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Expression of microRNAs in the hypothalamus of pregnant and non-pregnant goats

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Abstract: MicroRNAs (miRNAs) play a significant role in animal reproduction by regulating the expression of protein-coding genes. The hypothalamus regulates the pregnancy cycle changes in goats; however, the action mechanism of miRNAs in this regulation remains to be investigated. In this study, we performed RNA sequencing of hypothalamus samples to establish a comprehensive miRNA profiling of pregnant and non-pregnant goats. A total of 384 miRNAs were identified in the hypothalamus of pregnant goats, of which 239 were newly discovered, and 390 miRNAs were detected in the hypothalamus of non-pregnant goats of which 192 were novel miRNAs. In addition, a total of 280 differentially expressed miRNAs are characterized, of which 171 were known miRNAs and 109 were novel miRNAs. Functional enrichment suggests that the predicted target genes of differentially expressed miRNAs may be involved in the reproductive process. This preliminary study revealed that let-7f-5p, miR-99a-5p and miR-100-5p may be involved in the hypothalamic regulation of pregnancy cycle changes in goats. These data provide a basic reference for subsequent studies on the regulatory role of miRNAs in mammalian pregnancy.

Keywords: miRNA; RNA-seq; reproduction; differential expression

MicroRNAs (miRNAs) are a group of endogenous non-coding RNAs of 18 to 22 nucleotides that regulate post-transcriptional gene expression by inhibiting the translation of mRNA or influencing mRNA degradation. MiRNAs have been shown to be involved in many physiological processes, including hormone secretion, metabolism, tumourigenesis, cell proliferation, differentiation, and apoptosis (Tang et al. 2019; Zhang et al. 2020). MiRNAs are also relevant to the physiological processes of goats and are involved in the regulation of muscle growth, gonadal development (Niu et al. 2016), breast development (Chen et al. 2015), and hair follicle development (Li et al. 2016). Investigation of the functional mechanisms of miRNAs has been the focus of goat reproductive regulation research (Zhu et al. 2016). The reproductive function of mammals is regulated by the

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endocrine or reproductive axis, which is known as the "hypothalamic-pituitary-gonadal axis". The hypothalamus is located at the base of the brain and regulates the stimulation of reproduction by nerves, hormones and the environment (Dagklis et al. 2015). The hypothalamus stimulates the pituitary to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH) through the secretion of gonadotropin-releasing hormone (GnRH) to regulate gonad hormone secretion, thereby regulating reproductive activity (Lee et al. 2008). RNA sequencing (RNA-seq) technology is a powerful tool for the investigation of genes in various species such as humans, rats, cattle, and goats (Quan et al. 2019). RNA-seq has been widely used to identify non-coding RNAs in mammalian reproductive tissues. However, the reproductive miRNAs in the goat hypothalamus have not been explored by RNA-seq (quantitative) technology.

In this study, miRNAs were sequenced and analysed in the hypothalamus of pregnant and non-pregnant goats. MiRNAs that may be involved in the regulation of pregnancy were screened to provide basic data for subsequent studies of the mechanism of goat pregnancy regulation by the hypothalamus.

MATERIAL AND METHODS

Animals and tissues

The six Anhui white goats used in this experiment were obtained from Hefei Boda Animal Husbandry Technology Development Co., Ltd. To exclude the influence of other factors on the experiment, the physical condition and age of each experimental goat were essentially the same, and the feeding and management systems of the breeding farm were unified. Six hypothalamic samples were obtained from three pregnant (60 days, synchronized oestrus) and three non-pregnant Anhui white goats. The goats were approximately three and a half years old and experienced three pregnancies. After female goats were sacrificed, the hypothalamic tissues were harvested and stored in liquid nitrogen. Then, the samples were transferred to a freezer at –80 °C for long-term storage. All methods of animal tissue collection conformed to the standards of the ethics committee of Anhui Agricultural University, Anhui, China (permit No. AHAU20101025).

RNA extraction and small non-coding RNA sequencing

Total RNA of pregnant and non-pregnant goats was extracted from hypothalamic samples using RNAi Plus (TaKaRa, Shiga, Japan). To minimize naturally occurring transcriptomic differences between individual animals, total RNA from three different goats, including the hypothalamic tissues of the pregnant (PH) and non-pregnant goat (NH) groups, were combined in equal amounts to form a composite sample. Subsequently, total RNA was assayed using an RNA Nano 6000 Assay Kit (Agilent Technologies, Santa Clara, CA, USA) and an Agilent 2100 Bioanalyzer (Thermo Fisher Scientific, MA, USA). An aliquot of 3 μg of total RNA per sample was used as input material for generation of a small RNA library. Sequencing libraries were generated using a NEBNext®Multiplex Small RNA Library Prep Set for Illumina® (NEB, Ipswich, MA, USA) following the manufacturer's recommendations, and the index codes were added to attribute sequences to each sample (Ling et al. 2020). The library quality was assessed by an Agilent 2100 Bioanalyzer System (Thermo Fisher Scientific, Waltham, MA, USA) using a high-sensitivity DNA chip. The clustering of the index-coded samples was performed with a TruSeq PE Cluster Kit v3-cBot-HS (Illumina, San Diego, CA, USA) on a cBot Cluster Generation System and the library preparations were sequenced on an Illumina HiSeq 2500 platform in Shenzhen Huada Gene Technology Co., Ltd. (Shenzen, China). Finally, 150-bp single-end reads were generated.

Bioinformatics analysis

Raw data in the fastq format were initially processed by custom Perl and Python scripts. Raw data were filtered to remove the reads containing poly-*N* (with 5' adapter contaminants and poly A, T, G or C, without 3' adapter or the insert tag) and low-quality reads to obtain the clean data. Data quality and length statistics were analysed using the filtered data. The total distribution, all small non-coding RNAs (sRNAs), and various types of sRNA were compared and annotated using the NCBI GenBank (ftp.ncbi.nlm.nih.gov/genbank/) and Rfam (rfam.janelia.org). Sequences were compared to the data

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in the sRNA and miRBase (www.mirbase.org/ftp.shtml) databases using BLAST to identify known mi-RNAs for subsequent analysis and compare miRNAs that were not annotated. Reads per million (RPM) was used as a unit for estimation of the levels of miRNAs. Differential expression analysis of two stages was performed by the DESeq R package v3.1.2 (www.r-project.org). *P*-value < 0.05 was considered to define differential expression of miRNA. Differential expression levels of miRNAs were compared using the $\log_2$-ratio (fold change, FC) scatter plots (Kanehisa et al. 2008). |FC| > 2 was defined as an increase in the expression, and |FC| < 0.5 was considered a decline; 0.5 ≤ |FC| ≤ 2 was defined as equal expression. Cluster software v3.0 (Stanford University, Stanford, CA, USA) was used to perform the clustering based on the $\log_2$ values of the multiple significantly differentially expressed miRNAs. RNAhybrid software (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid) was used to predict the target genes of miRNAs. Functions and pathways of the target genes of differentially expressed miRNAs were acquired using the Gene Ontology (GO, geneontology.org) and Kyoto Encyclopaedia of Genes and Genomes (KEGG, www.genome.jp/kegg/) databases. GO analysis used GOseq-based Wallenius non-central hypergeometric distribution, which can be adjusted for a gene length bias. KOBAS software was used to calculate the enrichment of the candidate target genes in the KEGG pathway. Pathways with FDR ≤ 0.05 were considered to be significantly enriched (Khanum et al. 2007).

Reverse transcription quantitative real-time PCR

To verify the accuracy of the RNA sequencing data, six miRNAs were randomly selected for reverse transcription quantitative real-time PCR (RT-qPCR). Total RNA (1 μg) was reverse transcribed using Easy Script One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, Peking, China). The cDNA template was reverse transcribed using a dedicated reverse transcription primer (RT-primer, Table 1) designed by miRNA Design v1.01 (Vazyme Biotech Co., Ltd. Nanjing, China). Specific upstream primers for RT-qPCR were designed based on the sequence of each selected miRNA (Table 1). The downstream primers were universal primers. The primers were synthesized by Biotech Biotechnology Co., Ltd. (Shanghai, China). *U6* (NCBI reference sequence: KM401595.1) was used as a control. Master Mix was used to perform RT-qPCR on Step One Plus Real-Time PCR System (ABI, Waltham, MA, USA) according to the manufacturer's instructions. The PCR mixture (total volume of 20 μl) included 10 μl of SYBR Green Mix (Tolo Biotech, Shanghai, China), 2 μl of cDNA, 0.4 μl of forward primer (10 μM), 0.4 μl of reverse primer (10 μM) and 7.2 μl of ddH_2O. The template was denatured at 95 °C for 5 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 30 s and an extension at 60 °C for 30 s; then, the reaction was heated at a rate of 0.2 °C per 1 s to 95 °C. Each sample was assayed in triplicate.

Table 1. Primers for reverse transcription (RT) and RT quantitative real-time PCR (RT-qPCR) of microRNAs[1]

microRNA	RT-primer	RT-qPCR-primer
chi-let-7f-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACTAT	F: CGCGCGTGAGGTAGTAGATTGT R: AGTGCAGGGTCCGAGGTATT
chi-miR-423-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAGTC	F: GTGAGGGGCAGAGAGCGA R: AGTGCAGGGTCCGAGGTATT
chi-miR-708-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCCAGC	F: GCGCGAAGGAGCTTACAATCTA R: AGTGCAGGGTCCGAGGTATT
chi-miR-130a-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGCCCT	F: CGCGCAGTGCAATGTTAAA R: AGTGCAGGGTCCGAGGTATT
chi-miR-145-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGAT	F: CGGTCCAGTTTTCCCAGGA R: AGTGCAGGGTCCGAGGTATT
chi-miR-99a-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACAAGA	F: GCGAACCCGTAGATCCGA R: AGTGCAGGGTCCGAGGTATT
U6	TTGCGCAGGGGCCAT	F: CTCGCTTCGGCAGCACATA R: TTGCGCAGGGGCCAT

[1]The sequence of cDNA template is 5'-NNNN+GTCGTATCCAGTGCGAATACCTCGGACCCTGCACTGGATACGAC-3', NNNN indicate the mature sequence of microRNAs

https://doi.org/10.17221/113/2020-CJAS

Table 2. The classification of total small RNA tags by Solexa sequencing

Type	NH		PH	
	count	%	count	%
total_reads	12 125 000		11 696 869	
high_quality	12 081 583	100	11 648 943	100
3'adapter_null	45 289	0.37	55 322	0.47
insert_null	2 533	0.02	587	0.01
5'adapter_contaminants	6 988	0.06	8 477	0.07
smaller_than_18nt	238 121	1.97	239 669	2.06
polyA	116	0.00	125	0.00
clean_reads	11 788 536	97.57	11 344 763	97.39

NH = non-pregnant goat hypothalamus; PH = pregnant goat hypothalamus

The relative expression of miRNA was calculated using the $2^{-\Delta\Delta Ct}$ method. The SPSS v19.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of the data. Data are presented as the mean ± SD, and the differences were considered significant if the *P*-value was < 0.05.

RESULTS

Overview of sRNA sequencing of goat hypothalami

The filtered data passed the sequencing quality assessment, and the statistical sequencing results were used to identify a total of 12 125 000 original sequences in the non-pregnant goat hypothalamus (NH), and a total of 11 696 869 original sequences in the pregnant goat hypothalamus (PH). After removal of impurities, 11 788 536 pure reads were obtained in the NH library, and 11 344 763 pure reads were obtained in the PH library (Table 2). The length of the sequences of the NH library was mainly between 18 and 27 nt, and the length of the sequences of the PH library was mainly between 20 and 24 nt (Figure 1).

The sequence length distribution indicated that the sequencing libraries in this experiment contained various miRNAs. The number and types of publicly available sequences and types of novel miRNAs in NH and PH libraries are shown in Figure S1 [in electronic supplementary material (ESM); for the supplementary material see the electronic version] (Table S1 in ESM). A total of 22 082 507 publicly available sequences were detected in the two libraries, accounting for 95.46% of the sequences. The number of the sequences in the NH library was 659 839, accounting for 66.78%, and the number of the sequences in the PH library was 193 513, accounting for 23.25%.

sRNA classification notes

All sRNAs and various types of RNAs were compared. A total of 638 609 pure sequences were identified in NH, and a total of 276 425 pure sequences were present in PH. MiRNAs accounted for 26.08%

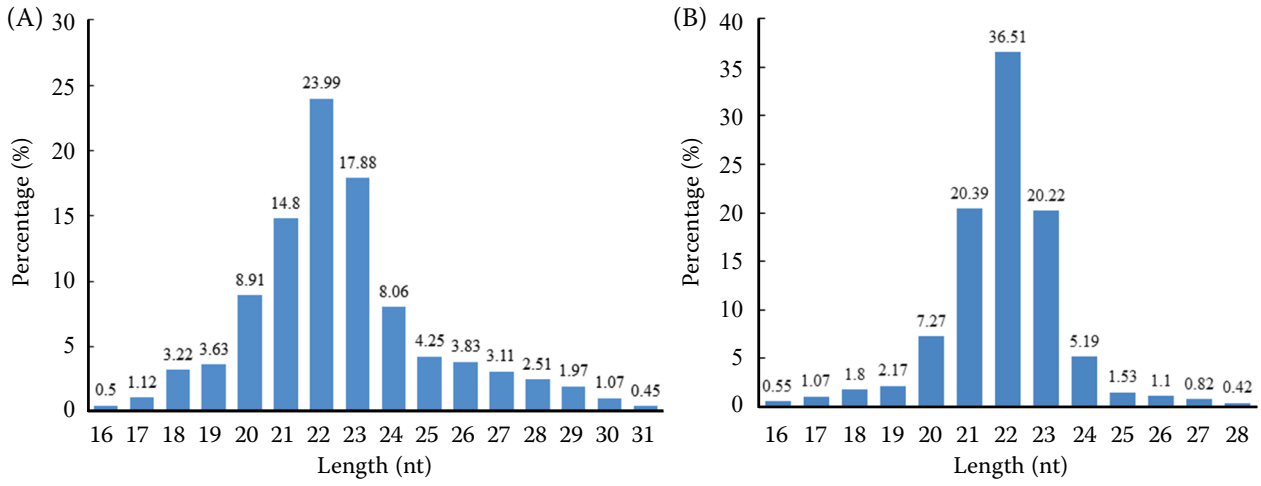

Figure 1. Distribution of sequence lengths of the sequencing results

(A) Frequency distribution of microRNA sequence lengths in the hypothalamus of the non-pregnant goat group (NH); (B) frequency distribution of microRNA sequence lengths in the hypothalamus of the pregnant goat group (PH)

https://doi.org/10.17221/113/2020-CJAS

Table 3. Expression of the most abundant known microRNAs

microRNA	NH		microRNA	PH	
	expressed	SD		expressed	SD
chi-miR-100-5p	252 015	31 617.409	chi-let-7f-5p	764 667	96 153.541 5
chi-let-7f-5p	218 829	27 453.945 1	chi-miR-100-5p	666 475	83 806.325 6
chi-miR-99b-5p	161 849	20 305.323 2	chi-miR-99b-5p	375 361	47 200.009 3
chi-miR-9-5p	152 285	19 105.438 7	chi-miR-9-5p	343 258	43 163.197
chi-miR-125a-5p	89 370	11 212.220 9	chi-miR-125a-5p	280 437	35 263.730 1
chi-miR-127-3p	39 592	4 967.150 6	chi-miR-127-3p	112 377	14 130.917 8
chi-miR-125b-5p	36 659	4 599.181	chi-miR-125b-5p	108 230	13 609.450 6
chi-miR-148a-3p	24 181	3 033.710 6	chi-let-7e-5p	65 164	8 194.088 9
chi-miR-10b-5p	23 373	2 932.340 1	chi-miR-99a-5p	60 480	7 605.096 3
chi-let-7c-5p	20 605	2 585.071 2	chi-miR-148a-3p	57 538	7 235.152 6

NH = non-pregnant goat hypothalamus; PH = pregnant goat hypothalamus

and 49.11% of the sequences in NH and PH, respectively. However, the miRNAs sequence types accounted for only 0.48% and 1.33% of sequences in NH and PH libraries, respectively. Most of the remaining sequences were mainly classified as unknown sequences. The results show that the expression of known miRNAs was higher than that of novel miRNAs (Figure S2 and Table S2 in ESM).

Predicted novel miRNAs

The sequences were compared with the data from the sRNA and miRBase (www.mirbase.org/ftp.shtml) databases by BLAST to identify novel miRNAs. A total of 390 and 384 known miRNAs were identified in NH and PH, respectively. A total of 192 novel and 239 novel miRNAs were detected in NH and in PH, respectively.

Differential analysis of known and novel miRNAs

To identify the differences between the two libraries of known and novel miRNAs, specifically expressed miRNAs were analyzed (Tables 3 and 4, Figure 2). The known miRNAs included 171 differentially expressed miRNAs (Table S3 in ESM); 152 miRNAs were co-expressed; 14 miRNAs were specifically expressed in NH, and 5 miRNAs were specifically expressed in PH. The novel miRNAs included 109 differentially expressed miRNAs (Table S4 in ESM); 26 miRNAs were co-expressed in both libraries; 23 novel miRNAs were specifically expressed in NH, and 60 miRNAs were specifically expressed in PH. Most of miRNAs in both libraries had low expression levels. The PH-specific expression of novel-mir-176 had 2 523 RPM, novel-mir-244 had 1 044 RPM, novel-mir-280 had

Table 4. Expression of the most abundant novel microRNAs

microRNA	NH		microRNA	PH	
	expressed	SD		expressed	SD
novel-mir-159	12 897	1 618.038	novel-mir-159	37 607	4 728.916
novel-mir-55	2 822	354.043 7	novel-mir-55	18 133	2 280.146
novel-mir-61	793	99.488 5	novel-mir-176	2 523	317.256 3
novel-mir-109	344	43.157 7	novel-mir-61	2 245	282.299
novel-mir-53	186	23.335 3	novel-mir-244	1 044	131.278 4
novel-mir-62	139	17.438 7	novel-mir-280	1 041	130.901 2
novel-mir-104	119	14.929 6	novel-mir-53	384	48.286 3
novel-mir-8	108	13.549 5	novel-mir-280	334	41.999 0

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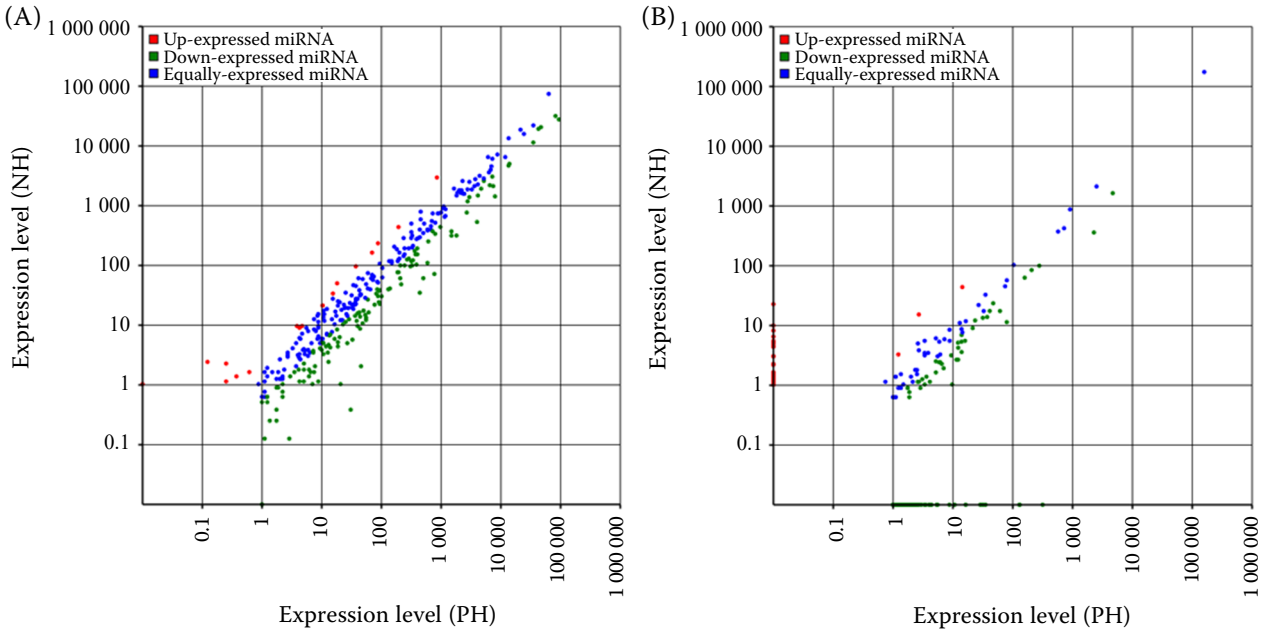

Figure 2. Scatter plot of the expression of differential microRNAs (miRNAs)
(A) Known miRNAs; (B) novel miRNAs
NH = non-pregnant goat hypothalamus; PH = pregnant goat hypothalamus

1 041 RPM, and the majority of other miRNAs were expressed relatively little.

Known and unknown differentially expressed miRNAs were clustered, and miRNAs with similar expression patterns were clustered with each other. In the cluster diagram, the expression level of green miRNAs in PH was higher than that in NH, while the trend of red miRNAs was opposite (Figure S3 in ESM).

Functional enrichment analysis of predicted target genes of miRNAs

To investigate the functions of miRNAs in the hypothalamus, the target genes of known and novel miRNAs differentially expressed were predicted using the RNAhybrid and TargetScan software. Gene Ontology enrichment analysis of all predicted target genes of differentially expressed miRNAs was performed (Figure 3). Gene Ontology terms of target genes of known and unknown miRNAs were involved in biological functions related to reproduction, including biological regulation, cell killing, biological process regulation and response to stimulation. Enrichment in reproduction and reproductive process was abundant. Additionally, the predicted target genes were subjected to KEGG pathway analysis, and the top 20 pathways of the enriched pathways are shown as bubble charts (Figure 4). The results showed that the target genes of differentially expressed known and unknown miRNAs are related to the pentose phosphate pathway, Jak-STAT signalling pathway, galactose metabolism, and neurotrophin signalling pathway. Notable enrichment was detected in the reproduction-related pathways, such as the TGF-beta signalling pathway, Wnt signalling pathway, MAPK signalling pathway, insulin signalling pathway and other pathways which are not shown in Figure 4.

RT-qPCR validation of differentially expressed miRNAs

To validate data from RNA-seq, we studied differentially expressed miRNAs using qRT-PCR. Six miRNAs were randomly selected, and the results are shown in Figure 5. The RT-qPCR results showed that the expression levels of chi-let-7f-5p, chi-miR-99a-5p, chi-miR-423-5p and chi-miR-130a-3p in PH were higher than those in NH, and an opposite trend was observed in the case of chi-miR-708-5p and chi-miR-145-5p. These results were consistent with the sequencing data.

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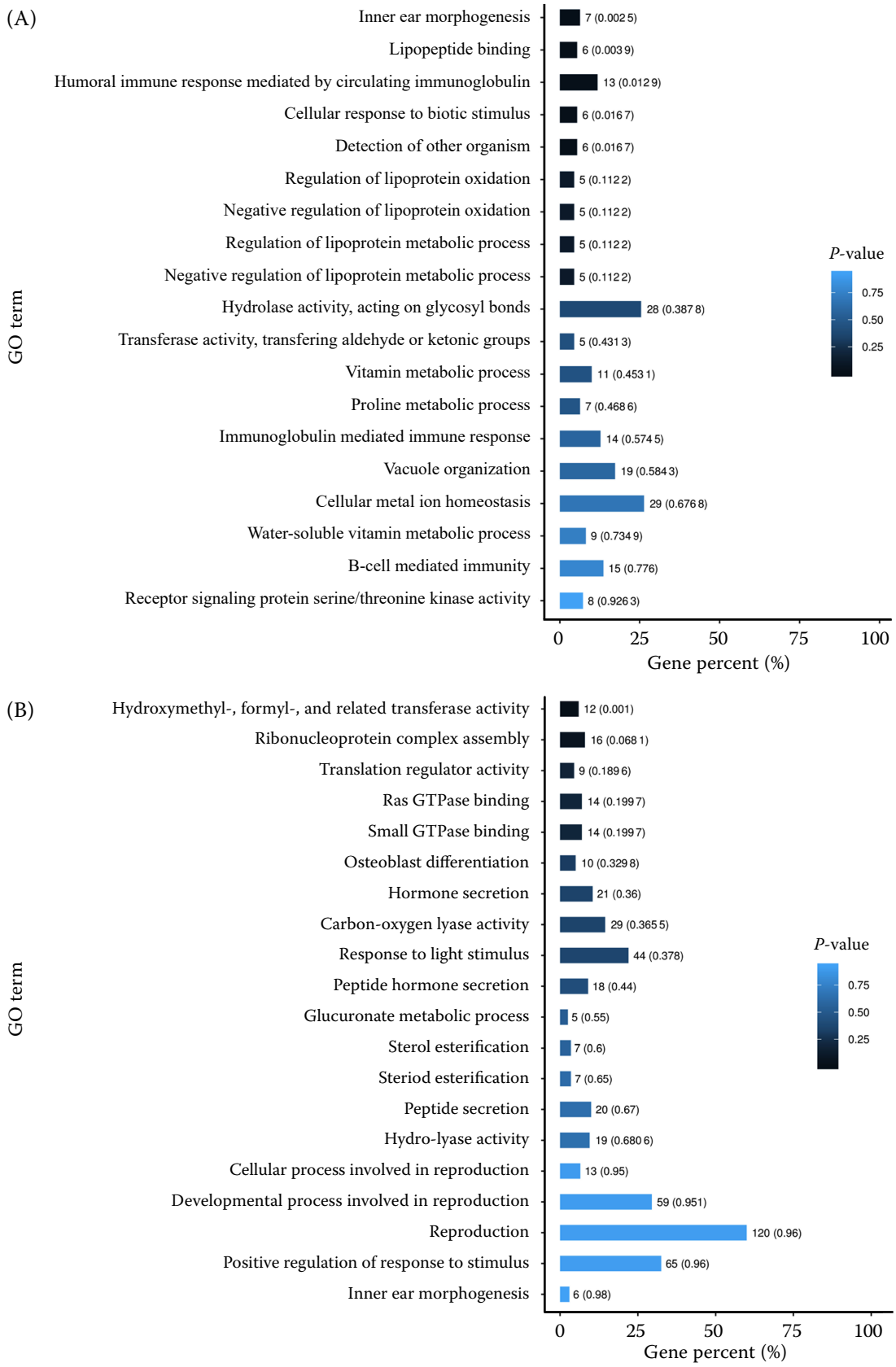

Figure 3. Bar chart for GO enrichment analysis of the target genes of differentially expressed microRNAs
(A) Predicted target genes of known microRNAs; (B) predicted target genes of novel microRNAs

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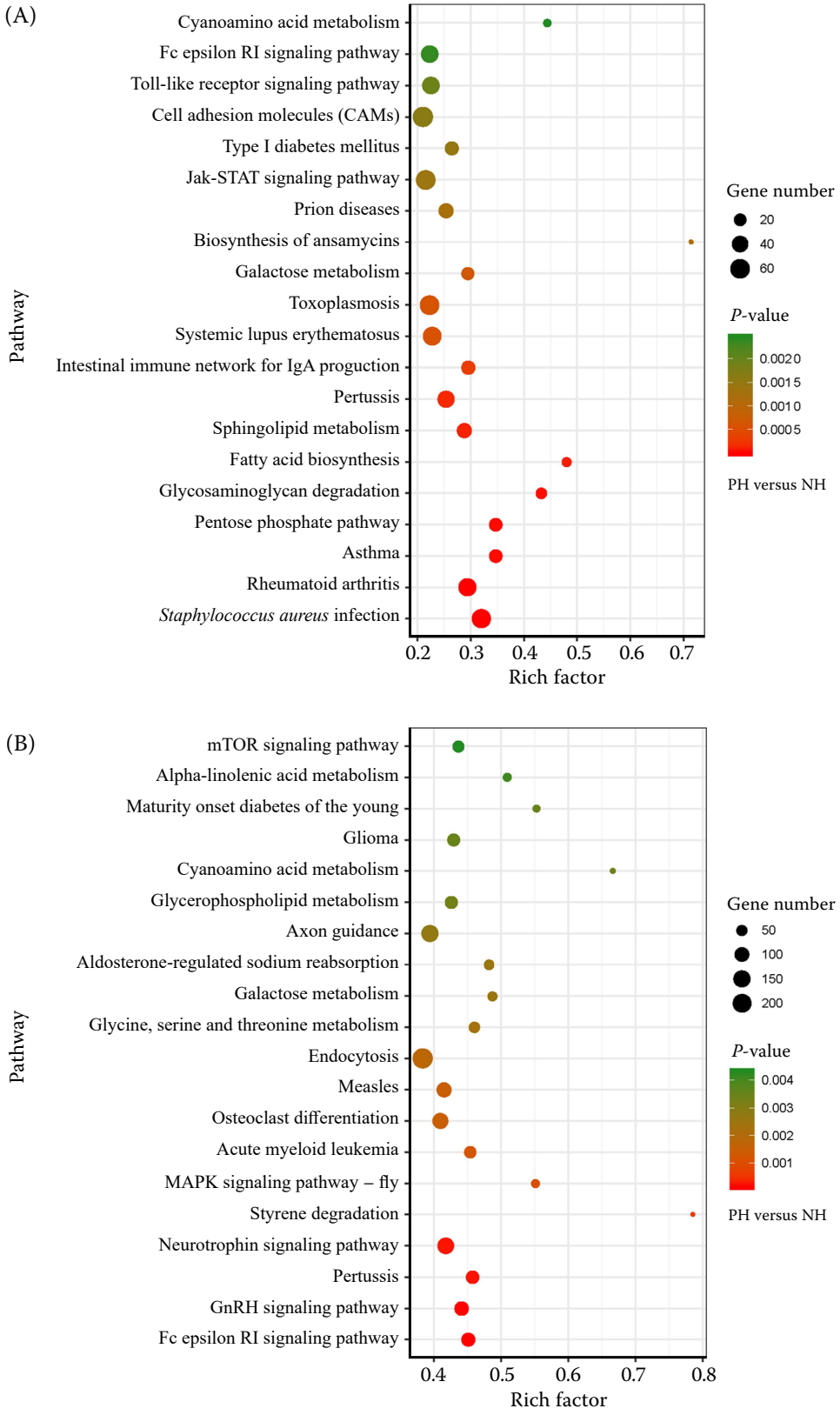

Figure 4. Top 20 KEGG pathway enrichment of the target genes of differentially expressed microRNAs
(A) Predicted target genes of known microRNAs; (B) predicted target genes of novel microRNAs
NH = non-pregnant goat hypothalamus; PH = pregnant goat hypothalamus

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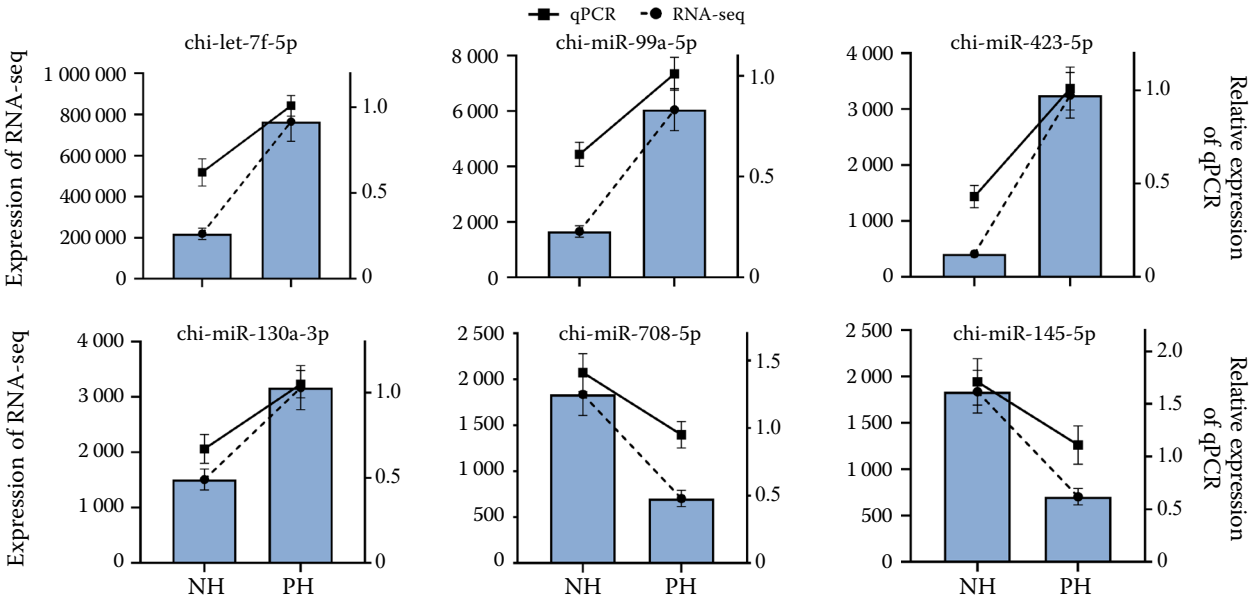

Figure 5. Validation of the RNA-seq (quantification) results by RT-qPCR

Results represent the mean (± SD) of three experiments. The *P*-values between the non-pregnant goat hypothalamus (NH) and pregnant goat hypothalamus (PH) groups were less than 0.05

DISCUSSION

The economic benefit of goats is mainly determined by total productivity, and the productivity of female goats is dependent on fertility and kid size (McBride et al. 2012). Reproductive functions, such as reproductive activities, are regulated by the endocrine or reproductive axis known as the "hypothalamic-pituitary-gonadal axis". The hypothalamus is an important part of this reproductive axis. Therefore, the hypothalamic tissues of goats were collected during pregnancy and non-pregnancy for RNA sequencing to identify reproduction-related miRNAs in the hypothalamus. These data provide a basic reference for subsequent studies on the regulatory role of miRNAs in mammalian pregnancy.

A total of 280 differentially expressed miRNAs were identified, of which 171 were known miRNAs and 109 were novel miRNAs. The expression of two miRNAs, miR-7 and miR-7b, was reported to be enriched in the hypothalamus of adult mice (Bak et al. 2008). In the rat hypothalamus, let-7 family, miR-136, miR-125a, miR-138, miR-124a, miR-338, let-7c, miR-7a/b, and miR-212 expression levels were high (Amar et al. 2012). Moreover, miR-16, miR-21, and miR-148a were the most abundant in the porcine hypothalamus (Haack et al. 2019). Our data are consistent with the results on the enrichment of the miR-7 and let-7 families in mice and rats, both families were expressed in excess of 10 000 and were up-regulated during pregnancy. Let-7f knockdown in endocervical epithelial cells affected the expression of many transcription factors (e.g. C/EBP beta), which are important regulators of immune responses (Ayyar and Reddy 2017). Expression of let-7f and miR-99a was different in the ovaries of prolific and non-prolific goats, and let-7f and miR-99a may affect follicular development (Zi et al. 2017). Moreover, let-7f was differentially expressed at the highest level in the follicular and luteal phase ovaries of goats (Zhu et al. 2016). In this study, the expression levels of let-7f and miR-99a were significantly differentially expressed in the hypothalamus of pregnant versus non-pregnant goats. The expression of miR-100-5p in NH was the highest and was significantly higher than the expression in PH. These data indicate that let-7f-5p, miR-99a-5p and miR-100-5p may be involved in the regulation of reproductive function in the hypothalamus.

Then, functional analysis of known and novel miRNAs was performed. The results of GO analysis showed enrichment in only four functions, including lipopeptide binding, inner ear morphogenesis, humoral immune response mediated by circulating immunoglobulin and cellular response to a bi-

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otic stimulus. The hypothalamus is the location of growth hormone-releasing hormone (GRHR) synthesis in the brain. Lipopeptide is an antagonist of GRHR and inhibits the effects of GRHR (Zarandi et al. 2006). In addition, the immune-related function was enriched in the hypothalamus during pregnancy, and immune stress regulates reproduction (Segner et al. 2017). Moreover, these functions are related to reproduction and the reproductive process.

Finally, the target genes of the known and novel miRNAs were analysed by KEGG. The genes were included in many pathways related to reproduction but were not enriched. For example, the genes participated in the MAPK signalling pathway, Wnt signalling pathway, insulin signalling pathway, and other pathways. MAPK signalling is an essential pathway in the hypothalamus that regulates the synthesis and secretion of GHRH (Fu et al. 2015). GHRH significantly stimulates progesterone production and cAMP accumulation in rat ovarian granulosa cells in a dose-dependent manner. GHRH has an effect on ovarian steroid production in rats and is involved in the regulation of the key steroid production steps related to progesterone degradation (Zhang et al. 2018). Most GH cells have GHRH receptors, indicating that GHRH is partially involved in the regulation of growth hormone (GH). Notably, *GH* is a predicted target gene of known differentially expressed miRNAs, including chi-mir-1, chi-mir-122, chi-mir-140, chi-mir-206 and chi-miR-29a-3p. GH is vital for the ovary and directly regulates gonadotropin-dependent and independent functions (Aguiar-Oliveira et al. 2017; Ob'edkova et al. 2017). GH effects on the ovaries before pregnancy influence gametogenesis and steroid production (Choe et al. 2018). During pregnancy, the GH system is regulated by placental growth hormone that increases during the growth of the placenta and stimulates IGF1 levels in the pregnant animals, leading to a decline in pituitary GH secretion (Vila and Luger 2018). The classic Wnt signalling pathway plays a central and multifaceted role in the regulation of the growth, pattern, differentiation and nucleation of the hypothalamus (Benzler et al. 2013). Additionally, brain-derived insulin production in the hypothalamus is regulated by the Wnt/β-catenin signals (Lee et al. 2016). The insulin level is an essential factor for the regulation of blood sugar, and the normal insulin level is one of the cornerstones for prevention of various risks, such as congenital malfor mations during pregnancy, overgrowth of the foetus, and premature birth (Ringholm et al. 2019). Our data indicate that miRNA-targeted genes are associated with the insulin signalling pathway in the hypothalamus.

CONCLUSION

The results of this study indicate that miRNAs in the goat hypothalamus are differently expressed in pregnancy versus non-pregnancy. The results indicate that let-7f-5p, miR-99a-5p and miR-100-5p are probably involved in the regulation of reproductive function in the goat hypothalamus, and provide a basic reference for additional studies on the regulatory role of miRNAs in mammalian pregnancy.

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Conflict of interest

The authors declare no conflict of interest.

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