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Molecular Characterization and Differential Expression of Two Prostaglandin E Synthase 2 Orthologs in *Coilia nasus*

Gangchun Xu[1,2], Fukuan Du[2], Yuyu Wang[2], Yan Li[2], Zhijuan Nie[2] and Pao Xu[1,2,*]

[1]*Fisheries College, Nanjing Agricultural University, Wuxi 214128, China*
[2]*Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, Jiangsu, 214081, China*

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GX performed the experiments, analyzed the data, wrote the manuscript. FD, YW, YL and ZN prepared samples used in this study. PX conceived and designed the project.

Key words
***Coilia nasus*, Prostaglandin E synthase 2 gene, Gene expression.**

ABSTRACT

The estuarine tapertail anchovy (*Coilia nasus*) is a widely-distributed and commercially-important aquaculture species. In this study, two prostaglandin E synthase 2 (*PTGES2*) orthologs were identified. The full length ortholog *PTGES2a* was 1,575 bp and contained a 1,167-bp Open Reading Frame that encoded a protein of 388 amino acids. The 5′ and 3′ untranslated regions were 269 bp and 139 bp, respectively. The full length ortholog *PTGES2b* was 1,457 bp and contained a 729-bp ORF that encoded a protein of 242 amino acids. The 5′ and 3′ untranslated regions were 402 bp and 326 bp, respectively. One polyadenylation signal (AATAAA) was present 14 nucleotides upstream of the poly(A) tail in both the *PTGES2a* and the *PTGES2b* gene. The full-length genomic DNA sequence of *PTGES2a* is 3,222 bp long, and composed of seven exons and six introns. The full-length genomic DNA sequence of *PTGES2b* is 1,908 bp long, and composed of six exons and five introns. *PTGES2a* was strongly expressed in the gills and liver while *PTGES2b* was strongly expressed in the gills and testis. Expression of both *PTGES2a* and *PTGES2b* decreased from stage III to stage V and *PTGES2a* was significantly more highly expressed than *PTGES2b*. In the spawning process, the expression of *PTGES2a* did not significantly change, remaining at a low level. In contrast, *PTGES2b* expression significantly increased during spawning. These results provide basic knowledge of the new *PTGES2s* of *C. nasus*.

INTRODUCTION

Prostaglandin E synthase 2 (*PTGES2*) is a membrane-associated enzyme, which catalyses the conversion of prostaglandin (PG) H2 to PGE2 (Mattila *et al*., 2009). This protein is thought to be targeted to the Golgi apparatus as well as the mitochondrion within the cell. Microsomal PTGES2 (mPTGES2) has been crystallized with the anti-inflammatory drug indomethacin (IMN) (Yamada *et al*., 2005). mPTGES2 exists as a dimer (Yamada *et al*., 2005), with its N-terminal attached to the lipid membrane and the two hydrophobic pockets connected to form a V shape and located at the bottom of a large cavity for IMN binding.

The *PTGES2* protein functions as one step of the PG synthesis pathway, which forms a component of the overall lipid synthesis mechanism in the human body. The activity of *PTGES2* is thought to be increased in the presence of sulfhydryl compounds, in particular dithiothreitol.

Model organisms have been used in the study of *PTGES2* function. A conditional knockout mouse line (Miller *et al*., 2010) was generated as part of the International Knockout Mouse Consortium program—a high-throughput mutagenesis project to generate and distribute animal models of disease to interested scientists—at the Sanger Institute (Collins *et al*., 2007; Dolgin, 2011). Male and female animals underwent a standardized phenotypic screen to determine the effects of deletion (Van *et al*., 2011). Twenty-two tests were carried out on mutant mice, but no significant abnormalities were observed (Gerdin, 2010).

As reported in fish, *PTGES2s* are involved in female reproduction, especially in follicular development and ovulation (Sun *et al*., 2006). In zebrafish, recent studies have shown that PGE2 is involved in regulation of oocyte maturation,ovulation (Lister and Van der Kraak, 2008, 2009) and gonad differentiation (Pradhan and Olsson, 2014, Biol Reprod). In contrast, studies in goldfish (*Carassius auratus*) have shown that PGE2 stimulates testosterone production in testis *in vitro,* suggesting that PGE2 may be involved in the control of steroidogenesis in the goldfish testis (Wade and Van der Kraak, 1993; Wade *et al*., 1994; Jørgensen *et al*., 2010).

Estuarine tapertail anchovy (*Coilia nasus,* junior synonym *C. ectenes*) is widely distributed in the Yangtze

* Corresponding author: xup13806190669@163.com
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River, the coastal waters of China, Korea, and the Ariake Sound of Japan (Jiang *et al*., 2012). It is a commercially important species due to its nutritional value and is regarded as a delicacy. However, excessive fishing and changes in aquatic ecology have almost caused the extinction of the species in the middle reaches of the Yangtze River (Zhang *et al*., 2005; Yang *et al*., 2017). The ovary development of *C. nasus* is population-asynchronous (Xu *et al*., 2016), in which development is synchronous within individuals, but asynchronous between different individuals within the same population. This results in a complex population structure, with fish with different stages of ovary development present in the pond at the same time. Spawning thus occurs over a long period in *C. nasus*.

Multiple transcript variants have been found for this gene, but no orthologs have been reported. In *C. nasus*, two *PTGES2s* were found in the ovarian transcriptome, and after validation by amplification, these two transcripts were demonstrated to come from different genome loci, which are *PTGES2* orthologs. Consequently, in this study, using molecular cloning, sequencing and differential expression of the two *PTGES2* genes from *C. nasus*, we evaluated the expression during ovary development and spawning.

MATERIALS AND METHODS

Experimental animals

Coilia nasus were adapted to a 7.0 m × 5.0 m × 1.0 m aquarium with a water temperature of 24.5 ± 1.0°C, pH 7.8, and dissolved oxygen concentration of 9.2 ± 0.5 mg O_2/L dechlorinated and aerated water. Fish were fed twice daily, at 7:00 AM and 5:00 PM. At the start of the experiments all fish appeared healthy.

Cloning and sequencing of C. nasus *PTGES2a and PTGES2b*

Total RNA of *C. nasus* ovary was purified using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and treated with RNase-free DNase (Promega, Madison, WI, USA). First-strand cDNA was synthesized from RNA using Moloney murine leukaemia reverse transcriptase (TaKaRa Bio Inc., Shiga, Japan). Fragments of the *PTGES2a* and *PTGES2b* genes were identified in the ovarian transcriptome data. Primers were designed based on the identified sequences, and then amplified from *C. nasus* ovarian cDNA using the primer pairs refer to Table I. The genomic DNA of the *PTGES2a* and *PTGES2b* genes was amplified from its genomic DNA using the primer pair as above. The resulting fragments were separated on a 1.0% agarose gel and purified using the Axygen DNA gel extraction kit (Axygen, Union City, CA, USA). The purified fragments were cloned into the pMD-18T vector (TaKaRa) by the TA cloning strategy and sequenced (BGI, Shenzhen, China). The 3′ ends of the *PTGES2a* and *PTGES2b* cDNA were obtained using the rapid amplification of cDNA ends (RACE) approach (Zhuan Dao, Wuhan, China).

Table I.- Sequences of primers used in this study.

Primer	Sequence	Usage
PTGES2a-1	GATGGATGGACTACAAGCATTTGACGA	3'RACE
PTGES2a-2	GAGAACACCAAGATCAAGCCCTGG	3'RACE
PTGES2b-1	CCATGCAGAAGGTGATCCGGGAGC	3'RACE
PTGES2b-2	ACTGATGATTTCAGCGCTGTGGA	3'RACE
153S	AGGCACTGGGTGATGTTGAATGAG	Forward primer for *PTGES2b genomic DNA*
154S	GGTTCCAGTAAAGTTCCCATTCTTGTTA	Forward primer for *PTGES2b genomic DNA*
155A	TCATCAAATGCCTGTAGCCCCTC	Reverse primer for *PTGES2b genomic DNA*
156A	GAAGAAGAACATTGTAAACGCTCCCAC	Reverse primer for *PTGES2b genomic DNA*
157S	GCCAGGGTGATGGGATGTGC	Forward primer for *PTGES2a genomic DNA*
158S	TTCTACCCACCAGCAACTCAACGA	Forward primer for *PTGES2a genomic DNA*
159A	GGGCGTGCGGTAGACATTCG	Reverse primer for *PTGES2a genomic DNA*
160A	CATAAACTTCCTGTGCTTGCCCA	Reverse primer for *PTGES2a genomic DNA*
167S	TGATGTTGAATGAGATAGCCCTTGA	Forward primer for *PTGES2b* RT-qPCR
167A	AAAAGCCAGTCATCTGCCCAA	Reverse primer for *PTGES2b* RT-qPCR
168S	CATGCGGAAGGAAATTAAGTGGT	Forward primer for *PTGES2a* RT-qPCR
168A	GTCGTTGAGTTGCTGGTGGGT	Reverse primer for *PTGES2a* RT-qPCR
42S	TGATTGGGACTGGGGATTGAA	Forward primer for 18sRNA RT-qPCR
42A	TAGCGACGGGCGGTGTGT	Reverse primer for 18sRNA RT-qPCR

Table II.- GenBank accession numbers of PTGES2 used in this study.

Protein	Accession No.
Homo sapiens PTGES2	13376617
Pan troglodytes PTGES2	350537055
Macaca mulatta PTGES2 isoform 2	297271234
Mus musculus PTGES2	260763900
Rattus norvegicus PTGES2	157822395
Danio rerio PTGES2	41053638
Salmo salar PTGES2	XP_013998234.1
Cynoglossus semilaevis PTGES2 isoform X2	XP_008333868.1
Cynoglossus semilaevis PTGES2	XP_008333867.1
Xiphophorus maculatus PTGES2	XP_014326022.1
Fundulus heteroclitus PTGES2	XP_012720762.1
Cyprinodon variegatus PTGES2	XP_015224766.1
Kryptolebias marmoratus PTGES2	XP_017283273.1
Poecilia formosa PTGES2	XP_007547493.1
Stegastes partitus PTGES2	XP_008285271.1
Austrofundulus limnaeus PTGES2	XP_013870069.1
Scleropages formosus PTGES2	KPP67828.1
Callorhinchus milii PTGES2	AFK11574.1
Salmo salar PTGES2 isoform X2	XP_014027157.1
Clupea harengus PTGES2	XP_012673252.1
Salmo salar PTGES2 isoform X1	XP_014027156.1
Sinocyclocheilus rhinocerous PTGES2	XP_016372450.1
Sinocyclocheilus anshuiensis PTGES2	XP_016315128.1
Sinocyclocheilus graham PTGES2	XP_016147256.1
Oreochromis niloticus PTGES2	XP_005451551.1
Pundamilia nyererei PTGES2 isoform X1	XP_005730632.1
Oreochromis niloticus PTGES2	XP_005451550.1
Neolamprologus brichardi PTGES2	XP_006797764.1
Pundamilia nyererei PTGES2 isoform X2	XP_005730633.1
Maylandia zebra PTGES2	XP_004555760.1
Haplochromis burtoni PTGES2 isoform X1	XP_005949007.1
Haplochromis burtoni PTGES2 isoform X2	XP_005949008.1
Nothobranchius furzeri PTGES2 isoform X1	XP_015815743.1
Nothobranchius furzeri PTGES2 isoform X2	XP_015815744.1
Larimichthys crocea PTGES2	XP_010748983.1
Takifugu rubripes PTGES2	XP_003974720.1
Astyanax mexicanus PTGES2	XP_007235931.1

Analysis of nucleotide and amino acid sequences

The nucleotide and predicted amino acid sequences of *PTGES2a* and *PTGES2b* were analyzed using DNA figures software (http://www.bio-soft.net/sms/index.html). The similarity of PTGES2s from *C. nasus* to PTGES2s from other organisms was analysed using the BLASTP search program (http://www.ncbi.nlm.nih.gov/blast). The domain structures were predicted using the SMART program (http://smart.embl-heidelberg.de/). The amino acid sequence was compared with those of PTGES2s from other species using CLUSTALX 1.83 (http://www.ebi.ac.uk/clustalW/) and GeneDoc (http://www.nrbsc.org/gfx/genedoc/). The phylogenetic tree was constructed using MEGA 3.1 (http://megasoftware.net; Table II).

mRNA expression profiles of PTGES2s in different tissues

For tissue distribution analysis of *PTGES2s*, total RNA was extracted from the gill, liver, spleen, kidney, head kidney, brain, ovary, testis and intestine from healthy *C. nasus* using TRIzol Reagent (Invitrogen). Samples of each tissue were obtained from five individuals and analyzed in triplicate to control for inter-individual differences.

First-strand cDNA was synthesized using the ReverTra Ace® qPCR RT kit (Toyobo, Japan), and Real Time - quantitative Polymerase Chain Reaction (RT-qPCR) was employed to detect the *PTGES2s* expression profiles using *18sRNA* as the reference gene. The RT-qPCR primers as that in Table I, which shared similar Tm values and were designed to amplify fragments of 103 bp, 97 bp and 114 bp, respectively. Before the RT-qPCR, the primers efficiency for these genes have been validated. RT-qPCR was performed on the ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) using 2× SYBR green real-time PCR mix (TaKaRa). PCR amplification was performed using the following cycling parameters: 94°C for 2 min; followed by 40 cycles of 15 s at 94°C, 15 s at 60°C, and 45 s at 72°C. The expression of target genes was calculated as relative fold-changes with the $2^{-\Delta\Delta CT}$ method.

mRNA expression profiles of PTGES2s in ovary development and ovulation

Three 60.0 m × 20.0 m × 2.0 m ponds were each stocked with 1,000 juvenile *C. nasus*. The fish were acclimatised to the ponds for approximately 3 years before the experiments. The stocking density was 37.5 g/m^2, and 71.0% of the fish survived until the end of the experiment. Seventy fish in each pond were euthanized to examine and analyse ovarian development. Ovaries of stage III, stage IV and stage V were removed, placed in liquid nitrogen, and stored at -80°C for subsequent analysis. The female fish used in current study were analyzed using histology (HE section) and scanning electron microscopy to determine stage III, IV, and V (Xu *et al*., 2016). The mean length (± standard error) was 286.76 ± 12.24 mm and the mean mass was 101.83 ± 12.82 g for all fish (n = 26) sampled in this experiment.

Fig. 1. Nucleic acid sequence and deduced amino acid sequence of PTGES2a. Capital letters above the nucleotide sequences indicate the corresponding amino acid sequence. The start codon (ATG) and stop codon (TGA) are boxed. The motif associated with mRNA instability (ATTTA) is in bold font and underlined. SMR domain is shade with green, GST-N3 domain is shade with blue, Leucine Rich Region domain is shade with yellow, GST-C3 domain is shade with pink, Glutaredoxins domain is underlined with wavy line, alpha helix are underlined with red line and beta sheets are underlined with dotted line.

Another 70 sexually mature female and male fish were placed together into spawning ponds, and sampled at 0, 0.5, 8, and 16 h, five female fish at each sample point. First-strand cDNA synthesis and RT-qPCR were then carried out as described above. Samples of each tissue were obtained from five individuals and analyzed in triplicate to control for inter-individual differences.

RESULTS

Cloning and sequence characterization of the PTGES2a and PTGES2b genes

The full length *PTGES2a* was 1,575 bp long and contained a 1,167-bp ORF that encoded a protein of 388 amino acids (Fig. 1). The 5′ and 3′ untranslated regions were 269 bp and 139 bp, respectively. The full length *PTGES2b* was 1,457 bp and contained a 729-bp ORF that encoded a protein of 242 amino acids (Fig. 2). The 5′ and 3′ untranslated regions were 402 bp and 326 bp, respectively. One polyadenylation signal (AATAAA) was present 14 nucleotides upstream of the poly(A) tail in both *PTGES2a* and *PTGES2b* genes (NCBI accession number: KY132103 and KY132104).

The *PTGES2a* gene is composed of seven exons and six introns. The full-length genomic DNA sequence of *PTGES2b* is 1,908 bp long. It is composed of six exons and five introns. All exon/intron junctions conform to the splicing consensus sequence (GT-donor/AG-acceptor) rule.

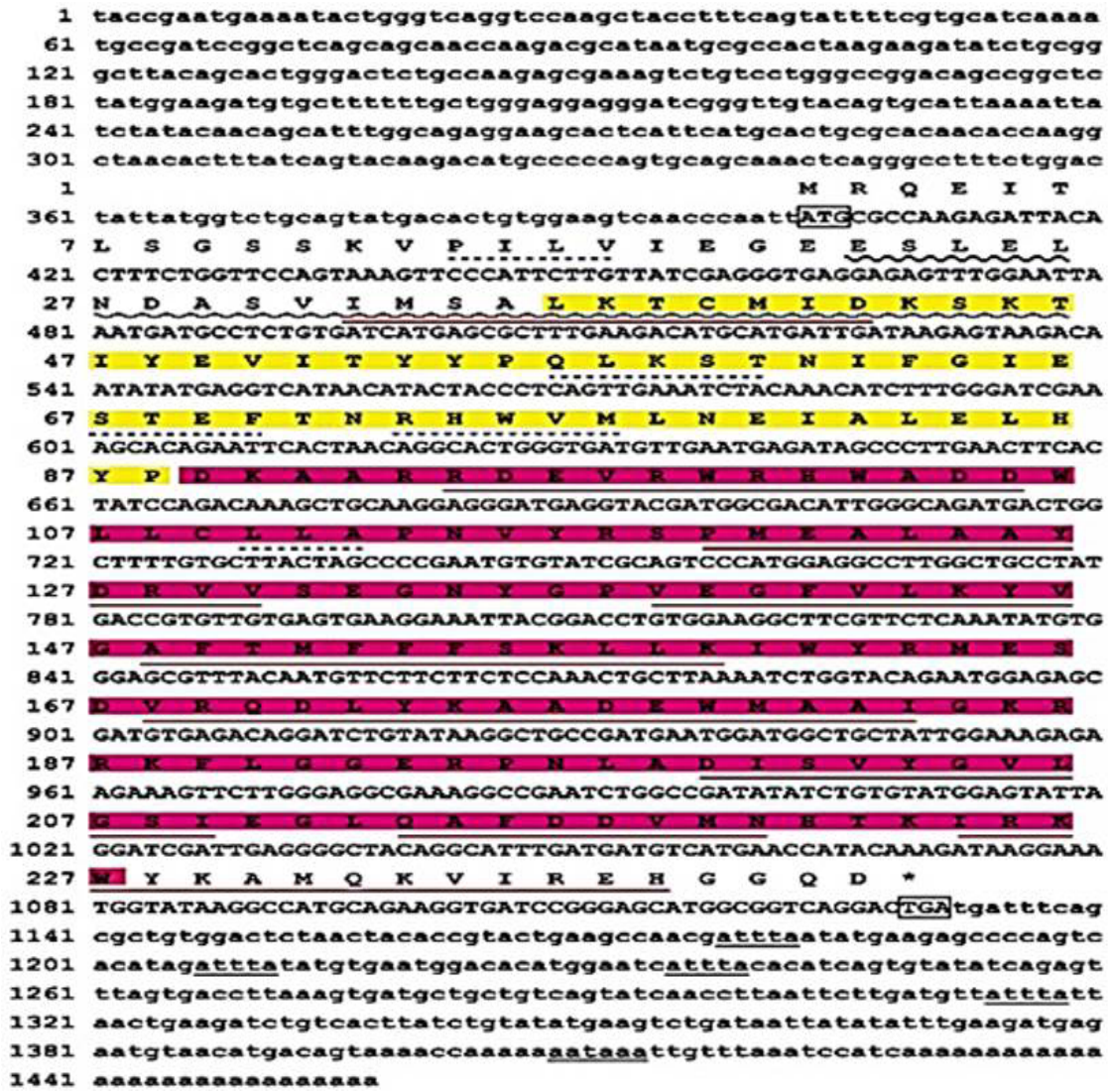

Fig. 2. Nucleic acid sequence and deduced amino acid sequence of PTGES2b. Particular sequences are indicated as described above. The poly(A) signal sequence (AATAA) is in bold font and double underlined. The domains marked as that in Figure 1.

The PTGES2a protein was predicted to contain an SMR domain, a GST-N3 domain, a glutaredoxin domain, a leucine-rich repeat domain and a GST-C3 domain (Fig. 1). The PTGES2b protein was predicted to contain a glutaredoxin domain, a leucine-rich repeat domain and GST-C3 domain (Fig. 2).

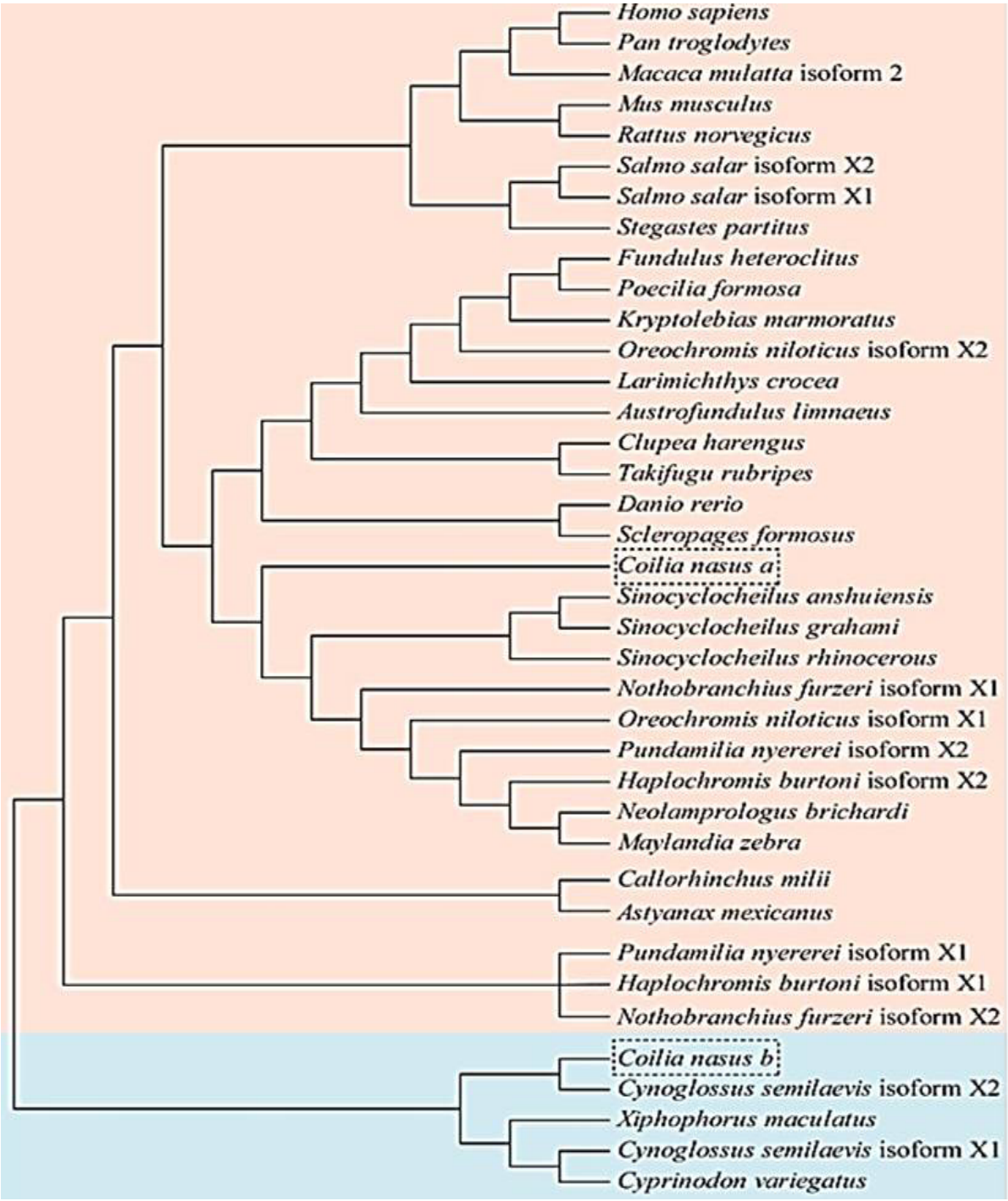

Fig. 3. Phylogenetic analysis of PTGES2s and other reported PTGES2 sequences from vertebrates. The phylogenetic tree was constructed by the neighbour-joining method using MEGA 3.1. The GenBank accession numbers of the analyzed PTGES2 sequences are listed in Table II.

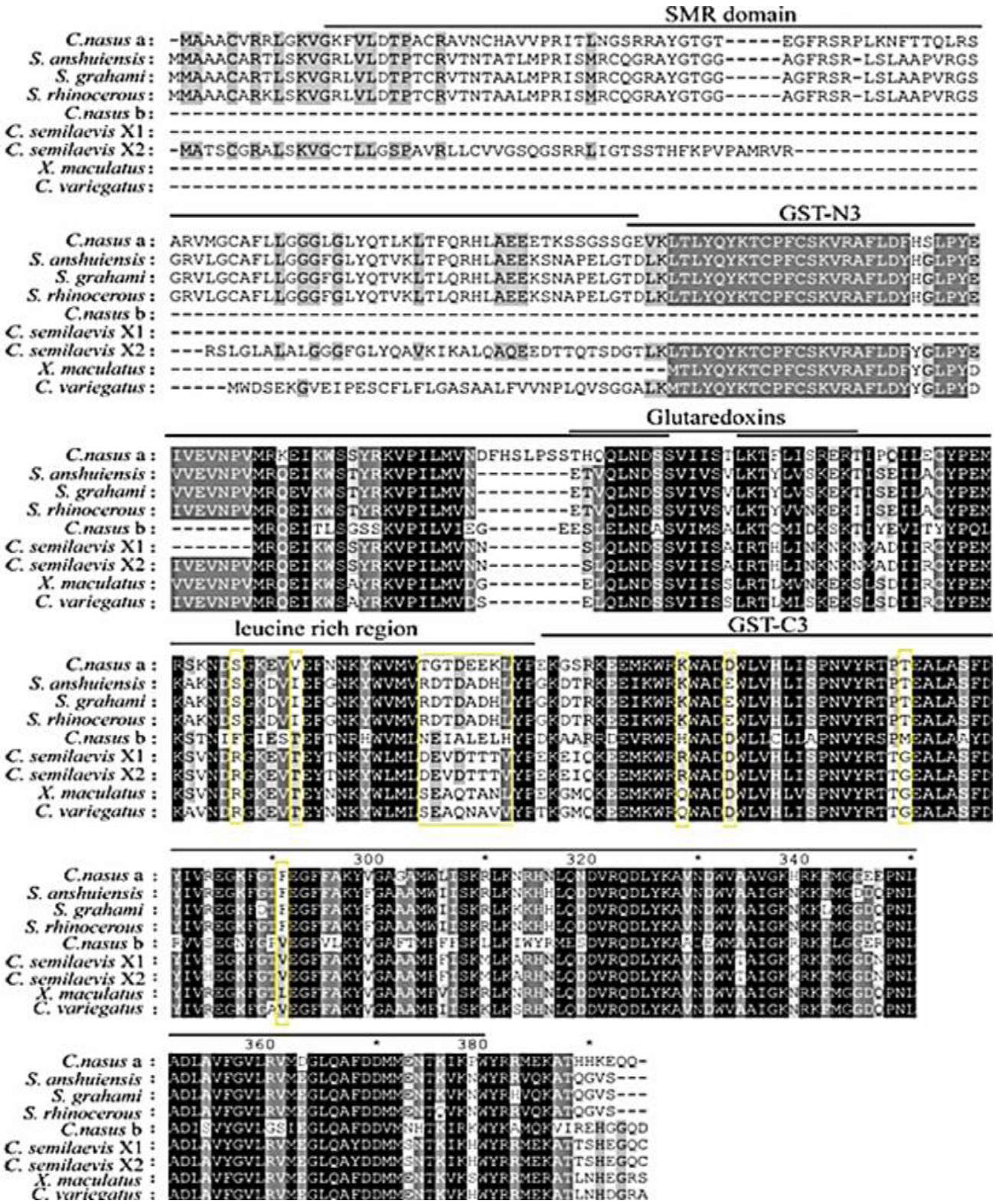

Fig. 4. Alignment of the deduced amino acid sequence of PTGES2a and PTGES2b. Completely conserved residues across all species are aligned and shaded in black. The conserved residues in PTGES2a or PTGES2b are boxed.

Homology analysis of PTGES2s

The phylogenetic tree of the PTGES2s proteins consists of two major branches: PTGES from *Homo sapiens*, *Macaca mulatta*, *Pan troglodytes*, *Mus musculus, Rattus norvegicus*, *Salmo salar isoform* X1, *S. salar isoform* X2, *Stegastes partitus*, *Fundulus heteroclitus*, *Oreochromis niloticus* isoform X2, *Larimicthys crocea*, *Astrofundulus limnaeus*, *Clupea harengus*, *Takifugu rubripes*, *Danio. rerio*, *Scleropages formosus*, *Sinocyclocheilus anshuiensis*, *Sinocyclocheilus grahami*, *Sinocyclocheilus rhinocerous*, *Nothobranchius furzeri* isoform X1, *Oreochromis niloticus* isoform X1, *Pundamilia nyererei* isoform X2, *Haplochromis burtoni* isoform X2, *Neolamprologus brichardi*, *Maylandia zebra*, *Callorhinchus milii*, *Astyanax mexicanus*, *Pundamilia nyererei* isoform X1, *H. burtoni* isoform X1, *N. furzeri* isoform X2 clustered into the PTGES2a like subgroup, while PTGES from *Cynoglossus semilaevis* isform X2, *Xiphophorus maculates*, *C. semilaevis* isoform X1 and *Cyprinodon variegates* clustered into the PTGES2b subgroup (Fig. 3).

In order to compare the PTGES2a and PTGES2b sequence, multiple alignments were created using CLUSTALX. Identity of the PTGES2a deduced amino acid sequence was observed with those of homologs from other species: *S. anshuiensis* (72.2%), *S. grahami* (71.4%) and *S. rhinocerous* (72.0%). The PTGES2b deduced amino acid sequence showed identity with those of homologs from other species: *C. semilaevis* isoform X2 (53.3%), *X. maculates* (51.2%), *C. semilaevis* isoform X1 (52.9%) and *C. variegates* (51.2%). The similarity between PTGES2a and PTGES2b was 50.4%. There was no SMR domain or GST-N3 domain in PTGES2b (Fig. 4).

The three-dimensional structures of PTGES2a and PTGES2b are shown in Figure 5. The differences between the PTGES2a and PTGES2b structures were in the SMR and GST-N3 domains, which are involved in attachment to the lipid membrane, and are missing in PTGES2b.

Tissue expression profiles of PTGES2a and PTGES2b

The mRNA expression profiles of *PTGES2a* and *PTGES2b* transcripts were assessed in different tissues by RT-qPCR using *18sRNA* as the reference gene. In healthy fish, *PTGES2a* was strongly expressed in the gills and liver, while expression was comparatively higher in the intestine, testis and muscle, and lower in the brain, spleen, head kidney, kidney and heart (Fig. 6A). *PTGES2b* was strongly expressed in the gills and testis, comparatively higher in the brain, liver, intestine and kidney, and lower in the spleen, head kidney, heart and muscle (Fig. 6A).

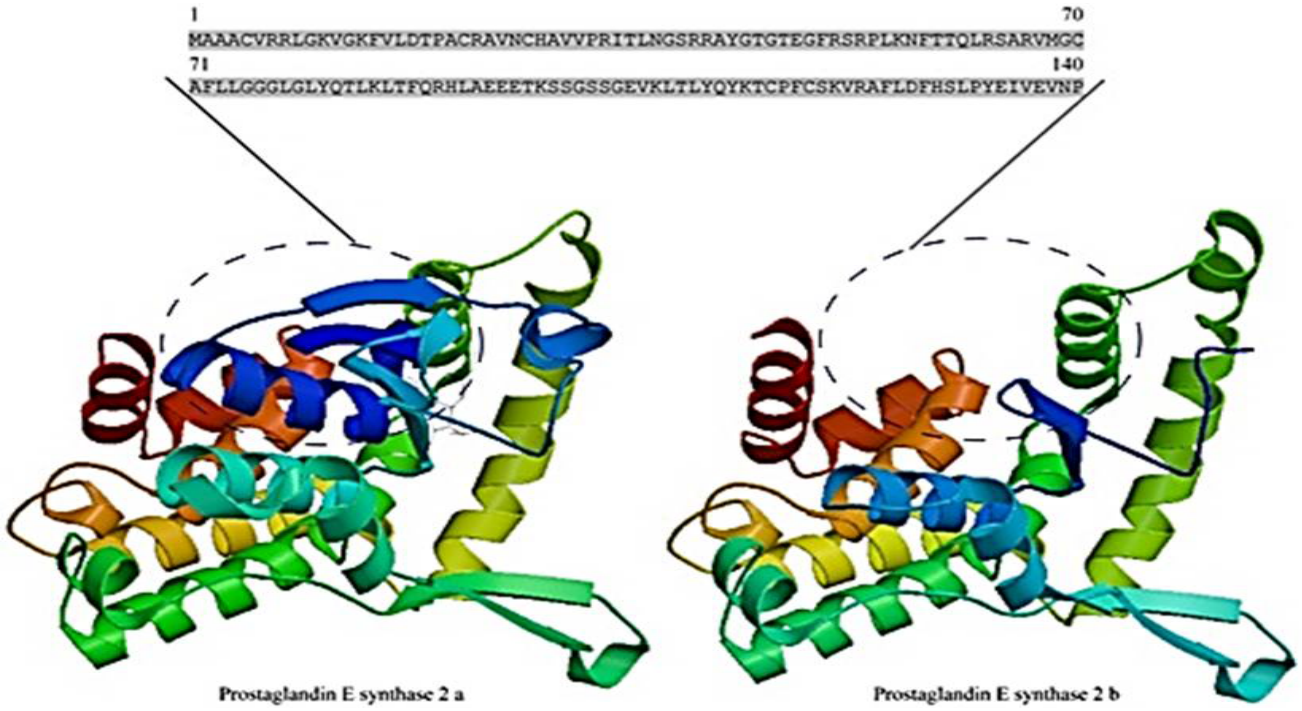

Fig. 5. The three-dimensional structures of PTGES2a and PTGES2b. This diagram was generated by SWISS-MODEL online software. The differences between the PTGES2a and PTGES2b structures are indicated by a dotted ellipse, and the corresponding amino acid sequences are shown.

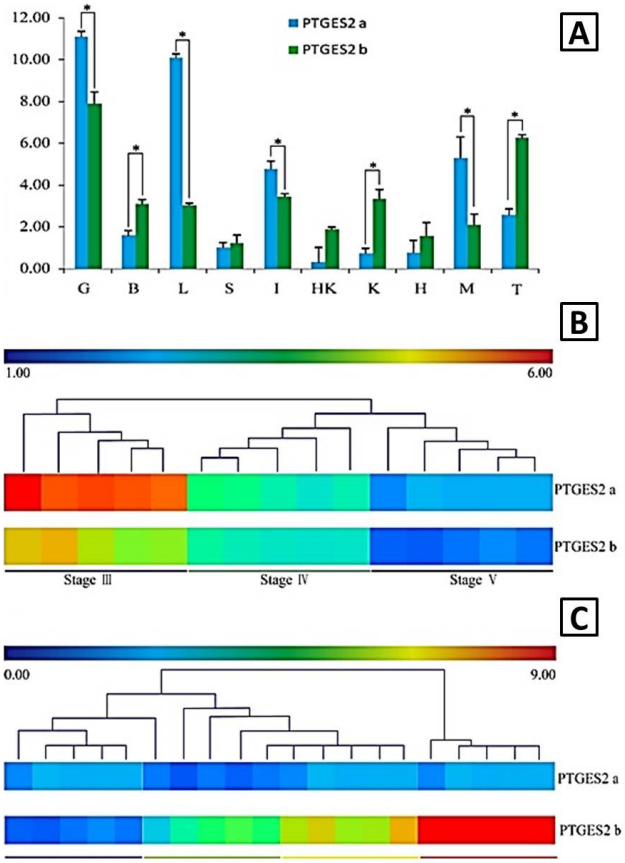

Fig. 6. The expression profiles of *PTGES2a* and *PTGES2b* mRNA. A, RT-qPCR analysis of the expression profiles of *PTGES2a* and *PTGES2b* mRNA in different *Coilia nasus* tissues. Data are expressed as the ratio of *PTGES2a* mRNA expression in the tissue to its expression in the spleen (mean ± SD); B, expression profiles of *PTGES2a* and *PTGES2b* in the ovary at different stages. Data are expressed as the ratio of *PTGES2b* mRNA at different stages to expression in stage V (Log 2 transformed); C, expression profiles of *PTGES2a* and *PTGES2b* during the spawning process. Data are expressed as the ratio of *PTGES2a* mRNA expression to expression in the controls (0 h, Log 2 transformed).

PTGES2a and PTGES2b responses to ovary development and spawning

The RT-qPCR results revealed that expression of *PTGES2a* gradually decreased from stage III to stage V, which showed 10.9- and 2.5-fold decreases compared to stage III. *PTGES2b* also decreased from stage III to stage V, which exhibited 4.9- and 3.2-fold decreases compared to stage III. Moreover, the expression of *PTGES2a* was significantly higher than that of *PTGES2b* (Fig. 6B, $P<0.05$).

In the spawning process, the expression of *PTGES2a* did not show any significant changes, remaining at low expression levels. In contrast, the expression of *PTGES2b* significantly increased during the spawning process, showing a 1.2-, 1.8- and 156.2-fold increase in expression after 0.5, 8 and 16 h of stimulation compared to controls (Fig. 6C).

DISCUSSION

In this study, based on the ovary transcriptome data, two *PTGES2*-like sequences were identified and then the complete sequences were obtained by RACE. As recorded in GenBank, many species of fish expressed *PTGES2* isoforms. In order to determine whether these two sequences were *PTGES2* isoforms or orthologs, the genomic DNA sequences were amplified in the genome. The results showed that the two *PTGES2* cDNAs were not isoforms, but rather they were orthologs. Consequently these genes were named *PTGES2a* and *PTGES2b*.

Most of the reported sequences showed greater similarity to PTGES2a*;* only those of *C. semilaevis* isoform X2, *X. maculates*, *C. semilaevis* isoform X1 and *C. variegates* were similar to PTGES2b (Fig. 3). When their secondary structure was compared, the results showed that the SMR domain and the GST-N3 domain were only found in PTGES2a (Fig. 4). The SMR domain is an approximately 90-residue which is a hydrophobic domain. This domain could assist the protein associated with the Golgi membrane (Murakami *et al*., 2003; Moreira and Philippe, 1999), so missing this domain make PTGES2b cannot bind to membrane. As a result, PTGES2b could be a cytosolic protein, while PTGES2a is a membrane associated protein. In eukaryotes, GST domains are often contained in glutathione S-transferases (GSTs), which participate in the detoxification of reactive electrophillic compounds by catalysing their conjugation to glutathione (Morgenstern, 2005; Josephy, 2010). The GST domain is also found in S-crystallins from squid, and proteins with no known GST activity, such as the eukaryotic elongation factors 1-gamma and the HSP26 family of stress-related proteins, which include auxin-regulated proteins in plants and stringent starvation proteins in *Escherichia coli* (Armstrong, 1997; Eaton and Bammler, 1999; Galina-Polekhina *et al*., 2001). A mutagenesis study indicates that Cys^{110}-X-X- Cys^{110} in GST-N3 domain is essential for the enzymatic activity, missing this domain could decrease the enzymatic activity in *Bos taurus* (Tanikawa *et al*., 2002). But its activity could activated by various SH-reducing reagents, such as dithiothreitol, GSH and β- mercaptoethanol (Watanabe *et al*., 2003). In summary, the protein structure indicates that PTGES2a could bind to membrane, and its activity is independent SH-reducing

reagents. While PTGES2b is a cytosolic protein and its activity is dependent on SH-reducing reagents. As the reported in human, PGE2 synthesized by cytosolic PTGES2 tended to act on reproductive processes (Murakami and Kudo, 2006), so we speculate that PTGES2b in *Coilia nasus* may be highly expressed in reproductive tissue.

Consistent with our sequence analysis result, *PTGES2a* was found to be highly expressed in the gills and liver, but *PTGES2b* was strongly expressed in the gills and testis. Moreover, the expression of the two genes showed significant differences in the gills, brain, liver, intestine, kidney, muscle and testis. Previous reported studies have revealed that PTGES2 is highly expressed in the brain and liver of *Mus musculus* and *Homo sapiens* (Toshiya *et al*., 1987; Kosaka *et al*., 1994; Tanikawa *et al*., 2002; Yang *et al*., 2006). The pattern of *PTGES2a* in this study was more consistent with this finding.

Ovary development can be defined as an oocyte maturation process. In this process, a set of nuclear, cytoplasmic and molecular changes occur that enables the oocyte to be fertilized normally (Kane, 2003; Gilchrist and Thompson, 2007). Prostaglandins are biologically-active lipid mediators that are involved in the regulation of many reproductive events such as ovulation, corpus luteum regression, implantation and establishment of pregnancy (Karim and Hillier, 1979). The key regulatory step in PG biosynthesis is the enzymatic conversion of the fatty acid precursor by PG endoperoxide synthase, PTGS1 and PTGS2, into PGG. This is then reduced to an unstable endoperoxide intermediate, PGH, and sequentially metabolized by cell-specific isomerases (PGE synthases (PTGES) or PGF synthases (PTGFS)) to produce PGE or PGF, respectively (Wang and Dey, 2005). Then PGE2 mediates oocyte maturation and even subsequent early embryo development. In this study, expression of *PTGES2*a and *PTGES2b* was highest in stage III, then gradually declined in stage IV, and decreased to its lowest level in stage V. This result is consistent with that in cattle. The reported study also revealed the dynamics of *PTGS2* gene expression during oocyte maturation. Basal expression of *PTGS2* stayed at a high level during early oocyte development, then declined during the middle oocyte development stage and remained unchanged at the oocyte stage (Marei *et al*., 2014). These results suggest that *PTGES2*a may play a more important role in the early stage of oocyte development.

In the ovulation process, there is no significant change in the expression of *PTGES2a*, but the expression of *PTGES2b* is significantly increased. These results suggest that there are some functional differences between *PTGES2a* and *PTGES2b* in the ovulation process. As mentioned above, *PTGES2a* and *PTGES2b* both play important roles in early oocyte development, but *PTGES2b* also takes part in ovulation; however this issue requires further study.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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