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Research Article

Sox9 gene expression during ontogeny stages and various tissues of Persian sturgeon, *Acipenser persicus* (Borodin, 1897)

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Abstract: Sox9 is a protein-coding gene that plays a critical role during organogenesis and regulation of the male gonad development among vertebrates. Knowledge about the expression of Sox9 during larval development can help to complete the lack of the information on its possible function in sturgeon fishes. Due to the importance of the Persian sturgeon as a valuable candidate species for aquaculture and the importance of Sox9 gene in vertebrate evolution, in this study, Sox9 cDNA was characterized and the expression pattern during various ontogeny stages (unfertilized egg to 210 days post hatching - dph) and among ten different tissues (gill, pyloric, spleen, gonad, kidney, intestine, heart, skin, liver, muscle) was analyzed using RT-PCR method. Results indicated that Sox9 mRNA was not detect in unfertilized and fertilized egg. However, first expression was measured at 1 dph then Sox9 mRNAs decreased in a gradual manner during the 1 to 50 dph and declined to minimal level at 210 dph (P<0.05). Also, Sox9 mRNA was present in most tissues with the highest expression in the gill and pyloric, weakly expression in the liver and gonad and no expression in the muscle. Our findings suggest that Sox9 was not applicable in sex determination in early developmental stages and probably it can be implicated in other developmental activities such as chondrogenesis.

Keywords: Chondrogenesis, Embryonic stages, q-RT-PCR, Sex determination, Sturgeons.

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Introduction

Acipenseriformes fish are highly valuable species in aquaculture that hold a basal position in the evolutionary system of fishes and vertebrates. Until now, the 27 species of sturgeon, are considered endangered or threatened, those occur in the Caspian Sea, are in crisis and listed in CITES (Pourkazemi 2006). The Persian sturgeon, *Acipenser persicus* (Borodin, 1897) is one of the species that inhabits mostly the southern part of the Caspian Sea and due to economic value and early maturation compared to Beluga, has remarkable export and aquaculture value. The male and female of the Persian sturgeon reach sexual maturity at least 8 and 12 years, respectively (Yarmohammadi et al. 2011). Also, in most sturgeons, even after maturity, have no apparent sexual signs to identify male and female (Doroshov et al. 1997). Hence, finding a solution to determine male and female individuals in early stages will be a great help to sturgeon farming and caviar production industry. Moreover, the mechanisms involved in sex determination of sturgeon is still poorly understand.

Because the pathways of sex determination and differentiation share many conserved mechanisms among vertebrates, the investigation on the sex-determining pathway in one vertebrate can provide

insight to the pathways of many other vertebrates (Devlin & Nagahama 2002; Graves & Peichel 2010). Although mechanisms for sex determination vary from purely environmental influences to strict genetic regulation, the sex-determining pathways are surprisingly conserved within vertebrates from mammals to teleosts (Pandian 2012). Several researches have been conducted to finding a precise molecular biomarker for the sex determination of sturgeon (McCormick et al. 2008; Hale et al. 2010; Keyvanshokooh & Gharaei 2010; Yarmohammadi et al. 2011). Indeed, there is a lack of knowledge in sex-specific genes and related genes in sturgeon species.

In some mammalian biological models such as mouse, rabbit and also human, Y-chromosomal gene Sry (sex-determining region on the Y chromosome) plays a major role in testis formation (Kashimada & Koopman 2010). Gonads without a Y-chromosome and therefore without Sry develop as ovaries, while Sry protein induces testis differentiation in XY gonads (Siegfried 2010). Sox proteins contain a SRY-related high mobility group (HMG) box DNA-binding domain. In general, Sox-transcription factors play a key role in embryonic development of vertebrates and are also major determinants of stem cell behavior (Guth & Wegner 2008). The Sox family consists of nine groups (A-H) while the Sox9 belongs to the SoxE (Sox8, Sox9 and Sox10) gene family (Wegner 1999). Sox9 has been connected to male sexual development across several teleosts including zebrafish, medaka, rainbow trout and rice field eel (Takamatsu et al. 1997; Yokoi et al. 2002; Zhou et al. 2003; Jørgensen et al. 2008). Recent efforts revealed that sturgeon Sox9 was highly similar to other vertebrates, especially in the HMG box (Hett et al. 2005; Berbejillo et al. 2012).

Previous studies shown the involvement of Sox9 in sexual development of sturgeon but their results emphasized on advanced stages while its function remaining unclear in early developmental stages. We found it interesting to study Sox9 gene during ontogeny stages in Persian sturgeon, one of the most valuable species for produce of luxury caviar in Caspian-sea region. To reach of this goal, we obtained partial mRNA sequences of Sox9 and analyzed its relative gene expression in developmental stages and various tissues of *A. persicus*. This study provides fundamental information for analyzing the function of Sox9 in both development process and sex determination in sturgeon fishes.

Materials and Methods

Rearing condition: This study was performed at the International Sturgeon Research Institute (ISRI) during spawning season of Persian sturgeon (fall and winter 2014–2015). All Persian sturgeon fish were artificially propagated from wild brood stocks were caught from the Caspian Sea and reared in the propagation and rearing department of ISRI from unfertilized egg to 210 dph. Larvae from the day of hatching to 30 dph were reared at density of 40 larvae L^{-1}. After 30 dph, sturgeon was reared at the density of 50 fish m^{-3}. The Persian sturgeon larvae were fed live *Artemia nauplii* from 8 to 18 dph and then commercial starter feeds (Biomar, France). The water quality (temperature, pH, dissolved oxygen conditions) was controlled every day. The average water temperature was 22.0±0.82°C, pH 7.3±0.08, dissolved oxygen concentration 8±0.36mg L^{-1}. A 12 h light: 12 h dark photoperiod was applied during the entire experiment.

Samples collection: Different ontogeny stages (unfertilized egg - 1, 4 and 7-days post fertilization - 1, 3, 6, 14, 50 and 210-day post hatching) and ten tissues (gill, pyloric, spleen, gonad, kidney, intestine, heart, skin, liver, muscle) were collected. Fish were anaesthetized using clove powder (300mg L^{-1}). From 1 to 50 dph whole fish were sampled. On 210 dph gonad were dissected for subsequent analysis. Samples were immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

RNA isolation and Reverse Transcription: Total RNA was separately extracted from different ontogeny stages and tissues (50mg pooled sample) using BIOZOL reagent (Bioflax-Bioer, China)

Table 1. Primers used for RT-PCR and Gene discovery of Sox9 and RPL6 gene.

Gene	Sequences (5′→3′) (Forward/ Reverse)	TM(°C)	Product size (bp)	application
Sox9	GAAGCGTCCTTTCGTTGAAG AAGATCGCAGTGGGTGAGAT	58	210	Gene discovery
Sox9	AGCAGCAAAAACAAGCCTCA AGCTCCGCGTTGTGAAGAT	60	162	Real-time PCR
RPL6	AGCTGGGCAAGCCCAACACC TGGTGGAAGGCCAGGTCGCT	60	127	Real-time PCR

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  1  V  E  E  A  E  R  L  R  V  Q  H  K  K  D  H  P  D  Y  K  Y  20
  1 CGTTGAAGAGGCAGAGAGACTGAGGGTGCAGCACAAGAAAGATCACCCCGATTACAAGTA 60

 21  Q  P  R  R  R  K  S  V  K  N  G  Q  N  E  A  E  D  G  S  E  40
 61 CCAGCCGAGGAGAAGGAAGTCAGTGAAGAACGGGCAGAACGAAGCTGAAGACGGATCTGA 120

 41  Q  S  H  I  S  P  T  A  I  F  K  A  L  Q  Q  A  D  S  S  H  60
121 GCAAAGTCACATCTCACCCACTGCGATCTTCAAAGCGCTGCAACAGGCCGATTCCTCTCA 180

 61  S  A  S  S  M  S  E  V  H  S  70
181 CTCTGCGTCCAGCATGAGCGAGGTGCATTC 210
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Fig.1. cDNA nucleotide (GenBank Accession no.: KP300013.1) and predicted amino acid sequences of the Persian sturgeon Sox9 Nucleotides are indicated above and numbered to the left of each lane. The deduced amino acid sequence is shown above the nucleotide sequence. Amino-acids are numbered to the left of each lane (lower row).

according to producer recommendations. Quantity and quality of RNA were assessed by Nanodrop ND-1000 spectrophotometer (Nanodrop Technology, Wilmington, DE, USA) and agarose gel electrophoresis respectively. Samples were adjusted to a final concentration of 500ng/µL and 4µL of total RNA were reverse transcribed to cDNA.

cDNA was synthesized with using 4µg of *DNase* treated total RNA using a RevertAid Reverse Transcriptase kit (Thermo Scientific, Germany) with 1µL oligo $(dT)_{18}$. Complete removal of contaminating DNA was verified by PCR amplification of RNA samples using gene specific primers as a no-amplification control (NAC) [Cq (NAC control)−Cq (cDNA synthesis)>10] (Yarmohammadi et al. 2017). The resulting first-strand cDNA product was used as a template to amplify genes using a quantitative real time polymerase chain reaction (qRT-PCR) strategy.

Sequencing: The Sox9 cDNA was amplified by PCR. The specific forward and reverse primers for Sox9 genes (Sox9-F and Sox9-R) were designed according to highly conserved nucleotide regions of known Sox9 sequences from other sturgeons, including *A. sinensis* (KJ526295.1), *A. baerii* (KJ526295.1), *A. schrenckii* (AY573261.1), *Acipenser schrenckii* (AY581214.1), *A. sturio* (AY788912.1), *A. schrenckii* (AY627286.1) available on GenBank, a feature of the NCBI website, using the Oligo v5 and Primer 3 software (http://frodo.wi.mit.edu/). Ribosomal protein L6 (RPL6) transcripts were used as the housekeeping gene for normalization of Sox9 mRNA transcription (Akbarzadeh et al. 2011). The genes and primers are listed in Table 1.

All PCRs were performed on an Eppendorf thermal cycler (Mastercycler ep gradient, 96 plus, eppendorf, Germany). The fragment amplified for Sox9 gene were purified using the high pure PCR purification kit (Roche, Mannheim, Germany), and subsequently the amplicons were cloned with the CloneJET ^{TM} PCR Cloning Kit (Fermentas, France) and sequenced at a commercial sequencing facility (Bioneer, Daejeon, South Korea) in both directions. Sequence specificity was confirmed via a

Table 2. Percent identity of Acipenser persicus Sox9 sequence to other species.

Scientific name	% Identity	Accession number
Acipenser baerri	100	EU241882.1
Acipenser schrenckii	97	AY581214.1
Acipenser sinensis	97	KJ526295.1
Gallus gallus	79	AB012236.1
Danio rerio	80	NM_131643.1
Oncorhynchus mykiss	77	AB006448.1
Cyprinus carpio	80	AY956415.1
Mus musculus	78	NM_011448.4
Canis familiaris	77	AY237827.1
Homo sapiens	79	NM_000346.3

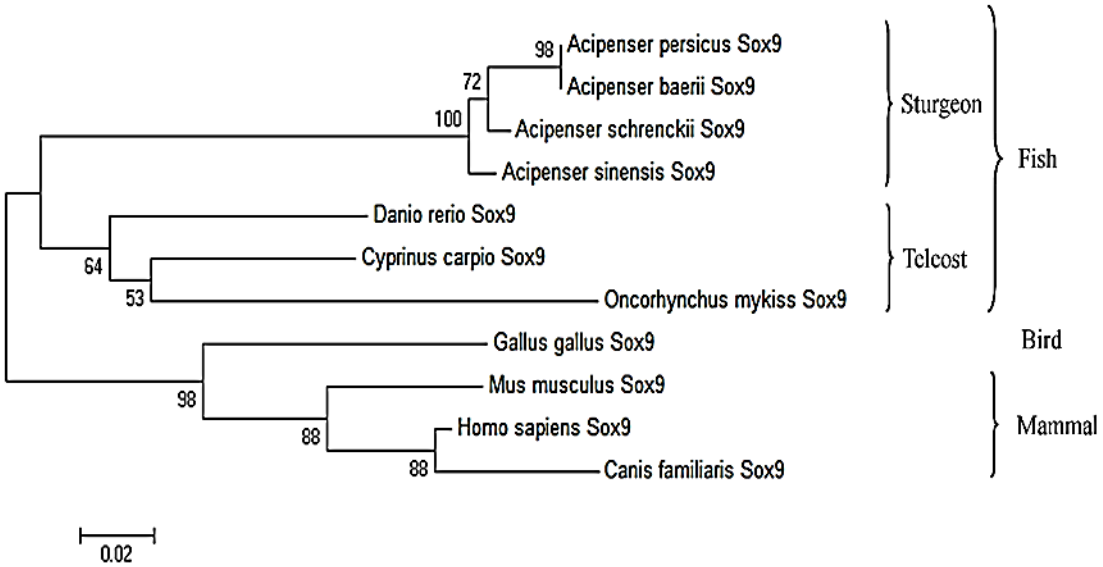

Fig.2. Phylogenetic analysis of Sox9 sequences inferred using the neighbor-joining method in vertebrates. Bootstrap values are indicated (1,000 replicates). The analysis involved 11 nucleotide sequences. Evolutionary analyses were conducted in MEGA6.

comparison of the homology to other sturgeon Sox9 sequences in the BLASTN database (http://blast.ncbi.nlm.nih.gov) (Altschul et al. 1990). The Partial cDNA sequences for Sox9 was characterized and deposited in the National Center for Biotechnology Information (http://www.ncbi. nlm. nih.gov/) (Fig. 1).

Phylogenetic analysis: The sequence of Sox9 gene was aligned with those from their vertebrate homologous by using CLUSTAL W (Trinity College Dublin, Dublin, Ireland). Phylogenetic trees based on the aligned sequences were constructed by the neighbor-joining method from 11 vertebrate species using the MEGA6 software (Table 2) (Tamura et al. 2013) and the bootstrapping test was performed with 1000 replications (Fig. 2).

Real time PCR: Relative *A. persicus* Sox9 mRNA expression levels were assessed by qRT-PCR in the CFX96 Real-Time PCR Detection System (Bio-Rad, USA). Reactions were performed in 12µl solution, with 1x SYBR® Green PCR master mix (Bioer-Bioflux), 2.5µM of ROX reference dye, 0.2µM of each primer with 2ng of cDNA template using standard protocol. The reaction conditions for the real-time PCR were: pre-denaturation at 94°C for 1 min; denaturation at 94°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 40 s; 40 cycles were performed. Melting curve analysis was performed after PCR to check the specificity and the identity of the target and absence of primer dimers

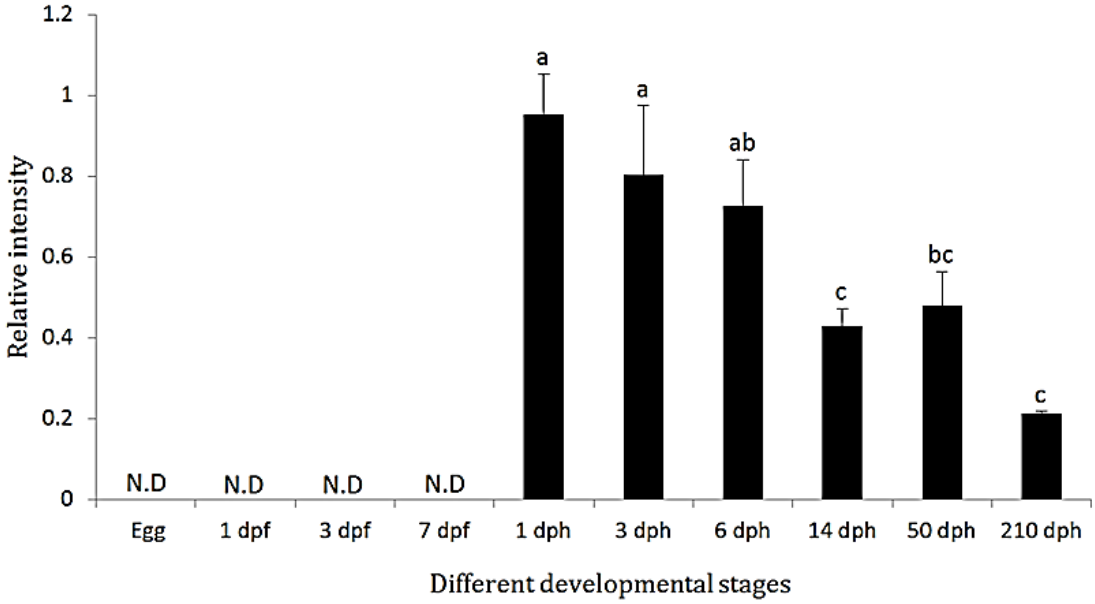

Fig.3. Expression of Sox9 during ontogeny stages of *Acipenser persicus*. RPL6 was used as RT-PCR control. The Sox9 gene expression values measured by Real Time PCR and Livak method. Data expressed as mean±SEM of each developmental stage. Differences between subscripts indicate significantly different means at (P<0.05).

and a no-template control (NTC) was included with each assay to verify that PCR master mixes were free of contamination (NTC>39) (Yarmohammadi et al. 2014). Dilution curves generated by serial dilutions (1:10) of cDNA were used to calculate amplification efficiencies. All samples were amplified in triplicates. RPL6 was chosen as housekeeping gene and it was used in experiment for data normalization due to its constant expression through different developmental stages (Akbarzadeh et al. 2011). The $2^{-\Delta\Delta Ct}$ method for relative gene expression analysis described by Livak & Schmittgen (2001) was used to calculate the gene expression values.

Statistical analysis: All quantitative data were presented as Mean±SEM. A Kolmogorov-Smirnov test was used to assess for normality of distributions. Normalized gene expression data passed Leven test for homogeneity of variance before one-way ANOVA. In cases where variances were not uniform, Statistical differences were estimated by Kruskal-Wallis test followed by post hoc Mann-Whitney *U* testes (a nonparametric analysis of variance) to compare the individual groups, and a probability level P<0.05 was used to indicate significance. All statistics were performed using SPSS 16.0 (SPSS, Chicago, IL, USA).

Results

Sox9 mRNA and phylogenetic analysis: To isolate the Persian sturgeon Sox9 cDNA, cDNA fragments were amplified by PCR using the two pairs of primers designed according to genomic fragment of other sturgeon Sox9 reported in the previous studies. The partial mRNA sequence of Persian sturgeon Sox9 was identified and submitted to the GenBank (accession no. KP300013.1). The isolated Sox9 cDNA is 210 bp long that encoding a 70 aa protein (Fig. 1). The identity percentage of Sox9 partial sequence was 97–100% compared to other sturgeon species and 77–80% compared to other teleosts (Table 2). The tree shows a high homology of Persian sturgeon Sox9 with other sturgeon species followed by other bony fish species, and finally other vertebrates (Fig. 2).

Ontogenic expression of Sox9 mRNA: To study the ontogeny of Sox9, the expression level was determined in 10 different developmental stages starting from unfertilized eggs to 210 days post-hatched larvae by qPCR using RPL6 as the internal

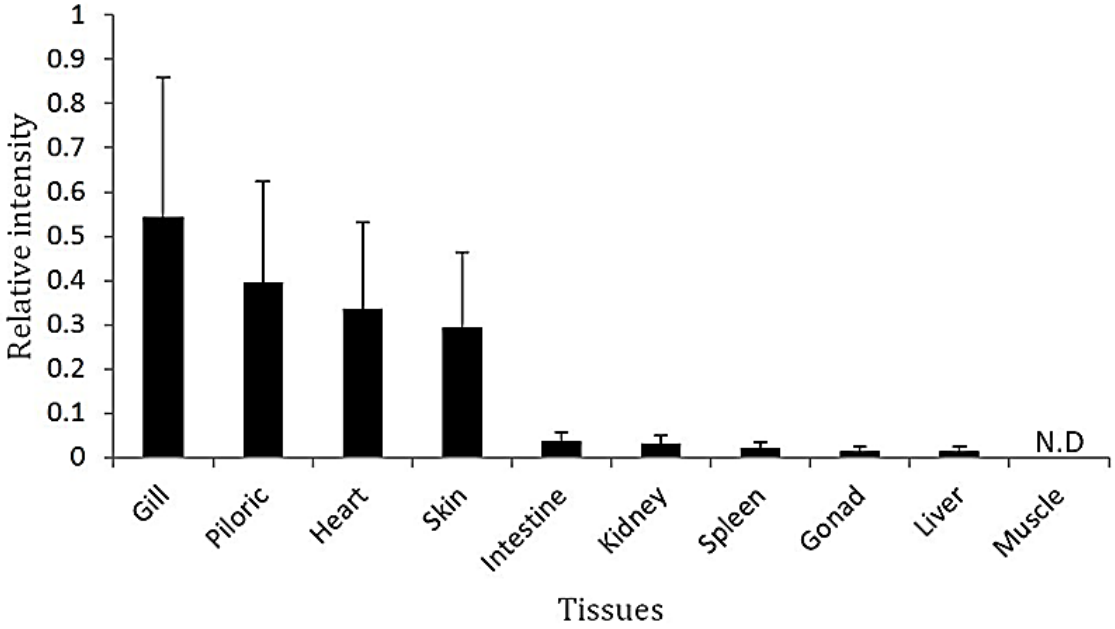

Fig.4. Expression of Sox9 in different tissue of *Acipenser persicus* juvenile. RPL6 was used as RT-PCR control. The Sox9 gene expression values measured by Real Time PCR and calculated by Livak method. Data expressed as mean±SEM of each tissues.

control (Fig. 3). No detection was found in initial embryonic stages including unfertilized and fertilized eggs (1 to 7 days post fertilizing). However, the first transcription of the Sox9 was detected after hatching. Sox9 mRNA expression level was not very stably expressed in larval stages as the expression level was found to be varied until 210 dph. Relative mRNA expression of Sox9 was highest at 1 to 3 days post-hatching and then Sox9 level decreased in a gradual manner during the 6 to 50 dph and declined to minimal level at 210 dph.

Tissue expression of Sox9 mRNA: The expression profile of Sox9 was determined in ten different tissues of *A. persicus* juveniles by q-RT-PCR using RPL6 as the internal control. The mRNA transcript of Sox9 could be detected in most tissues tested with the highest expression observed in gill followed by pyloric. Moderate level of Sox9 expression could be detected in heart and skin, whereas low transcript abundance was observed in intestine, kidney, spleen, gonad and liver (Fig. 4).

Discussion

Iran is one of the most famous producers of premium caviar in the world, and the Persian sturgeon is among main aquaculture candidate species in Caspian Sea. Gender identification is essential evaluation for the caviar industry, and early sex determination is economically desirable that leads to raise greater numbers of females with limited aquaculture resources. Previous reports on sex-determining mechanism in sturgeon suggested that these groups of fishes are gonochoristic and sex is genetically determined (Keyvanshokooh & Gharaei 2010). According to failure in DNA-based methods to finding a sex-specific marker (Keyvanshokooh & Gharaei 2010), the alternative method is based on sexual candidate genes expression. The Sox9 known as potential candidate gene that may connect to gonadal sex determination and differentiation in fishes. In the present study, for the first time the partial mRNA sequence of Sox9 of Persian sturgeon was cloned, and its expression was analyzed during ontogeny stages and among different tissues.

In numerus teleosts such as zebrafish, medaka, carp and rainbow trout reported two forms of Sox9 (Sox9a, and Sox9b), whereas observations on sturgeon, no evidence was detected for Sox9

duplication (Takamatsu et al. 1997; Chiang et al. 2001; Yokoi et al. 2002; Hett et al. 2005; Du et al. 2007). The sequencing results showed that Sox9 of Persian sturgeon share high levels of homology with those of other sturgeon species. Phylogeny analysis revealed three distinct branches, including fish, bird and mammals which fish group consists of two separate branches of sturgeon fish and the teleost fish. Further phylogenetic analysis in Atlantic and Siberian sturgeon illustrate that a basal position for sturgeon Sox9 during evolution (Hett et al. 2005; Berbejillo et al. 2012). Last investigation on great sturgeon (*Huso huso*) showed that the Sox9 mRNA shares high similarity to other sturgeon species while there is considerable homology relative to other vertebrates (Yarmohammadi et al. 2017). The distinctive position of the Sox9 sequence in the phylogeny tree has indicated that the sturgeon Sox9 differ from that of teleosts fishes and other vertebrates, including mammal and bird.

At present, there are a few studies focused on Sox9 expression during ontogeny of sturgeon. Our finding showed that the expression of Sox9 decreased gradually during embryogenesis and expression was highest after hatching time. Apparently, this pattern was observed during the early development of some teleost fish such as lambari fish, medaka, stickleback and zebrafish (Adolfi et al. 2015; Klüver 2007; Klüver et al. 2005). The spatiotemporal expression of Sox9 in medaka was mainly in craniofacial cartilaginous structures and the pectoral fins, while in stickleback in the mandibular and hyoid arches (Klüver et al. 2005). Strykowski (2011) studied the Sox9a expression throughout embryogenesis of Mangrove rivulus (*Kryptolebias marmoratus*) and suggested that it may involve in cartilage forming. Hence, these results may also provide evidence for involvement of Sox9 in the development of cartilage structures in sturgeon.

Chondrogenesis is reported as one of the main functions of Sox9 among vertebrates. Acipenseriformes are ancient fishes with a largely cartilaginous endoskeleton, a feature that is unique among the fishes. Sox9 is the initial identified nuclear factor that already expressed in mesenchymal condensations prior to chondrogenesis, and is importance for subsequent differentiation into chondrocytes (Franke 2017). Cartilage forming in *A. persicus* started just after hatching time and then to complete absorption of yolk-sac reserves, development of chondrogenesis related to feeding, swimming and respiratory activity occurred in larvae (Eshaghzadeh et al. 2018). Higher expression in the hatching time suggests that the Sox9 is likely to be involved in cell differentiation such as cartilage forming rather than sex determination as described in Russian sturgeon larvae (Kamaszewski et al. 2017). To confirm if the expression pattern of Sox9 in embryonic stages is directly related to chondrogenesis, future functional experiments have to be performed.

In the current study, the transcription of Sox9 was found in most organs, as previously reported on other sturgeons (Table 3) (see Chen et al. 2006; Berbejillo et al. 2013; Burcea et al. 2018), although there were no significant variations in the expression among tissues. Additionally, the tissues expression of the Sox9 has been studied in some species of teleosts that results suggest that this gene has a multi-functional role in fishes and that Sox9 pathways remain to be established in each case (Klüver et al. 2005; Du et al. 2007; Liu et al. 2007; Luo et al. 2010).

Differences in expression domains of Sox9 observed between species of sturgeon is likely due to species specific specialization of function or could simply be a reflection of physiological condition of the tested animals, because the time of season, the age of the samples, maintenance conditions, and even nutritional considerations can all affect gene expression (McCormick et al. 2008). The conclusions of Sox9 expression in various tissues are not consistent even in the same species (Luo et al. 2010). Therefore, we focused on its expression pattern in gonad.

Failed attempts to isolate a teleostean equivalent to the mammalian testis determining factor SRY

Table 3. Tissue expression of Sox9 in different sturgeon species.

Tissues	Persian sturgeon	Amur sturgeon	Siberian sturgeon	Best Beluga hybrid sturgeon
Gill	+++	N.R	++	N.R
Pyloric	++	N.R	N.R	N.R
Heart	++	+	N.R	N.R
Skin	++	N.R	N.R	N.R
Spleen	+	+	N.R	N.R
Kidney	+	++	+	+
Liver	+	++	+	+
Intestine	+	N.R	N.R	N.R
Muscle	-	N.R	+++	-
Eye	N.R	+++	N.R	N.R
Brain	N.R	+	+	N.R
Embryo	++	+	N.R	N.R
Testis	+++*	+++	++	++
Ovary	++*	++	+	++
Undifferentiated	+	N.R	+	N.R

N.R: No reported, +++: High expression, ++: Medium expression, +: Low expression, -: No expression, *: Unpublished data.

identified a SRY-related gene, Sox9 in fish, which is involved in mammalian sex determination down-steam of SRY (Morrish & Sinclair 2002). The previous study in teleost showed that Catfish Sox9a was expressed in testis, while Sox9b in ovary, as observed in zebrafish (Chiang et al. 2001; Raghuveer & Senthilkumaran 2010). In medaka, Sox9 was expressed in the ovary and Sox9a2 in the testis (Yokoi et al. 2002). In lambari fish and olive flounder Sox9 had higher expression in the testis (Adolfi et al. 2015; Wen et al. 2011). Moreover, Zhoue et al. (2003) showed that Sox9 was expressed in ovary, testis and ovatestis of rice field eel (*Monopterus albus*). Accordingly, these results suggest the possible role of Sox9 in sex determination and differentiation but the pattern expression in gonads seems to be somewhat diversified among fish species.

In current study, the Sox9 transcription was detected in undifferentiated gonad of Persian sturgeon (at 7 months old). Similarly, the same results of Sox9 expression was also noticed in juveniles of Russian sturgeon (Hagihara et al. 2014). In Siberian sturgeon, two group of fishes with either high or low Sox9 expression was observed (Vizziano-Cantonnet et al. 2016). On the contrary, in Great sturgeon, Sox9 mRNA was not expressed in undifferentiated gonad, although the low level of Sox9 was measurable during maturity stages I to III (Yarmohammadi et al. 2017). Analysis of immature gonads of Russian sturgeon by Western blot revealed that Sox9 protein was detectable in cytoplasm of all Primordial germ cells (Kamaszewski et al. 2017). Previously, the sexual dimorphism of Sox9 were reported in late stages of maturity in several sturgeon species including *A. gueldenstaedtii*, *A. schrenckii*, *A. baerii* and *H. huso* (Berbejillo et al. 2013; Jin et al. 2015; Chen et al. 2016; Yarmohammadi et al. 2017). However, through protein localization, researcher found that Sox9 play crucial role during gonad differentiation and Sox9 protein translocate from the cytoplasm to the nucleus in male gonads, while in female gonad, its localization remains cytoplasmic (Kamaszewski et al. 2017). Our results and previous studies confirm that the weakly expression of Sox9 in undifferentiated gonads of young fishes is not related with sex determination and possibly have role during final process Of masculine gonad differentiation in this basal fish.

This study characterizes the partial-length of Sox9 gene from *A. persicus*. The expression patterns of Sox9 in tissues of immature individuals suggest that

Sox9 is not applicable in the early determination of the sex. Besides, it seems that the Sox9 expression is related with organogenesis and chondrogenic activity at early life stages of sturgeon. Results suggests that the function of Sox9 in the cartilage formation is conserved among vertebrates, while that in the gonad is different. These results will offer useful information for further research of the Sox9 in chondrogenesis of fishes.

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مقاله پژوهشی

بیان ژن Sox9 در بافت‌های مختلف و طی مراحل اولیه رشد در تاس‌ماهی ایرانی *Acipenser persicus* (Borodin, 1897)

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چکیده: ژن Sox9 یک عامل کدکننده پروتئین بوده که در میان مهره‌داران نقش مهمی در اندام‌زایی و تکامل اولیه گناد جنس نر ایفا می‌نماید. دانش درباره الگوی بیان ژن Sox9 طی مراحل تکامل لاروی می‌تواند به تکمیل کمبود اطلاعات در زمینه عملکرد احتمالی این ژن در ماهیان خاویاری کمک کند. با توجه به اهمیت تاسماهی ایرانی به عنوان گونه ای باارزش و کاندید آبزی‌پروری و اهمیت ژن Sox9 در تکامل مهره‌داران، در این مطالعه، توالی نسبی cDNA ژن Sox9 شناسایی شد و الگوی بیان آن طی مراحل جنینی مختلف (تخم لقاح نیافته تا ۲۱۰ روز پس از تفریخ) و در میان ۱۰ بافت مختلف (آبشش، پیلوریک، طحال، گناد، کلیه، روده، قلب، پوست، کبد، ماهیچه) به وسیله روش RT-PCR مورد آزمایش قرار گرفت. نتایج نشان داد که mRNA ژن Sox9 در در تخم لقاح‌نیافته و لقاح‌یافته یافته نمی‌شود. با این حال، اولین بیان ژن در ۱ روز پس از تفریخ مشاهده شد، سپس mRNA های ژن Sox9 در بازه زمانی ۱ تا ۵۰ روز پس از تفریخ طی روندی نزولی کاهش یافت و در ۲۱۰ روز پس از تفریخ به کمترین سطح رسید ($P<0/05$). همچنین mRNA ژن Sox9 در اغلب بافت‌ها حضور داشت، بیشترین بیان ژن در آبشش و پیلوریک، کمترین بیان ژن در کبد و گناد و عدم بیان ژن در ماهیچه مشاهده شد. یافته‌های ما پیشنهاد می‌کنند که ژن Sox9 برای تعیین جنسیت طی مراحل اولیه تکامل کاربردی نیست و احتمالاً این ژن می‌تواند در فعالیت‌های تکاملی دیگری نظیر غضروف‌سازی مشارکت داشته باشد.

کلمات‌کلیدی: غضروف‌سازی، مراحل جنینی، واکنش q-RT-PCR، تعیین جنسیت، ماهیان خاویاری.