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# Comparison of the pituitary gland transcriptome in pregnant and non-pregnant goats (*Capra hircus*)

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**Abstract**: Pregnancy is strictly regulated by neuronal and hormonal factors with an essential role being played by the pituitary gland. We screened for differentially expressed genes (DEGs) in the pituitary that function in goat gestational development. Pregnant (AWGp) and non-pregnant Anhui white goats (AWGn) were analysed by deep-sequencing technology. A total of 12 774 092 and 13 872 327 clear reads were obtained in the AWGp and AWGn libraries, respectively. A total of 2593 genes were labelled as significantly differentially expressed in AWGp compared to AWGn, including 2158 upregulated genes and 435 downregulated genes. These genes included follicle stimulating hormone beta (*FSHB*) and luteinizing hormone beta (*LHB*), which showed an involvement in reproductive regulation and downregulation (AWGp vs AWGn). Quantitative real-time PCR (qPCR) results validated the DEG data. Subsequent gene ontology analysis indicated that a large number of these DEGs function in cellular processes, cell structures, and cell binding. The DEGs were also found by Kyoto Gene and Genomic Encyclopaedia analysis to be significantly enriched in 54 pathways, including the GnRH and TGF-beta signalling pathways that affect cell proliferation and hormone secretion. These data also identify genes that may play a role in pregnancy and reproduction in the goat and thus provide avenues for future research.

**Keywords**: differentially expressed genes; hypophysis; Anhui white goats; RNA-Seq

The Anhui white goat (AWG) is a local Chinese breed (*Capra aegagrus hircus*) that has become a model for studying goat fertility due to its early maturity and high fertility (Ling et al. 2017). The hypothalamic–pituitary–gonadal (HPG) axis strictly controls the reproductive endocrine system and reproductive activity in mammals. The pituitary gland, as the principal endocrine organ, receives signals from the brain and secretes various peptide hormones (Gorski et al. 2017), including growth hormone (GH), prolactin (PRL), somatostatin (SST), luteinizing hormone (LH), and follicle-stim-

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ulating hormone (FSH). These pituitary hormones are stored in the secretory granules and released by regulated exocytosis into the bloodstream to strictly regulate developmental and physiological processes including metabolism, growth, development, reproduction and lactation (Burow et al. 2019). Moreover, the expression, synthesis, and secretion of these hormones are regulated by neuroendocrine feedback systems (Adams et al. 2018). The pituitary gland is therefore a key to elucidating the molecular mechanisms underlying reproduction.

Pregnancy is a complex physiological process that is regulated and controlled by many factors, including a complex network of genes (Agaoglu et al. 2015). The pituitary gland also plays an essential role in this process. The differences in pituitary activity and endocrine characteristics between pregnancy and non-pregnancy have been clearly described (Kota et al. 2013). In pregnancy, pituitary FSH and LH secretion decreases, thereby preventing the development of follicles, temporarily stopping ovulation, and gradually increasing progesterone secretion to maintain pregnancy (Findlay et al. 2019). These differences may be related to the differential regulation of genes and the transcriptome under different physiological environments. It is also possible that many different genes are essential for the functioning of the endocrine homeostatic systems of the pituitary gland. Notably however, the current information on the global gene profiles in the animal pituitary is insufficient. Achieving a greater understanding of the molecular genetic mechanisms of goat reproduction and their possible application in improving goat fecundity will require detailed knowledge of the pituitary genotypes at different breeding stages.

RNA sequencing technology (RNA-Seq) is a powerful technique for studying genes across species such as humans, rat, cattle, and goat (Dadaneh et al. 2018). This method has been widely applied to identifying differentially expressed genes (DEGs) in mammalian reproductive tissues, such as porcine ovaries, bovine granulosa cells and others (Hatzirodos et al. 2017; Vuong et al. 2018; Yang et al. 2018). However, few studies to date have explored the DEGs associated with prolificacy traits in the goat using RNA-Seq (quantification) technology.

In our present study, the gene expression profiles in the pituitaries of non-pregnant and pregnant AWGs (AWGn and AWGp) were determined by RNA-Seq and compared. The results of this analysis make a significant contribution to elucidating the molecular mechanisms that function in pregnancy, and identify genes that were not previously known to regulate goat reproduction and may have future breeding applications in this commercially important animal.

## MATERIAL AND METHODS

***Animals and samples.*** Anhui white goats were raised at Anhui Agricultural University under consistent feeding conditions. Three pregnant (AWGp) and three non-pregnant (AWGn) female goats were randomly selected for pituitary sampling (including the anterior pituitary and posterior pituitary). The pituitaries were immediately collected after sacrifice in liquid nitrogen and stored at –80°C. All sampling of animal tissues conformed to the standards of the Ethics Committee of Anhui Agricultural University, Anhui, P.R. China (permit No. AHAU20101025).

***RNA preparation.*** Tissues were ground under liquid nitrogen and total RNA was then extracted using TRIzol$^{TM}$ Reagent (Invitrogen, USA) in accordance with the manufacturer's protocol. RNA concentrations were determined using an Agilent 2100 Bioanalyzer (Agilent, USA) and all samples were stored at –80°C.

***Sequencing and data analysis.*** To minimize naturally occurring transcriptomic differences between individuals, RNA samples from three different goats in each of the AWGp and AWGn groups were combined in equal amounts to form a mixed sample. The total RNA samples were first treated with Ambion$^{TM}$ DNase I (Invitrogen) to degrade any possible DNA contamination. Then the digestion products were purified with magnetic beads. After that, mRNA was enriched using the oligo(dT) magnetic beads (for eukaryotes). Mixed with the fragmentation buffer, mRNA was fragmented into short fragments (about 200 bp). cDNA was then synthesized using the mRNA fragments as templates (NEB, USA). The double-strand cDNA was purified with magnetic beads. Then the end repair was performed. After the previous step, adaptors were ligated to the ends of these fragments. Next, ligation products were selected by size and purified on Tris-acetate-EDTA (TAE) agarose gel. Finally, the fragments were enriched by PCR amplification, then purified with magnetic

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beads and dissolved in the appropriate amount of Epstein-Barr solution. The ligation products were depurated using agarose gel electrophoresis, amplified by PCR, and further purified using magnetic beads to obtain libraries. The sizes and concentrations of the molecules in the constructed library were determined using the Agilent 2100 Bioanalyzer. The cDNA libraries were finally sequenced using the Ion Proton platform at the Beijing Genomics Institute (BGI, Beijing, China). Contaminant reads were screened and deleted from the original reads in accordance with the experimental design and bioinformatics guidelines, and if the read lengths were less than the threshold (30 bp) (Kerpedjiev et al. 2014), SOAPaligner/soap2 software was used to position the clean reads into the goat reference gene sequence (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/704/415/GCF_001704415.1_ARS1/GCF_001704415.1_ARS1_rna.fna.gz) and the goat reference genome sequence (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/704/415/GCF_001704415.1_ARS1/GCF_001704415.1_ARS1_genomic.fna.gz) to authenticate the gene expression modes for the AWGp and AWGn libraries.

***Analysis of differentially expressed genes.*** Gene expression was calculated by the Reads per kilo base per million mapped reads (RPKM) method to identify DEG between AWGp and AWGn (Lowe et al. 2017). The differential expression of the two libraries was compared using fold-change and *P*-value. The formulas were shown as follows:

$$RPKM\ (A) = 10^9\ C/NL$$

where:

*RPKM* (*A*) = gene *A* expression level
*C* = alignment number of reads with gene *A*
*N* = total number of reads that are solely aligned with the reference gene
*L* = numbers of base pairs in gene *A*

Fold-change = log2(Pregnant-Pituitary-RPKM/
/Non-pregnant-Pituitary-RPKM)

*P*-value formula:

$$2\sum_{i=0}^{i=y} p(i|x) \quad \text{or}$$

$$2 \times \left(1 - \sum_{i=0}^{i=y} p(i|x)\right)\left(if \sum_{i=0}^{i=y} p(i|x) > 0.5\right)$$

$$p(y|x) = \left(\frac{N_2}{N_1}\right)^y \frac{(x+y)!}{x!\,y!\left(1+\frac{N_2}{N_1}\right)^{(x+y+1)}}$$

where:

$N_1$, $N_2$ = total clean reads
*x*, *y* = normalized expression level of a designated gene in the non-pregnant and pregnant goat pituitary library, respectively

The threshold *P*-value in tests was decided by the false discovery rate (FDR) and the FDR value was adjusted for analysis.

The DEGs were calculated using an algorithm developed by the Beijing Genomics Institute (BGI), which was based on the method of Audic and Claverie (1997). Then, the absolute values of log2 ratio ≥ 1 and FDR ≤ 0.001 were used to calculate whether or not the gene expression differs.

***Verification by real-time quantitative PCR (qPCR).*** RNA-Seq (quantitative) data were verified by qPCR analysis of 8 randomly selected DEGs. The primers for these genes were designed using Primer Express 5.0 program (Table 1). Data normalization was carried out using *GAPDH* as the reference gene. One μg of total RNA from each sample was reverse-transcribed into cDNA using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGene Biotech, China) in accordance with the manufacturer's instructions. qPCR was then performed using SYBR Master Mix (ToloBio, China). Each reaction system had a volume of 20 μl, including 10 μl of 2× Q3 SYBR Master Mix (high ROX, Tolo Bio, China), 1 μl of template cDNA (obtaining each cDNA by reverse transcription of 1 μg of total RNA), 0.4 μl of each primer (10 mM) and 8.2 μl of $ddH_2O$. The amplification protocol was as follows: 95°C for 30 s followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. Each experiment was repeated three times. Gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method and expressed as a mean ± standard deviation. Significant differences were analysed using the *t*-test with SPSS software Version 17.0. A *P*-value of ≤ 0.05 indicated a statistically significant difference.

***GO function enrichment and KEGG pathway analyses of DEGs.*** All DEGs were first mapped to the terms in the gene ontology (GO) database (http://www.geneontology.org/). The determination of a significantly enriched GO terminology

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Table 1. Sequences of the primers used for qPCR analysis

| Gene | NCBI reference sequence | Primer sequence | Amplicon (bp) |
|---|---|---|---|
| *FOXG1* | XM_018066618.1 | S: 5'-GGAGATAGGAAAGAGGTGAAA-3'<br>A: 5'-GTGGTGGTTGTCGTTCTG-3' | 90 |
| *SST* | NM_001287555.1 | S: 5'-AACCAGACAGAGAACGAT-3'<br>A: 5'-CTCCAGCCTCATTTCATC-3' | 75 |
| *NPY1R* | XM_013964443.2 | S: 5'-TAAACGGGCCAAAAGACGGA-3'<br>A: 5'-TTTCCCTGGCATCTTGGTGG-3' | 72 |
| *STMN2* | XM_005689118.2 | S: 5'-ATCTGCTCTTGCTTCTACC-3'<br>A: 5'-GATTTGCTTCACTTCCATATCA-3' | 75 |
| *CRH* | XM_005689029.3 | S: 5'-CTCCTCCGAGAAGTCTTG-3'<br>A: 5'-CAACAGTTTCCTATTGCTATGA-3' | 75 |
| *RYR3* | XM_018053769.1 | S: 5'-CTCTGGTCAGGTCGCATACC -3'<br>A: 5'-TTACCACGTCGTCTGACTGC -3' | 73 |
| *LHB* | XM_018062755.1 | S: 5'-TGTCTGTATCACTTTCACCAC-3'<br>A: 5'-CAGGATGACAGGCAGCAC-3' | 76 |
| *DRD2* | XM_018059765.1 | S: 5'-CCCTGTTGCCTCTGCCTTAG-3'<br>A: 5'-GTTACAGAGTGTGGGGCCTG-3' | 89 |
| *GAPDH* | XM_005680968.3 | S: 5'-GCATCGTGGAGGGACTTATGAC-3'<br>A: 5'-CGGCAGGTCAGATCCACAAC-3' | 238 |

S = sense primer, A = antisense primer

first required a calculation of the number of genes for each term and a screen for the DEGs using a hypergeometric test. The calculated *P*-value was corrected by Bonferroni and a GO term with a corrected *P*-value ≤ 0.05 was defined as being significantly rich in DEGs.

The Kyoto Encyclopaedia of Genes and Genomes (KEGG) is one of the most commonly used bioinformatics databases in the world and has been used to identify metabolic pathways or signal transduction pathways that are significantly enriched in DEGs via pathway enrichment analysis (Kanehisa et al. 2007). The formula used for KEGG calculations is the same as that in the GO analysis. Pathways with a Q-value ≤ 0.05 are considered significant. The Q-value is the corrected *P*-value, ranging from 0 to 1, and the smaller the value, the greater the intensity.

## RESULTS

***Sequencing data summary***. Raw reads of approximately 6.56 Gb and 7.44 Gb were obtained in the AWGp and AWGn libraries, respectively. The total base pairs produced were 1 635 349 367 and 1 896 299 119, respectively, in these two pituitary cDNA libraries using the Ion Proton sequencing platform. After trimming adapters and filtering out low-quality reads, 12 774 092 and 13 872 327 clean reads from the pituitary libraries of AWGp and AWGn, respectively, were ultimately retained and further analysed.

All clean reads were located using goat reference genes and genomic sequences to identify the genes corresponding to these reads in each library. The results indicated that 98.15% and 97.70% of the reads from the AWGn and AWGp libraries, respectively, corresponded to goat genomic sequences, whereas only 73.28% and 68.72% from these libraries could be assigned to reference genes. Moreover, 33.85% and 32.35% of the reads from the AWGn and AWGp libraries matched the reference genes exactly, with approximately 42% of the reads (AWGn 43.52%; AWGp 41.78%) matching the genome exactly. In the case of unique matches, the reads slightly above 48% (AWGn 53.05%; AWGp 48.61%) and above 90% (AWGn 91.97%; AWGp 90.51%) corresponded to the reference genes and genome, respectively.

***Identification of genes detected in the goat pituitary in pregnancy and non-pregnancy***. A total of 20 863 genes were identified in the two libraries, of which 17 195 genes were co-expressed. In addition, 2269 genes were found to be specifically expressed in the pituitary gland in AWGp

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and 1399 genes in AWGn. Approximately 17% of the genes total found in the AWGn and AWGp samples ranged from 70% to 90%, with 28% genes and 25% genes, respectively, covering 90–100% of the AWGn and AWGp libraries, which indicated that the read distribution trends between the two libraries were the same. Calculations were then done of the expression levels of all test genes from AWGn and AWGp using the RPKM method to search for genes that may be involved in reproductive regulation. Approximately 96% of the gene expression levels were below 100 RPKM and slightly more than 0.5% of the genes were expressed at more than 500 RPKM in the two libraries.

***Identification of differentially expressed genes detected in the goat pituitary in pregnancy and non-pregnancy.*** A total of 2593 genes were identified using the absolute values of FDR ≤ 0.001 and log2 ratio ≥ 1 for assessing significant differences in the expression between the two libraries. We thereby identified 2158 upregulated and 435 downregulated genes in AWGp compared with AWGn. Some genes were found to be involved in reproductive regulation such as follicle stimulating hormone beta (*FSHB*), luteinizing hormone beta (*LHB*), estrogen receptor (*ESR1* and *ESR2*) and others. Among these DEGs, 183 genes were expressed only in AWGp, and 9 genes showed expression in AWGn only. Some of the DEGs found to be involved in reproductive regulation were of particular interest, including forkhead box G1 (*FOXG1*), C-C motif chemokine ligand (*CCL8*), SMAD family member 6 (*SMAD6*), C-X3-C motif chemokine receptor 1 (*CX3CR1*), and integrin subunit alpha 4 (*ITGA4*) among others (Table 2).

***Validation of 8 randomly selected differentially expressed genes by RT-qPCR.*** As shown in Figure 1, in AWGp compared to AWGn the RT-qPCR analysis indicated that the expression of *FOXG1*, corticotrophin-releasing hormone (*CRH*), somatostatin (*SST*) and stathmin-2 (*STMN2*), neuropeptide Y receptor Y1 (*NPY1R*), and ryanodine receptor 3 (*RYR3*) was upregulated, whereas the expression of *LHB* and dopamine receptor D2 (*DRD2*) was downregulated. The expression trends of these genes were consistent with those detected by RNA-Seq (quantitative) results.

***GO and KEGG functional classifications.*** Gene ontology (GO) provides a comprehensive description of the properties of genes and their functional

Table 2. Details of the differentially expressed genes associated with reproduction in Anhui white goats

| Gene ID | Description | Log2 ratio (AWGp/AWGn) | *P*-value |
|---|---|---|---|
| gi\|548513805\|ref\|XM_005695258.1\| | gi\|471377604\|ref\|XP_004376611.1\|/2.50398e-125/PREDICTED: forkhead box protein G1, partial (FOXG1) | 15.83143 | 8.37E-178 |
| gi\|548507006\|ref\|XM_005693214.1\| | gi\|426237138\|ref\|XP_004012518.1\|/1.43084e-50/PREDICTED: c-C motif chemokine 8 (CCL8) | 12.80693 | 1.12E-10 |
| gi\|548526534\|ref\|XM_005699374.1\| | gi\|296472089\|tpg\|DAA14204.1\|/0/TPA: glutamate receptor delta-1 subunit-like (GRID1) | 5.213249 | 4.81E-64 |
| gi\|548514617\|ref\|XM_005695559.1\| | gi\|426249090\|ref\|XP_004018284.1\|/1.29639e-175/PREDICTED: CX3C chemokine receptor 1 (CX3CR1) | 1.790284 | 1.16E-46 |
| gi\|548454192\|ref\|XM_005676216.1\| | gi\|426220769\|ref\|XP_004004586.1\|/0/PREDICTED: integrin alpha-4 (ITGA4) | 1.377698 | 1.17E-06 |
| gi\|548496275\|ref\|XM_005690030.1\| | gi\|21914206\|gb\|AAM81325.1\|AF522070_1/3.07839e-76: follicle stimulating hormone beta-subunit (FSHB) | –2.10071 | 4.73E-75 |
| gi\|548487473\|ref\|XM_005687236.1\| | gi\|301769265\|ref\|XP_002920047.1\|/0/PREDICTED: steroidogenic factor 1-like (NR5A1) | –1.77111 | 9.21E-25 |
| gi\|548469328\|ref\|XM_005681305.1\| | gi\|410957011\|ref\|XP_003985128.1\|/3.89588e-113/PREDICTED: pituitary homeobox 2 isoform 5 (PITX2) | –1.7167 | 7.10E-06 |
| gi\|548449381\|ref\|XM_005674794.1\| | gi\|44829051\|emb\|CAD70238.1\|/1.7577e-166: pituitary specific POU domain transcription factor 1 (POU1F1) | –1.4077 | 3.78E-16 |
| gi\|548454408\|ref\|XM_005676322.1\| | gi\|440900738\|gb\|ELR51808.1\|/1.70145e-143: inhibin beta B chain, partial (INHBB) | –1.11251 | 9.40E-10 |

AWGp = pregnant Anhui white goats, AWGn = non-pregnant Anhui white goats

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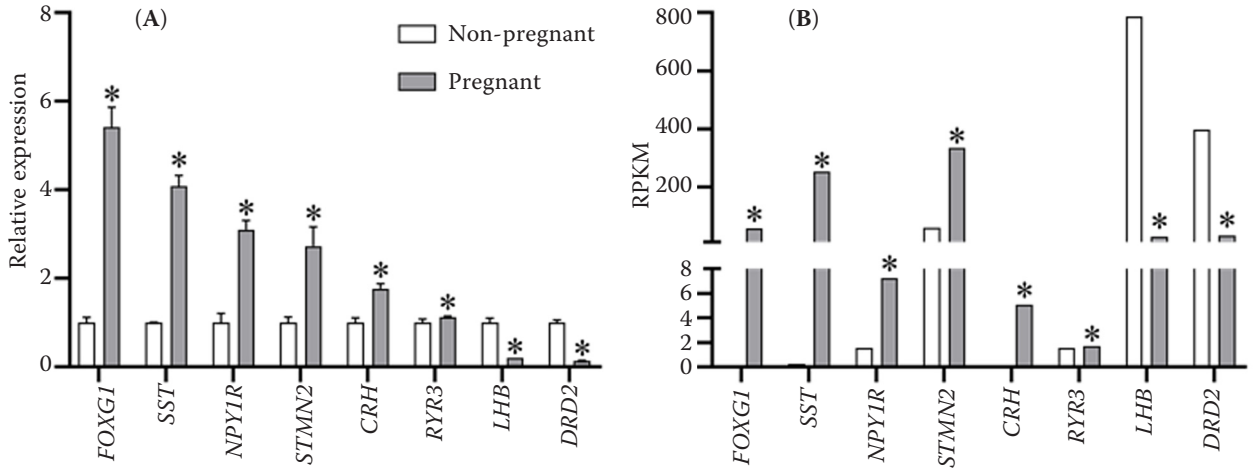


Figure 1. Validation of the RNA-Seq (quantification) results by qPCR: (**A**) relative expression levels of mRNA were detected by qPCR, three replicates; (**B**) gene expression levels were determined by RPKM

Results represent mean ± standard deviation of three experiments: No column indicates that no reads were obtained during this period

*$P \leq 0.05$

products in different organisms. It has thus become an internationally standardized and widely used gene function classification system (Miao and Luo 2013). GO covers cellular components, molecular functions, and biological processes. In our present analysis, DEGs showed significant enrichment

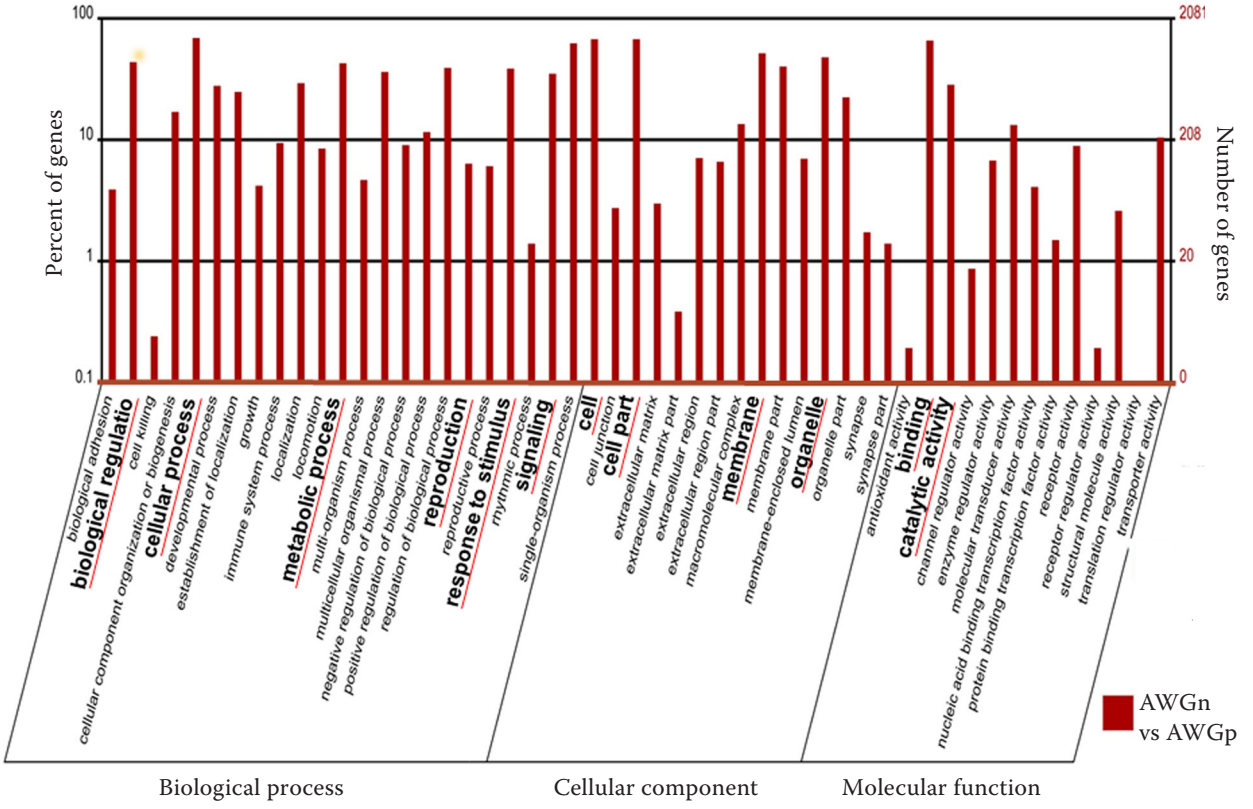


Figure 2. Histogram of gene ontology classifications. Results were summarized into three main categories: biological process, cellular component, and molecular function. Cellular process, binding, cell and cell part were predominant in each of these three main categories, respectively. Gene ontology terms related to reproduction are underlined in red

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under several GO terms, as shown in Figure 2, indicating that these genes participate in many core biological activities including reproduction. DEGs associated with the regulation of reproduction and the cell cycle in this analysis included *FOXG1*, adenylate cyclase activating polypeptide 1 (*ADCYAP1*), cyclin B2 (*CCNB2*), 24-dehydrocholesterol reductase (*DHCK24*), *SMAD6*, inhibitor of DNA binding 2 (*ID2*), bone morphogenetic protein 2 (*BMP2*) and cyclin D2 (*CCND2*).

Pathway analysis was then performed using the KEGG pathway database to predict the metabolic pathways and signal transduction pathways with a significant enrichment of our DEGs. In total, 2086 DEGs were mapped to 241 pathways. The largest category was "Neuroactive ligand–receptor interaction", which had 94 DEGs (Figure 3), most of which were upregulated and some of which participated in the regulation of reproduction, cell cycle, cell proliferation and hormone secretion, such as neuroactive ligand–receptor interaction, focal adhesion, ECM–receptor interaction, VEGF signalling pathway, GnRH signalling pathway, and TGF-beta signalling pathway.

## DISCUSSION

The present analysis showed that 2593 genes were expressed differentially in pregnant and non-pregnant goats. The GO analysis revealed that these DEGs were involved in many biological processes, including the reproductive regulation, cell proliferation, and hormone secretion that are of interest

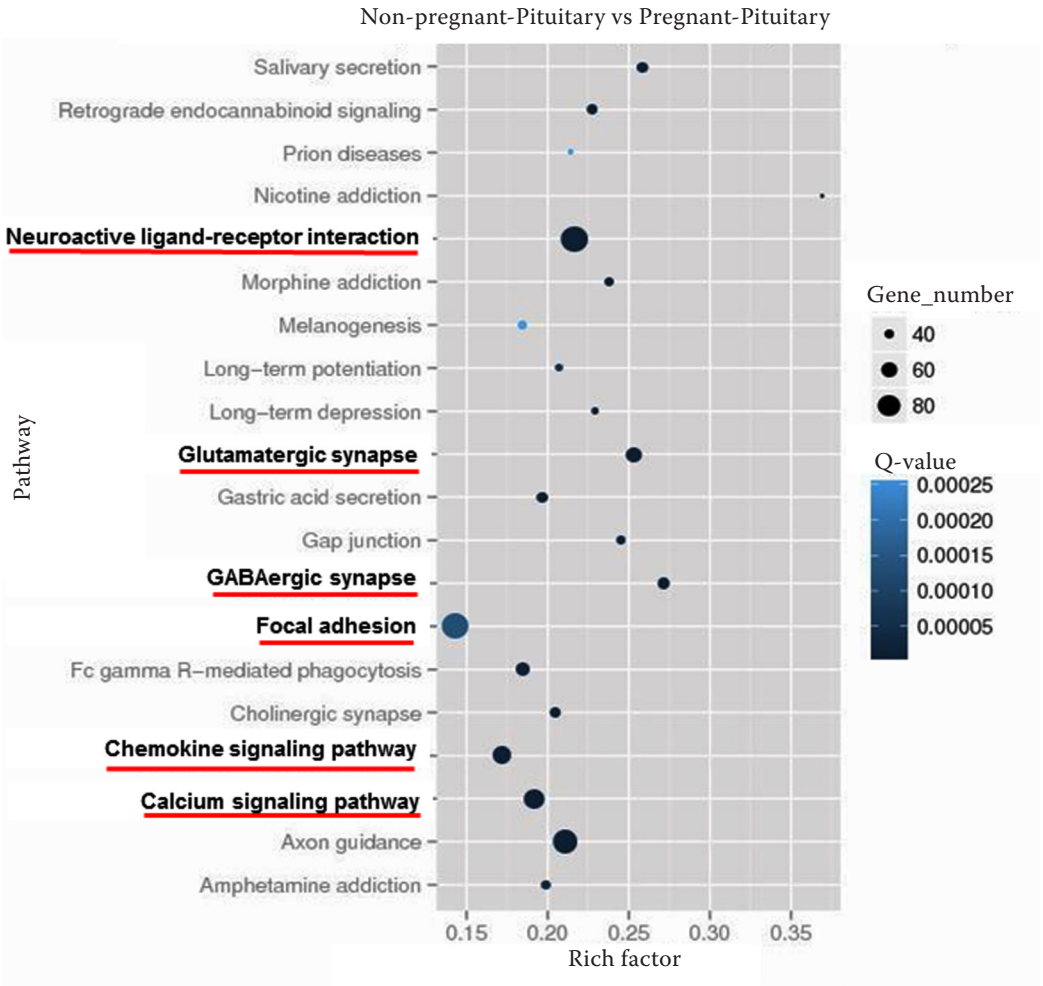


Figure 3. Top 20 statistics of pathway enrichment for all differentially expressed genes

Q-value is the corrected *P*-value ranging from 0 to 1, with a lower value indicating a greater intensity. Pathways involved in regulating reproduction, cell cycle, cell proliferation, and hormone secretion are underlined in red

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in relation to animal pregnancy. Genes such as *FSHB*, *LHB*, *GNRHR*, *CNTNAP2*, and *STMN2,* that may play a role in goat reproduction and pregnancy maintenance, were identified in this analysis and were therefore of considerable interest. FSH and LH, which are composed of a common alpha subunit (α-GSU) and unique beta subunits (FSHB and LHB), are two essential pituitary hormones that have an important effect on the development and maintenance of pregnancy. The production of FSH and LH is principally regulated by gonadotropin-releasing hormone (*GNRH1*) and via feedback from gonadal factors (such as estradiol, progesterone, and inhibin) (Haughian et al. 2013). Our present data indicate that the transcriptional levels of *FSHB*, *LHB*, *GNRHR*, estrogen receptor (*ESR1* and *ESR2*), and inhibin beta (*INHBB*) are significantly lower in the pituitary glands during pregnancy in the goat. This is consistent with our finding of the downregulated expression of *FSHB*, *LHB*, *GNRHR*, *ESR1/2* and *INHBB* in our exploration of factors affecting follicular development, ovulation, and pregnancy in the goat. The expression profiles of these relevant signal molecules were further analysed to better understand the transcriptional regulation of *FSHB* and *LHB* in the pituitary. It is known that some significant changes in hormone signalling molecules regulate the expression of *FSH* and *LH*, such as *BMP2*, glutamate ionotropic receptor delta type subunit 1 (*GRID1*) and dopamine receptor D1 (*DRD1*). Notably, these genes were identified to be upregulated in the pituitary gland of the AWGp involved in this study. Our results have therefore indicated that these signalling molecules may also participate in the regulation of *FSHB* or *LHB* production in the goat pituitary gland during pregnancy.

The transcriptional levels of other neuropeptide signalling molecules, such as growth hormone releasing hormone receptor (*GHRHR*), thyrotropin-releasing hormone receptors (*TRHR*), thyroid stimulating hormone receptor (*TSHR*), cholecystokinin (*CCK*), and *CRH*, exhibited significant changes in the AWGp pituitary gland. This implies that these signalling molecules may have an impact on goat pregnancy via neuroendocrine regulation. The expression level of stathmin-2 (*STMN2*) was also found to be significantly elevated in the AWGp pituitary gland. Stathmin plays major roles in the control of the microtubule cytoskeleton, neuronal differentiation, and hormone secretion (Chauvin and Sobel 2015). Recent reports have also indicated that the gene expression of *STMN2* is increased significantly in the pituitary of a goose in the laying period and in a high-yielding egg strain of this animal (Luan et al. 2013).

Our present study is significant as it is the first to report that *STMN2* is expressed in the pituitary of the goat. Its role in the regulation of goat reproduction remains to be explored in a future study. Synaptotagmin-1 (SYT1) is an integral membrane protein that plays a vital role in hormone secretion and is an abundant and evolutionarily conserved factor (Moghadam and Jackson 2013). Luan et al. (2014) previously found in geese that *SYT1* expression is upregulated during laying, which may affect the reproductive hormone secretion and ovulation associated with the breeding and spawning of female geese. Remarkably, our present study results have indicated that *SYT1* is upregulated in the AWGp pituitary, indicating that it may also affect the regulation of hormone secretion during goat reproduction.

In addition to the aforementioned genes, some of the identified DEGs in the goat pituitary were involved in signal transduction pathways that may have functional effects on the reproductive process of goats. These included pituitary transcription factors (such as *POU1F1* and *PITX2*), steroidogenic factor 1 (*NR5A1*), and G-protein-coupled receptors (*DRD2*, *VIPR1*, *NPY1R*, and *MTNR1A*). The pituitary-specific transcription factor (*POU1F1*) is essential for multiple hormone-secreting cell types and plays a pivotal role in regulating the expression of *PRL*, *GH*, and *TSH* (Yamamoto et al. 2011). Paired-like homeodomain transcription factor 2 (*PITX2*) is a member of the paired-like homeodomain transcription factor (*PITX*) family and plays a vital role in cell proliferation and differentiation (Xiao et al. 2013). It has also been reported that the *PITX2* gene regulates the expression of the *POU1F1* gene (Davis et al. 2010). Notably, the expression levels of *POU1F1* and *PITX2* in the AWGp pituitary were found to be decreased in our present analyses, which may indicate that these two genes are involved in the negative feedback regulation of certain pituitary hormones during pregnancy. Steroidogenic factor 1 (*SF-1*, also known as *NR5A1*) is a member of the nuclear receptor superfamily and is classified as an orphan receptor. It can regulate steroidogenesis, development and cell differentiation and also

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activate the expression of *CGA*, *LH* (*LHB*), *FSH* and *GNRHR* along with co-factors such as early growth response 1 (*EGR1*) and *PITX1* (Yazawa et al. 2015). However, *NR5A1* and *EGR1* were found in our present analysis to be downregulated in the pituitary gland of AWGp. Hence, these two genes may play an active role in pregnancy maintenance. Dopamine receptor D2 (*DRD2*) is a member of the dopamine receptor gene family. There is good evidence that dopamine exerts inhibitory effects on PRL secretion via *DRD2* at the pituitary level by antagonizing vasoactive intestinal peptide (VIP) (Al Kahtane et al. 2003). Numerous studies have documented that DRD2 is involved in reproductive hormone secretion and reproductive behaviour (Wang et al. 2014). A decrease in *DRD2* expression during AWG pregnancy may cause the upregulation of *PRL* expression. Researchers previously found that *VIPR1* was involved in the regulation of reproductive activity in chicken (Zhou et al. 2008). Recently, Bukowski and Wasowicz (2015) also reported the expression of *VIPR1* in porcine female reproductive tissues (such as the uterus, oviduct and ovary). In our present study, *VIPR1* showed upregulated expression in the AWGp pituitary gland, which implies that it has a promoting effect on reproductive activities in goat. Neuropeptide Y (NPY), acting through its receptors (NPY1R, NPY2R and NPY5R), participates in the regulation of various physiological functions, including the release of gonadotropins (Huang et al. 2014). Melatonin regulates animal reproduction through the binding to its receptors (MTNR1A, MTNR1B and MTNR1C) (Li et al. 2013). Our present data have revealed that *NPY1R* and *MTNR1A* are upregulated in the AWGp pituitary gland, which may indicate their role in regulating goat reproductive activities and maintaining pregnancy.

We explored the biological functions of the identified DEGs in the goat pituitary using both GO annotation and KEGG pathway analysis. We thereby identified 54 significantly enriched pathways. Interestingly, the neuroactive ligand–receptor interaction was the most prominently enriched pathway in our analysis, and is related to neuronal function. In addition, some of the hormone signalling molecules that we identified were found to participate in the neuroactive ligand–receptor interaction pathway, such as *FSHB*, *LHB* and *GNRHR*, and were therefore of particular interest. Most of the DEGs found to participate in this pathway were upregulated genes. This indicated that the neuroactive ligand–receptor interaction pathway may have important functional roles in pituitary development and pregnancy maintenance. However, the specific relationships in this regard remain unclear and require further research and exploration. Notably, the pathways found to be involved with some of the DEGs in this study are known to be related to reproduction, such as the calcium signalling pathway, focal adhesion, dopaminergic synapse, ECM–receptor interaction, VEGF signalling pathway, and GnRH signalling pathway. The interactions between these signalling networks require further exploration and verification.

## CONCLUSION

We have identified 20 863 genes in the pituitary libraries of pregnant and non-pregnant goats, of which 17 195 genes were co-expressed. In addition, 2269 of these genes were specially expressed in the pituitary gland in the AWGp subjects and 1399 genes in AWGn. Data displayed 2593 significantly differentially expressed genes with upregulated expression of 2158 genes and downregulated expression of 435 genes during pregnancy in the goat. These DEGs showed a possible involvement in hormone secretion, pregnancy maintenance, follicular development and other processes of interest. KEGG analysis mapped 2086 of these DEGs onto 241 pathways, with 54 pathways showing significant enrichment. We verified randomly selected DEGs by qPCR to confirm the reliability of the data.

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