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Ovary transcriptome profiling in high- and low-yielding Chinese Chahua laying chickens

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Abstract: This study aimed to explore the underlying reasons for the differences in egg production in Chinese Chahua chickens. The Chahua chicken population was divided into two groups, namely a high-yielding group (H) and a low-yielding group (L). The egg-laying performance, ovarian morphology, histological characteristics, and serum hormone concentrations in the H and L groups were analysed. The results revealed that there were significant differences ($P < 0.01$) between the two groups for the age at the first egg and the number of eggs. The H group showed significantly more ($P < 0.01$) hierarchical follicles and small yellow follicles than the L group. While several secondary follicles and primary follicles were observed in the ovaries of the H group, only a few secondary follicles were observed in the ovaries of the L group. The serum concentrations of the follicle-stimulating hormone, the luteinising hormone, and oestradiol were significantly higher in the H group compared to that in the L group ($P < 0.01$). Next, we sequenced and analysed the ovarian expression profiles in the two groups using RNA-seq. The transcriptome sequencing revealed 875 differentially expressed genes (DEGs) between the H and L groups. Of the 875 DEGs, 624 were up-regulated, and 251 were down-regulated. The DEGs were mapped to 31 Gene Ontology (GO) terms and 15 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for the ovarian tissue. The KEGG pathway analysis mainly revealed the involvement of focal adhesion, cytokine-cytokine receptor interaction, and the TGF-beta signalling pathway, while the GO analysis mainly showed involvement of the hormonal activity, extracellular matrix, and extracellular region. Our results showed that understanding the differences in the gene expressions between the ovarian tissues of high- and low-yielding hens may provide a useful reference for improving the egg-laying performance in Chahua chickens.

Keywords: egg-laying performance; histological characteristics; hormones in serum; ovarian follicle; reverse transcription-qPCR

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The Chahua chicken is a popular breed in China, which is a rare tropical primitive breed domesticated and bred from the red *Gallus gallus*. It is found in the tropical or subtropical regions of China's southern border. The main characteristics of this breed involve early sexual development, coarse feeding tolerance, strong disease resistance, and tender flesh.

The egg-laying performance is an important characteristic in poultry production of laying hens (Kim et al. 2004). The ovary is the female reproductive organ that produces and releases eggs. The main functional structure of the ovary is the follicle. Follicular development begins from the original follicle and grows about 400 times to form a mature follicle. The development process includes recruitment, selection, dominance, growth, and maturation before ovulation (Regan et al. 2018). Based on the diameter, follicles are generally divided into small white follicles (SWFs), < 2 mm in diameter, large white follicles (LWFs), 2~4 mm in diameter, small yellow follicles (SYFs), 4~8 mm in diameter, and hierarchical follicles (HFs), > 8 mm in diameter (Hocking 2009). To maintain continuous ovulation, a SYF is selected and entered into the HF layer each day, which then begins to grow rapidly and eventually differentiate (Onagbesan et al. 2009). Each stage, from follicle collection to ovulation in poultry, is a rigorous process. The follicle reserve at each stage may affect the ovulation cycle and ultimately affect the rate of ovulation (Ghanem and Johnson 2018). The ovary function of the follicular development and normal ovulation is mainly maintained by the follicle-stimulating hormone (FSH), the luteinising hormone (LH), and oestradiol (E2) (Du et al. 2020).

Recently, RNA sequencing has attracted vast attention and has been used to study the gene expression, regulation, and exploration of new genes for breeding (Sun et al. 2020; Zou et al. 2020). Additionally, RNA-sequencing analysis has been used to explore different and new genes related to the egg-laying performance in both the hypothalamus and pituitary gland of chickens (Wang and Ma 2019). Moreover, high-throughput sequencing has been used to study the mechanism of egg production in chicken ovaries. For instance, differentially expressed genes (DEGs) were analysed in the ovarian tissue of Jinghai yellow chickens, revealing low and high egg-production at the age of 43 weeks, along with identifying five important candidate genes and some signalling pathways related to the egg production (Zhang et al. 2019). In Luhua chicken ovaries, ten candidate genes, including kynureninase (*KYNU*), tryptophan 2,3-dioxygenase (*TDO2*), and aralkylamine *N*-acetyltransferase (*AANAT*), have been identified related to the egg production (Mishra et al. 2020). In another study, Mu et al. (2021) identified four important candidate genes, including the prolactin receptor (*PRLR*), proopiomelanocortin (*POMC*), and galanin receptor 1 (*GALR1*), in the ovaries of the high and low egg-producing chickens. Hence, transcriptome sequencing can be used to provide a fast and accurate method for the genetic selection and as a complement to traditional breeding techniques.

In this study, we subjected three ovarian tissues, each from high-yield (H) and low-yield (L) Chahua hens at the age of 43 weeks, for transcriptome analysis, which generated mRNA profiles of the ovaries. Next, a comparative analysis of the mRNA transcriptomes was performed. Also, the ovarian morphology, histological characteristics, serum hormone concentrations and expressions of the selected genes were analysed. The resulting data could be used to further understand the functioning of chicken ovarian tissues and to also study the mRNA regulation mechanism in poultry.

MATERIAL AND METHODS

Ethics committee statement

The animal protocol was approved by the Animal Ethics Committee of the Yunnan Agricultural University, and it followed the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Experimental animals

The Chahua chickens used in this experiment were obtained from Xishuangbanna Chahua chicken Industry Development Co., Ltd. During the whole experiment, the feeding conditions and nutrition levels were maintained per the standard commercial hen food with *ad libitum* access to water. We selected 1 848 hens of the same generation and individually raised them in a single cage that was housed separately. Hens entering the egg-

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laying stage (at 16 week of age) were exposed to a 16 h/8 h light/dark regimen at a temperature of 13–25 °C. The production of eggs was counted in the hens aged between 16 and 43 weeks, sorted by ascending order. The top 10% of the hens with the greatest egg production were considered the H group, while the last 10% of the hens with lowest egg production were considered the L group.

Analysis of egg-laying performance

We recorded the age at the first egg (AFE), the body weight at the first egg (BWF), the number of eggs (EN), and the egg weight (EW) values of 184 H and 184 L chickens. The difference was analysed using a t-test with a significance level of $P = 0.05$. The Yang Ning model (Liu et al. 2021) was adopted to simulate the egg production curve of the H and L groups using the formula:

$$y(t) = a \times \exp(-b \times t)/\{1 + \exp[-c \times (t - d)]\} \quad (1)$$

where:

$y(t)$ – egg production rate at t week(s);
a, b, c, d – undetermined parameters;
exp – natural base number;
t – age of the egg production week.

The optimal parameters (a, b, c, and d) of the model and the corresponding fitting degree R^2 were calculated using the SPSS software v25.0 (IBM Corp., Armonk, NY, USA). Finally, the egg production characteristics of the Chahua chickens were inferred based on the egg production curve.

Analysis of the ovarian morphology, histological characteristics, and hormones in the serum

At the age of 43 weeks, 24 hens (12 hens each selected randomly from the 184 H and 184 L groups) were slaughtered, and their ovarian tissues were photographed, which was followed by counting the follicle grade. The ovarian tissues were sampled in the following two ways: one was quickly frozen in liquid nitrogen for the subsequent RNA extraction; the other part was fixed in 10% paraformaldehyde and dehydrated after 24 h. Next, it was embedded in paraffin, sectioned, and stained with haematoxylin and eosin staining. The sections were then observed using a microscope. Next, 2 ml of venous blood was collected from the 48 hens (24 hens each selected randomly from the 184 H and 184 L groups). Later, the clotted blood was centrifuged, the serum was collected and stored at –20 °C. The serum concentrations of FSH, LH, and E2 were measured using chicken-specific enzyme-linked immunosorbent assay (ELISA) kits (Yuanye Bio-Technology, Shanghai, CN) according to the manufacturer's protocols.

Transcriptome sequencing: RNA extraction, library construction, and sequencing

A RNeasy Mini Kit (Takara, Beijing, CN) was used to extract the total RNA from six ovarian tissues (three tissues, each selected randomly from the 184 H and 184 L at the age of 43 weeks). Agarose gel electrophoresis was used to check the degradation degree of the RNA. A NanoPhotometer® (IMPLEN, Munich, Germany) was used to determine the purity of the RNA, while an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) was used to check its integrity. A NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, Ipswich, MA, USA) was used to build the cDNA libraries, while the quality of the library obtained from the six samples was analysed using the Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

Quality control, alignment, and annotation

Illumina's CASAVA v1.8 (Illumina, Inc., San Diego, CA, USA) was used to identify the imaging data, which was measured by the high-throughput quenchers. The imaging data were then transformed into sequence data (reads) in the FASTQ format. Initially, in-house Perl scripts were used to process the raw FASTQ data. To filter the raw data, we removed the reads with adapter, indeterminate base information, and low-quality reads. Meanwhile, the Q20, Q30, and GC content of the clean data were calculated. The chicken's genome sequence was downloaded from the genome website (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF_000002315.4_Gallus_gallus-5.0_genomic.gff.gz). Hisat2 v2.0.5 was used to build an index of the

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reference genome, while clean reads were selected to map the reference genome.

Bioinformatics Analysis

The gene expression levels were estimated using the number of normalised FPKM (fragments per kilobase of exon model per million mapped reads). The PheatMap package v1.0.12 was used to perform hierarchical clustering in the R package (https://cran.r-project.org/), while the Origin software v2019b (Origin Software, San Clemente, CA, USA) was used to perform the principal component analysis (PCA) of the FPKM. Pearson's correlation was used to calculate the correlations of the FPKM between the samples using the Origin software v2019b. A differential expression analysis was performed between the two combinations using DESeq2 R package v1.16.1 based on the raw read count. The *P*-value was adjusted using the Benjamini-Hochberg method (Benjamini and Hochberg 1995), while the corrected *P*-value < 0.05 and $|\log_2 \text{foldchange}| > 2$ were used as the threshold for detecting the significant differential expression. The GO enrichment analysis (Yu et al. 2012) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the DEGs were carried out using ClusterProfiler v3.4.4. A *P*-value < 0.05 indicated the significant enrichment of the DEGs in the GO term and the KEGG pathway.

Verification by reverse transcription-qPCR (RT-qPCR)

To verify the accuracy of transcriptome sequencing data, nine candidate genes were randomly selected for real-time quantitative polymerase chain reaction reverse transcription (RT-qPCR) validation. An RNeasy Mini Kit (Takara, Beijing, China) was used to extract the total RNA from six ovarian tissues; each picked from both the H and L groups. The purity and concentration of the total RNA were evaluated using a NanoPhotometer® (Implen GmbH, München, Germany), while the integrity of the total RNA was evaluated using 1% agarose gel electrophoresis. The RT-qPCR was performed using a PrimeScript™ RT reagent Kit with a gDNA Eraser (Takara, Beijing, China) according to the manufacturer's instructions. The procedure was as follows: step 1: the genomic DNA was removed using a 10 µl reaction volume containing 2 µl of 5×gDNA Eraser Buffer, 2 µl of gDNA Eraser, 1 µg of Total RNA, and RNase free dH_2O up to 10 µl. The PCR reaction programme was set at 42 °C for 2 minutes. Step 2: the cDNA was synthesised using a 20 µl reaction volume containing 10 µl of the reaction liquid of step 1, 1 µl of PrimeScript RT Enzyme Mix I, 1 µl of RT Primer Mix, 4 µl of 5×PrimeScript Buffer 2, and 4 µl of RNase free dH_2O. The PCR programme was set at 37 °C for 15 min and 85 °C for 5 seconds. Step 3: the real-time PCR was performed using a 20 µl reaction volume containing 10 µl of SYBR@ Premix Ex TaqII (2X), 0.8 µl of forward primer (10 µM), 0.8 µl of reverse primer (10 µM), 0.4 µl of ROX Reference II, 2 µl cDNA, and 6 µl of dH_2O. The PCR programme was set as follows: 94 °C for 30 s to activate the reaction. Then, 94 °C for 15 s for denaturation, 56 °C for 30 s for annealing, and finally 72 °C for a 30 s extension for 40 cycles. Beta-actin (*ACTB*) was used as the internal control for the normalisation of the results, while the $2^{-\Delta\Delta CT}$ method was used to determine the relative expression. All the primer sequences are listed in Table S1 in electronic supplementary material (ESM; for the supplementary material see the electronic version).

RESULTS

Analysis of the egg-laying performance

Table 1 shows the egg-laying performance of the 184 H and 184 L chickens, which indicates the aver-

Table 1. Egg laying performance in the high-yielding (H) and low-yielding (L) chickens

	AFE (days)		BWF (g)		EW (g)		EN	
Chickens	H	L	H	L	H	L	H	L
Mean value	140.11 ± 15.16	155.31 ± 10.35	1 010.93 ± 135.89	994.42 ± 154.01	41.40 ± 6.78	42.99 ± 3.21	116 ± 5.10	38 ± 7.42
P-value	1.23×10^{-12}**		0.326		0.06		5.056×10^{-187}**	

AFE = age at first egg; BWF = body weight at first egg; EN = number of eggs; EW = egg weight

**$P < 0.01$

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age AFE, BWF, EW, and EN of both groups. The AFE and EN of the H chickens were significantly different from that of the L chickens ($P < 0.05$) (Table 1). Figure 1 shows the optimal parameters of the H and L chickens (where, *a*, *b*, *c* and *d*) corresponding to the R^2 and Yang Ning model expressions. The growth stage, inflection point, and stable yield stage (high yield stage) of the H chickens were found to be 16–25 weeks, the 26th week, and 27–43 weeks, respectively, with the stable yield stage showing a slight decrease. In the L chickens, the growth stage and the inflection point were 16–25 weeks and the 26th week, respectively. However, during the stable yield stage (27–43 weeks), the declining trend of the egg-laying rate curve was found to be steeper than that of the H group (Figure 1).

Analysis of the ovarian morphology, histological characteristics, and hormones in the serum

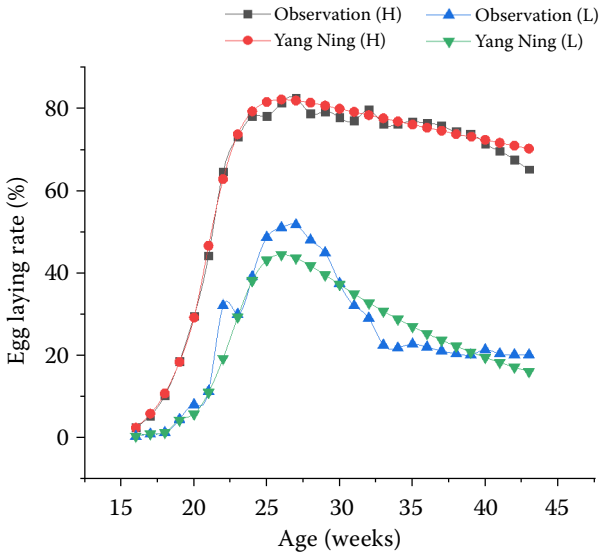
The comparison between the ovaries of the H and L groups showed a larger volume of ovaries covered with more HF and alternative SYF in the H group (Figure 2A), but a comparatively smaller volume with no or fewer HF and SYF in the L group (Figure 2A). Significantly more HF and SYF were found in the H group than those in the L group (Figure 2B, $P < 0.01$). The ovarian tissue sections of both the H and L groups were observed under the same magnification microscope, which revealed many secondary follicles (SFs, > two layers of granulosa cells) and primary follicles (PFs, one layer of cuboidal granulosa cells) in the ovaries of the H group (Figure 2C), but only a few SFs in the ovaries of the L group (Figure 2D). Our results showed that the serum concentrations of the FSH, E2, and LH were significantly higher in the H group compared to that of the L group ($P < 0.05$) (Table 2).

Data summary of the ovary transcriptome in the Chahua hens

We constructed six ovarian cDNA libraries of the Chahua chickens, including both the high- and low-yielding laying hens. Each library had more than 45 million raw reads. To filter out the low-quality and linker sequences, we retained more than 44 million clean reads in each sample. These clean reads involved quality levels of not less than 97.32% in the Q20 and 93.08% in the Q30 (Table S2 in ESM). The FASTQ files were submitted to the Sequence Read Archive database with the BioProject ID PRJNA666971.

Overview of the ovary transcriptome

The Illumina RNA-Seq produced about 16 359 mRNAs, whose genomic characterisations were depicted by analysing the alignment regional distribution, the distribution of the expression level, and the expression level of the mRNAs in both the high- and low-yielding laying hens. Our results showed that the ratio of the H group mapping to the exon region was lower than that of



Model	Yang Ning			Model	Yang Ning		
Equation	$y(t) = a \times \exp(-b \times t)/$ $/\{1 + \exp[-c^*(t-d)]\}$			equation	$y(t) = a \times \exp(-b \times t)/$ $/\{1 + \exp[-c^*(t-d)]\}$		
Adj. R^2	0.978			adj. R^2	0.969		
		value	standard error			value	standard error
Egg laying rate (H)	*a*	108.032	4.291	egg laying rate (L)	*a*	262.415	34.823
	b	0.01	0.001		*b*	0.065	0.004
	c	0.839	0.062		*c*	0.813	0.073
	d	20.844	0.08		*d*	23.007	0.167

Figure 1. Egg-laying curves for the high-yielding (H) and low-yielding (L) chickens

The H and L Yang Ning model expressions are: $y(t) = 108.032 \times \exp(-0.01 \times t)/\{1 + \exp[-0.839 \times (t - 20.844)]\}$ ($R^2 = 0.978$) and $y(t) = 262.415 \times \exp(-0.065 \times t)/\{1 + \exp[-0.813 \times (t - 23.007)]\}$ ($R^2 = 0.969$), respectively; where: t is the age of the egg production week, $y(t)$ is the egg production rate at t week(s)

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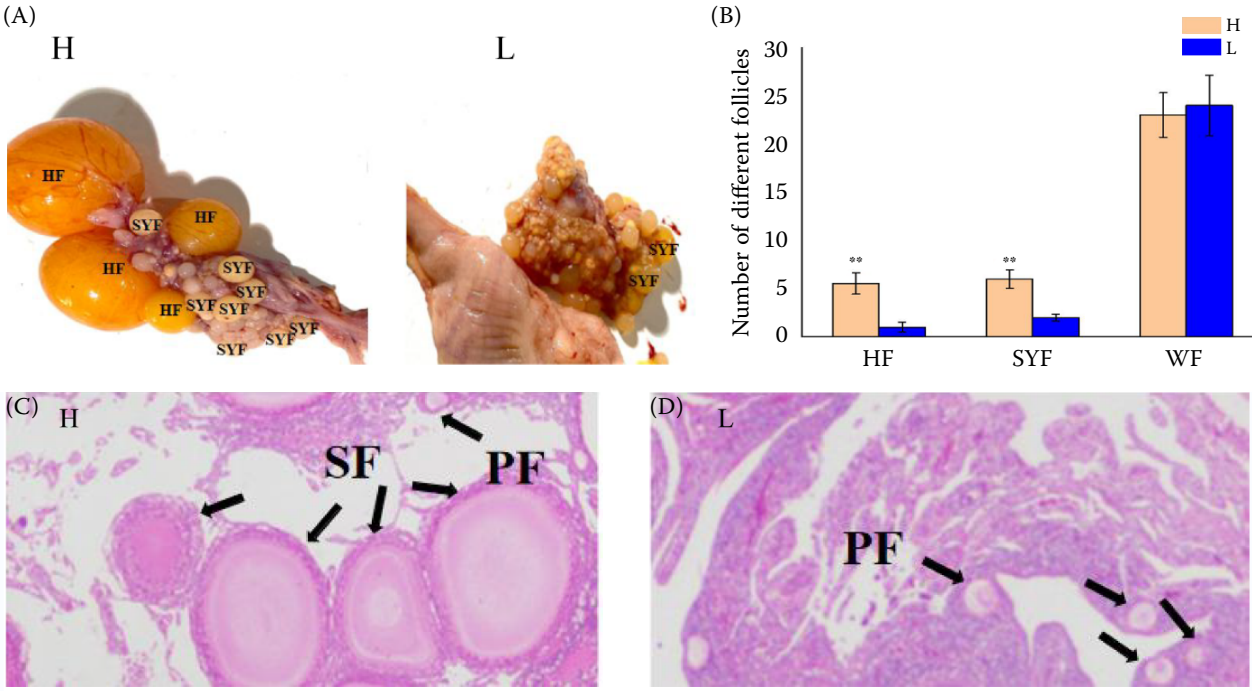

Figure 2. Ovarian morphological and histological difference between the high-yielding (H) and low-yielding (L) chickens

(A) Morphological characteristics in the ovary of the H and L chickens. (B) The number of different follicles in the H and L chickens. (C) Histological characteristics in the H and (D) L chickens (H&E staining at 40×)

HF = hierarchical follicles, > 8 mm in diameter; PF = primary follicles; SF = secondary follicles; SYF = small yellow follicles, 4–8 mm in diameter; WF = white follicles, 1–4 mm in diameter

$^{**}P < 0.01$

Table 2. Serum concentrations of the follicle stimulating hormone (FSH), luteinising hormone (LH) and oestradiol (E2) in the high-yielding (H) and low-yielding (L) chickens

	FSH (mIU/ml)		E2 (pg/ml)		LH (mIU/ml)	
Chickens	H	L	H	L	H	L
Mean value	9.07 ± 0.32	8.04 ± 0.8	84.64 ± 3.2	82.04 ± 4.2	2.05 ± 0.06	1.96 ± 0.06
P-value	3.21×10^{-14}**		0.003**		0.046*	

$^{*}P < 0.05$; $^{**}P < 0.01$

the L group, while the ratio of the H group mapping to the intron region and the intergene region was higher than that of the L group (Figure S1A in ESM). However, both the H and L groups were similar in the distribution of their expression levels (Figure S1B in ESM) and also in the expression of the mRNAs (Figure S1C in ESM). Figures S1D–F in ESM present the expression profiles of the mRNAs, where the six mRNA samples were separated into two clusters based on their egg production. Of the 16 359 genes, 893 and 1 140 genes were specifically expressed in the H and L groups, respectively, while 14 326 genes were co-expressed in both groups (Figure S1D in ESM). According to the PCA, six mRNA samples were also distinguished with two components, where the variance was 82.7% (Figure S1E in ESM). The expression patterns of the mRNAs were verified in both the H and L group by calculating Pearson's correlation value. Similarly, in the sample correlation analysis, mRNAs were also grouped into two clusters based on the H and L group. The expression patterns of the samples in the group were found to be similar (Figure S1F in ESM), except for the correlation between the individual H4 and H1, which was not so good. The correlation coefficient among the other intra groups was greater than 0.8, while the correlation coefficient between the groups was less

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than 0.8. The selection of the experimental samples was considered relatively consistent and reliable.

Analysis of the differentially expressed genes and function enrichment

A total of 875 DEGs were identified in the ovaries of the high- and low-yielding laying Chahua hens, which included 624 up-regulated and 251 down-regulated genes (Table S3 in ESM, Figure 3A). The expression patterns of the genes in the samples highlighted the reproducibility and credibility of the data, which is shown in the FPKM hierarchical clustering map of DEGs (Figure 3B).

The biological functions of the DEGs were identified using a gene ontology (GO) analysis. A total of 31 significantly enriched GO terms (P-value < 0.05) were obtained, where about eight significant terms were found enriched in the biological process (BP) mainly involved in the protein targeting, response to oxidative stress, anion transport, etc. In the cellular component (CC), four significant terms were enriched, with the main involvement in the extracellular region, extracellular space, extracellular matrix, etc. In the Molecular Function (MF), 19 significant terms were found to be enriched with the main involvement in the hormonal activity, 3',5'-cyclic-nucleotide phosphodiesterase activity, metalloendopeptidase activity, etc. (Figure 4A, Table S4 in ESM). The genes corresponding to the extracellular region, extracellular space, extracellular matrix, and hormone activity are shown in Table 3.

To further identify the major biochemical, metabolic, and signal transduction pathways of the DEGs, a KEGG pathway enrichment analysis was performed. At a threshold P-value < 0.05, 15 significant KEGG pathways were obtained that were mainly involved in the focal adhesion, cytokine-cytokine receptor interaction, TGF-beta signalling pathway, etc (Figure 4B and Table S5 in ESM). The genes corresponding to the above-mentioned KEGG pathways are shown in Table 3.

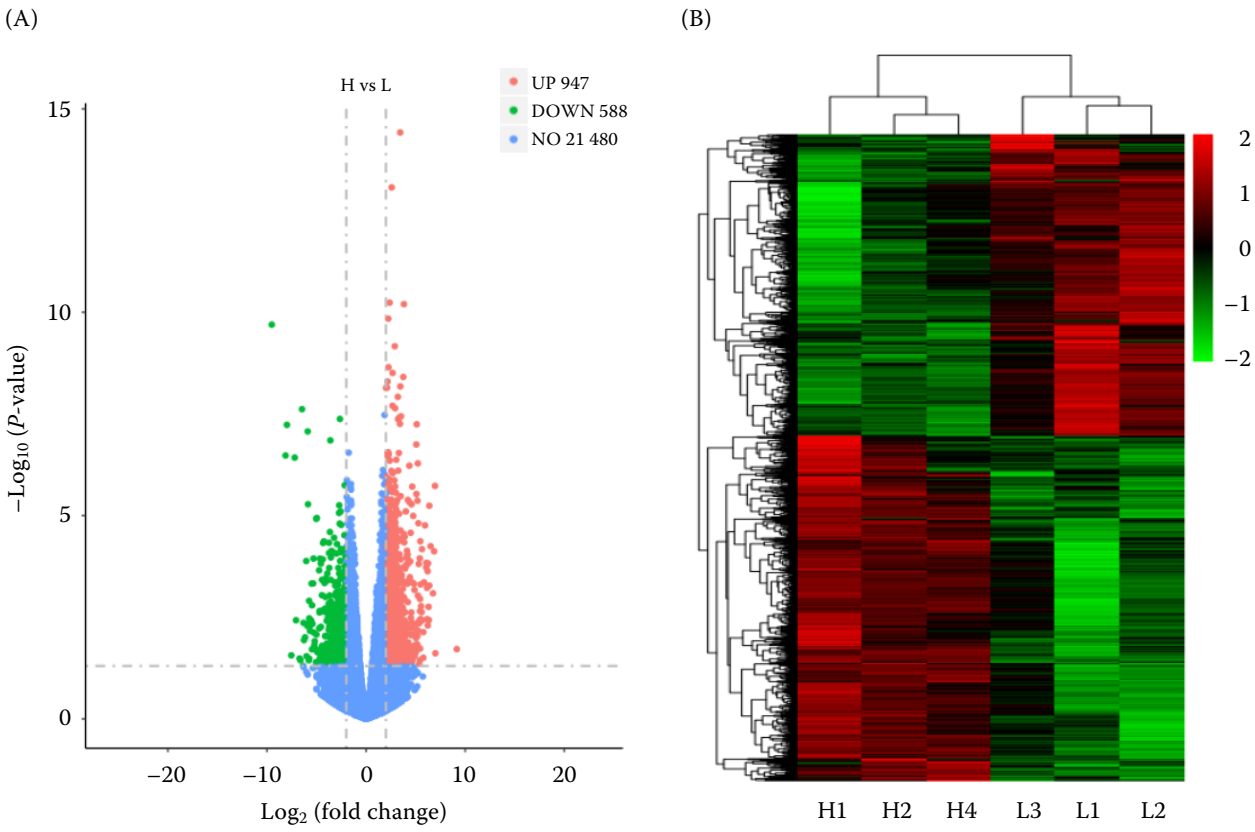

Figure 3. Differentially expressed genes (DEGs)

(A) Volcano plot of the DEGs. (B) Hierarchical cluster analysis of the DEGs

coloured bars = expression levels; green blocks = genes with the lowest expression levels; H = high-yielding hens; L = low-yielding hens; red blocks = overexpressed genes

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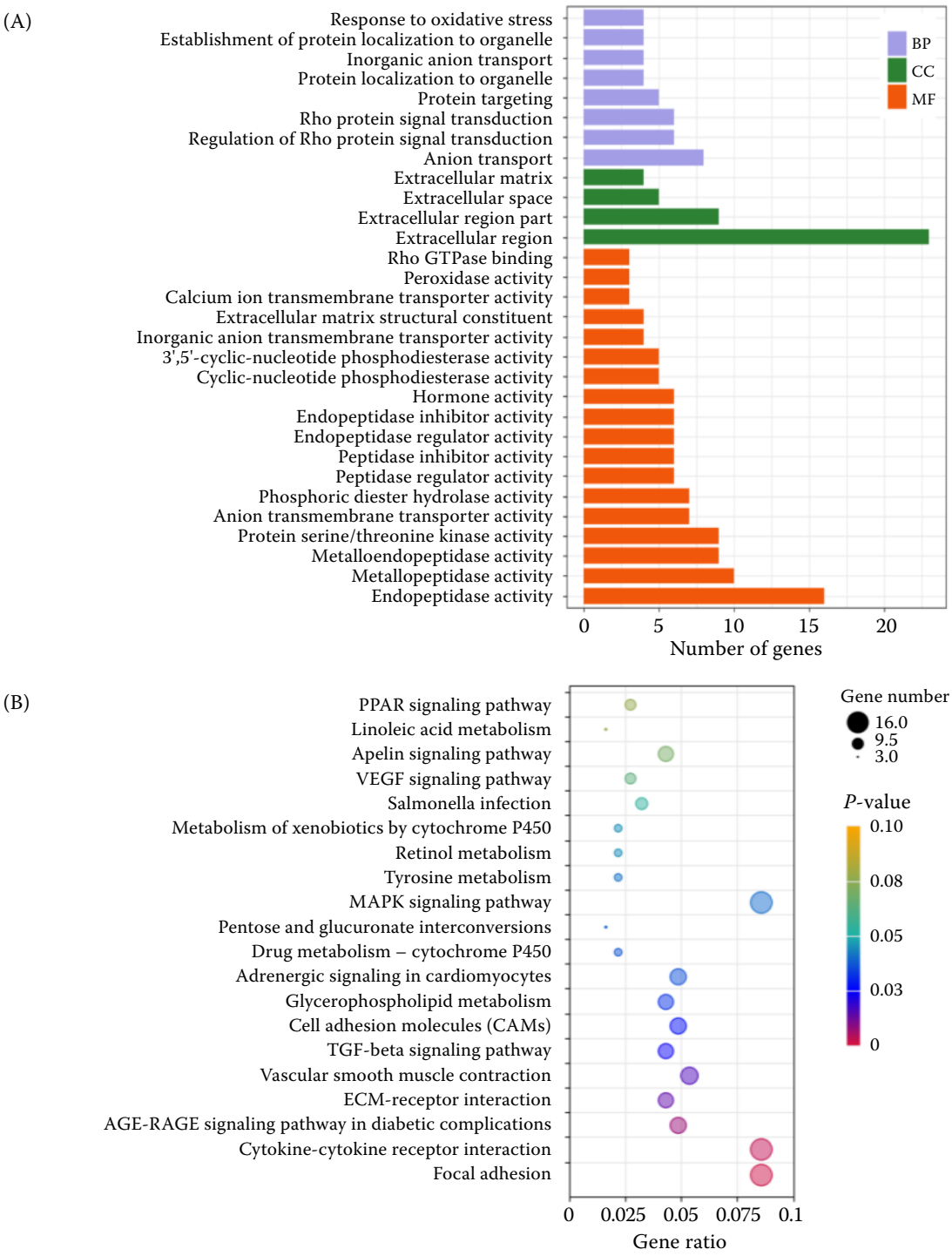

Figure 4. Differentially expressed genes (DEGs) functional enrichment in the ovary
(A) Top 30 of the GO analysis of the DEGs. (B) Top 20 of the KEGG analysis of the DEGs
BP = biological process; CC = cellular component; MF = molecular function

Verification of differentially expressed genes by RT-qPCR

We selected nine candidate genes randomly for a RT-qPCR analysis, which included seven up-regulated genes (*MMP10*, *PTGS2*, *MMP9*, *MMP13*, *HGF*, *PTHLH*, *BMPR2*) and two down-regulated genes (*FOXA2*, *CDH1*) (Figure 5). For two genes (*MMP9*, *FOXA2*), there are relatively great differences between the RNA-seq and the RT-qPCR.

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Table 3. List of partially representative differentially expressed genes

Pathways	Genes	Description	Log_2 FC	FDR
Extracellular region, extracellular space, extracellular matrix	*MMP13*	matrix metallopeptidase 13	4.20	0.005 2
	MMP10	matrix metallopeptidase 10	5.47	4.28×10^{-05}
	MMP9	matrix metallopeptidase 9	5.27	1.54×10^{-05}
Hormone activity	*SST*	somatostatin	−2.47	0.001 3
	PTHLH	parathyroid hormone like hormone	2.22	0.006 5
	RNP	renal natriuretic peptide	−2.30	0.006 5
Focal adhesion	*HGF*	hepatocyte growth factor	3.06	0.002 4
Focal adhesion, TGF-beta signalling pathway	*THBS1*	thrombospondin 1	3.57	0.000 37
Cytokine-cytokine receptor interaction, TGF-beta signalling pathway	*BMPR2*	bone morphogenetic protein, receptor type 2	2.06	0.005 8
TGF-beta signalling pathway	*GREM1*	gremlin 1, DAN family BMP antagonist	3.19	0.000 78

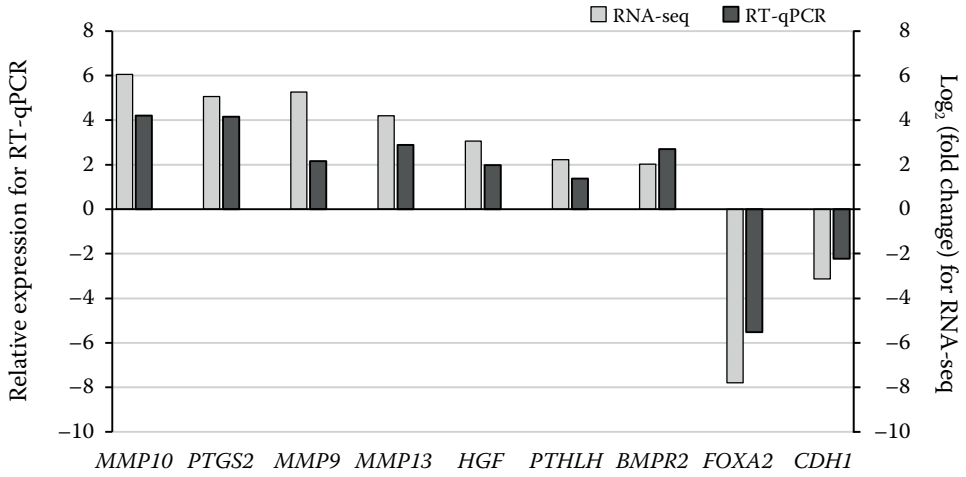

Figure 5. Validation of nine differentially expressed genes by RT-qPCR

Gene names are given in Table 3. Additional gene names are: *PTGS* (prostaglandin endoperoxide synthase 2), *FOXA2* (forkhead box A2) and *CDH1* (cadherin 1)

DISCUSSION

The most concerning endocrine mechanism related to the egg production performance is the Hypothalamic-pituitary-gonad (HPG) axis. On stimulation, the hypothalamus secretes a gonadotropin-releasing hormone (GnRH) mediated by its receptor (GnRHR), which then stimulates the pituitary to secrete FSH and LH. These hormones are required for maintaining the normal function of the ovary (Du et al. 2020). Several previous studies have focused on uncovering the genes and molecular mechanisms related to the egg-laying performance (Qin et al. 2015; Azmal et al. 2019). Unfortunately, the DEGs and key pathways regulating the egg-laying performance across the ovaries have not yet been discovered.

A comparative analysis of the egg production traits of the L and H groups showed that the AFE and EN were significantly different between the two groups. The AFE of the H group was significantly earlier than that of the L group. Since the speed of ovarian maturity determined the AFE (Chen et al. 2007), the ovarian maturity of the H group was found to be earlier than that of the L group, which may also cause differences in the egg production of the Chahua chickens at 43 weeks of age. Upon maturity and ovulation of F1 (the largest follicle) in the HF, F2 (the second-largest follicle) and F3 (the third-largest follicle) are considered the new F1 and F2 follicles. Additionally, SYF is selected to become the smallest F6 (the least mature follicle) (Luc et al. 1996), and HF does not generally become atresia (Gosden and Spears 1997). The increased

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HF and SYF in the H group might also be one of the important reasons for the differences in the egg production. Previous studies have shown that follicle storage may affect the egg production rate (Zou et al. 2020). We found a significantly better egg production rate in the H group between 16 and 43 weeks than that of the L group, which may be directly related to the SYF follicle and the HF reserves. Previous studies have also shown that poultry with a high egg production had more follicles, which was closely related to the LH and FSH levels (Yang et al. 2019). In our study, the levels of FSH, E2, and LH were found to be significantly higher in the high-yielding hens than in the low-yielding hens. Therefore, the FSH, E2, and LH levels might be one of the immediate causes affecting the egg yield of Chahua chickens.

The transcriptome analysis showed 875 DEGs in the ovaries of the H and L groups, with 624 up-regulated and 251 down-regulated genes. These DEGs in the ovaries of the H and L groups were significantly enriched by the hormonal activity, extracellular matrix, extracellular region, and extracellular region part. The hormone activity was closely linked to the egg-laying performance in the chickens, with its main involvement in the HPG axis (Bedecarrats et al. 2016). Several DEGs encode the *SST*, *PTHLH* and *RNP* genes, which are involved in the hormonal activity. *PTHLH* plays an important role in the selection of chicken follicles by stimulating cell proliferation and steroidogenesis (Guo et al. 2019). Some DEGs code the *MMP9*, *MMP13*, and *MMP10* genes, which are involved in the extracellular matrix, extracellular region, and extracellular region part. The extracellular matrix is closely related to animal reproduction, especially the development of the ovary. The follicle development, maturation, ovulation, luteal formation, and degeneration of the reproductive cycle of the ovary are all dependent on the periodic degradation of the extracellular matrix and tissue reconstruction (Curry and Osteen 2003). Studies have shown that the extracellular matrix in response to FSH exhibits a great relationship with the development of the ovarian follicles (Ingman et al. 2000), which may play an important role in the regulation of the egg-laying performance. Therefore, the extracellular matrix, in response to the hormonal activity, may be involved in the development of ovarian follicles and regulation of the SYF and HF reserves, affecting the ovulation cycle and, thus, influencing the egg production of Chahua chickens. The *MMP9* and *MMP13* genes were found to influence the ovary and uterus during the oestrous and menstrual cycles (Curry and Osteen 2003).

The KEGG pathway analysis showed that products of the DEGs that were identified in the ovaries of the H and L groups are mainly involved in the signalling pathways, such as the focal adhesion, cytokine-cytokine receptor interaction, and TGF-beta signalling pathway. Focal adhesion plays an important role in oocyte-follicle communication and reproduction (Tahir et al. 2021). In this pathway, HGF and THBS1 regulate granulosa cell steroidogenesis and suppress apoptosis in non-ovarian cells (Uzumcu et al. 2006; Zhu et al. 2019). The TGF-beta signalling pathway can control the follicle-stimulating hormone receptor (FSHR), signalling a reduction in ovarian granulosa cell apoptosis (Du et al. 2016). This pathway also involves the *BMPR2*, *THBS1*, and *GREM1* genes. The *BMPR2* gene affects the suppression of the *BMP15* gene in the production of FSH-induced progesterone (Edwards et al. 2008). The *GREM1* gene plays an important role in the granulosa cell proliferation and steroidogenesis of hen ovarian pre-hierarchical follicles (Qin et al. 2020). The cytokine-cytokine receptor interaction is also a significant pathway contributing to the production of eggs. Studies have indicated that the functions of the cytokine-cytokine receptor interaction are related to reproduction (Zhang et al. 2019). Since the *BMPR2* gene is involved in this pathway, the responsible gene may be considered a candidate affecting the production of eggs. Our results suggest that these pathways have been enriched, indicating their functions in the regulation of chicken egg production. However, our results are not consistent with those of Zhang et al. (2019) and Mu et al. (2021), who also conducted an RNA-seq analysis in chicken ovaries. Zhang et al. (2019) identified four known pathways related to reproduction. Among them, the focal adhesion and cytokine-cytokine receptor interaction pathways alone showed similarities with our results. Although Mu et al. (2021) reported nine pathways, no pathways overlapped with our results. These conflicting results may be attributed to the differences in the chicken breed used in the respective experiments. While we used Chahua chickens, Zhang et al. (2019) used Jinghai yellow chickens, and Mu et al. (2021) used Changshun green-shell laying hens.

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CONCLUSION

In summary, the variations in the egg production between the H and L Chahua chickens were closely related to the AFE, the EN, the number of the SYF and HF, and the hormones in the serum. The differentially expressed candidate genes, GO terms, and the regulating pathways associated with the reproductive functions in the chicken ovaries were identified using transcriptome analysis. Our research findings may provide better understanding of the transcriptional basis of high and low production rates of eggs in Chahua chicken. However, since the functionalities of these genes have not been verified, further investigations are needed to study more reproductive signals from ovarian tissues in connection with the reproductive function in breeder hens. In conclusion, these results not only provide a theoretical basis for the research on the regulatory mechanism of egg production, but can also identify candidate genes for genetic improvement.

Conflict of interest

The authors declare no conflict of interest.

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