PSACF medium decreased with increasing duration of incubation and the highest fertilization rate was observed after 12 h of incubation; however, this difference was not significant.

The correlations between culture media (SIS, RM2, L-15 and PSACF) and fertilization rate of Persian sturgeon (*Acipenser persicus*) are presented in table 2. As shown in Table 2, the relationship between SIS, RM2 and L-15 media with fertilization rate was significantly reversed ($P<0.05$). Additionally, the highest correlation was observed between fertilization rate and SIS medium ($R^2 = -0/763$ (

Table 1. Fertilization rate of Persian sturgeon (*Acipenser persicus*) oocytes in the SIS, RM2, L-15 and PSACF medium

Incubation medium	12 hr	24 hr	30 hr
SIS	84 ± 5.65^a	51.60 ± 29.2^{ab}	8.3 ± 14.43^b
RM2	83 ± 7.07^a	23.50 ± 33.23^b	30 ± 25.93^b
L-15	83 ± 7.07^a	34.40 ± 33.85^{ab}	26.25 ± 22.50^b
PSACF	70.50 ± 24.74^a	45.50 ± 32.80^a	16.66 ± 14.43^a

Different letters denote a significant difference in average of fertilization rate at the same row ($P<0.05$)

) $P < 0.05$).

Linear regression equations and relationship between incubation time and fertilization rate of oocytes of SIS, L-15, PSACF and RM2 media are presented in Figure 1. There was a significant inverse relationship between both parameters ($R^2 = 0.5816$; $P < 0.05$). There were significant relationships between fertilization rate of oocytes of L-15 ($R^2 = 0.3989$; $P < 0.05$), PSACF ($R^2 = 0.4246$; $P < 0.05$) and RM2 ($R^2 = 0.4179$; $P < 0.05$) media and incubation time. As incubation time increased, lower values for fertilization rate of oocytes of SIS, L-15, PSACF and RM2 media were observed. The highest correlation with fertilization rate was observed in oocytes incubated in SIS medium.

The linear correlation between sexual hormones levels (17- β estradiol, progesterone and testosterone) and fertilization rate of *Acipenser persicus* is presented in Figure 2. No significant correlations were observed between 17- β estradiol ($R^2 = 0.0134$), progesterone ($R^2 = 0.0159$) and testosterone ($R^2 = 0.0652$) and fertilization rate ($P > 0.05$).

Discussion

Artificial reproduction of sturgeon is based on the hormonal stimulation of gamete maturation (Goncharov *et al.*, 2001). The objective of induced ovulation with hormone injection in aquaculture is to produce a large supply of high quality eggs. The stripping time in mature fish affects egg fertility, embryo survival and hatching rates and successful larval development (Lee & Yang, 2002). Therefore, creating a reliable technique for obtaining a large number of high quality eggs is important in larvae production of sturgeons (Omoto *et al.*, 2005).

Studying the response of Persian sturgeon oocytes to 17 α , 20 β -Dihydroxyprogesterone hormone by in vitro maturation, significant differences were found in the fertilization rate of oocytes incubated in SIS medium after 12 and 30 h. In SIS medium and also the other media, fertilization rate decreased with increasing duration of incubation, so that fertilization rate after 30 h of incubation in SIS medium was 8.3 %, RM2 30 %, L-15 26.25 % and PSACF 61 %.

A relatively strong correlation was found between fertilization rate and the time of progesterone-induced in vitro oocyte maturation in Persian sturgeon. The results reported in figure 1 showed that there was a significant inverse relationship between incubation time and fertilization rate in the four investigated media. Therefore, if eggs are not collected artificially from ovulated fish, the quality of eggs will critically decrease and fail to fertilize. Moreover, accurate prediction of both maturation and ovulation of brood fish following hormone injection is a key factor for the artificial propagation of farmed sturgeons. This observation appeared promising to be used as a quantitative characteristic of the reaction of oocytes to 17 α , 20 β -

Dihydroxyprogesterone for estimating the physiological status of the oocytes and as an index of the reproductive potential of the females selected for breeding. This criterion will contribute to the improvement of breeding management of Persian sturgeon.

Culture medium composition affects the hormonal induction of oocyte maturation (Goncharov, 2003). In order to gain insights into the importance of medium composition on incubation of oocytes, the effects of medium type and also its relationship with fertilization rate were evaluated. SIS medium was designed on the basis of blood ionic composition of Siberian sturgeon (*Acipenser baeri brandt*) and the obtained results supported the high efficiency of this medium. For simulating ovarian fluid, its mean composition was determined. Based on the results, a specific PSACF (Persian Sturgeon Artificial Coelomic Fluid) was designed that can be made available to hatchery operators. According to our results, SIS medium has a better performance than PSACF medium. Abe *et al.* (2010) evaluated the role of protein (bovine serum albumin) on in vitro maturation, ovulation and incubation of eggs from Japanese eel, *Anguilla japonica*, but found little effect (Abe *et al.*, 2010). Garczynski and Goetz (1997) identified elevated levels of ovulatory proteins in the coelomic fluid of rainbow trout, secreted by the granulosa layer. However, the proposed function of these anti-proteolytic proteins was deemed to protect eggs from bacterial infection, rather than contributing to maintaining viability. Also, Lahnsteiner (2007) concluded that ovarian fluid proteins poorly correlated with egg quality.

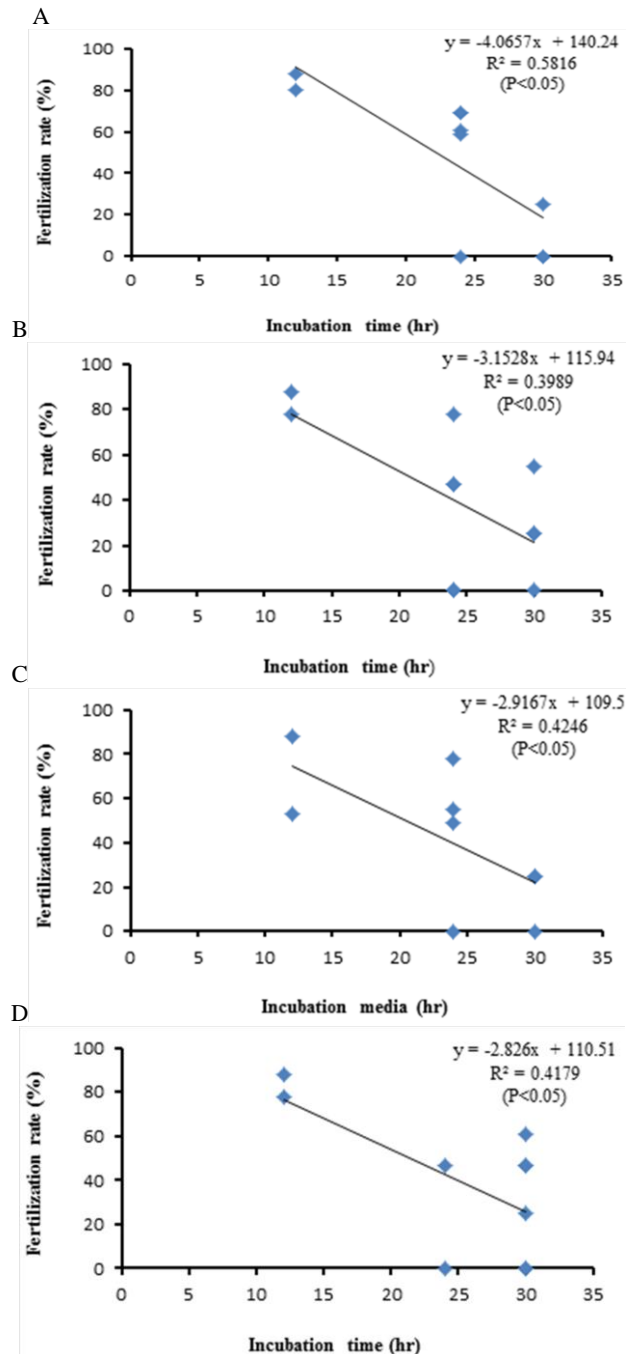
L-15 medium contains all components necessary for supporting the metabolism of isolated cells. Also, the Ringer solution modified with 0.5 g of sodium bicarbonate becomes more effective on GVBD and this effect of the medium is related to the direct influence of sodium bicarbonate on the oocyte rather than the changes of the medium physicochemical properties (Goncharov, 2003). In SIS, L-15 and PSACF media, no differences were found between fertilization rate of oocytes after 12 and 24 h of incubation, but in the RM2 medium, a significant difference was observed between fertilization rate of oocytes incubated for 12 and 24 h; therefore, the time required for 17 α ,20 β -Dihydroxyprogesterone-induced maturation is affected by the medium composition.

Three sex steroid hormones, 17 β -estradiol (E2), 11-ketotestosterone (11-KT), and 17 α , 20 β -dihydroxy-4-pregnen-3-one (DHP), are well established as primary estrogen, androgen, and progestin in teleost fish, respectively. In vitro and in vivo assays suggest that 11-KT and E2 play primary roles in previtellogenic and growth of oocytes, respectively, whereas DHP is essential for induction of final oocyte maturation (Kazeto *et al.*, 2011). A number of studies have shown that gonadal steroids act via a classical negative feedback loop to prevent

Table 2. Correlation between culture media and fertilization rate of Persian sturgeon (*Acipenser persicus*)

	PSACF	L-15	RM2	SIS
Fertilization rate	-0.652	-0.632*	-0.646*	-0.763*

*(P<0.05)

**Figure 1.** The relationship among the fertilization rate and incubation time of SIS medium (A), L-15 medium (B), PSACF medium (C) and RM2 medium (D) during in vitro maturation of Persian sturgeon (*Acipenser persicus*) oocytes induced by 17 α ,20 β -Dihydroxyprogesterone.

GTH release from the mammalian pituitary. In this regard, testosterone was shown to inhibit GTH secretion by impairing pulsatile release of GnRH. Testosterone has also been shown to stimulate GTH

secretion by acting directly at the level of the pituitary. Both negative and positive effects of gonadal steroids on GTH production have also been demonstrated in teleost species (Habibi *et al.*, 1998;

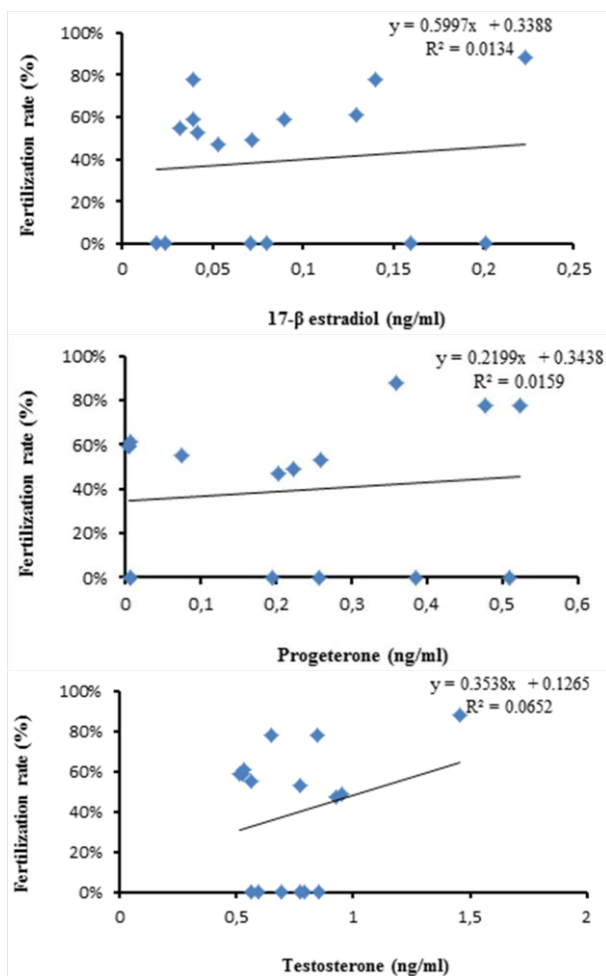


Figure 2. The relationship among sexual hormones, 17-β estradiol (A), progesterone (B) and testosterone (C) and fertilization rate of Persian sturgeon (*Acipenser persicus*) during reproductive migration to the south-east of Caspian Sea.

Aramli *et al.*, 2014). In the present study, blood samples were collected before injecting females with acetone-dried sturgeon pituitary and so plasma levels of sex steroids were recorded low prior to vitellogenesis. During vitellogenesis, there is a gradual increase in plasma E2 levels in females with matching patterns of testosterone (T). Plasma E2 levels peak towards the end of vitellogenesis and they decline rapidly in the maturation phase. Plasma T levels decline as oocyte maturation proceeds, whereas plasma maturation-inducing hormone (DHP) levels rise rapidly (Heidari *et al.*, 2010). Lutes (1985) reported that 17,20β-P stimulated 100% GVBD in white sturgeon follicles incubated for 24 h at a concentration of 31 ng.ml⁻¹, while 17-OH progesterone, progesterone (P4), and deoxycorticosterone stimulated 100% maturation at 62 ng.ml⁻¹.

Conclusion

The comparison of the obtained results regarding the application of four media- L-15, SIS, RM2 and PSACF- demonstrates that the relative efficiency of

each medium in supporting the reaction of oocytes to 17α, 20β-Dihydroxyprogesterone hormone depends on medium composition and hormone concentration. Our results support the role of 17α, 20β-Dihydroxyprogesterone as a maturation inducing steroid (MIS) in Persian sturgeon, and indicate that postvitellogenic oocytes of Persian sturgeon have the ability to respond to this steroid and to undergo final maturation in the presence of 17α, 20β-Dihydroxyprogesterone at 1 μg.ml⁻¹ concentration. Similar results were reported by Williot (1997). The difference in steroid potencies in various studies is probably related to the bioassay design, including the incubation time and the developmental stage of collected follicles.

References

- Abe, T., Ijiri, S., Adachi, S. and Yamauchi, K. 2010. Development of an in vitro culture system for the production of larvae from immature ovarian follicles of Japanese eel (*Anguilla japonica*). Fish Science, 76: 257–265. doi: 10.1007/s12562-010-0216-8.
- Aramli, M.S., Kalbassi, M.R., Nazari, R.M. 2014. Sex steroid levels of Persian sturgeon, *Acipenser persicus*,

- Borodin, 1897, males in negative and positive responding to LH-RH-analogue. *J. Appl. Ichthyol.* 30: 18–19. doi: 10.1111/jai.12347.
- Azari Takami, G. 2009. *Breeding and Cultivation of Sturgeon Caviarian Fish.* (2nd ed.). Tehran University Press, Tehran, Iran.
- Bahmani, M., Kazemi, R. and Donskaya, P. 2001. A comparative study of some hematological features in young reared sturgeons, *Acipenser persicus* and *Huso huso*. *Fish Physiology and Biochemistry*, 24: 135–140. doi: 10.1023/A:1011911019155.
- Billard, R. and Lecointre, G. 2001. Biology and conservation of sturgeon and paddle fish. *Fish Biology and Fisheries*, 10: 355–392. doi: 10.1023/A:1012231526151.
- Dumont, H.J. 1998. The Caspian Lake: history, biota, structure, and function. *Limnology and Oceanography*, 43: 44–52.
- Garczynski, M. and Goetz, F.W. 1997. Molecular characterization of a ribonucleic acid transcript that is highly upregulated at the time of ovulation in the brook trout, *Salvelinus fontinalis* ovary. *Biology of Reproduction*, 57: 856–864. doi: 10.1095/biolreprod57.4.856.
- Goncharov, B.F. 2003. Application of the model system of hormonal stimulation of the sturgeon oocyte maturation and ovulation in vitro for solving some fundamental and applied problems. *Russian Journal of Developmental Biology*, 34: 75–83. doi: 10.1023/A:1023392112217.
- Goncharov, B.F., Williot, P. and Le Menn, F. 2001. Comparison of the effects of gonadotropic preparations of the carp and stellate sturgeon pituitaries on in vivo and in vitro oocyte maturation in the siberian sturgeon, *Acipenser baeri Brandt*. *Russian Journal of Developmental Biology*, 5: 320–327. doi: 10.1023/A:1012364702687.
- Habibi, H.R. and Huggard, D.L. 1998. Testosterone regulation of gonadotropin production in goldfish. *Comparative Biochemistry and Physiology*, 119: 339–344. doi:10.1016/S0742-8413(98)00022-X.
- Heidari, B., Roozati, S.A. and Yavari, L. 2010: Changes in plasma levels of steroid hormones during oocyte development of Caspian Kutum, *Rutilus frisii kutum*. *Animal Reproduction*, 4:373–381.
- Kazanskii, B. N., Feklov, Yu.A., Podushka, S.B. and Molodtsov, A.N. 1978. Express-Method for Estimation of the Degree of Gonad Maturity in Sturgeon Breeders. *Ryb Khozvo*, 2: 24–27.
- Kazeto, Y., Tosaka, R., Matsubara, H., Ijiri, S. and Adachi, S. 2011. Ovarian steroidogenesis and the role of sex steroid hormones on ovarian growth and maturation of the Japanese eel. *Journal of Steroid Biochemistry and Molecular Biology*, 43: 1–6. doi:10.1016/j.jsbmb.2011.03.013.
- Lahnsteiner, F. 2007. First result on a relation between ovarian fluid and egg proteins of *Salmo trutta* and egg quality. *Aquaculture Research*, 38: 131–139. doi: 10.1111/j.1365-2109.2006.01633.x.
- Lee, W.K. and Yang, S.W. 2002. Relationship between ovarian development and serum levels of gonadal steroid hormones, and induction of oocyte maturation and ovulation in the cultured female Korean spotted sea bass, *Lateolabrax maculatus*. *Aquaculture*, 207: 196–183. doi:10.1016/S0044-8486(01)00728-1.
- Lutes, P.B. 1985. Oocyte maturation in white sturgeon, *Acipenser transmontanus*: some mechanisms and applications. In: *North American Sturgeons*. pp. 87–92. Edited by F.P. Binkowski & S.I. Doroshov. Dr. W. Junk Publishers, Dordrecht, Netherlands.
- Lutes, P.B., Doroshov, S.I., Chapman, F. Harrah, J., Fitzgerald, R. and Fitzpatrick, M. 1987. Morpho-physiological predictors of ovulatory success in white sturgeon, *Acipenser transmontanus* Richardson. *Aquaculture*, 66: 43–52. doi:10.1016/0044-8486(87)90282-1.
- Matsuyama, M., Ohta, K., Morita, S., Hoque, M.M., Kagawa, H. and Kambegawa, A. 1998. Circulating levels and in vitro production of two maturation-inducing hormones in teleost: 17 α ,20 β -dihydroxy-4-pregnen-3-one and 17 α ,20 β ,21-trihydroxy-4-pregnen-3-one, in a daily spawning wrasse, *Pseudolabrus japonicus*. *Fish Physiology and Biochemistry*, 19: 1–11. doi:10.1023/A:1007761729290.
- Meunpol, O., Iam-Pai, S., Suthikrai, W. and Piyatiratitivorakul, S. 2007. Identification of progesterone and 17 α -hydroxyprogesterone in polychaetes (*Perinereis* sp.) and the effects of hormone extracts on penaeid oocyte development in vitro. *Aquaculture*, 270: 485–492. doi:10.1016/j.aquaculture.2007.05.031.
- Moghim, M., Vajhi, A.R., Veshkini, A. and Masoudifard, M. 2002. Determination of sex and maturity in *Acipenser stellatus* by using ultrasonography. *Journal of Apply Ichthyology*, 18: 325–328. doi: 10.1046/j.1439-0426.2002.00423.x.
- Mohagheghi Samarini, A., Mojazi Amiri, B., Soltani, M., Nazari, R. M., Kamali, A. and Naghavi, M.R. 2011. Effects of storage duration and storage temperature on viability of stored ova of kutum, *Rutilus frisii kutum* in ovarian fluid. *African Journal of Biotechnology*, 10:12309–12314.
- Mojazi Amiri, B., Maebayashi, M., Omoto, N., Adachi, S. and Yamauchi, K. 2001. In vitro oocyte maturation in a hybrid sturgeon, Bester: changes in the germinal vesicle breakdown and 17, 20 β -dihydroxy-4-pregnen-3-one production. *Journal of Agriculture Science Technology*, 3: 199–207.
- Nazari, R.M., Sohrabnejad, M. and Ghomi, M.R. 2009. The effect of maternal size on larval characteristics of Persian sturgeon, *Acipenser persicus*. *Aquaculture Research*, 40: 1083–1088. doi: 10.1111/j.1365-2109.2009.02202.x.
- Omoto, N., Maebayashi, M., Adachi, S., Arai, K. and Yamauchi, K. 2005. The influence of oocyte maturational stage on hatching and triploidy rates in hybrid (bester) sturgeon, *Huso huso × *Acipenser ruthenus*. *Aquaculture*, 245: 287–294. doi:10.1016/j.aquaculture.2004.11.008.*
- Semenkova, B., Bayunova, L.V., Webb, M.A.H., Kolmakov N.N., Romanov, A.G. and Barannikova, L.A. 2006. Effect of progestins on germinal vesicle break down in sturgeon follicles in vitro. *Journal of Apply Ichthyology*, 22: 353–357. doi: 10.1111/j.1439-0426.2007.00983.x.
- Sohrabnezhad, M., Kalbassi, M.R., Nazari, R.M. and Bahmani, M. 2006. Short-term of Persian sturgeon, *Acipenser persicus* ova in artificial media and coelomic fluid. *Journal of Apply Ichthyology*, 22: 395–399. doi: 10.1111/j.1439-0426.2007.00993.x.
- Taati, M., Jafaryan, H. and Mehran, B. 2011. Effects of extenders, temperature and holding duration on short-

- term storage of unfertilized eggs of Common Carp, *Cyprinus carpio*. Australian Journal of Basic and Applied Sciences, 5: 146–152.
- Talbott, M., Van Eenennaam, P., Linares-Casenave, J., Doroshov, I., Guy, S., Struffenegger, P. and Webb, A.H. 2011. Investigating the use of plasma testosterone and estradiol-17 β to detect ovarian follicular atresia in farmed white sturgeon, *Acipenser transmontanus*. Aquaculture, 315: 283–289. doi:10.1016/j.aquaculture.2010.10.041.
- Webb, A.H., Feist, W., Trant, M., Van Eenennaam, S., Fitzpatrick, B., Schreck. and Doroshov I. 2002. Ovarian steroidogenesis in white sturgeon, *Acipenser transmontanus* during oocyte maturation and induced ovulation. General and Comparative Endocrinology, 129: 27–38. doi:10.1016/S0016-6480(02)00508-7.
- Williot, P. 1997. Effects of incubation media on maturation of isolated ovarian follicles of siberian sturgeon, *Acipenser baeri Brandt* induced by sturgeon gonadotropic preparation or 17 α ,20 β -Dihydroxyprogesterone. Comparative Biochemistry and Physiology, 3: 285–293. doi:10.1016/S0742-8413(97)00178-3.