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**Original Article**

# Identification and Functional Prediction of Lncrnas Associated with Oocyte Development in the Donkey

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**Abstract:**
Long non-coding RNAs (lncRNAs) play key regulatory roles in various biological processes. However, the importance and molecular regulatory mechanisms of lncRNAs in donkey oocyte maturation remain to be further investigated. This study used published transcriptomic data from the germinal vesicle (GV) phase and metaphase II (MII) phase donkey oocytes to identify lncRNAs and obtained 4,352 novel lncRNAs. Compared with the coding genes, the novel lncRNAs and the known lncRNAs exhibited some typical features, such as shorter transcript length and fewer exons. A total of 3,805 coding genes and 569 lncRNAs were differentially expressed between the GV and MII stages. The differentially expressed genes were found to be involved in various biological processes related to oocyte development. The potential target genes of differentially expressed lncRNAs were predicted in cis and trans manner. Functional analysis of lncRNA targets showed that some lncRNAs may act on potential target genes involved in oocyte maturation. This study provides valuable information for further investigation of the molecular mechanisms of oocyte development in donkeys, serving as a theoretical basis for improving the in vitro maturation rate and ultimately benefiting from the expansion of the donkey population and the conservation of biodiversity and genetic resources.

**Keywords: donkey, lncRNA, oocyte maturation, functional analysis, regulatory network**

## 1. Introduction

The donkey (Equus asinus) is a descendant of the African wild ass and is a common domestic beast of burden [1, 2]. It can also provide meat and milk, and particularly donkey-hide gelatin, mostly composed of collagen, a traditional Chinese medicinal material [3]. The donkey has become somewhat redundant as a beast of burden, and the global donkey population has been significantly reduced after agricultural and industrial development and mechanization [4]. The number of donkeys also decreases in China, as the demand for donkey-hide gelatin products increases [5]. There is now a need to address aspects of donkey breeding to preserve the species.

The use of animal-assisted reproductive technology (ART), such as in vitro fertilization (IVF) and embryo transfer, will help improve the efficiency of donkey farming [6-9]. However, a limitation of this technology with donkeys is that the in vitro oocyte maturation rate is relatively low [10]. Therefore, a deep understanding of the characteristics of donkey oocyte development is needed, focusing on the meiotic maturation of oocytes from germinal vesicle (GV) to metaphase II (MII). The period from GV–MII marks the maturation of an oocyte, both nuclear and cytoplasmic [11, 12]. Studying the key regulatory

factors of oocyte development during this period, and the precise and meticulous regulatory signaling pathways is of utmost significance for improving the in vitro maturation rate of donkey oocytes.

Long non-coding RNA (lncRNA) is a class of non-coding RNAs with a length greater than 200 nucleotides. Increasing evidence suggests that lncRNA plays important roles in various biological processes, such as embryonic development [13, 14], gene expression regulation [15, 16], reprogramming [17, 18], and genomic imprinting [19, 20]. Additionally, many lncRNAs are involved in regulating oocyte development. For example, knockdown of lncRNAs OOSNCR1, OOSNCR2, and OOSNCR3 in bovine immature oocytes results in a decreased rate of blastocyst development and reduces the expression of genes associated with oocyte competencies, such as nucleoplasmin 2 (NPM2), growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), and JY-1 in MII oocytes [21]. The lncRNA PWRN1 affects ovarian follicular development by regulating the function of granulosa cells [22]. In another example, the knocking down of lncRNA Rose results in abnormalities in mouse oocyte cytokinesis and impaired preimplantation embryo development [23]. The lncRNA ZNF674-AS1 regulates granulosa cell glycolysis and proliferation by interacting with ALDOA [24]. However, the roles of lncRNAs in donkey oogenesis are still far from being fully investigated.

This study used published transcriptome data from the germinal vesicle (GV) phase and metaphase II (MII) phase oocytes of Biyang donkey to identify lncRNAs, and conducted differential expression studies of coding genes and lncRNAs, constructing an expression regulatory network, which can provide a theoretical basis for the protection of donkey germplasm resources.

## 2. Materials and Methods

### 2.1 Data Sources

The GV phase and MII phase oocytes RNA-seq data from Biyang donkeys were obtained from a previously published study and downloaded from the NCBI's GEO database (PRJNA763991) [6]. The donkey gene annotations were downloaded from https://ftp.ensembl.org/pub/release-112/gtf/equus_asinus. Moreover, the Non-Redundant Protein Sequence Database (NR) was downloaded from ftp://ftp.ncbi.nih.gov/blast/db/. The uniref90 database was downloaded from https://ftp.uniprot.org/pub/databases/uniprot/uniref/uniref90.

### 2.2 RNA-Seq Reads Mapping and Transcriptome Assembly

The quality of sequencing reads was evaluated by FastQC command. The raw reads were filtered and trimmed by Trimmomatic (version 0.39) with default parameters. The clean reads were then mapped to the donkey reference genome (ASM1607732v2) by HISAT2 v2.2.1 with the default parameters. StringTie (version 2.2.1) was used to assemble the mapped reads with default parameters. Then, the merge tool of StringTie was used to merge the 6 assembled transcript files (GTF format) of the two groups into a nonredundant transcriptome. In addition, by using the assembled GTF file, the StringTie software was used to estimate the expression levels of genes and transcripts in all samples for subsequent studies with the parameters "-e" and "-B".

### 2.3 LincRNAs Identification Pipeline

The pipeline for lincRNA (long intergenic non-coding RNA) identification was as follows (Fig. 1): (1) Retained those transcripts with the 'u' category, categorized by using gffcompare, which indicated intergenic transcripts. (2) According to the merged GTF file, the transcripts with single exons and less than 200 bp in length were removed. (3) The CPC2, CNCI, PLEK, and LGC

were used to assess the protein-coding potential of complete transcript sequences; the transcripts that cannot encode proteins based on protein-coding potential were retained. (4) The HMMER was used to identify the transcripts translated in all six possible frames with homologs that were concluded in any of the known protein family domains in the Pfam database, and transcripts that matched the Pfam hit (E-value < 1e-5) were excluded. (5) BLASTX program was used to filter out any transcripts that have similarities to known proteins in the NCBI NR and UniRef90 databases (E-value < 1e-5). (6) Reserve transcripts with FPKM values greater than 0 in at least one sample.

### 2.4 Comparisons between lncRNAs and Protein-Coding Transcripts

We selected the transcripts annotated as "protein-coding" in the gene annotation file, and the obtained lncRNAs were screened with "known" and "novel" by the "blastn" command. The transcript length, exon length, and exon number of lncRNAs were compared with those of protein-coding transcripts.

### 2.5 Analysis of Differentially Expressed Genes (DEGs) and Differentially Expressed lncRNAs (DELs)

DESeq2 tool was used to perform differential expression analysis of protein-coding genes and lncRNAs between the GV group and the MII group. |log2 fold change |≥2 and adjusted p-value (padj) <0.01 were used to screen differentially expressed genes and lncRNAs.

### 2.6 Prediction of Potential Target Genes

We predicted the molecular functions of protein-coding genes regulated by RNA in cis and trans manner. Firstly, the neighboring protein-coding genes nearby DELs (<100 kb) were identified based on cis-prediction principles using Bedtools. For trans regulation of DELs, we calculated the Pearson correlation coefficient (r) between DELs and protein-coding genes. We selected protein-coding genes for which the Pearson correlation coefficient $|r| \geq 0.99$, p-value$\leq 0.001$, as the potential target genes of DELs.

### 2.7 Functional Enrichment Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed by cluster Profiler. The p-value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1 Summary of RNA-seq Data Mapping and Transcript Assembly in Oocytes

The individual samples in the groups were named GV1, GV2, GV3, MII1, MII2, and MII3. The clean reads were mapped to the donkey reference genome using HISAT2. Approximately 91.79%-96.67% of the clean reads from each library were mapped to the donkey reference genome, and 83.04%-91.47% of the reads were uniquely mapped to the genome. Then, the transcriptome was assembled for each library by StringTie, and all transcripts were synthesized into nonredundant transcripts using StringTie-Merge. After merging the non-redundant transcripts, about 0.94% (10,584 of 113,006) of the transcripts were intergenic transcripts. The 4,352 putative lincRNAs were obtained according to the illustration shown in Fig. 1.

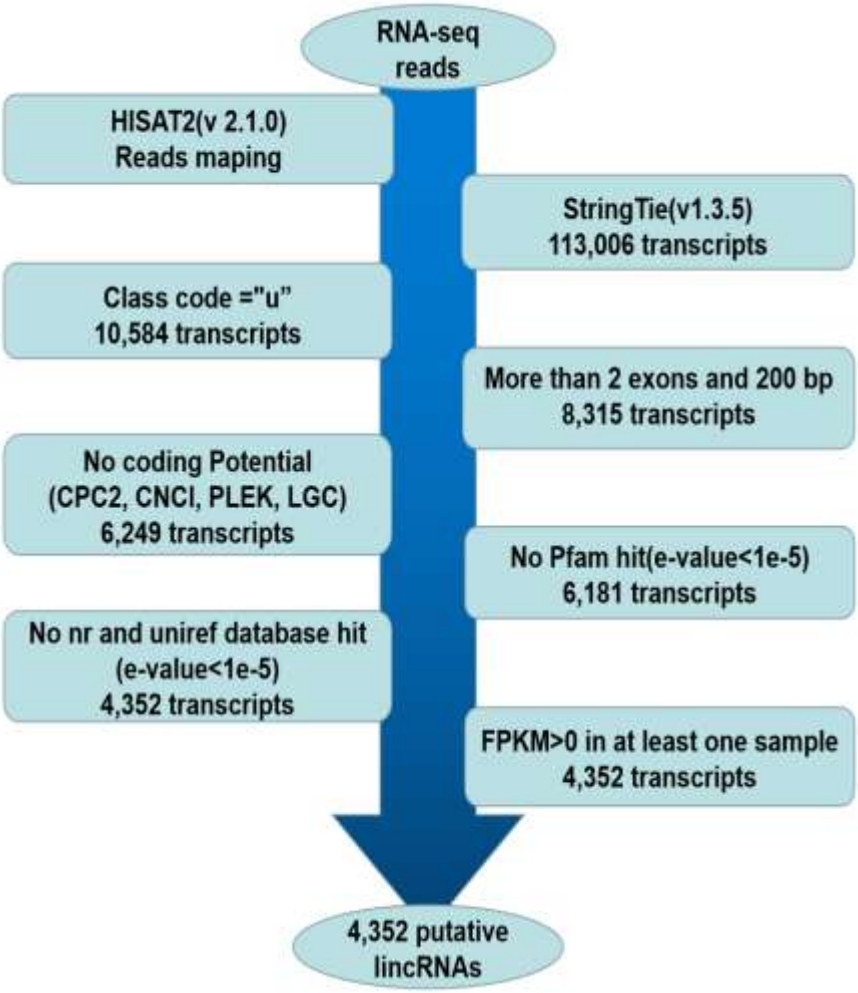


**Fig. 1 The pipeline for the identification of putative lincRNAs in this study. The frames in the direction of the arrow show the filtering process and the number of screened transcripts. "u": Unknown, intergenic transcript; CPC2: Coding Potential Calculator 2; nr: non-redundant; FPKM: fragments per kilobase of transcript per million mapped reads.**

### 3.2 Comparison of Coding Genes and lncRNA Features

According to the assembled transcriptome, the characteristics of lncRNA and protein-coding transcripts were compared. A total of 51,952 protein-coding transcripts corresponding to 21,494 protein-coding genes annotated in donkeys were acquired. The donkey annotation file also contains 8,712 known lncRNA transcripts corresponding to 4,877 lncRNA genes.

The average transcript length of the protein-coding transcripts (2,679 bp) was longer than that of the novel lncRNA transcripts (1,325 bp) and the known lncRNA transcripts (2,046 bp) (Fig. 2A). In terms of average exon length, the novel lncRNA is 374 bp in length, shorter than the known lncRNA (632 bp), but longer than the protein-coding transcripts (267 bp) (Fig. 2B). Additionally, we found that the average number of exons in the protein-coding transcripts was 10.0, which is significantly higher than that of the novel lncRNA (3.5) and the known lncRNA (3.2) (Fig. 2C).

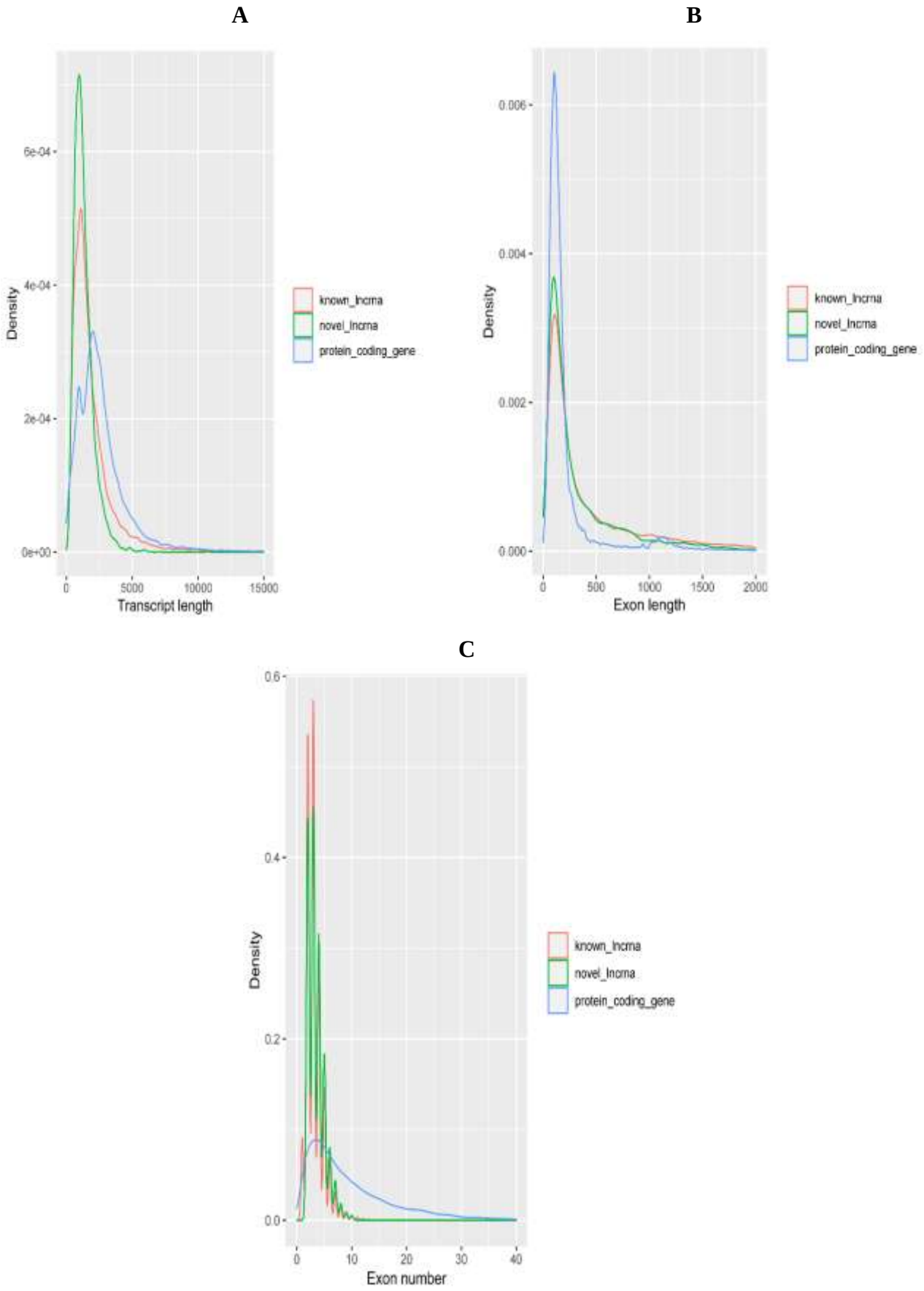


**Fig. 2 Comparison of the characteristics of protein-coding genes and lncRNA genes. (A) Comparison of transcript length, (B) Comparison of exon length, (C) Comparison of exon number.**

### 3.3 Differential Expression Analysis of Coding Genes and lncRNAs

We performed differential expression analysis of coding genes and lncRNAs to explore their potential biological functions. A total of 3,805 differentially expressed genes (DEGs) were obtained by comparing the GV group with the MII group in the oocyte samples. Among these, 709 genes were upregulated in the MII group, and 3,096 were downregulated (Fig. 3A). Additionally, 569 differentially expressed lncRNAs (DELs) were identified, with 164 lncRNAs upregulated and 405 lncRNAs downregulated in the MII group compared to the GV group (Fig. 3B).

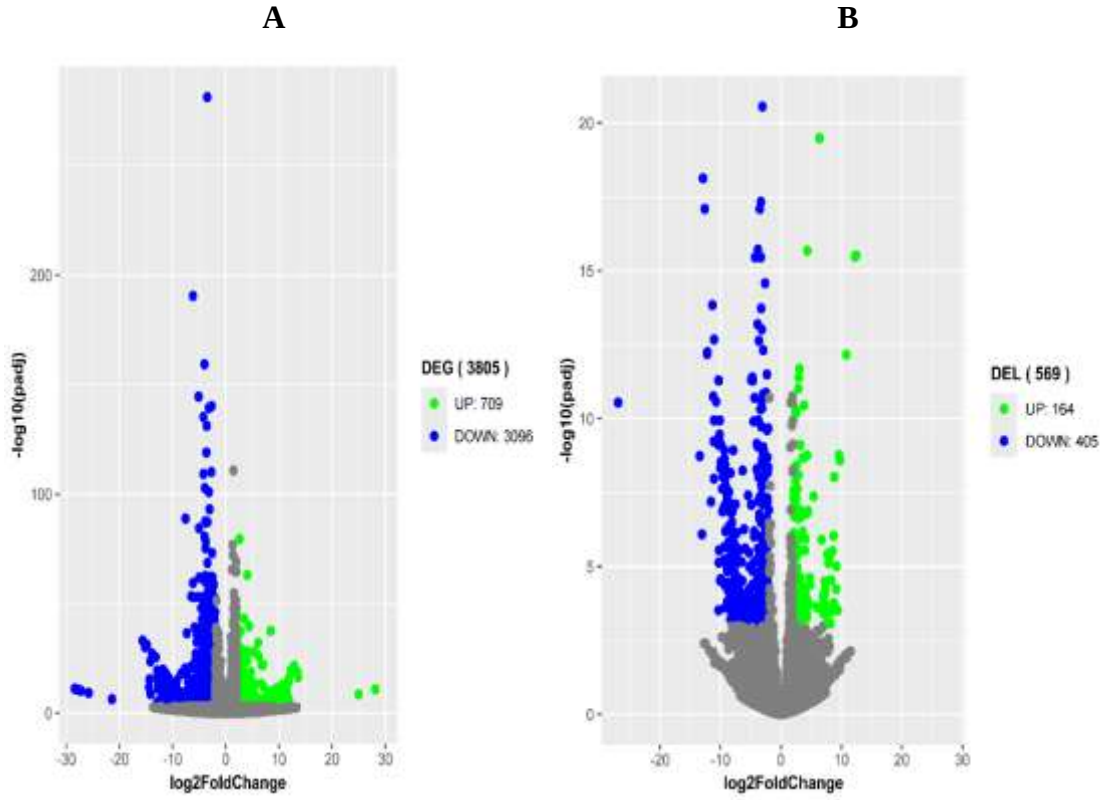


**Fig. 3 Volcano plots in analyzing differentially expressed genes (DEGs) (A) and differentially expressed lncRNAs (DELs) (B) of MII vs. GV stage oocytes. The green plot represents upregulated expression in the MII group; the blue plot represents downregulated expression in the MII group; and the gray plot represents no significance.**

### 3.4 Functional Analysis of Differentially Expressed Genes (DEGs)

We performed GO and KEGG analyses to investigate the function of DEGs. The GO analysis of DEGs showed that ncRNA metabolic process, ribosome biogenesis, ribonucleoprotein complex biogenesis, rRNA metabolic process, and rRNA processing were the most abundant terms in the biological process category. In terms of the cellular component category, mitochondrial protein-containing complex, mitochondrial inner membrane, ribonucleoprotein complex, organelle inner membrane, and mitochondrial envelope, while ligase activity, catalytic activity, acting on RNA, catalytic activity, acting on DNA, ATP-dependent activity, acting on DNA, and DNA helicase activity were most prevalent in the molecular function. KEGG analysis indicated that DEGs were significantly enriched in 53 KEGG pathways, of which the Cell cycle was related to oocyte development. Additionally, some other

pathways were related to oocyte development, such as the mTOR signaling pathway, Notch signaling pathway, AMPK signaling pathway, PI3K-Akt signaling pathway, Hippo signaling pathway - multiple species, Insulin signaling pathway, Hippo signaling pathway, Oocyte meiosis, Progesterone-mediated oocyte maturation, TGF-beta signaling pathway, Ovarian steroidogenesis, Toll-like receptor signaling pathway, Wnt signaling pathway, MAPK signaling pathway, ErbB signaling pathway, Steroid hormone biosynthesis, and Estrogen signaling pathway.

### 3.5 Prediction and Functional Analysis of lncRNA Target Genes

We identified a total of 2,577 PTGs corresponding to 569 DELs as potential target genes (PTGs) regulated by lncRNAs in cis (<100 kb). Among these genes, 427 PTGs were differentially expressed and were referred to as DEPTGs. GO and KEGG analyses were performed on expressed protein-coding genes transcribed near lncRNA (< 100 kb) to explore the function of putative lncRNAs. The results indicated that 60 of the 427 DEPTGs were significantly involved in 104 biological processes (Fig. 4A). Of them, 43 DEPTGs significantly participated in 3 pathways (Fig. 4B). Some other pathways are related to oocyte development, namely the Hippo signaling pathway-multiple species, Notch signaling pathway, AMPK signaling pathway, PI3K-Akt signaling pathway, Hippo signaling pathway, and Wnt signaling pathway.

In this study, we conducted the trans analysis to find the PTGs that were significantly correlated ($|r| \geq 0.99$, $p \leq 0.001$) with the DELs. In total, 3,389 PTGs were highly correlated with 564 DELs. All PTGs are differentially expressed and are referred to as DEPTGs. GO enrichment analysis showed that 901 DEPTGs were enriched in 368 biological processes (Fig. 4C). In biological processes, some GO terms were significantly associated with oocyte development, such as reproductive system development, response to progesterone, reproductive structure development, and mitotic cell cycle process. Additionally, 515 DEPTGs were enriched in 50 pathways, of which the Cell cycle was related to oocyte development (Fig. 4D). Other pathways involved in oocyte development, have also been identified, including the Hippo signaling pathway, Notch signaling pathway, TGF-beta signaling pathway, Insulin signaling pathway, Oocyte meiosis, AMPK signaling pathway, Progesterone-mediated oocyte maturation, PI3K-Akt signaling pathway, Ovarian steroidogenesis, Hippo signaling pathway - multiple species, Wnt signaling pathway, ErbB signaling pathway, Steroid hormone biosynthesis, Toll-like receptor signaling pathway, MAPK signaling pathway, and Estrogen signaling pathway.

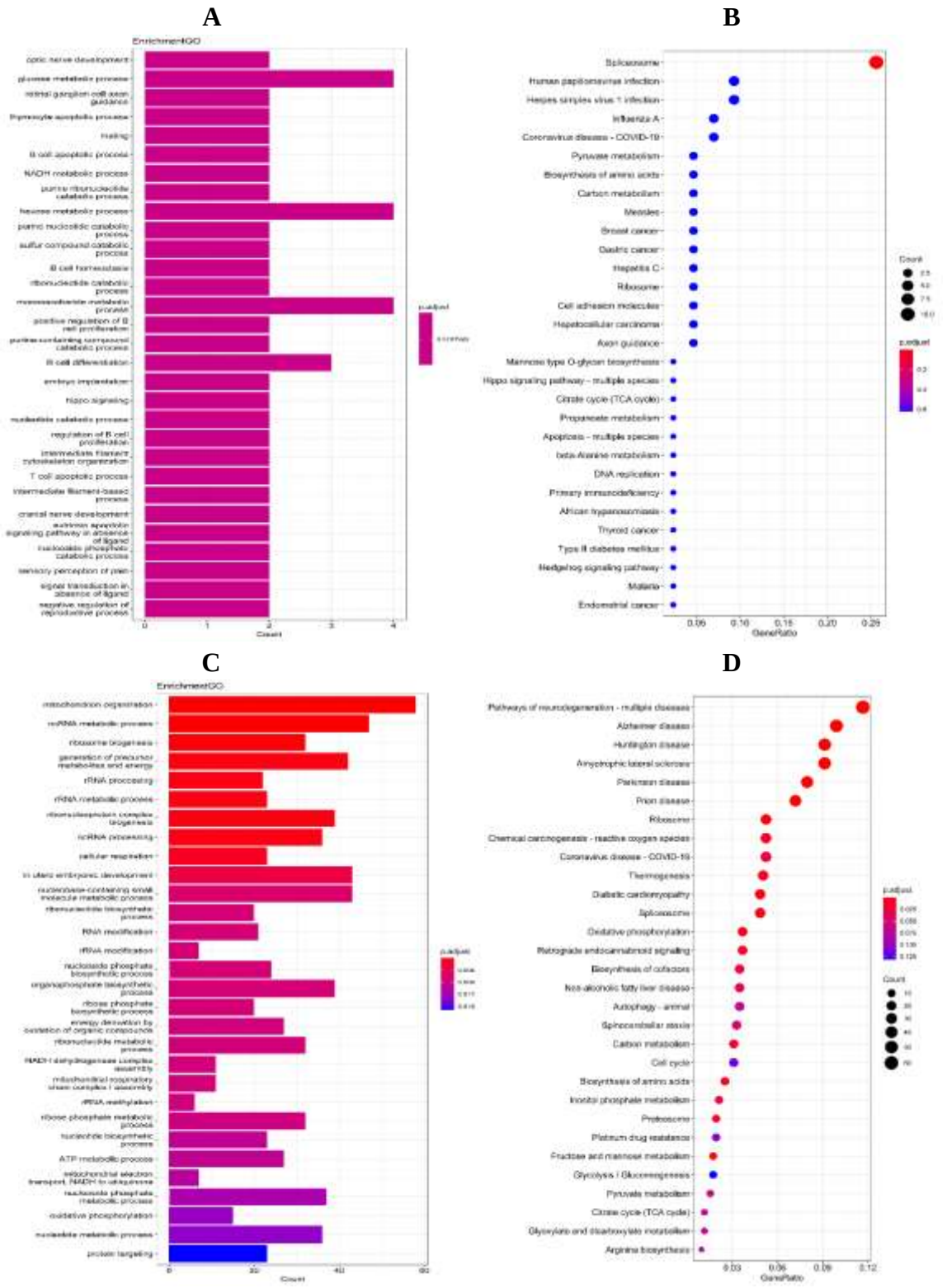


**Fig. 4 Gene Ontology and pathway analysis of DEPTGs of DELs. (A) Gene Ontology analysis of cis-regulated targets DEPTGs, (B) Pathway enrichment of cis-regulated targets DEPTGs, (C) Gene Ontology analysis of trans-regulated targets DEPTGs, (D) pathway analysis of trans-regulated targets DEPTGs. PTGs, potential target genes; DELs, differentially expressed lncRNAs; DEPTGs, differentially expressed potential target genes.**

A detailed examination was conducted to ascertain lncRNA–protein-coding gene pairs belonging to co-localization (cis action) and

expression correlation (trans action) relationships. This examination identified 172 lncRNA–protein coding gene pairs that fulfilled these criteria. However, no regulatory network associated with oocyte development was identified.

## 4. Discussion

The present study used transcriptome sequencing of the GV and MII phases of oocytes from Biyang donkeys to investigate genes and lncRNAs related to oocyte development. A total of 21,494 protein-coding genes, 8,712 known lncRNAs, and 4,352 novel lincRNAs were obtained. We compared known and novel lncRNAs with donkey protein-coding genes and found that known and novel lncRNAs have shorter transcript lengths, longer exon lengths, and fewer exon numbers than protein-coding transcripts, which is consistent with some previous studies [25, 26].

In this study, the differential expression analysis of coding genes and lncRNAs during the transition from GV to MII stage was uncovered. The results showed that a total of 3,805 DEGs and 569 DELs were significantly changed. To clarify the mechanism of lncRNAs in oocyte development, we selected DELs and DEGs for subsequent analysis. The GO results of DEGs showed that the involvement of a significant number of differentially expressed genes in oocyte development-related biological processes, including reproductive system development, ovarian follicle development, reproductive structure development, female gonad development, development of primary female sexual characteristics, multicellular organismal reproductive process, female sex differentiation, negative regulation of reproductive process, and gonad development. Moreover, KEGG revealed that Cell cycle, mTOR signaling pathway, Notch signaling pathway, AMPK signaling pathway, PI3K-Akt signaling pathway, Hippo signaling pathway-multiple species, Insulin signaling pathway, Hippo signaling pathway, Oocyte meiosis, Progesterone-mediated oocyte maturation, TGF-beta signaling pathway, Ovarian steroidogenesis, Toll-like receptor signaling pathway, Wnt signaling pathway, MAPK signaling pathway, ErbB signaling pathway, Steroid hormone biosynthesis, and Estrogen signaling pathway were likely involved in the regulation of oocyte fate during the transition from GV to MII.

Previous studies have indicated that lncRNAs can regulate gene expression in a cis-acting manner [27, 28]. Protein-coding genes transcribed near lncRNAs (< 100 kb) were screened to predict the function of DELs. A total of 2,577 PTGs were identified, corresponding to 569 DELs. Here, with the enrichment of cis-regulated genes (DEPTGs), we uncovered the cis-regulatory pathway of lncRNAs; several cis-regulatory pathways of key lncRNAs were identified, which included Hippo signaling pathway - multiple species, Notch signaling pathway, AMPK signaling pathway, PI3K-Akt signaling pathway, Hippo signaling pathway, and Wnt signaling pathway.

MSTRG.18086.3 and ENSEAST00005044753 acted on IL7 and CDC37 (members of the PI3K-Akt signaling pathway), respectively, in the cis form. Previous studies have shown that the PI3K-Akt signaling pathway regulates oocyte development [29]. Another DEL ENSEAST00005047205 was found to target TEAD4, which plays a key role in the Hippo signaling pathway- multiple species. Previous studies have also shown that the Hippo signaling pathway regulates oocyte development [30]. Furthermore, DEL MSTRG.5416.1 was found to target DTX2, which engages in the Notch signaling pathway. The Notch signaling pathway regulates oocyte development [31]. Moreover, DEL ENSEAST00005068214 was found to target CSNK1A1, which is involved in Wnt signaling pathway. The Wnt signaling pathway has also been found to be involved in the regulation of oocyte development [32, 33]. These findings suggested that lncRNAs may act on their neighboring protein-coding genes in the cis form to regulate oocyte development.

LncRNAs can not only regulate the expression of neighboring protein-coding genes through a cis mechanism, but also regulate the expression of genes located on other chromosomes via a trans mechanism [34]. The analysis of PTGs by trans-acting lncRNAs was conducted, and a total of 53,809 interaction relationships were detected in the trans form between 564 differentially expressed lncRNAs and protein-coding genes in the donkey genome. Our results indicated that the trans-regulated genes (DEPTGs) involved in Cell cycle, Hippo signaling pathway, Notch signaling pathway, TGF-beta signaling pathway, Insulin signaling pathway, Oocyte meiosis, AMPK signaling

pathway, Progesterone-mediated oocyte maturation, PI3K-Akt signaling pathway, Ovarian steroidogenesis, Hippo signaling pathway - multiple species, Wnt signaling pathway, ErbB signaling pathway, Steroid hormone biosynthesis, Toll-like receptor signaling pathway, MAPK signaling pathway, and Estrogen signaling pathway.

An intriguing observation is that 14 DELs were found to target MAPK3. Furthermore, MAD2L2 was targeted by six DELs. Moreover, six DELs were all found to act on ANAPC2. Additionally, three DELs, MSTRG.18538.2, MSTRG.666.2, and ENSEAST00005059539, were all found to target ADCY7. DEL MSTRG.6705.10 was also found to target CCNB2. Importantly, all these target genes were involved in Oocyte meiosis and Progesterone-mediated oocyte maturation, which are the key pathways associated with oocyte development [35-37]. These lncRNAs may regulate the key signaling pathways of Oocyte meiosis and Progesterone-mediated oocyte maturation during donkey oocyte development, as reflected by their targeted genes.

An interesting observation is that 16 DELs were all found to target PPP2R5D. Furthermore, PPP2CA was targeted by seven DELs. Moreover, three DELs MSTRG.20775.2, MSTRG.24835.2, and MSTRG.6236.17 were all found to act on YWHAB. Additionally, two DELs, MSTRG. 35 21.5 and ENSEAST00005064852 were all found to target PPP2CB. Notably, all these target genes engage in Oocyte meiosis, which is the key pathway associated with oocyte development. These lncRNAs may regulate the key signaling pathway, Oocyte meiosis during donkey oocyte development, as reflected by their targeted genes.

Twenty-three DELs were all found to target KIF22, which engages in Progesterone-mediated oocyte maturation. Two DELs, ENSEAST00005047912 and ENSEAST00005074506, were found to act on GNAI1, which is involved in Progesterone-mediated oocyte maturation. These lncRNAs may regulate the key signaling pathway, Progesterone-mediated oocyte maturation during donkey oocyte development, as reflected by their targeted genes.

An integrated regulatory network was constructed in the present study to further elucidate the interaction between lncRNAs and their target genes. The network indicated that each putative lncRNA and its potential target gene appeared to represent remarkably diverse regulatory relationships among them. Three central node genes (PPP2R5D, KIF22, and MAPK3) were detected in the integrated network. All three central node genes were determined to be regulated by more than 10 lncRNAs in the trans form. Interestingly, 10 DELs (MSTRG.18128.1, MSTRG.23603.1, MSTRG. 458 0.1, ENSEAST00005038101, ENSEAST000050 40 302, ENSEAST00005042156, ENSEAST 000 0505 0688, ENSEAST00005062704,ENSEAST000 0507 1576, ENSEAST00005079578) acted on both PPP2R5D (member of Oocyte meiosis) and KIF22 (member of Oocyte meiosis and progesterone-mediated oocyte maturation pathway) in a trans manner. Furthermore, MAPK3 (member of the progesterone-mediated oocyte maturation pathway) and KIF22 (members of the progesterone-mediated oocyte maturation pathway) were also found to be regulated by several of the same lncRNAs, including MSTRG.21241.1, MSTRG.24422.1, MSTRG.8981.1, ENSEAST0 0005043811, and ENSEAST00005069034. These lncRNAs may regulate target genes involved in oocyte development pathways through trans-acting mechanisms.

## 5. Conclusion

We identified 4,352 putative lncRNAs and analyzed the characteristics of lncRNAs compared with protein-coding genes in donkey oocytes from the GV to MII stages in the study. We observed numerous differentially expressed lncRNAs and protein-coding genes in the MII group compared to the GV group. Some differentially expressed genes are involved in various biological processes related to oocyte development. Functional enrichment analysis of potential target genes by DELs revealed that some lncRNAs may act on potential target genes, participate in oocyte maturation processes, and regulate oocyte development during GV-MII. As a result, we suggest that multiple lncRNA-mRNA networks may be involved in key signaling pathways throughout oocyte differentiation during the GV-to-MII stage transition. This study provides considerable understanding regarding the maturation of donkey oocytes. It also serves as a theoretical basis for improving the in vitro maturation rate of donkey oocytes, intending to

benefit the expansion of the donkey population and conservation of biodiversity and genetic resources.

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**Data Availability Statement:** The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

**Conflict of Interest Statement**: The authors declare no conflicts of interest.

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**References**

1. Todd E T, Tonasso-Calviere L, Chauvey L, et al. (2022) The genomic history and global expansion of domestic donkeys. *Science*, 377 (6611):1172-1180. https://doi.org/10.1126/science.abo3503.
2. Wang C, Li H, Guo Y, et al. (2020) Donkey genomes provide new insights into domestication and selection for coat color. *Nat Commun*, 11(1): 6014. https://doi.org/10.1038/s41467-020-19813-7.
3. Bennett R and Pfuderer S (2020) The Potential for New Donkey Farming Systems to Supply the Growing Demand for Hides. *Animals (Basel)*, 10(4). https://doi.org/10.3390/ani10040718.
4. Zhang F L, Zhang S E, Sun Y J, et al. (2022) Comparative Transcriptomics Uncover the Uniqueness of Oocyte Development in the Donkey. *Front Genet*, 13(839207. https://doi.org/10.3389/fgene.2022.839207.
5. Seyiti S and Kelimu A (2021) Donkey Industry in China: Current Aspects, Suggestions and Future Challenges. *J Equine Vet Sci*, 102(103642. https://doi.org/10.1016/j.jevs.2021.103642.
6. Li Z, Song X, Yin S, et al. (2021) Single-Cell RNA-Seq Revealed the Gene Expression Pattern during the In Vitro Maturation of Donkey Oocytes. *Genes (Basel)*, 12(10). https://doi.org/10.3390/genes12101640.
7. Li Y, Gu Y and Ao X (2025) Nano selenium and plant extracts supplementation enhanced reproductive performance of parity-2 sows. *Sci Rep*, 15(1): 9678. https://doi.org/10.1038/s41598-025-92981-y.
8. Liu Y, Guo X, Fan J, et al. (2024) CREBRF regulates apoptosis and estradiol via ISG15/ISGylation in pig granulosa cells. *Free Radic Biol Med*, 225(445-455. https://doi.org/10.1016/j.freeradbiomed.2024.10.287.
9. Khan M Z, Khan A, Huang B, et al. (2024) Bioactive Compounds Protect Mammalian Reproductive Cells from Xenobiotics and Heat Stress-Induced Oxidative Distress via Nrf2 Signaling Activation: A Narrative Review. *Antioxidants (Basel)*, 13(5). https://doi.org/10.3390/antiox13050597.
10. Goudet G, Douet C, Kaabouba-Escurier A, et al. (2016) Establishment of conditions for ovum pick up and IVM of jennies oocytes toward the setting up of efficient IVF and in vitro embryos culture procedures in donkey (Equus asinus). *Theriogenology*, 86(2): 528-35. https://doi.org/10.1016/j.theriogenology.2016.02.004.
11. Eppig J J (1996) Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. *Reprod Fertil Dev*, 8(4): 485-9. https://doi.org/10.1071/rd9960485.
12. Zhao Z H, Meng T G, Li A, et al. (2020) RNA-Seq transcriptome reveals different molecular responses during human and mouse oocyte maturation and fertilization. *BMC Genomics*, 21(1): 475. https://doi.org/10.1186/s12864-020-06885-4.
13. Zhang K, Huang K, Luo Y, et al. (2014) Identification and functional analysis of long non-coding RNAs in mouse cleavage stage

embryonic development based on single cell transcriptome data. *BMC Genomics*, 15(1): 845. https://doi.org/10.1186/1471-2164-15-845.

14. Li H, Wang Z, Zhao B, et al. (2023) Sperm-borne lncRNA loc100847420 improves development of early bovine embryos. *Anim Reprod Sci*, 257(107333. https://doi.org/10.1016/j.anireprosci.2023.107333.
15. Ransohoff J D, Wei Y and Khavari P A (2018) The functions and unique features of long intergenic non-coding RNA. *Nat Rev Mol Cell Biol*, 19(3):143-157. https://doi.org/10.10 38/nrm.2017.104.
16. Natarajan P, Shrinivas K and Chakraborty A K (2023) A model for cis-regulation of transcriptional condensates and gene expression by proximal lncRNAs. *Biophys J*, 122(13): 2757-2772. https://doi.org/10.1016/j.bpj.2023.05.032.
17. Loewer S, Cabili M N, Guttman M, et al. (2010) Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. *Nat Genet*, 42 (12): 1113-7. https://doi.org/10.1038/ng.710.
18. He X Y, Fan X, Qu L, et al. (2023) LncRNA modulates Hippo-YAP signaling to reprogram iron metabolism. *Nat Commun*, 14(1): 2253. https://doi.org/10.1038/s41467-023-37871-5.
19. Prickett A R and Oakey R J (2012) A survey of tissue-specific genomic imprinting in mammals. *Mol Genet Genomics*, 287(8): 621-30. https://doi.org/10.1007/s00438-012-0708-6.
20. Di Michele F, Chillon I and Feil R (2023) Imprinted Long Non-Coding RNAs in Mammalian Development and Disease. *Int J Mol Sci*, 24(17). https://doi.org/10.3390/ijms241713647.
21. Current J Z, Chaney H L, Zhang M, et al. (2024) Characterization of bovine long non-coding RNAs, OOSNCR1, OOSNCR2 and OOSNCR3, and their roles in oocyte maturation and early embryonic development. *Reprod Biol*, 24(3):100915. https://doi.org/10.1016/j.repbio.2024.100915.
22. Zhang T, Zhang J, Yang G, et al. (2024) Long non-coding RNA PWRN1 affects ovarian follicular development by regulating the function of granulosa cells. *Reprod Biomed Online*, 48(5): 103697. https://doi.org/10.1016/j.rbmo.2023.103697.
23. Iyyappan R, Aleshkina D, Zhu L, et al. (2021) Oocyte specific lncRNA variant Rose influences oocyte and embryo development. *Noncoding RNA Res*, 6(2): 107-113. https://doi.org/10.1016/j.ncrna.2021.06.001.
24. Li D, Wang X, Li G, et al. (2021) LncRNA ZNF674-AS1 regulates granulosa cell glycolysis and proliferation by interacting with ALDOA. *Cell Death Discov*, 7(1): 107. https://doi.org/10.1038/s41420-021-00493-1.
25. Chen G, Cheng X, Shi G, et al. (2019) Transcriptome Analysis Reveals the Effect of Long Intergenic Noncoding RNAs on Pig Muscle Growth and Fat Deposition. *Biomed Res Int*, 2019(2951427. https://doi.org/10.1155/2019/2951427.
26. Gong Y, He J, Li B, et al. (2021) Integrated Analysis of lncRNA and mRNA in Subcutaneous Adipose Tissue of Ningxiang Pig. *Biology (Basel)*, 10(8). https://doi.org/10.3390/biology10080726.
27. Shi G, Chen L, Chen G, et al. (2019) Identification and Functional Prediction of Long Intergenic Non-coding RNAs Related to Subcutaneous Adipose Development in Pigs. *Front Genet*, 10(160. https://doi.org/10.3389/fgene.2019.00160.
28. Chen L, Shi G, Chen G, et al. (2019) Transcriptome Analysis Suggests the Roles of Long Intergenic Non-coding RNAs in the Growth Performance of Weaned Piglets. *Front Genet*, 10(196. https://doi.org/10.3389/fgene.2019.00196.
29. Bezerra M E S, Barberino R S, Menezes V G, et al. (2018) Insulin-like growth factor-1 (IGF-1) promotes primordial follicle growth

and reduces DNA fragmentation through the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signalling pathway. *Reprod Fertil Dev*, 30(11): 1503-1513. https://doi. org /10.1071/RD17332.

30. Clark K L, George J W, Przygrodzka E, et al. (2022) Hippo Signaling in the Ovary: Emerging Roles in Development, Fertility, and Disease. *Endocr Rev*, 43(6): 1074-1096. https://doi.org/10.1210/endrev/bnac013.
31. Li L, Shi X, Shi Y, et al. (2021) The Signaling Pathways Involved in Ovarian Follicle Development. *Front Physiol*, 12(730196. https://doi.org/10.3389/fphys.2021.730196.
32. Li L, Ji S Y, Yang J L, et al. (2014) Wnt/beta-catenin signaling regulates follicular development by modulating the expression of Foxo3a signaling components. *Mol Cell Endocrinol*, 382(2): 915-25. https://doi.org/10.1016/j.mce.2013.11.007.
33. Hernandez Gifford J A (2015) The role of WNT signaling in adult ovarian folliculogenesis. *Reproduction*, 150(4): R137-48. https://doi.org/10.1530/REP-14-0685.
34. Feng H, Liu T, Yousuf S, et al. (2022) Identification and analysis of lncRNA, miRNA and mRNA related to subcutaneous and intramuscular fat in Laiwu pigs. *Front Endocrinol (Lausanne)*, 13(1081460. https://doi.org/10.3389/fendo.2022.1081460.
35. Jiang Y, He Y, Pan X, et al. (2023) Advances in Oocyte Maturation In Vivo and In Vitro in Mammals. *Int J Mol Sci*, 24(10). https://doi.org/10.3390/ijms24109059.
36. Pei Z, Deng K, Xu C, et al. (2023) The molecular regulatory mechanisms of meiotic arrest and resumption in Oocyte development and maturation. *Reprod Biol Endocrinol*, 21(1): 90. https://doi.org/10.1186/s12958-023-01143-0.
37. Shimada R and Ishiguro K I (2024) Female-specific mechanisms of meiotic initiation and progression in mammalian oocyte development. *Genes Cells*, 29(10): 797-807. https://doi.org/10.1111/gtc.13152.