

Transcriptomic analysis reveals molecular mechanisms underlying follicle selection in Zi geese

DONGYU LIU^{1,2}, HAOYUAN ZHAI¹, XIAOYU WU¹, QIUJU WANG^{1,2}, YAPING CHEN¹

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

²Key Laboratory of Exploration and Innovative Utilization of White Goose Germplasm Resources in Cold Regions of Heilongjiang Province, Daqing 168319, P. R. China

Received 22.06.2026

Accepted 09.09.2026

Liu D. Y., Zhai H. Y., Wu X. Y., Wang Q. J., Chen Y. P.

Transcriptomic analysis reveals molecular mechanisms underlying follicle selection in Zi geese

Summary

This study analyzed transcriptomic differences between SYF and F6 follicles in Zi geese. A total of 4,505 DEGs were identified, indicating strong transcriptional reprogramming during follicle selection. Enriched pathways were related mainly to ECM-receptor interaction, focal adhesion, and neuroactive ligand-receptor signaling, which highlights coordinated regulation of structural remodeling and endocrine signaling. Key genes involved in ECM remodeling (FN1, COL1A1), signaling (SRC, MAPK8), and neuroendocrine regulation (NPY, DRD1) were significantly altered. In addition, genes related to follicular dominance and steroidogenesis (NR5A2, AMH, FST, INHBB) showed marked expression changes, suggesting a shift from inhibitory control to follicular maturation. Overall, follicle selection in geese is driven by integrated ECM, signaling pathways, and endocrine networks, providing candidate genes for improving reproductive performance.

Keywords: Zi goose, follicle selection, transcriptome, ECM-receptor interaction, granulosa cell

The goose industry constitutes an important sector of waterfowl production. However, breeding programs for geese are less developed than those for chickens and ducks, and their reproductive performance remains relatively low. Domestic geese are seasonal breeders and generally exhibit lower egg production than other poultry species, with most breeds producing only a few dozen eggs annually. Consequently, limited reproductive capacity has become one of the major factors restricting the sustainable development and economic competitiveness of the goose industry (10).

Among these breeds, the Zi goose (originating from Heilongjiang Province, China) is recognized as one of the more productive indigenous goose breeds and has considerable economic value in waterfowl production systems (13). Under standard management conditions, it demonstrates relatively high laying performance, although substantial individual variation in reproductive capacity is commonly observed (4).

Follicular development is a highly coordinated process regulated by endocrine and local factors. Only a small proportion of follicles are selected to enter the preovulatory hierarchy, whereas the majority undergo atresia (1, 6). During each ovulatory cycle, one small yellow follicle (SYF) is recruited into the follicular hierarchy and subsequently undergoes rapid growth until ovulation. This process, known as follicle selection, is a pivotal event in establishing follicular hierarchy and maintaining continuous egg production. Therefore, follicle selection is regarded as one of the key determinants of reproductive efficiency in birds (14). Despite their importance, the mechanisms responsible for selecting one follicle and directing the others toward atresia have not been fully elucidated, either in mammals or in avian species.

Previous studies have demonstrated that follicle selection is accompanied by extensive changes in gene expression and cellular function. In chickens, several signaling pathways and regulatory molecules, including transforming growth factor- β (TGF- β), insulin-like growth factor binding proteins (IGFBPs), and extracellular matrix-related factors, have been implicated in granulosa cell differentiation and follicular maturation (8). Recent advances in single-cell transcriptomics have further revealed that granulosa cell differentiation

*This work was supported by the Guiding Science and Technology Plan Project of Daqing City (zd-2023-57); Longjiang Science and Technology Talent Spring Goose Support Program for Young Talents (No. CYQN24048); Key Project of Heilongjiang Provincial "Excellent Young Teachers Basic Research Support Program" (2024ZR164A02); Heilongjiang Province Double First-class Characteristic Discipline Platform Project (HLJTSXK2022); Heilongjiang Bayi Agricultural University Talent Research Start-up Fund (XYB202012, XYB202206); Heilongjiang postdoctoral project (LBH-Z21074).

during follicle selection is associated with coordinated activation of multiple signaling pathways, including phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), mitogen-activated protein kinase (MAPK), and transforming growth factor- β (TGF- β) pathways (12). However, compared with chickens and mammals, the molecular mechanisms underlying follicle selection in geese remain poorly understood.

In the present study, RNA sequencing (RNA-seq) was performed to compare the transcriptomic profiles of SYF and hierarchical F6 follicles in Zi geese. Differentially expressed genes (DEGs) and enriched biological pathways associated with follicle selection were identified through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes analyses (KEGG). The findings of this study provide new insights into the molecular mechanisms governing follicle selection and offer potential candidate genes for improving egg production through molecular breeding.

Material and methods

The study protocol was approved by the Ethics Committee of Science and Technology of Heilongjiang Bayi Agricultural University (Daqing, China) (Number: DWKJXY2023054).

Three 8-month-old laying Zi geese were selected from an individually housed flock at Daqing Beifang Goose Farm. Relevant production records, including laying performance, were continuously monitored. Three birds with comparable body weights (3.1 ± 0.2 kg) and similar laying performance were selected for sampling to minimize inter-individual variation. However, the sampling procedure was not synchronized to a precisely defined time point relative to oviposition, and no experimental synchronization of the physiological status of the birds was performed. SYF and hierarchical F6 follicles were collected from the same three birds, with one representative F6 follicle collected from each bird. Geese were euthanized following national animal welfare guidelines. Ovaries were immediately excised, rinsed in cold PBS, snap-frozen in liquid nitrogen, and stored at -80°C until RNA extraction.

Tab. 1. Primer sequences used for RT-qPCR analysis of selected DEGs

Gene	NCBI reference sequence	Sequence (5'-3')	Product length	DEGs
NR5A2	XM_083846757.1	F: GACCGAATGCGTGGTGGTAGA R: CCTTTGGAGGCAGAATGGATG	191 bp	Up
CRHR2	XM_083825464.1	F: TGCTCTTGCCCATAGTTCTCC R: AGGCGTTTCTTGTTGGTGTGT	234 bp	
SFRP2	XM_083836413.1	F: ATCATCCAGCCGTGCCACTCG R: TTCTTGACAGCGTCGCAGACC	203 bp	
INHBB	XM_048072198.2	F: GTCCGCCTCTATTTCTTCATCTCG R: CAGCCCTCACATTGAACATCCA	296 bp	Down
FST	XM_013180613.3	F: CCGCTGCCAGGTCTCTACAA R: CGGGTCCACAGTCCACGTTCT	188 bp	
CCNB1	XM_083816021.1	F: GTTGGTATCACGGCTATGTTCCCTT R: CAGGGCTTGAGAACTTTATCTC	141 bp	
GAPDH	XM_067004670.1	F: TGATGCTCCCATGTTCTGATG R: GTGATGGCATGGACAGTGGT	168 bp	

RNA extraction and library preparation. Total RNA was extracted using TRIzol reagent (Life Technologies, USA) according to the manufacturer's instructions. RNA quality and concentration were assessed with a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and Agilent 2100 Bioanalyzer. Six libraries (three SYF and three F6) were prepared using the Illumina TruSeq RNA Sample Preparation Kit and sequenced on an Illumina HiSeq platform to generate 150 bp paired-end reads.

Read processing and alignment. Raw reads were processed using fastp v0.18.0 to remove adapters, low-quality reads, and reads with more than 10% unknown nucleotides. Clean reads were aligned to the goose reference genome (NCBI_GCF_002166845.1) using HISAT2 v2.1.0. Mapping statistics including the total mapped reads, GC content, Q20, and Q30 values were calculated to assess sequencing quality.

Differential expression analysis. Gene expression levels were quantified from mapped read counts, and FPKM values were calculated for expression profiling. Genes with FPKM < 1 in all samples were filtered out prior to differential expression analysis. DESeq v1.20.0 was used to identify DEGs between SYF and F6 follicles. Read counts were normalized by the median-of-ratios method implemented in DESeq, and differential expression was assessed using the negative binomial model with follicle type (SYF vs. F6) as the experimental factor. Genes with a false discovery rate (FDR) < 0.05 and an absolute $\log_2$ fold change ($|\log_2\text{FoldChange}| \geq 1$) were considered differentially expressed.

Functional annotation and pathway analysis. DEGs were annotated for GO terms and KEGG pathways using GO.db v3.14.0 and KOBAS v2.0, respectively. Enrichment significance was determined by adjusted P-value < 0.05 .

Reverse transcription quantitative PCR (RT-qPCR) validation. Six DEGs were randomly selected for quantitative validation using RT-qPCR, including three upregulated genes (NR5A2, CRHR2, SFRP2) and three downregulated genes (INHBB, FST, CCNB1) (Tab. 1). GAPDH was used as the internal reference gene. cDNA synthesis was performed using the PrimeScript RT reagent kit (Takara, Japan). RT-qPCR was conducted using TB Green® Premix Ex Taq™ (Takara) on a Bio-Rad iQ5 Real-Time PCR System. Each sample was assayed in triplicate. Relative expression levels were calculated by the $2^{-\Delta\Delta C_t}$ method.

The raw RNA-seq data generated in this study have been deposited in the NCBI BioProject database under accession number PRJNA1511459. The associated BioSample accessions are SAMN62356207, SAMN62356208, SAMN62356209, SAMN62356210, SAMN62356211, and SAMN62356212.

Results and discussion

Sequencing quality was high, with mapping rates ranging from 87.22% to 90.60%, which confirms the reliability of the RNA-seq data (Tab. 2). Pearson correlation analysis revealed high consistency

Tab. 2. Summary statistics of RNA-seq data from SYF and F6 follicles in Zi geese

Sample	Quality control sequence	Q20%	Q30%	GC (%)	Mapped reads (%)
SYF-1	41 425 520	98.90	96.72	47.62	89.78
SYF-2	44 910 920	98.85	96.48	47.31	90.60
SYF-3	48 503 440	97.75	93.04	47.54	90.25
F6-1	47 158 488	98.84	96.59	46.50	87.22
F6-2	40 722 240	98.76	96.25	47.02	87.74
F6-3	48 968 128	98.06	94.33	46.74	88.37

among biological replicates ($R^2 > 0.90$) both within and between SYF and F6 groups (Fig. 1A), indicating strong reproducibility and reliability of the samples for comparative transcriptomic analysis.

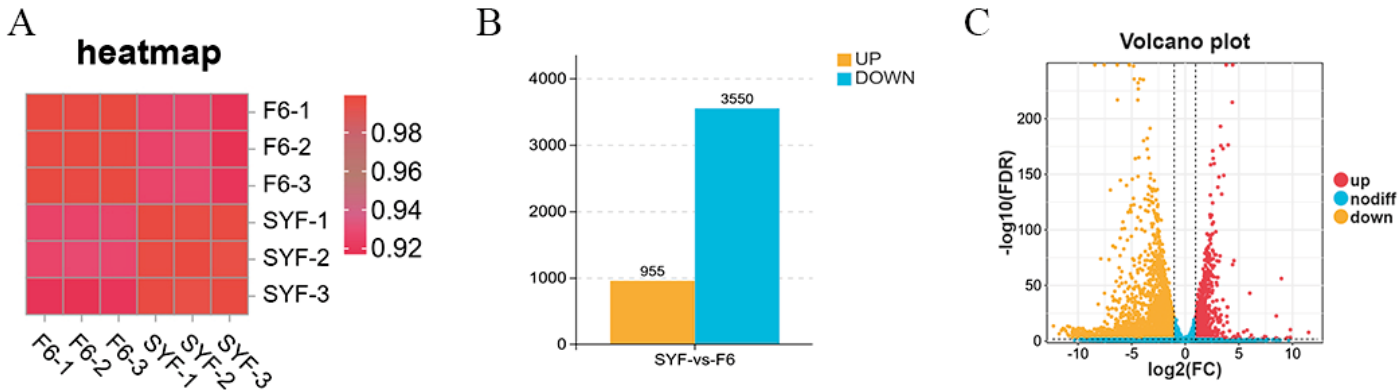


Fig. 1. Transcriptome profiling and differential expression analysis of SYF and F6 follicles. (A) Pearson correlation analysis among samples from SYF and F6 follicles; (B) Number of upregulated and downregulated DEGs identified between SYF and F6 follicles; (C) Volcano plot showing the distribution of DEGs

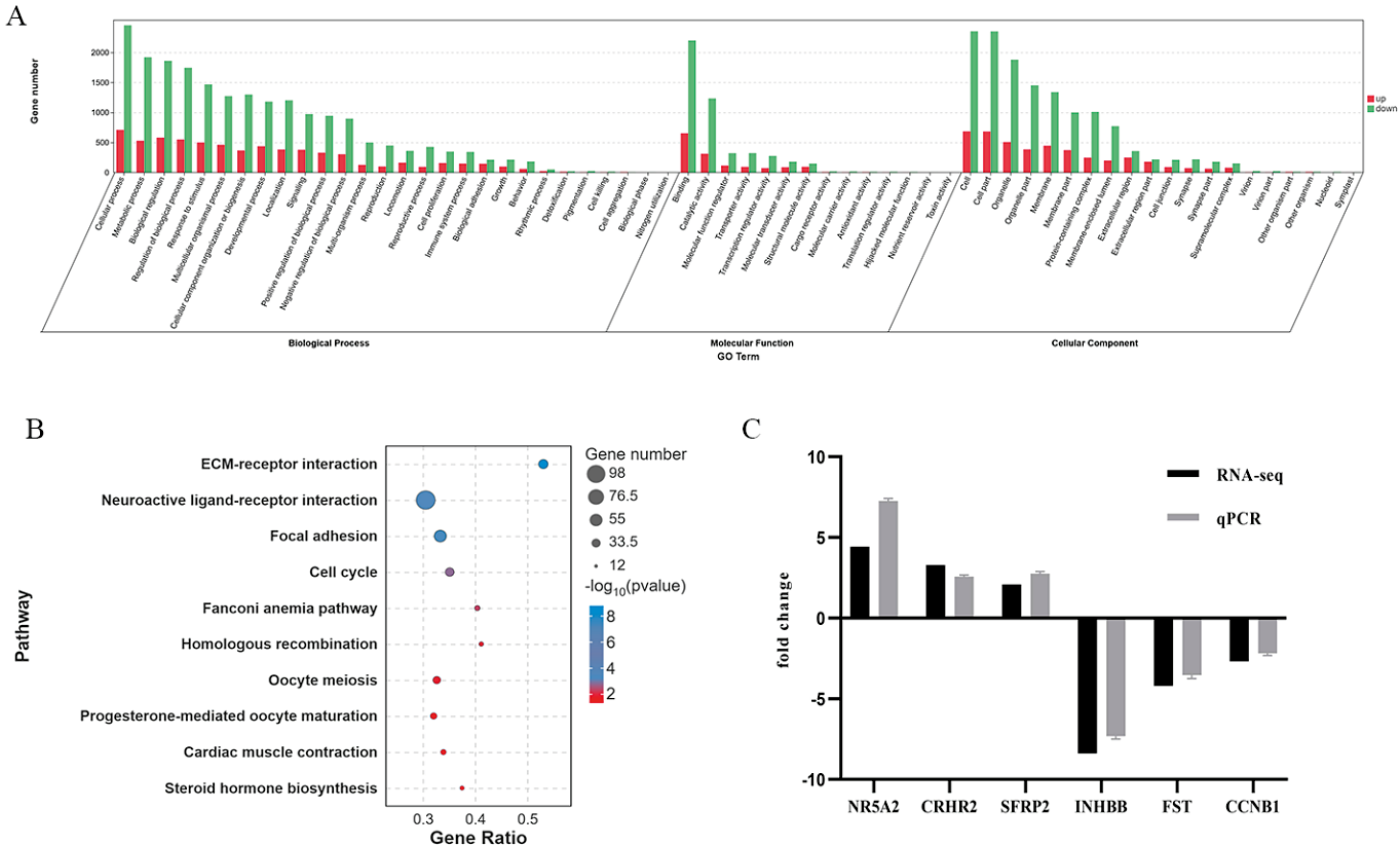


Fig. 2. Functional enrichment analysis and qRT-PCR validation of DEGs between SYF and F6 follicles. (A) GO classification of DEGs between SYF and F6 follicles; (B) KEGG pathway analysis of DEGs identified between SYF and F6 follicles; (C) Validation of RNA-seq data by qRT-PCR analysis of six selected genes

showing the greatest expression differences, DEGs were ranked according to their fold changes, and the top 10 upregulated and top 10 downregulated genes are presented in Tab 3.

GO classification analysis assigned DEGs to 63 functional categories within biological process (BP), cellular component (CC), and molecular function (MF) domains (Fig. 2A). In BP, genes were mainly involved in cellular process, metabolic process, biological regulation, and response to stimulus. In CC, they were enriched in membrane, organelle, cell junction, and extracellular regions, indicating active microenvironment remodeling. In MF, binding, transcription factor, transport, and antioxidant activities were enriched, suggesting coordinated regulation of transcription, transport, and redox balance during follicle development.

KEGG pathway analysis revealed a set of moderately enriched pathways (Fig. 2B). Among them, extracellular matrix (ECM)-receptor interaction and neuroactive ligand-receptor interaction showed relatively higher significance and gene coverage, suggesting their central roles in follicle selection. Other pathways, including focal adhesion, steroid hormone biosynthesis, and oocyte meiosis, provided additional support for coordinated regulation of structural remodeling and endocrine signaling during follicular transition.

RT-qPCR analysis of six DEGs confirmed the RNA-seq expression trends, validating the reliability of the transcriptomic data (Fig. 2C). Expression levels of upregulated genes were significantly higher in F6 follicles, while downregulated genes exhibited lower expression compared with SYF, consistent with sequencing results.

Follicle selection is a pivotal biological event in avian ovarian function, during which a single SYF is recruited into the hierarchical follicle pool and develops into a preovulatory follicle. This process determines the efficiency of follicular hierarchy formation and directly influences egg production performance (5). In this study, transcriptomic analysis identified 4,505 DEGs between SYF and F6 follicles in Zi geese, indicating that the transition from SYF to F6 is accompanied by extensive transcriptional reprogramming rather than changes in a limited number of follicle-associated genes. Among the enriched pathways, ECM-receptor interaction and focal adhesion were particularly prominent, suggesting that changes in cell-matrix interactions may be an important feature of the SYF-to-F6 transition (11). The extracellular matrix is not only a structural component of the follicle, but also a source of signals that can influence cell adhesion, differentiation, and intracellular signaling. Therefore,

Tab. 3. Top 10 upregulated and top 10 downregulated DEGs between SYF and F6 follicles ranked by fold change

Gene	Log2 fold change	FDR	Expression
PTN	4.46313131303792		Up-regulated
ELN	3.85507131913385		
NR5A2	4.42722021464512	4.95020612208775e-215	
NPC2	3.32713797458197	2.21044582283522e-193	
LOC106034204	4.0454103450063	1.07162092786731e-176	
SMOC1	3.36650883168069	4.70049258721081e-176	
LOC106034203	3.5580191951554	2.44983019460964e-173	
TAGLN	2.6004916211665	2.39009829134735e-171	
ACTG2	2.64652177371846	1.77236357717108e-164	
COL8A1	2.73682401661105	6.24555037965145e-160	
INHBB	-8.383472601		Down-regulated
TMOD1	-5.186014686		
AMH	-6.300791979		
CD24	-5.157185731		
LOC125185016	-7.517568273		
EPCAM	-4.776228406	1.29484630760221e-247	
FST	-4.189213251	5.2645767452383e-236	
NEFL	-4.721040146	5.83929369297833e-236	
LOC106047684	-3.867041148	2.20612577389525e-235	
ATP8A2	-4.351937208	1.55730443953978e-232	

the differential expression of ECM-related genes in the present study may reflect coordinated remodeling of the follicular microenvironment during follicle selection. The differential expression of FN1, COL1A1, ITGB5, and ITGB8 further supports the involvement of extracellular matrix organization and integrin-associated signaling in this transition. However, whether ECM remodeling actively contributes to follicle selection or occurs as a consequence of follicular maturation cannot be determined from the present transcriptomic data.

Focal adhesions provide a functional interface between extracellular matrix components and intracellular signaling pathways, which suggests that extracellular remodeling may be coupled with changes in cellular signaling during follicle selection (9). NR5A2 was markedly upregulated in F6 follicles. NR5A2 has been implicated in ovarian function and steroidogenic regulation in chickens (7), and its increased expression may reflect the greater functional specialization of hierarchical follicles. Conversely, INHBB, FST, and AMH were downregulated in F6 follicles. These genes are associated with intraovarian regulatory networks and TGF- β -related signaling involved in follicular development (2). Their differential expression suggests that the balance of local follicular regulatory signals changes during the SYF-to-F6 transition. The downregulation of CCNB1 and CCNB2 may further indicate a change in cellular state during follicular maturation, potentially reflecting a transition from a relatively proliferative prehierarchical state toward

greater cellular differentiation. These findings are generally consistent with previous studies in chickens, suggesting conserved mechanisms of follicle selection across avian species (3). Nevertheless, reduced expression of cell-cycle genes does not necessarily indicate a global reduction in follicular proliferation, and this interpretation requires direct assessment of cellular proliferation and differentiation.

Several limitations should be considered when interpreting the present findings. First, only three biological replicates were included, which limits statistical power and generalizability. Although SYF and F6 follicles were collected from the same individual geese, and birds with comparable body weight and laying performance were selected, a larger number of animals would provide stronger statistical support. Second, physiological status was not experimentally synchronized using a precisely defined interval relative to oviposition, and residual variation in the ovulatory cycle may have contributed to transcriptional differences. Finally, the present study identifies primarily transcriptional associations rather than causal mechanisms. Future studies using larger cohorts, more precisely controlled sampling, and functional validation will be necessary to confirm these findings.

Comparison with previous studies in chickens suggests that several molecular features identified in the present study may represent conserved mechanisms underlying avian follicle selection. In particular, the involvement of extracellular matrix organization, cell-matrix adhesion, and local endocrine regulation is consistent with the established importance of granulosa-cell signaling and follicular microenvironment remodeling during chicken follicle selection. However, the relative contribution of these pathways may differ between species. Geese exhibit lower laying frequency and stronger reproductive seasonality than chickens, and these physiological characteristics may impose distinct regulatory requirements on follicular recruitment and hierarchical development. Thus, the enrichment of similar signaling pathways in geese may reflect a conserved regulatory framework, whereas differences in gene expression patterns and pathway activity may contribute to species-specific reproductive traits.

In conclusion, follicle selection in Zi geese is associated with coordinated transcriptional changes in extracellular matrix remodeling, cell-matrix signaling, endocrine regulation, and cellular differentiation. The genes and pathways identified by this study provide candidate molecular mechanisms underlying the SYF-to-F6 transition and provide a basis for further functional studies of follicle selection and reproductive performance in geese.

References

1. Johnson A. L.: The avian ovary and follicle development: some comparative and practical insights. *Turkish Journal of Veterinary & Animal Sciences*, 2014, 38, 660-669, doi: 10.3906/vet-1405-6.
2. Knight P. G., Glister C.: Local roles of TGF-beta superfamily members in the control of ovarian follicle development. *Anim. Reprod. Sci.* 2003 Oct 15, 78 (3-4), 165-183, doi: 10.1016/s0378-4320(03)00089-7.
3. Li D., Zhong C., Sun Y., Kang L., Jiang Y.: Identification of genes involved in chicken follicle selection by ONT sequencing on granulosa cells. *Front Genet.* 2023 Jan, 13, 1090603, doi: 10.3389/fgene.2022.1090603.
4. Liu S., Yin J., Cong K., Yue S., Zhang Y., Sun J., Ren X., Jiang K., Liu Y., Zhao X.: Synergistic transcriptomic and metabolomic analyses in Zi geese ovaries with different clutch lengths. *Poult Sci.* 2025 Jul, 104 (7), 105210, doi: 10.1016/j.psj.2025.105210.
5. Ma Y., Yao J., Zhou S., Mi Y., Tan X., Zhang C.: Enhancing effect of FSH on follicular development through yolk formation and deposition in the low-yield laying chickens. *Theriogenology* 2020 Nov, 157, 418-430, doi: 10.1016/j.theriogenology.2020.07.012.
6. McGee E. A., Hsueh A.: Initial and cyclic recruitment of ovarian follicles 1. *Endocrine Reviews* 2000, 21 (2), 200-214, doi: 10.1210/edrv.21.2.0394.
7. Nie R., Tian H., Zhang W., Li F., Zhang B., Zhang H.: NR5A1 and NR5A2 regulate follicle development in chicken (*Gallus gallus*) by altering proliferation, apoptosis, and steroid hormone synthesis of granulosa cells. *Poult. Sci.* 2024 May, 103 (5), 103620, doi: 10.1016/j.psj.2024.103620.
8. Onagbesan O., Bruggeman V., Decuyper E.: Intra-ovarian growth factors regulating ovarian function in avian species: a review. *Anim. Reprod. Sci.* 2009 Apr, 111 (2-4), 121-140, doi: 10.1016/j.anireprosci.2008.09.017.
9. Woodruff T. K., Shea L. D.: The role of the extracellular matrix in ovarian follicle development. *Reprod. Sci.* 2007 Dec, 14 (8 Suppl), 6-10, doi: 10.1177/1933719107309818.
10. Yan X., Xu Y., Zhen Z., Li J., Zheng H., Li S., Hu Q., Ye P.: Slaughter performance of the main goose breeds raised commercially in China and nutritional value of the meats of the goose breeds: a systematic review. *J. Sci. Food Agric.* 2023 Jun, 103 (8), 3748-3760, doi: 10.1002/jsfa.12244.
11. Young J. M., McNeilly A. S.: Theca: the forgotten cell of the ovarian follicle. *Reproduction* 2010 Oct, 140 (4), 489-504, doi: 10.1530/REP-10-0094.
12. Zhang W., Chen X., Nie R., Guo A., Ling Y., Zhang B., Zhang H.: Single-cell transcriptomic analysis reveals regulative mechanisms of follicular selection and atresia in chicken granulosa cells. *Food Res. Int.* 2024 Dec, 198, 115368, doi: 10.1016/j.foodres.2024.115368.
13. Zhao X., Yue S., Zhang Y., Sun J., Peng F., Guo Z.: The TRHDE and TSHR genes regulate laying traits in domesticated Zi geese. *Curr. Issues Mol. Biol.* 2025 May 4, 47 (5), 331, doi: 10.3390/cimb47050331.
14. Zhou S., Ma Y., Zhao D., Mi Y., Zhang C.: Transcriptome profiling analysis of underlying regulation of growing follicle development in the chicken. *Poult. Sci.* 2020 Jun, 99 (6), 2861-2872, doi: 10.1016/j.psj.2019.12.067.

First author: Dongyu Liu, PhD, College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing 163319, P. R. China; Key Laboratory of Exploration and Innovative Utilization of White Goose Germplasm Resources in Cold Regions of Heilongjiang Province, Daqing 168319, P. R. China; e-mail: ldysuper@126.com