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Changes in Total Calcium of Persian Sturgeon, *Acipenser persicus* Follicles during Different Stages of Germinal Vesicle Migration

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ABSTRACT

The changes of total calcium content of Persian sturgeon, *Acipenser persicus* follicles were investigated during different stages of germinal vesicle migration. Total calcium content increased during maturation and ripening of the oocytes i.e. migration of nucleus toward the animal pole. According to data, the total calcium of follicles with Polarization Index (PI: the ratio of the distance of the germinal vesicle from the animal pole over the animal-vegetal oocyte diameter×100) less than 5.2 (group I) were significantly higher than those in groups with 5.7< PI< 8.1 (group II) and PI> 9.4 (group III). Also, there were no significant differences observed in total calcium content of follicles with PI> 9.4 (group III) and follicles retained for 20 days in body cavity with PI> 10.5 (group IV). As well, there was a significant negative relationship observed between PI values and total calcium content of follicles. It is concluded that calcium is accumulated during the final oocyte maturation in Persian sturgeon.

Keywords: Calcium, Germinal vesicle migration, Persian sturgeon.

INTRODUCTION

Calcium is vital to the development and growth of fish skeleton (bony structure), maintenance of osmotic equilibrium, muscular activity and functioning of the nervous system. It is also involved in blood coagulation and the regulation of hormonal secretions (calcitonin, prolactin, catecholamines, parathyroid, etc.) (Guillaume *et al.,* 2001).

In addition to the above functions, calcium could also be involved in oocyte maturation and fertilization of aquatic invertebrates as well as in vertebrates. In fertilization, calcium content rises suddenly from a specific point in the egg (intracellular stores), and then is expanded as an extensive calcium wave all over the cytosol, a phenomenon known as egg activation (Berridge, 1997; McDougall *et al.,* 2000; Bootman *et al.,* 2001; Marchant and Parker, 2001; Whitaker, 2005). In addition to intracellular stores, it hasbeen observed that the extracellular calcium could be used in this process (Deguchi Osanai, and Morisawa, 1996; Mcguiness *et al.,* 1996). This phenomenon, being simultaneous to the entrance of sperm into the egg, controls and mediates critical physiological functions. In this regard, calcium ion causes gene expression, prevention from occurrence of polyspermy, and causes embryonic development in eggs (Busa and Nuccitelli, 1985; Nuccitelli *et al.,* 1993; Lorca *et al.,* 1993; Morin *et al.,* 1994). Also, calcium causes activation of such enzymes as calmodulin (CAMK) which in turn inactivate cytostatic factor that shifts cell cycle from meiotic to mitotic phase (Lorca *et al*., 1993; Morin *et al*., 1994). Several

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studies have shown that calcium is essential for nuclear maturation of oocytes of aquatic invertebrates (Steinhardt *et al.,* 1974; Baker and Whitaker, 1978; O'Connor *et al.,* 1977; Hollinger *et al.,* 1977; Gilkey *et al.,* 1978). Nuclear maturation characterized by Germinal Vesicle Breakdown (GVB) can be induced either by adding extra calcium with ionophore A23187 (Wasserman and Masui, 1975; Schuetz, 1975) in the culture medium, or by direct application of calcium through ionophoresis on the oocyte (Hollinger *et al.,* 1977; Moreau *et al.,* 1976). In *Xenopus* (an Amphibian), it has been suggested that during stages of egg maturation, calcium is stored in intracellular storages and at fertilization, these calcium stocks are used by mature eggs to sustain global calcium wave (Wassim *et al.,* 2005). Persson *et al.* (1998) reported that during spawning migration, necessity of calcium is evident for gonad development and accumulates in gonads of Atlantic salmon, *Salmo salar,* gradually. Persian sturgeon, *Acipenser persicus,* is an anadorumous valuable chondrostei fish of Caspian Sea which during the few past decades many attempts have been made to augment their stocks in terms of supportive breeding in Iran. In this study, with regard to the importance of calcium in egg maturation, the changes of egg calcium ion of Persian sturgeon was investigated in stage IV of maturation, i.e. during nuclear migration to the animal pole.

MATERIALS AND METHODS

Sampling

The experiments were carried out at Dr. Beheshti Artificial Sturgeon Propagation and Rearing Center (BASPRC) near Sangar dam in Rasht Province, Iran, during spawning season from April to May in 2006. Female brooders of *A. persicus* (119–159 cm total length and 17–20.5 kg weight) were captured from the Sefidroud River as well as from Caspian Sea, and then transferred to the broodstock pond of the hatchery at BASPRC. The follicle sampling was done in two steps of:

Step 1

In this stage, the basis of the follicle sampling was polarization of germinal vesicle toward the micropile during oocyte maturation. In this regard, follicle batches were taken from 18 untreated female brooders by a sharp striated metal tube (the diameter of the tube fitting the diameter of follicle) (each follicle batch was taken from one female separately). Afterwards, from each follicle batch, 30 follicles, with the same diameter were sampled, then, according to Billard (2000) 5 follicles processed to determine Polarization Index (PI: the ratio of the distance of the germinal vesicle from the animal pole over the animal-vegetal oocyte diameter×100). Finally, according to PI value, the follicles were divided into the following three groups: group I: immature follicles having PIs more than 9.4; group II: follicles having PIs ranging from 5.7 to 8.1. and group III: follicles with PIs less than 5.2 (Table1).

Step 2

In this stage, the follicles were sampled from female brooders which in spite of remaining for 20 days in tanks had PIs more than 10.5. Indeed, the germinal vesicle migration was not commenced in the oocytes of these fish. This group was numbered as group IV (Table1).

Preparation of Eggs and Measurement of Total Calcium Content

After being rinsed with distilled water to remove blood and other wastes, follicles were dried for 48 hours at 40°C, using plus oven. Then the dried follicle batches were crushed and homogenized through mortar. Total calcium content of follicles was

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Table 1. Total calcium content of follicle samples and means related to total calcium content of groups (I, II, II and IV).

Groups	Polarization index	Total calcium content of each sample (µg gr^{-1}dry matter)	Mean calcium content of each group (µg gr^{-1}dry matter)
	3.1	278.6	
	3.2	279.6	
I	3.8	262.2	263.23±15.09 [a]*
	4.1	261	
	4.3	259.6	
	5.2	238.4	
	5.7	219.4	
	6.2	203	
II	6.9	207.6	206.5±10.42 [b]
	7.3	208.4	
	7.7	212.2	
	8.1	188.4	
	9.4	164.2	
	10.1	161.18	
III	10.4	162.4	159.26±4.81 [c]
	11.3	159.6	
	12.8	156.6	
	13.2	151	
	10.5	89.2	
	11.1	86.2	
IV	12.2	72.8	156.6 ±15.2 [a]
	12.9	70.8	
	13.4	73.6	
	13.7	77.4	

* Different letters indicate statistical significance at $P< 0.05$ among groups (Mean±SD).

measured according to Fagbuaro *et al.* (2006) through atomic absorption spectrophotometer.

Statistical Analysis

SPSS software was employed to analysis the data. Data were normal according to Kolmogorov–Smirnov test. All parameters were expressed as means along with standard deviations recordings. One-way ANOVA was employed to analyze data. Then, means were compared through Tukey test. Also, Linear and non-linear regression models were investigated using regression fits. Then, the relationship between the total calcium content of follicles and Polarization Index was presented as linear plot.

RESULTS AND DISCUSSION

During stages of germinal vesicle migration, total calcium content of follicles of Persian sturgeon increased from 151 µg calcium gr^{-1} of dry matter in follicles with PI value of 13.2 to 278.6 µg calcium gr^{-1} of dry matter in follicles with PI value of 3.1 (Table 1). The means of total calcium of follicles with Polarization Index less than 5.2 (group I) were significantly higher ($P> 0.05$) than those of groups with $5.7< PI< 8.1$ (group II) and $PI> 9.4$ (group III). Also, there was no significant difference ($P< 0.05$)

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observed in total calcium content of follicles with PI> 9.4 (group III) and follicles retained for 20 days in body cavity with PI> 10.5 (group IV) (Table 1).

As well, there was a negative relationship observed between different stages of germinal vesicle migration (different PI values) and the values of total calcium content of follicles. In this regard, with decreasing PI value from 13.2 to 3.1, the total calcium content of follicles increased constantly (Figure.1; $r^2 = 0.92$, $P< 0.05$).

Findings of the study revealed that during the germinal vesicle migration (different PI values) in Persian sturgeon follicles, calcium ion accumulates in follicles. This calcium increment is probably absorbed from blood vessels, the only external substrate in contact with follicles. As well, in follicles of group IV (PI> 10.5) in spite of remaining for 20 days in body cavity, no calcium accumulation occurred with the average calcium content being equal to that in follicles of group III (PI> 9.4). Several studies have reported that calcium is essential for nuclear maturation of oocytes of aquatic invertebrates (Steinhardt *et al.,* 1974; Baker and Whitaker, 1978; O'Connor *et al.,* 1977; Hollinger *et al.,* 1977; Gilkey *et al.,* 1978) as well as in vertebrates such as rat (Satish *et al.,* 1980). In fish, the maturation characterized by germinal vesicle breakdown (GVB) can be induced either by adding extra calcium with ionophore A23187 (Wasserman and Masui, 1975; Schuetz, 1975) in the culture medium, or by direct application of calcium by ionophoresis on the oocyte (Hollinger *et al.,* 1977; Moreau *et al.,* 1976). Therefore, in this study, the accumulation of calcium during germinal vesicle migration may be associated with the swelling and dissolution of the germinal vesicle membrane of the oocyte, i.e. germinal vesicle breakdown. Researches on oocyte maturation of fish have suggested that the activation of MPF protein (Maturation Promoting Factor) by cdckinase enzyme is responsible for GVBD (reviewed by Nagahama, 1994). Thus, because of the accumulation of calcium during germinal vesicle migration of Persian sturgeon, it appears that calcium plays a key role in the pathway of cdckinase activation and subsequently the formation of MPF or a MPF-Like protein in sturgeons. Also, in fertilization, the egg activation depends on calcium wave which is created by release of calcium from intracellular storages of egg (Berridge, 1997; McDougall *et al.,* 2000; Bootman *et al.,* 2001; Marchant and Parker,

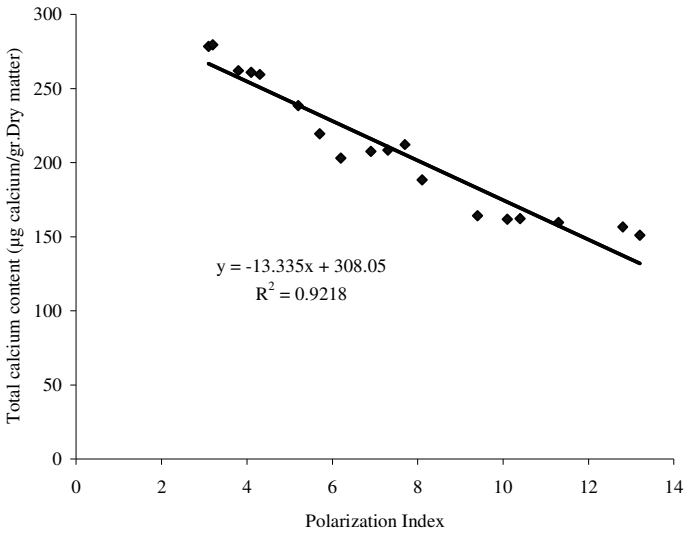

Figure1. Negative relationship between total calcium content of follicles and different values of Polarization Index (PI< 5 is for mature 5< PI< 8 for mid-mature and PI> 8 for immature follicles).

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2001; Whitaker, 2005). Therefore, the accumulated calcium during maturation may be used for calcium global wave at fertilization. According to the present data, the follicle batches of group IV did not show germinal vesicle migration and accumulation of calcium, in spite of remaining for 20 days in body cavity. This result emphases the relationship between maturation process and calcium uptake. At the end, it is concluded that the egg quality could be linked to its calcium content, since the mature eggs contained higher calcium content in comparison with immature eggs in Persian sturgeon.

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تغییرات محتوای کلسیم فولیکول های تاس ماهی ایرانی *Acipenser persicus* طی مراحل مختلف مهاجرت هسته

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چکیده

در این مطالعه تغییرات محتوای کلسیم کل فولیکول های تاسی ماهی ایرانی(*Acipenser persicus*) طی مراحل مختلف مهاجرت هسته بررسی شدند. مقادیر کلسیم کل طی رسیدگی تخمک ها یعنی مهاجرت هسته به سمت قطب حیوانی افزایش یافت. طبق نتایج، مقادیر کلسیم کل فولیکول های با شاخص قطبیت (شاخص قطبیت: نسبت فاصله هسته از قطب حیوانی به قطر تخمک (محور حیوانی- نباتی) × ۱۰۰) کمتر از ۵/۲ (گروه I) به طور معنی داری بالاتر از گروه های با شاخص قطبیت بین ۸/۱ - ۵/۷ (گروه II) و بالای ۹/۴ (گروه III) بودند. همچنین تفاوت معنی داری در مقادیر کلسیم کل بین فولیکول های با شاخص قطبیت بالای ۹/۴ و فولیکول های ۲۰ روز مانده در حفره شکمی(گروه IV) با شاخص قطبیت بالای ۱۰/۵ وجود نداشت. علاوه بر این همبستگی منفی و معنی داری بین مقادیر شاخص قطبیت و محتوای کلسیم کل فولیکول ها ثبت شد. نتایج این مطالعه بیان می کنند که کلسیم طی رسیدگی نهایی تخمک های تاس ماهی ایرانی در فولیکول ها تجمع می یابد.

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