Expression of Eleven Egg Performance-associated Genes in the Ovary of Zi Geese *Anser anser domestica*

Hong Ji^{1,2a}, Jianfa Wang^{2a}, Jingru Guo^{1,2}, Li Guo¹, Lin Zang¹, Dan Wang¹,
Huanmin Yang¹ and Juxiong Liu²

¹ College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing 163319, P. R. China

² College of Animal Science and Veterinary Medicine, Jilin University, Changchun 130062, P. R. China

^aThese authors contributed equally

The relative expression of eleven egg performance-associated genes including the *tau-crystallin/α enolase* (*ENO1*), ribosomal protein *S27a* (*RPS27A*), *calmodulin 1* (*CALM1*), *solute carrier family 25 A6* (*SLC25A6*), *arylsulfatase A* (*ARSA*), *syndecan 2* (*SDC2*), *ribosomal protein L10a* (*RPL10A*), *ornithine decarboxylase antizyme 1* (*OAZ1*), *cytochrome c somatic variant 2* (*CYCS*), *adenosine triphosphate synthase, H⁺ transporting, α subunit, isoform 1* (*ATP5A1*) and *the ring finger protein 130* (*RNF130*) in the ovaries of pre-laying and laying Zi geese were analyzed using quantitative real-time PCR (qRT-PCR). Comparison between pre-laying and egg-laying stages, the relative expression of *ENO1*, *RPS27A*, *SLC25A6*, *ARSA*, *SDC2*, *RPL10A*, *OAZ1*, *CYCS* and *RNF130* were significantly up-regulated ($P < 0.05$), whereas the *CALM1* and *ATP5A1* were highly significantly up-regulated ($P < 0.01$). This study established the primary foundation to understand the possible roles of these genes in the ovaries.

Key words: egg performance-associated genes, geese, ovaries, qRT-PCR

J. Poult. Sci., 50: 91–98, 2013

Introduction

Zi geese (*Anser anser domestica*) are excellent layers (around 100 per year) with a superior feed-to-egg conversion ratio (Kang *et al.*, 2009). This species breeds in Northeast China (Heilongjiang and Jilin provinces). In our previous study, in order to study the molecular genetic mechanisms of egg laying and improve laying performance, suppression subtractive hybridization (SSH) and reverse dot-blot were employed to identify egg laying performance-associated genes in the ovaries. We have discovered that the expression of 18 known and 8 unknown gene fragments were higher in the ovaries of egg-laying geese than pre-laying geese (Kang *et al.*, 2009). In present study, *tau-crystallin/α enolase* (*ENO1*), *ribosomal protein S27a* (*RPS27A*), *calmodulin 1* (*CALM1*), *solute carrier family 25 A6* (*SLC25A6*), *arylsulfatase A* (*ARSA*), *syndecan 2* (*SDC2*), *ribosomal protein L10a* (*RPL10A*), *ornithine decarboxylase antizyme 1* (*OAZ1*), *cytochrome c somatic variant 2* (*CYCS*), *adenosine triphosphate synthase, H⁺ transporting, α subunit, isoform 1* (*ATP5A1*) and *the ring finger protein 130* (*RNF130*), which have been

identified to be differentially expressed in the ovaries by SSH. Their expression were confirmed by using quantitative real-time PCR (qRT-PCR) in both egg-laying and pre-laying stages in the ovaries of the Zi geese. This results provided the primary foundation to understand the possible roles of these genes in egg formation in geese.

Materials and Methods

Geese and Tissue Collection

Twenty female Zi geese were selected on a local farm and were raised according to the program used in farm. All the geese were fed according to conventional farming (Kang *et al.*, 2009). Ten geese were killed by exsanguination at 5-month-old (average BW = 3.0 ± 0.2 kg) and the ovaries were treated at pre-laying stage. Another 10 geese were killed at 8-month-old (average BW = 3.5 ± 0.2 kg) and the ovaries were treated at egg-laying stage. Ovaries samples, which comprised the whole ovary including the small and large yellow follicles, were rapidly removed, wrapped in foil, frozen in liquid nitrogen, and stored at -70°C until analysis.

Extraction of Total RNA and Quality Control

Total RNA was prepared by the Trizol reagent (Invitrogen Corporation, CA, U.S.A.) according to the instructions of the manufacturer. The total RNA samples from pre-laying and laying ovaries ($n=10$, for each) were pooled separately. Genomic DNA was removed by DNase I (Takara Biotech-

Received: March 1, 2012, Accepted: October 15, 2012

Released Online Advance Publication: November 25, 2012

Correspondence: Dr. H.M. Yang, Heilongjiang Bayi Agricultural University, Room 3089, Sheng Wu Guan, 2 Xinyang Road, Daqing 163319, China.
(E-mail: yanghuanmin@yahoo.com.cn)

nology Company, Dalian, China) according to the instruction of manufacturer. Total RNA concentration and purity were determined by using a SmartSpecTM Plus spectrophotometer (Bio-Rad, CA, U.S.A.). Total RNA integrity was checked by electrophoresis on a denaturing agarose gel (Fig. 1a). All total RNA samples were stored at -70°C until analysis.

Reverse Transcription-PCR and qRT-PCR Analysis

In order to determine the expression of *ENO1*, *RPS27A*, *CALM1*, *SLC25A6*, *ARSA*, *SDC2*, *RPL10A*, *OAZ1*, *CYCS*, *ATP5A1* and *RNF130*, first-strand cDNA was generated from 3 μg total RNA pooled from pre-laying or laying geese ovaries ($n=10$) by using Superscript III (Invitrogen Corporation). Gene-specific primers were designed according to the cDNA sequences by using the Primer Premier 5.00 (Premier Biosoft International, CA, U.S.A.) and synthesized by Takara Biotechnology Company. The primers are listed in Table 1. In order to check the specificity of all primers further, PCR was performed on cDNA, and PCR products were analyzed on a 1% agarose gel (Fig. 1b). The qRT-PCR was performed in 50 μL reactions on a Line-Gen K Real-Time PCR Detection System and software (Bioer Technology, Hangzhou, China), using 1 μL of first-strand cDNA and SYBR Green PCR Master Mix (Takara Biotechnology Company). Thermal cycling was performed with

an initial denaturation step of 10 s at 94°C , followed by 45 cycles of 5 s at 94°C , 56 $^{\circ}\text{C}$ for 30 s, and then a final extension at 72°C for 20 s. Relative quantification of gene expression was performed in 3 replicates for each sample and normalized by β -actins (*ACTB*). As a control for genomic DNA contamination, an equivalent amount of total RNA without reverse transcription was tested for each sample per gene. A no-template control (NTC) was also included in each run for each gene. The quality of standard curves were judged by the slope of the standard curve and the square of the Pearson correlation coefficient (R^2). The PCR amplification efficiency of each primer pair was calculated from the slope of a standard curve using the following equation: Efficiency % = $(10^{(-1/\text{slope})} - 1) \times 100\%$ (Bustin *et al.*, 2009).

Statistical Analysis

The relative expression levels of *ENO1*, *RPS27A*, *CALM1*, *SLC25A6*, *ARSA*, *SDC2*, *RPL10A*, *OAZ1*, *CYCS*, *ATP5A1* and *RNF130* were calculated relative to *ACTB* (the normalizer) by using the comparative cycle threshold method (Livak and Schmittgen, 2001). The abundance of these genes in the ovaries of pre-laying geese were assigned a value of 1, and a Student's *t*-test was performed to determine the differential significance of the genes by comparing the values from the ovaries of laying geese to those ovaries of

Fig. 1. Confirmation of amplicon size and primer specificity of studied genes. (a) Agarose gel electrophoresis showing the 28S and 18S rRNA bands, Lane (Ln) 1, total RNA sample of pre-laying Zi geese; Ln 2, total RNA sample of laying Zi geese; (b) Agarose gel electrophoresis showing specific reverse transcription PCR products of the expected size for each gene. Ln A, DL2000 DNA marker; Ln 1, *ENO1*; Ln 2, *RPS27A*; Ln 3, *CALM1*; Ln 4, *SLC25A6*; Ln 5, *ARSA*; Ln 6, *SDC2*; Ln 7, *RNF130*; Ln 8, *RPL10A*; Ln 9, *OAZ1*; Ln 10, *CYCS*; Ln 11, *ATP5A1*; Ln 12, *RNF130*; Ln 13, *ACTB*.

Table 1. List of primers used for quantitative real-time PCR

Target	Accession number	Primers	Amplicon Length (bp)
<i>ENO1</i>	GE616638	F: 5' ACTTCAGGAACCCTCGCATCAACT 3' R: 5' GCTGCCCTCATCTTTCTAATACGG 3'	115
<i>RPS27A</i>	GE616644	F: 5' TGAAGACCCTCACGGGGAAAAC 3' R: 5' GTGCGTCCATCCTCTAACTGCT 3'	151
<i>CALM1</i>	GE616646	F: 5' ATGAGGTGCTTGGGTCAAATC 3' R: 5' CCTGTCAAAGACTCGGAATGC 3'	178
<i>SLC25 A6</i>	GE616648	F: 5' AAGGGCATCATCGATTGCGTAGTG 3' R: 5' GCGAAGTTGAGAGCTTGAGTTGGG 3'	116
<i>ARSA</i>	GE616650	F: 5' ACTTCACACAAGGTTCTTTTCACAG 3' R: 5' CAAATCATAGTTCTCAGCAGGGTCT 3'	131
<i>SDC2</i>	GE616652	F: 5' ACCTGTAGCTTCAGTGAGATTTGT 3' R: 5' GCAGCAACTTCTCAAAATACTGT 3'	154
<i>RPL10A</i>	GE616637	F: 5'AGAACTACGACCCGAGAAAAGATAA 3' R: 5'GCCAGCTTCTTCACCAACTTCT 3'	196
<i>OAZ1</i>	GE616645	F: 5'CAGGTGGGCGAGGGAATAGT 3' R: 5'GCATCTGTAAGCCTTGACTGGAC 3'	141
<i>CYCS</i>	GE616639	F: 5'ACAGATGCTAATAAGAATAAAGGTATCAC 3' R: 5'TAAATAAGGCAGCAGATAAATTCTACTTC 3'	193
<i>ATP5A1</i>	GE616649	F: 5'ACTTCAGAGACAGCGAAAGC 3' R: 5'CCAGCCTGAGTTTCAATGACA 3'	229
<i>RNF130</i>	GE616651	F: 5'TGAAGAACCTGTCACGATGACTCA 3' R: 5'AAAGACTAAAGAGCCCCGACTGAA 3'	177
<i>ACTB</i>	M26111.1	F: 5'-GCGGCATGCCACACCGTGCCCATCTATGAG-3' R: 5'-GCGAAGCTTGGCCATCTCTGCTCGAAGT-3'	205

pre-laying geese. The correlation was subjected to analysis of variance (ANOVA) of the SAS software. Differences were considered significant at $P < 0.05$ and extremely significant at $P < 0.01$.

Results

Only the samples with an A260/A280 ratio of 1.8–2.0 were chosen for analysis. Agarose gel electrophoresis (Fig. 1a) revealed that the 28S:18S ratio was approximately 2:1, which indicated that the samples are high quality RNA. Agarose gel electrophoresis (Fig. 1b) revealed that all primer pairs amplified a single PCR product with the expected size. The related information of the primers was listed in Table 2. The average value of the slope of the standard curve was in the range of $-3.6 \leq \text{slope} \leq -3.1$, corresponding to an amplification efficiency of 90–110 %. The average value of R^2 was ≥ 0.98 . The qRT-PCR experiment was set up according to the MIQE guidelines (Bustin *et al.*, 2009, 2010; Taylor *et al.*, 2010).

The qRT-PCR results showed that *ENO1*, *RPS27A*, *SLC25A6*, *ARSA*, *SDC2*, *RPL10A*, *OAZ1*, *CYCS* and *RNF130* were significantly up-regulated in the ovaries of laying geese compared with that of pre-laying geese ($P < 0.05$), whereas *CALM1* and *ATP5A1* were highly significantly up-regulated in the ovaries of laying geese compared with that of pre-laying geese ($P < 0.01$). The relative expression levels (Fig. 2) of *ENO1*, *RPS27A*, *CALM1*, *SLC25A6*, *ARSA*, *SDC2*, *RPL10A*, *OAZ1*, *CYCS*, *ATP5A1* and *RNF130* in the ovaries of laying geese increased by 2.34 ± 0.67 , 1.9 ± 0.46 , $4.31 \pm$

0.18 , 1.97 ± 0.47 , 1.98 ± 0.26 , 1.78 ± 0.23 , 10.36 ± 1.83 , 3.54 ± 0.21 , 4.35 ± 0.36 , 3.06 ± 0.21 , and 4.64 ± 0.29 folds respectively compared with those of the pre-laying geese.

In addition, the correlation of mRNA expression was also analyzed between the eleven genes (Table 3). The correlation between *ENO1* and *RPS27A*, *CALM1* and *ARSA* were positive strong ($R^2 = 0.915$, 0.818 and 0.901 ; $P < 0.05$); The correlation between *RPS27A* and *CALM1*, *ARSA* and *RPL10A* were positive strong ($R^2 = 0.847$, 0.974 and 0.812 ; $P < 0.05$); The correlation between *CALM1* and *ARSA*, *RPL10A*, *OAZ1*, *CYCS*, *ATP5A1* and *RNF130* were positive strong ($R^2 = 0.848$, 0.930 , 0.873 , 0.916 , 0.899 and 0.911 ; $P < 0.05$); The correlation between *SLC25A6* and *ARSA*, *ATP5A1* were positive strong ($R^2 = 0.811$, 0.841 ; $P < 0.05$); The correlation between *SDC2* and *CYCS* was positive strong ($R^2 = 0.833$; $P < 0.05$); The correlation between *RPL10A* and *OAZ1*, *CYCS*, *ATP5A1* and *RNF130* were positive strong ($R^2 = 0.987$, 0.986 , 0.985 and 0.886 ; $P < 0.05$); The correlation between *OAZ1* and *CYCS*, *ATP5A1* and *RNF130* were positive strong ($R^2 = 0.978$, 0.989 and 0.878 ; $P < 0.05$); The correlation between *CYCS* and *ATP5A1*, *RNF130* were positive strong ($R^2 = 0.956$, 0.924 ; $P < 0.05$); The correlation between *ATP5A1* and *RNF130* was positive strong ($R^2 = 0.866$; $P < 0.05$).

Discussion

Analysis of mRNA transcription in the ovaries is a very important method to find the relationship between gene function and egg-production (Yen *et al.*, 2006; Chen *et al.*,

Table 2. Analysis of each qRT-PCR

Target	Amplicon Length (bp)	Slope	Amplification efficiency (%)	S.D of efficiency	R ²	Average Ct of cDNA
<i>ENO1</i>	115	-3.14	108.19	0.009	0.998	22.92
<i>RPS27A</i>	151	-3.49	93.43	0.008	0.998	24.13
<i>CALM1</i>	178	-3.30	100.92	0.016	0.996	23.91
<i>SLC25A6</i>	116	-3.23	103.98	0.010	0.998	26.05
<i>ARSA</i>	131	-3.17	106.75	0.013	0.996	26.57
<i>SDC2</i>	154	-3.58	90.25	0.011	0.996	24.24
<i>RPL10A</i>	196	-3.29	101.35	0.012	0.998	23.87
<i>OAZ1</i>	141	-3.57	90.59	0.012	0.994	25.33
<i>CYCS</i>	193	-3.54	91.64	0.007	0.998	27.51
<i>ATP5A1</i>	229	-3.13	108.68	0.012	0.998	26.30
<i>RNF130</i>	177	-3.54	91.64	0.003	1.000	24.55
<i>ACTB</i>	205	-3.40	96.84	0.014	0.998	30.68

2007; Yang *et al.*, 2007; Kang *et al.*, 2009). This paper described the relative expression profiles of eleven egg-performance associated genes in ovaries of both pre-laying and laying Zi geese.

ENO1 is a key enzyme in the glycolytic pathway which catalyzes the reversible conversion of D-2-phosphoglycerate into phosphoenol-pyruvate in the second half of the glycolytic pathway (Avilán *et al.*, 2011). Recent studies have revealed that *ENO1* can be detected in many prokaryotic and eukaryotic cells, and localized in cytoplasm, cell surface and nucleus of various mammalian cells to possibly mediate distinct functions (Liu *et al.*, 2007). *ENO1* gene up-regulation in mRNA and/or protein levels have been observed in several tumors, including brain, breast, cervix, colon, eye, and so on (Liu *et al.*, 2007; Capello *et al.*, 2011).

ENO1 is associated with tumor development through a process known as the Warburg effect. Tumor tissues or other normal proliferative tissues consume more glucose by the pathway of glycolytic than normal cells and generate ATP by converting pyruvate to lactic acid, even in the presence of a normal oxygen supply (Vander *et al.*, 2009). This study found that the relative expression levels of *ENO1* in the ovaries of laying geese compared with those of pre-laying geese were increased by 2.34 ± 0.67 fold. It has been found that the mRNA concentrations of *ENO1* in the livers of laying geese were higher than pre-laying geese (Yen *et al.*, 2009). We propose that the rapid growth and development of avian ovarian follicle also need consume glucose by the pathway of glycolytic as the dynamics of avian ovarian follicle development. Further studies are needed to determine the role of *ENO1* in avian ovarian follicle growth and development.

Ribosomal protein is an essential component of the 40S subunit of the ribosome, and it was proved that it has an intimate relationship with protein synthesis (Shang *et al.*, 2005). Follicular morphous and function of the poultry in breeding season generate several changes, which indicated that the large amounts of proteins were synthesized not only to activate the maturation promoting factor (MPF) but also have a close relationship with the developmental capacity of

postfertilization megagametocyte. *Mus musculus* ribosomal protein gene *S29* and human follicular cell ribosomal protein gene *S25* and *S18* conspicuous differential expression in different stages of mammal follicular development (Dharma *et al.*, 2009). *RPS27A* was also found to be up-regulated in matured buffalo (*Bubalus bubalis*) oocytes (Kandil *et al.*, 2010). The up-regulation of *S27A* in laying season in our research shows that it may be involved in the protein synthesis during the follicle alveolar substance maturation process.

CALM1, which is a ubiquitous calcium-binding protein and mediator of the calcium signal, is known to regulate numerous cellular processes, including cell motility, proliferation, and intermediary metabolism (Toutenhoofd *et al.*, 1998). *CALM1* can activate the Ca^{2+} -transporting system in the avian shell gland for maintenance eggshell formation (Lundholm *et al.*, 1990). *CALM1* is also critical for up-regulation of steroidogenesis in the theca cells of hens (Levorse *et al.*, 1991). In addition, *CALM1* was highly expressed in the ovary of laying geese, which implies that *CALM1* may be involved in the process of egg laying in geese.

SLC25A6, which was expressed at high levels in the ovaries of laying geese, is localized to the inner mitochondrial membrane (Palmieri, 2004); these proteins were referred as mitochondrial transporter family. *SLC25A6* consists of proteins that function as transporters of a large variety of molecules (i.e. ATP, ADP, citrate, malate, Ornithine, citrulline, lysine, arginine, histidine, coenzyme A) (Fiermonte *et al.*, 2009). Therefore, the up-regulation of *SLC25A6* may be the result of high metabolic activity in the ovaries (Kang *et al.*, 2009).

ARSA is involved in hydrolase activity, catalytic activity, and cerebroside-sulfatase activity. *ARSA* almost located everywhere in organism, for example, *ARSA* exist in cytolysosome in somatic cell as lysosomal enzyme, butin the perforatorium as acrosomal enzyme and cytolemma surface of spermatozoa and ovum as membrane peripheral protein. *ARSA* keeps high conservation organic evolution and exist in almost animals including mammalia, fish and birds. The research showed that *ARSA* unfertilized only bind with ovum

Fig. 2. Expression of eleven egg performance-associated genes in the ovary of Zi geese. Expression of *ENO1*, *RPS27A*, *CALM1*, *SLC25A6*, *ARSA*, *SDC2*, *RPL10A*, *OAZ1*, *CYCS*, *ATP5A1* and *RNF130* genes in ovaries of pre-laying geese (prelay) compared with those of laying geese (lay). The expression of these genes were normalized to *ACTB*. Gene expression levels, calculated by the $\Delta\Delta$ threshold crossing point method, are presented in arbitrary units (AU). * Significantly different ($P < 0.05$) from prelay; ** highly significantly different ($P < 0.01$) from prelay. Values are means $\pm$ SD ($n = 10$).

but not with spermatovum (Wu, 2004), which suggested that the close correlation of *ARSA* and sperm-egg fusion may affect the egg laying. It's up-regulation in the ovary of laying geese suggests an association with laying performance (Kang *et al.*, 2009).

The syndecans are a family of cell-surface heparan sulfate proteoglycans that regulate cell behavior through the binding of extracellular matrix molecules and/or soluble ligands (Zimmermann and David, 1999). This interaction regulates cell-ECM adhesion, migration, cytoskeleton organization, and gene expression as the membrane protein of receptor

through signal transduction pathways (Park *et al.*, 2002). It is concluded that the up-regulation of *SDC2* in ovaries of laying Zi geese may affect follicular proliferation by regulating follicular maturation.

The *OAZ1* inhibits ornithine decarboxylase (*ODC*) and accelerates its degradation (Matsufuji *et al.*, 1995). *ODC* is a key enzyme in the biosynthesis of the polyamines and its activity is regulated in response to factors that stimulate cell proliferation (Schipper *et al.*, 2000). Steady-state *ODC* mRNA levels and enzyme activity were measured in tissues of chicks genetically selected for increased growth rate or for

Table 3. Analysis of the eleven genes correlation

		<i>ENO1</i>	<i>RPS27A</i>	<i>CALM1</i>	<i>SLC25A6</i>	<i>ARSA</i>	<i>SDC2</i>	<i>RPL10A</i>	<i>OAZ1</i>	<i>CYCS</i>	<i>ATP5A1</i>	<i>RNF130</i>
<i>ENO1</i>	R ²	1	0.915*	0.818*	0.540	0.901*	0.331	0.733	0.631	0.647	0.700	0.510
	P		0.011	0.047	0.268	0.014	0.521	0.097	0.179	0.165	0.121	0.301
<i>RPS27A</i>	R ²	0.915*	1	0.847*	0.794	0.974**	0.472	0.812*	0.746	0.751	0.800	0.613
	P	0.011		0.033	0.059	0.001	0.345	0.050	0.089	0.085	0.056	0.196
<i>CALM1</i>	R ²	0.818*	0.847*	1	0.722	0.848*	0.582	0.930**	0.873*	0.916*	0.899*	0.911*
	P	0.047	0.033		0.105	0.033	0.226	0.007	0.023	0.010	0.015	0.012
<i>SLC25A6</i>	R ²	0.540	0.794	0.722	1	0.811	0.441	0.773	0.798	0.739	0.841*	0.700
	P	0.268	0.059	0.105		0.050	0.382	0.072	0.057	0.093	0.036	0.121
<i>ARSA</i>	R ²	0.901*	0.974**	0.848*	0.811	1	0.306	0.756	0.686	0.686	0.765	0.618
	P	0.014	0.001	0.033	0.050		0.555	0.082	0.133	0.133	0.077	0.191
<i>SDC2</i>	R ²	0.331	0.472	0.582	0.441	0.306	1	0.769	0.790	0.833*	0.696	0.657
	P	0.521	0.345	0.226	0.382	0.555		0.074	0.062	0.040	0.125	0.156
<i>RPL10A</i>	R ²	0.733	0.812*	0.930**	0.773	0.756	0.769	1	0.987**	0.986**	0.985**	0.886*
	P	0.097	0.050	0.007	0.072	0.082	0.074		0.000	0.000	0.000	0.019
<i>OAZ1</i>	R ²	0.631	0.746	0.873*	0.798	0.686	0.790	0.987**	1	0.978**	0.989**	0.878*
	P	0.179	0.089	0.023	0.057	0.133	0.062	0.000		0.001	0.000	0.021
<i>CYCS</i>	R ²	0.647	0.751	0.916*	0.739	0.686	0.833*	0.986**	0.978**	1	0.956**	0.924**
	P	0.165	0.085	0.010	0.093	0.133	0.040	0.000	0.001		0.003	0.008
<i>ATP5A1</i>	R ²	0.700	0.800	0.899*	0.841*	0.765	0.696	0.985**	0.989**	0.956**	1	0.866*
	P	0.121	0.056	0.015	0.036	0.077	0.125	0.000	0.000	0.003		0.026
<i>RNF130</i>	R ²	0.510	0.613	0.911*	0.700	0.618	0.657	0.886*	0.878*	0.924**	0.866*	1
	P	0.301	0.196	0.012	0.121	0.191	0.156	0.019	0.021	0.008	0.026	

* $P < 0.05$; ** $P < 0.01$

egg production (Johnson *et al.*, 1995). Carmen *et al.* (2002) indicated that although elevated levels of *ODC* are not required for acute ovarian steroidogenesis, the preovulatory peak of ovarian *ODC* activity observed in the evening of proestrus may be critical for the establishment of a constitutive steroidogenic pathway and progesterone secretion by the corpus luteum during the diestrus stage of the murine estrous cycle. The up-regulation of *OAZ1* in ovaries of laying Zi geese may be the result of the increase of *ODC*. It may be hypothesized that *OAZ1* is associated with the secretion of reproduction hormone in geese to affect egg production.

CYCS gene encodes a small heme protein that functions as a central component of the electron transport chain in mitochondria. The encoded protein associates with the inner membrane of the mitochondrion where it accepts electrons from cytochrome b and transfers them to the cytochrome oxidase complex. This protein is also involved in initiation of apoptosis (Evans *et al.*, 1988). Therefore, it is not surprising that the level of expression of *CYCS2* was higher in the ovaries of laying geese in the present study.

ATP5A1, which encodes a protein that is involved in oxidative phosphorylation and the ATP synthetic pathway is a gene required for oxidative metabolism. The *ATP5A1* gene is essential for aerobic respiration (Schnauffer *et al.*, 2005), and its absence results in cell death (Baran *et al.*, 2007). Therefore, the expression level of *ATP5A1* was higher in the ovaries of laying geese is the result of high metabolic activity in the ovaries because of a great quantity of ATP generated by aerobic respiration.

Ring finger proteins could play an important role in energy

metabolism, protein synthesis, oocyte maturation, tumorigenesis (Fang *et al.*, 2003; Hirvonen-Santti *et al.*, 2004; Koyama *et al.*, 2008). In present study, the relative expression level of *RNF130* in the ovaries of laying geese increased by 4.64 ± 0.29 fold compared with those of pre-laying geese. It may be the result of high metabolic activity in the ovaries.

The correlation results suggested that there are certain synergistic effect among some proteins encoded by the above certain genes (positive strong correlation) in the mature process of follicle. For example, *CALM1* could regulate numerous cellular processes, and accordingly have strongly correlated with great majority of genes. In addition, some genes were closely associated with others such as *RPL10A* and *OAZ1*, *CYCS*, *ATP5A1*, *RNF130*, because they may be related to the energy metabolism and cell proliferation. But the reason why so much positive strong among some genes but no obvious correlation among others are an important problem for us to further pursue in the future.

In the present study, we are the first to show that the *ENO1*, *RPS27A*, *CALM1*, *SLC25A6*, *ARSA*, *SDC2*, *RPL10A*, *OAZ1*, *CYCS*, *ATP5A1* and *RNF130* mRNAs are transcribed and exhibited changes in pre-laying and laying Zi geese ovaries.

Acknowledgments

This work was supported by the Natural Science Foundation of HeiLongjiang Province of China (ZD201116). The authors would like to thank Mrs. Yibing Han, for her valuable time in providing language help. We also thank the anonymous reviewers for their valuable comments and suggestions.

References

- Avilán L, Gualdrón-López M, Quiñones W, González-González L, Hannaert V, Michels P A.M and Concepción JL. Enolase: A key player in the metabolism and a probable virulence factor of trypanosomatid parasites-perspectives for its use as a therapeutic target. *Enzyme Research*, 2011: 1–14. 2011.
- Baran AA, Silverman KA, Zeskand J, Koratkar R, Palmer A, McCullen K, Curran WJ, Edmonston TB, Siracusa LD and Buchberg AM. The modifier of Min 2 (Mom2) locus: Embryonic lethality of a mutation in the ATP5A1 gene suggests a novel mechanism of polyp suppression. *Genome Research*, 17: 566–576. 2007.
- Bustin SA, Benes V, Garson JA, Hellemans J, Huggett Jim, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J and Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, 55: 4611–622. 2009.
- Bustin SA, Beaulieu JF, Huggett J, Jaggi R, Kibenge F SB, Olsvik PA, Penning LC and Toegel S. MIQE précis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. *BMC Molecular Biology*, 11: 74. 2010.
- Capello M, Ferri-Borgogno S, Cappello P and Novelli F. α -enolase: a promising therapeutic and diagnostic tumor target. *FEBS Journal*, 287: 1064–1074. 2011.
- Carmen M. Bastida, Francisco T, Asunci on Cremades and Rafael Pe~nafil. The preovulatory rise of ovarian ornithine decarboxylase is required for progesterone secretion by the corpus luteum. *Biochemical and Biophysical Research Communications* 293: 106–111. 2002.
- Chen LR, Chao CH, Chen CF, Lee YP, Chen YL and Shiu YL. Expression of 25 high egg production related transcripts that identified from hypothalamus and pituitary gland in red-feather Taiwan country chickens. *Animal Reproduction Science*, 100: 172–185. 2007.
- Dharma SJ, Modi DN and Nandedkar TD. Gene expression profiling during early folliculogenesis in the mouse ovary. *Fertility and Sterility*, 91: 2025–2036. 2009.
- Evans MJ and Scarpulla RC. The human somatic cytochrome c gene: two classes of processed pseudogenes demarcate a period of rapid molecular evolution. *Proceedings of the National Academy of Sciences of the United States of America*, 85: 9625–9629. 1988.
- Fang S, Lorick KL, Jensen JP and Weissman AM. RING finger ubiquitin protein ligases: implications for tumorigenesis, metastasis and for molecular targets in cancer. *Seminars in Cancer Biology*, 13: 5–14. 2003.
- Fiermonte G, Paradies E, Todisco S, Marobbio CT and Palmieri F. A novel member of solute carrier family 25 (SLC25A42) is a transporter of coenzyme a and adenosine 3',5'-diphosphate in human mitochondria. *Journal of Biological Chemistry*, 284: 18152–18159. 2009.
- Hirvonen-Santti SJ, Sriraman V, Anttonen M, Savolainen S, Palvimäki JJ, Heikinheimo M, Richards JS and Janne OA. Small nuclear RING finger protein expression during gonad development: Regulation by gonadotropins and estrogen in the postnatal ovary. *Endocrinology*, 145: 2433–2444. 2004.
- Johnson R, Bulfield G, Taita A and Goddard C. Increased expression and activity of ornithine decarboxylase in chicks genetically selected for rapid growth rate. *Comparative Biochemistry and Physiology Part B, Biochemistry & Molecular Biology*, 110: 531–537. 1995.
- Kandil OM, Abdoon ASS, Phatsara C, Ghanem N, Hölker M, Schellander K and Tesfaye D. Transcriptional analysis of buffalo (*Bubalus bubalis*) oocytes during in vitro maturation using bovine cDNA microarray. *Reproduction in Domestic Animals*, 45: 63–74. 2010.
- Kang B, Guo JR, Yang HM, Zhou RJ, Liu JX, Li SZ and Dong CY. Differential expression profiling of ovarian genes in prelaying and laying geese. *Poultry Science*, 88: 1975–1983. 2009.
- Koyama S, Hata S, Witt CC, Ono Y, Lerche S, Ojima K, Chiba T, Doi N, Kitamura F, Tanaka K, Abe K, Witt SH, Rybin V, Gasch A, Franz T, Labeit S and Sorimachi H. Muscle ring-finger protein-1 (MuRF1) as a connector of muscle energy metabolism and protein synthesis. *Journal of Molecular Biology*, 376: 1224–1236. 2008.
- Levorso JM, Tilly JL and Johnson AL. Role of calcium in the regulation of theca cell androstenedione production in the domestic hen. *Reproduction*, 92: 159–167. 1991.
- Liu KJ and Shih NY. The role of enolase in tissue invasion and metastasis of pathogens and tumor cells. *Journal of Cancer Molecules*, 3: 45–48. 2007.
- Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25: 402–408. 2001.
- Lundholm CE. The distribution of calmodulin in the mucosa of the avian oviduct and the effect of p-p'-DDE on some of its metabolic parameters. *Comparative Biochemistry and Physiology Part C, Comparative Pharmacology*, 96: 321–326. 1990.
- Matsufuji S, Matsufuji T, Miyazaki Y, Murakami Y, Atkins JF, Gesteland RF and Hayashi S. Autoregulatory frameshifting in decoding mammalian ornithine decarboxylase antizyme. *Cell*, 80: 51–60. 1995.
- Palmieri F. The mitochondrial transporter family (SLC25): Physiological and pathological implications. *Pflügers Archiv European Journal of Physiology*, 447: 689–709. 2004.
- Park H, Kim Y, Lim Y, Han I and Eok-Soo Oh. Syndecan-2 mediates adhesion and proliferation of colon carcinoma cells. *Journal of Biological Chemistry*, 277: 29730–29736. 2002.
- Schipper RG, Penning LC and Verhofstad AA. Involvement of polyamines in apoptosis. Facts and controversies: Effectors or protectors? *Seminars in Cancer Biology*, 10: 55–68. 2000.
- Schnauffer A, Clark-Walker GD, Steinberg AG and Stuart K. The F1-ATP synthase complex in bloodstream stage trypanosomes has an unusual and essential function. *EMBO Journal*, 24: 4029–4040. 2005.
- Shang X, Cheng HH and Zhou RJ. Ribosomal protein genes highly expressed in swamp eel gonads. *Hereditas (Beijing)*, 27: 227–230. 2005.
- Taylor S, Wakem M, Dijkman G, Alsarraj M and Nguyen M. A practical approach to RT-qPCR-Publishing data that conform to the MIQE guidelines. *Methods*, 50: S1–S5. 2010.
- Toutenhoofd SL, Garcia F, Zacharias DA, Wilson RA and Strehler EE. Minimum CAG repeat in the human calmodulin-1 gene 5' untranslated region is required for full expression. *Biochimica et Biophysica Acta, Gene Structure and Expression*, 1398: 315–320. 1998.
- Vander Heiden MG, Cantley LC and Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*, 324: 1029–1033. 2009.
- Wu TT. The role of AS-A in sperm-egg fusion. *Chinese Journal of Andrology*, 18: 56–59. 2004.
- Yang KT, Lin CY, Liou JS, Fan YH, Chiou SH, Huang CW, Wu

- CP, Lin EC, Chen CF, Lee YP, Lee WC, Ding ST and Huang MC. Differentially expressed transcripts in shell glands from low and high egg production strains of chickens using cDNA microarrays. *Animal Reproduction Science*, 101: 113–124. 2007.
- Yen CF, Lin EC, Wang YH, Wang PH, Lin HW, Hsu JC, Wu LS, Jiang YN and Ding ST. Abundantly expressed hepatic genes and their differential expression in liver of prelaying and laying geese. *Poultry Science*, 88: 1955–1962. 2009.
- Yen CF, Lin HW, Hsu JC, Lin C, Shen TF and Ding ST. The expression of pituitary gland genes in laying geese. *Poultry Science*, 85: 2265–2269. 2006.
- Zimmermann P and David G. The syndecans, tuners of transmembrane signaling. *FASEB Journal*, 13 (Suppl.), S91–S100. 1999.