BF-1547
[1-8]

RESEARCH ARTICLE Indian Journal of Animal Research

Identification of Circular RNAs in the Ovaries of Hu Sheep and Local Fat-tailed Sheep during the Luteal Phase

Zhenhan Zhai[1], Jianhua Li[3], Binglei Zhang[1], Mengyao Wang[1], Hongxiang Ding[1], Lu Zhao[1], Saiqiao Wang[1], Caihong Wei[2], Yuqin Wang[1]

10.18805/IJAR.BF-1547

ABSTRACT

Background: Circular RNAs (circRNAs), a novel class of endogenous non-coding RNAs with covalently closed loop structures, play important role in mammalian growth and development by regulating gene expression and participating in various biological processes. The aim of this study was to identify the expression profiles of circRNAs in the ovaries of Hu sheep and local Fat-tailed sheep during the luteal phase to determine the molecular mechanisms underlying the effects of circRNAs on reproduction and follicular development in sheep.

Methods: To identify the differentially expressed circRNAs expressed in luteal phase conditions in Hu sheep and fat-tailed sheep, ovarian samples collected during the luteal phase were analyzed using high-throughput sequencing and subsequent transcriptomic approaches and bioinformatics analysis.

Result: A total of 2,875 differentially expressed circRNAs were identified by high-through sequencing. Gene Ontology terminology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis showed that the host genes were mainly involved in the secretion and metabolism of the various pathway. Quantitative real-time PCR was performed to validate the results of expression profiling. These results contributed to an improved understanding of circRNA and the biological mechanisms of ovarian reproduction in sheep.

Key words: CircRNA, Hu sheep, Ovary, RNA-Seq, Transcriptome.

INTRODUCTION

The ovary is a vital gonadal organ which has the functions of synthesis and secretion of sex hormones, endocrine and reproduction. The maturation and ovulation of primary follicles are regulated by hormones and signaling pathways (Johnson, 2015). During the post-ovulation, the antral follicle and surrounding tissues are reorganized to form a gland with an internal analysis function under the action of gonadotropin, called the corpus luteum (Wang *et al*., 2020a). The function of the corpus luteum is not only regulated by gonadotropins, but also closely related to cell proliferation and transformation, angiogenesis and metabolite transport in the corpus luteum. Guo *et al*. (2015) demonstrated that ERK1/2 and AMPK metabolic pathways are associated with luteinizing hormone. Moreover, genes, such as StAR, P450 (*CYP11A*) and 3β-HSD have appeared many times in many studies and they are the hallmark genes of ovarian corpus luteum development and follicular maturation and ovulation (Song *et al*., 2021).

In recent years, with the development of high-throughput sequencing, novel bioinformatics methods and related experimental validation, several classes of non-coding RNAs, such as circRNA, lncRNA and miRNA, have emerged and appear to play an important role in various biological processes (Chang *et al*., 2018; He *et al*., 2019; Chen *et al*., 2020; Zhou *et al*., 2020; Mei *et al*., 2021; Wu *et al*., 2021). CircRNA is different from linear RNA without 5′ cap and 3′ end poly-A tail. It is mainly produced by RNA alternative splicing or reverse splicing and has a closed loop structure with valence (Qu *et al*., 2015). Due to the special structure, compared with long non-coding RNA, circRNA could resist the degradation of RNase R, which was a particular path to identify circRNA (Suzuki and Tsukahara, 2014). Especially in terms of tissue specificity, spatiality and stability, circRNAs are abundant in eukaryotic cells (Chen and Yang, 2015). Jeck and Sharpless (2014) proved that circRNA was widely present in the cytoplasm. Xu and coworkers (2019) identified that circ-TCP1 act as competing endogenous RNAs (ceRNAs) that bind miR-183 that regulates porcine ovarian in biological processes related to litter size. In the recent studies, circRNAs have become markers for disease

[1]College of Animal Science and Technology, Henan University of Science and Technology, Luoyang, Henan 471023, China.
[2]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China.
[3]Center of Reproductive Medicine and Genetics, the Seventh Medical Center of PLA General Hospital, Beijing 100700, China.

Corresponding Author: Yuqin Wang, College of Animal Science and Technology, Henan University of Science and Technology, Luoyang, Henan 471023, China. Email: wangyq6836@163.com

How to cite this article: Zhai, Z., Li, J., Zhang, B., Wang, M., Ding, H., Zhao, L., Wang, S., Wei, C. and Wang, Y. (2022). Identification of Circular RNAs in the Ovaries of Hu Sheep and Local Fat-tailed Sheep during the Luteal Phase. Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1547.

Submitted: 30-05-2022 **Accepted:** 15-09-222 **Online:** 27-09-2022

diagnosis (Wang *et al*., 2019; Sheng *et al*., 2020). However, studies on sheep luteal ovaries were very few.

In this study, high-throughput transcriptomic circRNA sequencing was performed to reveal changes in the expression profile of circRNAs in luteal phase ovaries in two different breeds of sheep. An in-depth analysis to explore the potential biological functions of specific circRNAs on sheep ovary development was also undertaken. This study adds new comprehensive knowledge on circRNA expression in ovarian tissue, complements key pathways of follicular development in luteal phase ovaries and provides candidate circRNAs for further functional studies and biomarker evaluation.

MATERIALS AND METHODS

All experimental procedures involving sheep followed the policies and guidelines on the Henan University of Science and Technology Animal Welfare and Use Committee.

Experimental animal preparation

The experiment was conducted in 2019-9 and 2019-10 at Henan University of Science and Technology pastures. The geographical location belongs to the western Henan Province of China at 34°32′-34°45′N and 112°16′-112°37′E. Hu sheep and fat-tailed sheep were purchased from Luoning Nongben Biotechnology Co., Limited located in Luoning of Luoyang Province of China. Eight ewes with the same feeding and other management conditions, aged 3.5±0.40 years and weighing 50.5±2.0 kg, each ewes with three consecutive births and divided into OAL group (*Hu sheep,* n=4, litter size per ewe=3) and OZL group (local *Yuxi fat-tailed sheep,* n=4, litter size per ewe=1). Ovarian tissues were collected on day 5 of the luteal phase, washed with diethyl pyrocarbonate water (DEPC) and placed in lyophilized tubes, numbered, rapidly frozen in liquid nitrogen and stored in a -80°C refrigerator until follow-up experiments.

Extraction of RNA, construction of cDNA library and sequencing

Total RNA was extracted from the ovarian tissues using TRIzol reagent (Invitrogen). An amount of 5 µg of RNA from each sample was used to prepare the input material and the circRNA library was prepared according to the steps. 1) Pretreatment of 5µg of total RNA, enrichment of circRNA was digested with 3U of RNase R enzyme reagent (Gisai, Guangzhou, China) per µg of RNA. 2) the RNA pretreament using NEBNext Ultra™ Directional RNA Library Prep Kit (NEB, USA). 3) Use the BioAnalyzer 2100 instrument for library quality control and quantification. According to Illunima sequencing instructions, the library was denatured into single-stranded DNA molecules, the second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H, captured on Illunima flowcel, amplified in situ into clusters and selected fragments at 150 to 200 bp in length on an Illumian HiSeq sequencer. The sequencing work was performed by NovoHozyme Biotech.

Identification of differentially expressed circRNA

CircRNAs were detected and identified using find-circ (Version 1.1) software (Memczak *et al*., 2013) and CIRI2 (Version 1.2) (Gao *et al*., 2018). Circos (Version 0.62-1) software was used to map and display in co-expressed networks. The DESeq2 (Version 1.10.1) R software package was performed to analyze the data for differential expression. DESeq2 provides a model based on the negative binomial distribution to determine the regular statistics of differential expression in digital gene expression databases. In brief, we identified differentially expressed genes with |fold change|³2.0 and P<0.05 between the two groups as differentially expressed circRNAs.

RNase R treatment experiments

The RNA samples extracted from the ovarian tissue of Hu sheep were divided into two parts, one was treated with RNase R enzyme (RNase R+) and the other was used as a control (RNase R-). For the treated group of RNA (2.5 ng/ µL), 10 units of RNase R enzyme was added while for the control group, similar quantity of buffer was added and the control group added the same amount of buffer, incubated at 37°C for 30 min and then inactivated the enzyme at 70°C for 10 min and then proceeded to reverse transcription reaction directly.

qRT-PCR analysis and sanger sequencing

To verify the accuracy of the sequencing results, the ovis glyceraldehyde 3 phosphate dehydrogenase enzyme gene (GAPDH) was selected as the internal reference gene and the expression levels of eight randomly selected differentially expressed circRNAs were verified by applying real-time fluorescence quantitative PCR. Primers were designed using Oligo 7 software and the primer sequences of the eight circRNAs and the GAPDH internal reference gene are shown in Table 2. Then after, the RNA samples were extracted and the first strand of cDNA was synthesized was used as a template for real-time fluorescence quantitative PCR to detect the circRNAs expression level. The qPCR reaction mix for circRNA (20 µl) consisted of 10 µl of 2× ChamQ Universal STBR qPCR Master Mix, 0.4 µl each for upstream and downstream primers, 1 µl of cDNA and 8.2 µl ddH_2O. The qPCR reaction conditions were as follows: 95°C for 15 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. Each sample contains three technical replicates. The qPCR products were sent to Shanghai Biotechnology Co., Ltd for sequencing. According to the $2^{-\Delta\Delta}$Ct method was used to calculate the relative expression (Rao *et al*., 2013). Dependent sample *t*-test was performed to calculate relative expression and SPSS13.0 was used to analyze the significant difference.

Co-expression network of circRNA-miRNA construction

The Regrna 2.0 database (http://regrna2.mbc.nctu.edu.tw/) was performed to predict the regulation of miRNA by circRNA. The miRNA target sites in the exons of the circRNA locus were identified using miRanda (Version 3.3a) software.

Table 1: Reads quality of circRNA libraries.

Sample name	OAL	OZL
Total reads	92420022	92846510
Total mapped	80858124 (87.49%)	80298201 (86.48%)
Multiple mapped	16414220 (17.76%)	15743074 (16.96%)
Uniquely mapped	64443904 (69.73%)	64555127 (69.53%)
Read-1	32482132 (35.15%)	32610253 (35.12%)
Read-2	31961772 (34.58%)	31944874 (34.41%)
Reads map to '+'	32184185 (34.82%)	32331022 (34.82%)
Reads map to '-'	32259719 (34.91%)	32224105 (34.71%)
Non-splice reads	43784726 (47.38%)	48138023 (51.85%)
Splice reads	20659178 (22.35%)	16417104 (17.68%)
Reads mapped in proper pairs	60543684 (65.51%)	60302640 (64.95%)

According to the predicted binding site of miRNA to target gene of circRNA, the circRNA–miRNA interaction network was constructed using Cytoscape (Version 3.6.4) software for visual data analysis.

RESULTS AND DISCUSSION

Identification features and characteristics of circRNAs

The RNA sequencing was undertaken to detect the identities and abundances of circRNAs in luteal ovaries obtained from Hu sheep and fat-tailed sheep. As shown in Table 1, after excluding low-quality raw reads, a total of 92,420,022 and 92,846,510 clean reads from two groups (OAL and OZL), which mapping to the sheep reference genome (Oar-v4.0) of OAL and OZL were 80,858,124 (87.49%) and 80,298,201 (86.48%), the GC contents were 54.8% and 56.44%,

Table 2: Primers of circRNA for qRT-PCR.

Primer name	Primer sequence (5′→3′)	Product size (bp)	Tm
novel_circ_0014364	F: TTGGAAGCAGAGCAGCGGAG	150	60
	R: GCACCCAGCCTGAAGCAACA		
novel_circ_0004071	F:GGAAAGCCAGCACCAAGACCT	103	60
	R: ACACCAGCACGTTGTCCTCCT		
novel_circ_0011246	F: AGAAACGCCACCTCCTGGATAT	134	60
	R: TCCTGCTGGGAAGTTAGTGTTT		
novel_circ_0012521	F: TGGTAGTAGCACTGAGCCTGA	306	60
	R:TGGTAGTAGCACTGAGCCTGA		
novel_circ_0004711	F: GGCTGCCAACATCAGAGGTT	186	60
	R: TCTGGCTGAGCGAGCTGTAA		
novel_circ_0014084	F:TGAGGTTCGGGCAAGGTCCAT	107	60
	R:GGCAAGTGTCACAGCGGAGAAG		
novel_circ_0008333	F:GCTCCGAGGTCTTCATCACAGG	196	60
	R: ACCTTGCCCGTACAGGAAAGC		
novel_circ_0013661	F: TGCCCAGAGAATCAGCCAGG	156	60
	R: GCTCCCTCTGTTCTGCAAGG		
GAPDH	F:GTCGGAGTGAACGGATTTGG	196	60
	R:CATTGATGACGAGCTTCCCG		

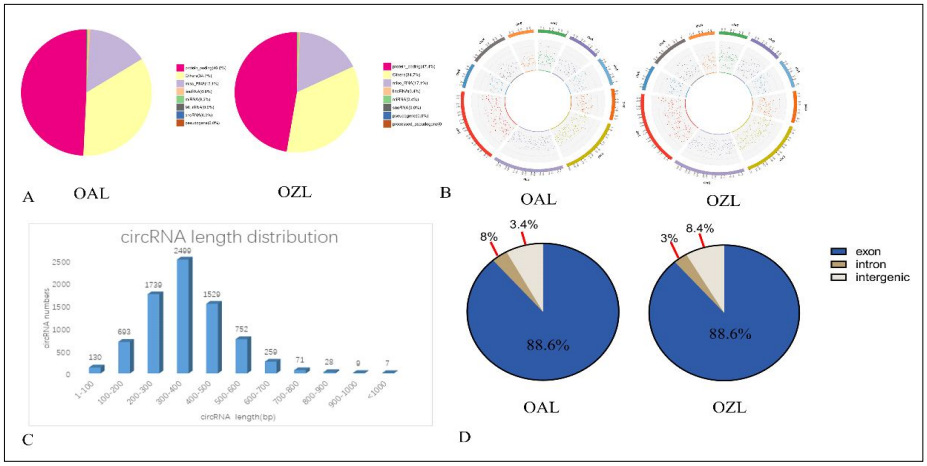

Fig 1: General characteristics of circRNAs in the OAL and OZL. (A) Different colors represent RNA categories and the percentage of reads in that category is compared in parentheses. (B) The density distribution of circRNA on chromosomes. (C) Length distribution of circRNAs. (D) the source regions of circRNAs.

respectively. The distribution of reads in known types of genes was shown in (Fig 1A). Totally, 7 711 circRNAs were identified together in the two groups, with 2,875 annotated circRNAs could be compared to the reference genome. In addition, 278 novel circRNAs were obtained in this experiment. The statistics of the density of each chromosome-circRNA show that circRNAs were mainly distributed on chromosomes 1 to 9 and X (Fig 1B,1C). Results of the present study revealed that the length of the sheared circRNAs were between 200 to 500 bp, which was consistent with the content of the previous study (Yang *et al*., 2020). These circular RNAs are mainly composed of three types (Fig 1D), introns (8%~3%), intergenic (3.4%~8.4%) and exons (both 88.6%). The sequencing results in the ovaries of previous studies, such as pig (Wang *et al*., 2021), goat (La *et al*., 2019) and sheep (Liu *et al*., 2021) animals, were similar to the results obtained in the present study.

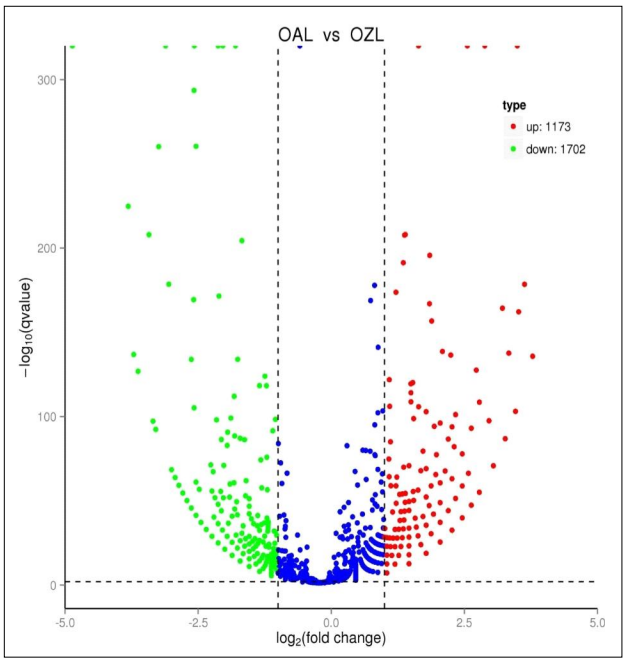

Fig 2: Difference circRNA volcano map**.** The X axis represents the fold change of circRNA expression and the Y axis represent the statistically significant degree of circRNA expression change. The scattered dots in the figure represent each circRNA, the blue dots represent circRNA with no significant differences and the red dots represent significantly up-regulated ones. Differential circRNA, green dots indicate significantly down-regulated differential circRNA.

Differentially expressed analysis of circRNAs

Using RNA-seq transcriptome data to analysis the transcriptional levels of circRNA in the ovaries of two sheep breeds. Based on the standard (|fold change|≥2.0 and P<0.05), there were we identified 2,875 differentially expressed circRNAs (DE-circRNAs) by DEseq2. A volcano map of the circRNA expression profiles among the OAL and OZL showed that the 1,173 DE-circRNAs were up- and 1,702 DE-circRNAs were down-regulated (Fig 2).

Enrichment analysis of differentially expressed host gene

In the GO analysis, as shown in Fig 3A, the top three biological processes consisted of the metabolic process (GO:0008152), organic substance metabolic process (GO:0071704) and cellular metabolic process (GO: 0044237). In terms of molecular function, OAL and OZL were mainly enriched in catalytic activity (GO:0003824), binding (GO:0005488), protein binding (GO:0005515), organic cyclic compound binding (GO:0097159) and heterocyclic

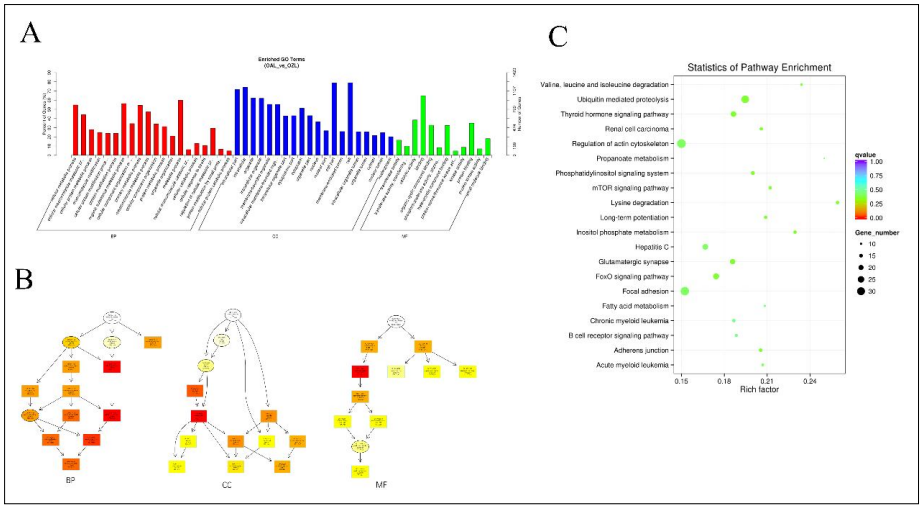

Fig 3: GO and KEGG analyses of differentially expressed circRNA source genes. (A) GO functional analysis of differentially expressed circRNA source genes in OAL vs. OZL. (B) GO enriched directed acyclic graph. BP, biological process; CC, cell component; MF, molecular function. (C) KEGG analysis of differentially expressed circRNA source genes in OAL vs. OZL. The X axis represents the name of the pathway and the Y axis represent the Rich factor. The size of the dot indicates the number of source genes in the pathway and the color of the dot corresponds to different q-value ranges.

compound binding (GO:1901363). The cellular components identified were significantly enriched in terms of the intracellular part (GO:0044424) and intracellular (GO:0005622). As shown in the Fig 3B, the three GO domains (cell composition, biological process and molecular function) were represented by a separate fundamental body term. All terms in a domain could trace their parent source to a root term. There might be many different paths through the intermediate terms to the ontology root. KEGG analysis showed that the host genes (STAT1 and UBE2L6) of the DE-circRNAs were mainly enriched in the ubiquitin mediated pathways, such as ubiquitin-mediated proteolysis and protein processing in endoplasmic reticulum; hormone metabolism-related pathways like thyroid hormone signaling pathway and propanoate metabolism (Fig 3C). The host gene, such as MAP9 (Microtubule-associated protein 9) and UBE2L6 also enrichment in GO terms. MAP9, located on chromosome 4q32.1, was a gene responsible for spindle assembly and cytokinesis (Zhang *et al*., 2020). For example, UBE2L6 could adsorb miR-146a-5p and inhibit the apoptosis of Mtb infected macrophages, which can be used as a potential biomarker of TB. The formation and dissipation of the luteal body provide energy reserves for follicular development, follicular recruitment, ovulation and other biological processes of Hu sheep (Wang *et al*., 2020b). Therefore, the results of GO and KEGG analyses provide a valuable resource for studying high yield in luteal phase ewes.

Co-expression network of circRNA-miRNA and function prediction

The miRNA binding sites of the selected eight differentially expressed circular RNAs were predicted by miRNA software and the relationship network of circRNA-miRNA was obtained. As shown in the Fig 4, a total of 35 nodes (including 27 miRNAs and 8 circRNAs) and 39 edges constitute the circRNA-miRNA interaction network. These differentially expressed circRNAs can regulate the expression of downstream target genes by antagonizing 28 miRNAs and its molecular mechanism needs to be further verified. Importantly, the predicted results indicate that new-circ-0013661 has an FC value of -2.5 in the OAL/OZL group and targets miR-23a and miR-23b, in addition miR-23b can act on its host gene STAT1 (ENSORARG00000013903). These predicted circRNAs are the focus of in-depth exploration.

Verification of candidate circRNAs using RT-qPCR and Sanger sequence

To verify the accuracy of the RNA-seq results, we randomly selected eight DE circRNAs and designed back-to-back divergent primers. The RNA incubated with RNase R was reverse transcribed to cDNA and the tissue expression levels of these eight circRNAs were determined by quantitative real-time PCR (qRT-PCR). Also, the results showed similar expression trends to our RNA-seq data (Fig 5A). The dorsal spore node was verified by Sanger sequencing of the products amplified by the PCR procedure (Fig 5B). It indicated that the loop RNA in this experiment had a specific loop structure. And the tissue expression levels of these eight circRNAs were determined by quantitative real-time PCR (qRT-PCR). Meanwhile, the results showed a similar expression trend with our RNA-seq data. The reverse shear point was verified by Sanger sequencing of the products amplified by the PCR procedure. This indicates that the loop RNA in this experiment has a specific loop structure.

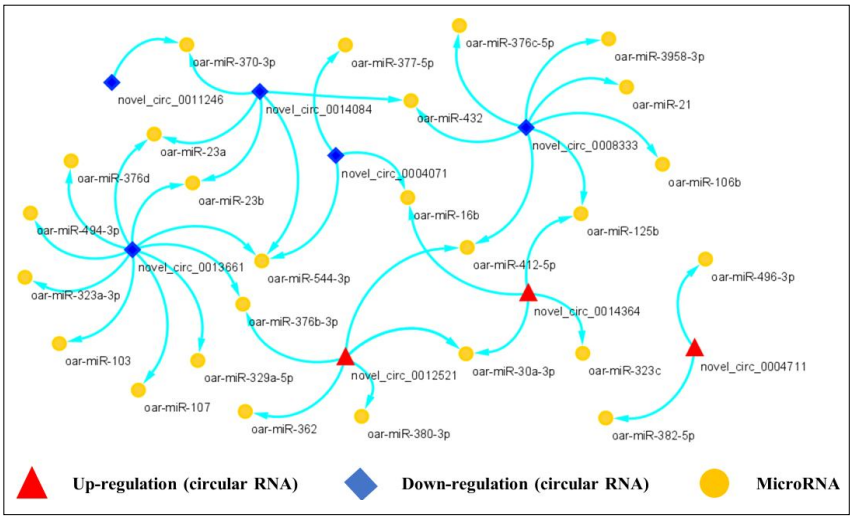

Fig 4: Interaction network assigned with the different expression circRNA and their potential target miRNA. The eight differentially expressed circRNAs and their potential target miRNA were used for their interaction network analysis. The red triangle represent up-regulation circRNA; the blue diamond represent down-regulation circRNA;the yellow circular represent miRNA.

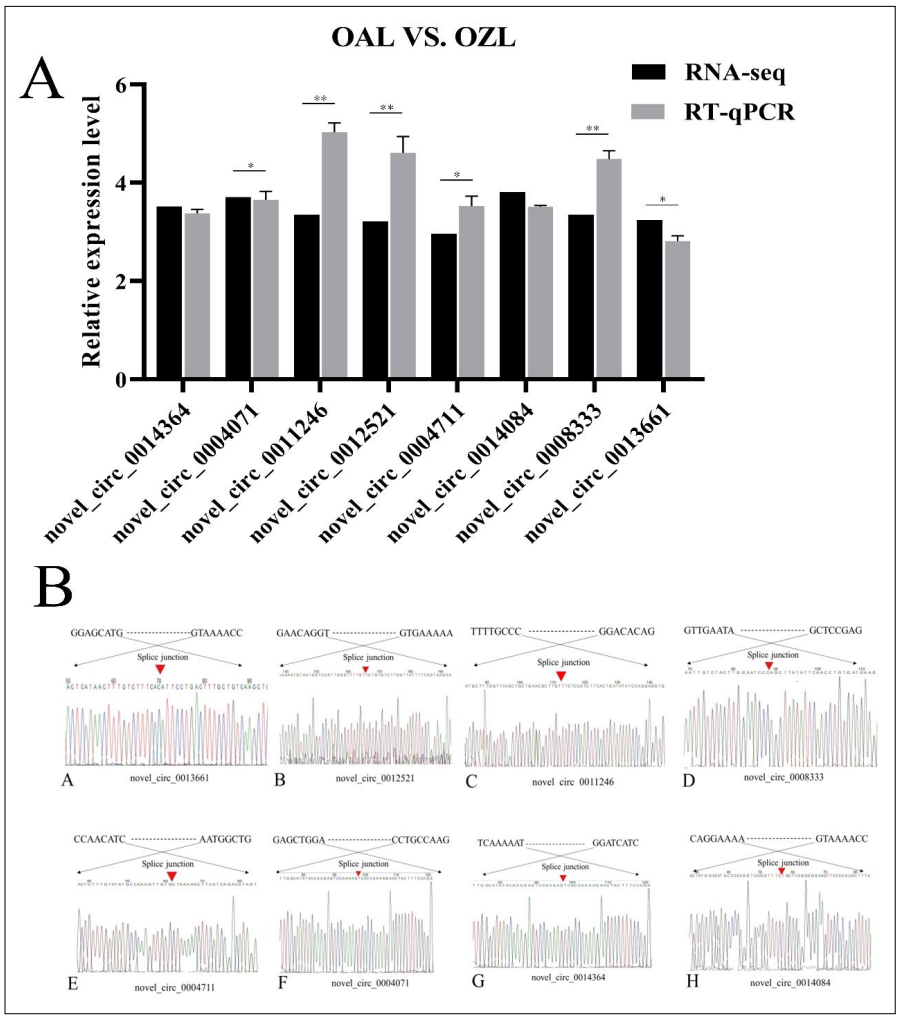

Fig 5: Validate the relative expression level of circRNA by qRT-PCR and Sanger sequencing. (A) Real-time qPCR analyses of differentially expressed circRNAs in RNA-seq and post RNA-seq. Values are shown as means ± SEM. *P<0.05 (B) Back-spliced junctions of the eight randomly selected circRNAs was confirmed by Sanger sequencing. The red trangle represents the back-spilced sites. Green and red horizontal lines indicate the 5′ and 3′ ends of the circRNA sequence, respectively.

Hansen *et al*. (2013) identified circRNA that contained binding sites of miRNA due to the miRNA response element (MRE), which acted as a competitive endogenous RNA (ceRNA) to modulate gene expression. The ceRNA hypothesis (Wu *et al*., 2021) states that circRNA can act as sponges for miRNAs and restrict the expression of miRNA target genes. In present study, miRanda software was to the identified miRNA target sites in sheep circRNAs. The ceRNA network had been constructed to explore further the role of the small RNAs that regulated in the ovary. Interestingly, the interaction network displays that oar-miR-16b, oar-miR-23a, oar-miR-23b, oar-miR-544-3p, oar-miR-376b-3p and oar-miR-432 are associated with two circRNAs. The miR-16 inhibited BCL-2 and the NF-KBI/MMp-9 signaling pathway, a tumor suppressor gene in glioma and invasiveness (Yang *et al*., 2014). The previous study detected that miR-544 inhibits cell proliferation, migration and invasion through down-regulating the 3′UTR of *PARK7* (Jin *et al*., 2016). The study showed that miR-544 regulated dairy goat male germline stem cell self-renewal by targeting *PLZF* (Song *et al*., 2015).

CONCLUSION

In this study, the results of transcriptome sequencing demonstrated the specific expression of circRNA in the ovaries of Hu sheep and local fat-tailed sheep. Functional enrichment analysis of significantly different circRNA host genes revealed that STAT1 (novel-circ-0004071) and UBE2L6 (novel-circ-0013661) were predicted to influence the molecular mechanism of ovarian development in sheep through the ubiquitination pathway. These results contribute to an improved understanding of circRNA and the biological mechanisms of ovarian reproduction in sheep.

ACKNOWLEDGEMENT

This research was supported by the China Agriculture Research System of MOF and MAR, CARS-38 and The

National Natural Science Foundation of China (31472095).

Conflict of interest

The authors declared that they have no conflict of interest.

REFERENCES

Chang, W.H., Wang, J.H., Zhang, Y., Wu, J.Y. (2018). Discovery of two novel miRNAs from the Ovis aries by a combinatorial approach of experiments and bioinformatics. Indian Journal of Animal Research. 52: 1155-1161. doi: 10.18805/ijar.B-721.

Chen, L.L., Yang, L. (2015). Regulation of circRNA biogenesis. RNA biology. 12: 381-388. http://doi.org/10.1080/15476286.2015.1020271.

Chen, W., Lv, X., Wang, Y., Zhang, X., Wang, S., Hussain, Z., Chen, L., Su, R., Sun, W. (2020). Transcriptional profiles of long non-coding RNA and mRNA in sheep mammary gland during lactation period. Frontiers in Genetics. 11: 946. http://doi.org/10.3389/fgene.2020.00946.

Cui, C., Han, S., Yin, H., Luo, B., Shen, X., Yang, F., Liu, Z., Zhu, Q., Li, D., Wang, Y. (2019). FOXO3 is expressed in ovarian tissues and acts as an apoptosis initiator in granulosa cells of chickens. Biomed Res Int. 2019: 6902906. http://doi.org/10.1155/2019/6902906.

Deng, L., Chen, Q., Xie, J., Wei, W., Hui, H. (2020). CircPUM1 promotes polycystic ovary syndrome progression by sponging to miR-760. Gene. 754: 144903. http://doi.org/10.1016/j.gene.2020.144903.

Gao, Y., Zhang, J., Zhao, F. (2018). Circular RNA identification based on multiple seed matching. Brief Bioinform. 19: 803-810. http://doi.org/10.1093/bib/bbx014.

Guo, N., Meng, C., Bai, W., Wei, Q., Shi, F., Davis, J.S., Mao, D. (2015). Prostaglandin F2α induces expression of activating transcription factor 3 (ATF3) and activates MAPK signaling in the rat corpus luteum. Acta Histochem. 117: 211-218. http://doi.org/10.1016/j.acthis.2014.12.008.

Hansen, T.B., Jensen, T.I., Clausen, B.H., Bramsen, J.B., Finsen, B., Damgaard, C.K., Kjems, J. (2013). Natural RNA circles function as efficient microRNA sponges. Nature. 495: 384-388. http://doi.org/10.1038/nature11993.

He, K., Li, W.X., Guan, D., Gong, M., Ye, S., Fang, Z., Huang, J.F., Lu, A. (2019). Regulatory network reconstruction of five essential microRNAs for survival analysis in breast cancer by integrating miRNA and mRNA expression datasets. Functional and Integrative Genomics. 19: 645-658. http://doi.org/10.1007/s10142-019-00670-7.

Jeck, W.R., Sharpless, N.E. (2014). Detecting and characterizing circular RNAs. Nature Biotechnology. 32: 453-461. http://doi.org/10.1038/nbt.2890.

Jin, S., Dai, Y., Li, C., Fang, X., Han, H., Wang, D. (2016). Micro RNA-544 inhibits glioma proliferation, invasion and migration but induces cell apoptosis by targeting PARK7. Am J. Transl Res. 8: 1826-1837.

Johnson, A.L. (2015). Ovarian follicle selection and granulosa cell differentiation. Poultry Science. 94: 781-785. http://doi.org/10.3382/ps/peu008.

Langmead, B., Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie2. Nat Methods. 9: 357-359. http://doi.org/10.1038/nmeth.1923.

Legnini, I., Timoteo, G.D., Rossi, F., Morlando, M., Briganti, F., Sthandier, O., Fatica A. *et al.* (2017). Circ-ZNF609 is a Circular RNA that can be translated and functions in myogenesis. Molecular cell. 66: 22-37 e9. http://doi.org/10.1016/j.molcel.2017.02.017.

La, Y., Tang, J., Di, R., Wang, X., Liu, Q., Zhang, L., Zhang, X., Zhang, J., Hu, W., Chu, M. (2019). Differential expression of circular RNAs in polytocous and monotocous uterus during the reproductive cycle of sheep. Animals (Basel). 9(10). doi: 10.3390/ani9100797.

Liu, A., Chen, X., Liu, M., Zhang, L., Ma, X., Tian, S. (2021). Differential expression and functional analysis of CircRNA in the ovaries of low and high fecundity hanper sheep. Animals (Basel). 11(7): 1863. doi: 10.3390/ani11071863.

Mei, B. and Liu, R. (2021). Identification of lncRNAs differentially expressed during natural and induced estrus in sheep. Indian Journal of Animal Research. 55(12): 1421-1429. DOI: 10.18805/IJAR.B-1347.

MacKay, L., Khadra, A. (2019). Dynamics of mechanosensitive nascent adhesion formation. Biophys J. 117: 1057-1073. http://doi.org/10.1016/j.bpj.2019.08.004.

Mao, X., Cai, T., Olyarchuk, J.G., Wei, L. (2005). Automated genome annotation and pathway identification using the KEGG orthology (KO) as a controlled vocabulary. Bioinformatics (Oxford, England). 21: 3787-3793.

Memczak, S., Jens, M., Elefsinioti, A., Torti, F., Krueger, J., Rybak, A., Maier, L. *et al.* (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 495: 333-338. http://doi.org/10.1038/nature11928.

Paczkowski, M., Schoolcraft, W.B., Krisher, R.L. (2014). Fatty acid metabolism during maturation affects glucose uptake and is essential to oocyte competence. Reproduction. 148: 429-439. http://doi.org/10.1530/REP-14-0015.

Qu, S., Yang, X., Li, X., Wang, J., Gao, Y., Shang, R., Sun, W., Dou, K., Li, H. (2015). Circular RNA: A new star of noncoding RNAs. Cancer Letters. 365: 141-148. http://doi.org/10.1016/j.canlet.2015.06.003.

Rao, X., Huang, X., Zhou, Z., Lin, X. (2013). An improvement of the 2ˆ(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostat Bioinforma Biomath. 3(3): 71-85. http://doi.org/10.1016/S0920-5489(99)92176-1.

Sheng, R., Li, X., Wang, Z., Wang, X. (2019). Circular RNAs and their emerging roles as diagnostic and prognostic biomarkers in ovarian cancer. Cancer Letters. 473: 139-147. http://doi.org/10.1016/j.canlet.2019.12.043.

Song, P., Yue, Q., Fu, Q., Li, X., Lim X,, Zhou, R., Chen, X., Tao, C. (2021). Integrated analysis of miRNA-mRNA interaction in ovaries of turpan black sheep during follicular and luteal phases. Reprod Domest Anim. 56: 46-57. http://doi.org/10.1111/rda.13848.

Song, W., Mu, H., Wu, J., Liao, M., Zhu, H., Zheng, L., He, X., Niu, B. *et al.* (2015). miR-544 regulates dairy goat male germline stem cell self-renewal *via* targeting PLZF. J. Cell Biochem. 116: 2155-2165. http://doi.org/10.1002/jcb.25172.

Suzuki, H., Tsukahara, T. (2014). A view of pre-mRNA splicing from RNase R resistant RNAs. International Journal of Molecular Sciences. 15: 9331-9342. http://doi.org/10.3390/ijms 15069331.

Wang, D., Luo, Y., Wang, G., Yang, Q. (2019). Circular RNA expression profiles and bioinformatics analysis in ovarian endometriosis. Mol Genet Genomic Med. 7: e00756. http://doi.org/ 10.1002/mgg3.756.

Wang, Q., Ma, X., Zhang, C. (2020). Effects of thyroid hormone on ovarian cell apoptosis in the rat. Reprod Fertil. 32: 1060-1066. http://doi.org/10.1071/RD20110.

Wang, J., Yang, Y., Xing, B., Chen, J., Lu, Q., Zhang, J., Ren, Q., Ma, Q., Guo, H., Cao, H. (2021). Castration induced circRNA expressional changes in subcutaneous adipose tissue of male pigs. Anim Sci J. 92(1):e13648. doi: 10.1111/asj.13648.

Wu, Z., Zhai, Z., Wang, M., Ding, H., Shi, H., Tian, Z., Zhang, X., Wang, Y., Wei, C., Li, F. (2021). Cloning, characterization and bioinformatics analysis of the sequences of miR-10a and miR-10b in sheep (Hu sheep). Indian Journal of Animal Research. 55(5): 522-529. DOI: 10.18805/IJAR.B-1208.

Xu, G., Zhang, H., Li, X., Hu, J., Yang, G., Sun, S. (2019). Genome-wide differential expression profiling of ovarian circRNAs associated with litter size in pigs. Frontiers in Genetics. 10: 1010. http://doi.org/10.3389/fgene.2019.01010.

Yang, T.Q., Lu, X.J., Wu, T.F., Ding, D.D., Zhao, Z.H., Chen, G.L., Xie, X.S., Li, B. *et al*. (2014). MicroRNA-16 inhibits glioma cell growth and invasion through suppression of BCL2 and the nuclear factor-kappaB1/MMP9 signaling pathway. Cancer Sci. 105: 265-271. http://doi.org/10.1111/cas. 12351.

Yang, T., Qiu, L., Bai, M., Wang, L., Hu, X., Huang, L., Chen, G., Chang, G. (2020). Identification, biogenesis and function prediction of novel circRNA during the chicken ALV-J infection. Anim Biotechnol. 1-11. doi: 10.1080/10495398 .2020.1856125.

Zhou, C.X., Ai, K., Huang, C.Q., Guo, J.J., Cong, H., He, S.Y., Zhu, X.Q. (2020). miRNA and circRNA expression patterns in mouse brain during toxoplasmosis development. BMC Genomics. 21: 46. http://doi.org/10.1186/s12864-020-6464-9.