Effect of body condition on follicle transcriptome in estrous goats

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Body condition or obesity has profound effects on estrus, follicle development, endocrine and conception in both livestock and human beings. To identify potential genes and pathways associated with follicle development in goats of different body condition scores (BCSs), the global gene expression levels of 2.0-3.5 mm follicles from 24 Yangtze River Delta white goats were analyzed by ribonucleic acid sequencing (RNA-Seq). A total of 2,019 differentially expressed genes (DEGs) were identified in obese goats compared with the control, with 381 up-regulated and 1,638 down-regulated. A total of 309 DEGs were associated with reproduction in top-15-cluster-frequency Gene Ontology (GO) terms, and they accounted for 47.3% of all DEGs in biological process classification (653). Using Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, several pathways relevant to reproduction and metabolism were enriched, such as cytokine-cytokine receptor interaction, steroid hormone biosynthesis, etc. Based on the results of GO analysis and KEGG analysis, eight DEGs related to reproduction and metabolism were selected for quantitative real-time polymerase chain reaction (qRT-PCR) assays. The results showed that the expression level of these genes exhibited similar trends to the expression pattern of RNA-Seq. In addition, no significant difference was found in the intrafollicular estradiol (E2) concentration between the obese group and the control. The concentrations of progesterone (P4) and leptin were lower, and a higher E2/P4 ratio was found in the obese group. In summary, the establishment of goat follicle transcriptome would provide a useful reference for the molecular mechanism of follicle development. The combined analysis of DEGs, enriched pathways and follicular fluid composition revealed that P4 and leptin might be potential biomarkers of follicle development in goats.
Effect of body condition on follicle transcriptome in estrous goats

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ABSTRACT

Body condition or obesity has profound effects on estrus, follicle development, endocrine and conception in both livestock and human beings. To identify potential genes and pathways associated with follicle development in goats of different body condition scores (BCSs), the global gene expression levels of 2.0-3.5 mm follicles from 24 Yangtze River Delta white goats were analyzed by ribonucleic acid sequencing (RNA-Seq). A total of 2,019 differentially expressed genes (DEGs) were identified in obese goats compared with the control, with 381 up-regulated and 1,638 down-regulated. A total of 309 DEGs were associated with reproduction in top-15-cluster-frequency Gene Ontology (GO) terms, and they accounted for 47.3% of all DEGs in biological process classification (653). Using Kyoto Encyclopedia of Genes and Genomes
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**Keywords:** follicle; body condition; transcriptome; oocyte; gene; pathway; goat
INTRODUCTION

Capra hircus is one of the most important livestock and the oldest economic domesticated species. The Yangtze River Delta white goat is an indigenous breed famous for prolificacy and pen-hair in Southeast China. Female reproductive performance is a key factor affecting the economic benefits of goats under stall-feeding conditions. However, an increasing number of reproduction disorders are associated with body condition, for example, delayed puberty, reduced ovulation rate and pregnancy rate, postpartum anestrous, etc. Female goats in lesser body condition have a shorter breeding season, more abnormal estrous cycles and fewer ovulations than do those in greater body condition (De Santiago-Miramontes et al., 2009). As a uniparous mammal, the goat is an ideal reproduction model for humans. Ovarian follicles are the basic structural and functional units of female reproductive biology and create a suitable microenvironment of oocyte maturation.

Researchers found that obesity has a profound effect on estrous, follicle development, endocrine and conception in both livestock and human beings (Sessions-Bresnahan et al., 2014). In addition, the body conditions of dairy cows affected the quality of the preimplantation embryos, and a body condition score (BCS) of over 3.0 resulted in a higher incidence of poor (fragmented) embryos (Makarevich et al., 2016). Obesity is associated with sub-optimal reproductive performance. It is understandable that the number of young women with elevated body mass indexes (BMIs) accessing assisted reproductive treatment (ART) is on the rise. Furthermore, increasing BMI was positively correlated to increasing rates of preterm delivery and increasing delivery rates of singleton term macrosomic offspring (>4000 g) (Deirdre et al., 2012). Polycystic ovarian syndrome (PCOS) represents one of the most common endocrine disorders that influence about 8% of reproductive women, who suffer from obesity and increased
cardiovascular risk (Al-Gareeb et al., 2016). Identifying new potential indicators is important for evaluating the developmental competence of follicles and oocytes (Mamo et al., 2011). Several metabolic hormones and cytokines in follicular fluid (FF) and plasma were related to follicular development. Some studies demonstrated that oocyte number correlated negatively to FF leptin and insulin, and positively to resistin (Várnagy et al., 2013). In women undergoing in vitro fertilization, higher adiponectin is a better biomarker of adequate follicular development than are body weight or BMI (Liu et al., 2005). In conclusion, obesity is an important factor that affects reproductive performance and follicle development.

To date, the molecular mechanism of body condition on the follicle development of a goat is not clear. Therefore, the aim of this study was to investigate the effects of body condition on gene expression levels of follicles in estrous goats by RNA-Seq. Several potential genes and pathways involved in the follicle development of obese goats were identified. The concentrations of estradiol (E₂), progesterone (P₄), leptin and adiponectin in follicular fluid were compared between the obese group and the control to find a potential biomarker of follicle development. This dataset provides a useful reference for the molecular mechanisms of follicle development and obesity in goats, and it contributes to the nutritional regulation of the body conditions of breeding goats.

**MATERIALS AND METHODS**

**Animals**

The Yangtze River Delta white goat is famous for prolificacy and pen-hair in Southeast China. A total of 72 female goats of the same age (9 months old) were raised by total mixed rations (TMRs, 60:40 roughage and concentrate) under stall-fed conditions. The flock was estrous diagnosed twice daily by male goats to identify the natural estrous ones. According to a five-
point scale BCS, 24 estrous goats were classified into two groups: BCS > 3.5 (the obese group, n=10) and BCS = 3 (the normal group, control group, n=14), respectively. All experimental procedures were licensed by the Department of Health, The People's Republic of China (PRC), in accordance with the Cruelty to Animals Act. All experiments were conducted in accordance with the ethical procedures and policies approved by the Nanjing Agricultural University Animal Care Committee (SYXK2011-0036). All efforts were made to minimize suffering.

**Follicle collection**

The 24 estrous goats were slaughtered at a local abattoir 8 h post estrous, and the ovaries were obtained immediately. Intact follicles were isolated and torn by two tweezers under a stereomicroscope. After measuring their diameters using vernier calipers, a single 2.0-3.5 mm follicle was frozen in liquid nitrogen immediately. Follicular fluid was aspirated by syringe from healthy, antral follicles (2.0-3.5 mm in diameter), transferred into 0.5 ml EP tubes and centrifuged at 3000 rpm for 10 min at 4°C. Then, supernatants were collected and frozen in a -80°C refrigerator for further hormone analysis.

**RNA extraction**

Total RNA of each follicle was extracted using the RNeasy Micro Kit (Qiagen, Germany) according to the manufacturer's protocol. The yield and purity of RNA were assessed by NANODROP. RNA had a 260/280 absorbance ratio of 1.8–2.0 to be used for following analysis. RNA integrity was assessed by gel electrophoresis using a 1% agarose gel.

**Illumina sequencing for transcriptome analysis**

To obtain complete gene expression information, pooled RNA samples from goats of similar BCS was used for transcriptome analysis. Total RNA samples from each follicles were pooled.
RNA-seq transcriptome libraries were prepared following the TruSeqTM RNA sample preparation Kit (Illumina, San Diego, CA). 5 μg of RNA from each group was used. The mRNA was isolated with poly(A) selection using oligo (dT) beads and fragmented into short fragments with fragmentation buffer. The cDNA was synthesized with buffer, dNTPs, RNase H, and DNA polymerase I, using the mRNA fragments as templates. The cDNA was eluted with EB buffer for end repair and poly (A) addition. Finally, sequencing adapters were ligated to the fragments. The cDNA fragments were purified by 2% agarose gel electrophoresis, followed by PCR amplification (15 cycles) using Veriti 96 Well Fast Thermal Cycler (Applied Biosystems) to create cDNA libraries. After quantification using TBS380, cDNA libraries were sequenced on the Illumina sequencing.

The goat genome was downloaded from the NCBI database CHIR_1.0 (http://www.ncbi.nlm.nih.gov/genome/?term =CAPRA%20HIRCUS). Sequencing-received raw image data was transformed into sequence data by Base Culling. Adaptor sequences, low-quality sequences, reads with many N, and reads <25nt were filtered out using SeqPrep (https://github.com/jstjohn/SeqPrep) and ConDeTri_v2.0.pl (http://code.google.com/p/condetri/downloads/detail?name= chir_1.0 ) software. Clean reads were mapped to the goat genome using Tophat (http://tophat.cbcb.umd.edu) software, allowing up to two base mismatches.

**Functional classification of DEGs and clustering analysis**

After mapping to the reference genome, the expression level of each gene obtained from the RNA-seq analysis was calculated and normalized to FPKM (reads per kilobase transcriptome per million mapped reads). After statistical analysis of FPKM for each gene using Cuffdiff
(http://cufflinks.cbcb.umd.edu) software (Trapnell et al., 2013), FDR (false discovery rate) < 0.05 and \(|\log2FC|>1\) were used as the threshold to judge the significance of difference. Filtered DEGs were visualized with Cummerbund (http://compbio.mit.edu/cummeRbund) software. Hierarchical clustering analysis was performed on the significant DEGs. Cluster distance was calculated using the complete linkage algorithm. The distance of samples and genes was evaluated via Spearman’s rank method and Pearson correlation analysis, respectively.

**GO and KEGG analyses**

To identify the biological pathways and functions, GO and KEGG analyses were performed on all detected genes and DEGs respectively. We obtained GO annotation terms and performed GO functional enrichment using Goatools (https://github.com/tanghaibao/goatools) software. KEGG pathway analysis was performed using KOBAS (http://kobas.cbi.pku.edu.cn/home.do) software against the KEGG database.

**Quantitative real-time PCR validation**

8 DEGs (prolactin receptor, PRLR; 3β-hydroxysteroid dehydrogenase, 3βHSD; Cholesterol; apoptotic peptidase activating factor 1, APAF1; phosphodiesterase 5A, PDE5A; insulin-like growth factor-I, IGF-I, Apolipoprotein A-I, Leptin-Receptor) from the transcriptome analysis list and reference genes were selected for reanalysis and comparison using qRT-PCR. Capra hircus sequence for these genes were retrieves from the NCBI database (http://www.ncbi.nlm.nih.gov/genome/?term=CAPRA%20HIRCUS) and used to design primers. The RNA was converted to cDNA using the Prime ScriptTM RT Master Mix kit (TaKaRa). Then the qRT-PCR was performed using SYBR® Premix Ex TaqTM II (TaKaRa) and a 7300 Real-Time PCR System (Applied Biosystems) according to the manufacturer’s protocol. Each
sample was run in triplicate. The primers used for validation were designed with Primer3 (http://primer3.ut.ee/) and GAPDH was used as an internal control (Table 1).

Hormone assay of follicular fluid

The concentration of E\(_2\), P\(_4\), leptin, adiponectin in follicular fluid were determined with the sheep ADP ELISA Kit (DRE-G1329c; Kmaels, US) following the manufacturer’s protocol. The range of standard curve was 6.25–100 ng/ml. All samples were run in triplicate in the same assay. Absorbance values were measured at 450 nm using DNM-9602A microplate reader.

Statistical analysis

The data were expressed as mean ± SEM. The differences of E\(_2\), P\(_4\), leptin and adiponectin in follicular fluid between the two groups were determined using Student’s t-tests. Other statistical analysis was performed by one-way analysis of variance (ANOVA) with multiple comparisons of the means with the help of SPSS (Version 17.0; SPSS Inc. Chicago, IL, USA). The differences were considered to be highly significant at p < 0.05.

RESULTS

Overview of Illumina sequencing and read assembly

We performed the de novo transcriptome sequencing of follicles using Illumina sequencing. The results (Table 2) indicated that 47.6 million clean paired-end (PE) reads were generated in the follicles of obese goats. Based on a sequence similarity search, 14,810 (80.44%) and 15,030 (81.64%) genes were identified in the obese goats and the control, respectively. A total of 47,591,664 clean reads were assembled into 15,919 unigenes consisting of 14,810 known genes and 1,109 new genes in the obese group. In addition, 48,868,968 clean reads were assembled into 15,030 unigenes consisting of 16,147 known genes and 1,117 new genes in the normal group.
Differentially regulated genes of follicles between obese and normal goats

A summary of differentially expressed genes (DEGs) from the global follicle transcriptome analysis is shown in Table 3. Compared with the control, 2,019 genes of the follicles of the obese goats were differentially expressed, with 381 up-expressed and 1,638 down-expressed.

GO analyses of DEGs

A total of 15,919 genes were summarized as three main GO categories (Table 3). A total of 5,852 genes were annotated to cellular components, 674 of which were differentially expressed. A total of 5,574 genes were annotated to molecular functions, 638 of which were differentially expressed. Finally, 5,734 genes were annotated to biological processes, 653 of which were differentially expressed. The top GO terms of DEGs significantly enriched are shown in Figure 1. In the molecular functions category, the top GO terms of DEGs significantly enriched were involved in binding (GO: 0005488), catalytic activity (GO: 0003824) and transport activity (GO: 0005215), etc. Regarding cellular components, cell part (GO: 0044464), cell (GO: 0005623) and organelle (GO:0043226) were the dominant subcategories. Within the biological process category, cellular process (GO: 0009987), metabolic process (GO: 0008152) and biological process (GO: 0050789) were the dominant subcategories.

The top 15 GO terms associated with reproduction in biological process classification are shown in Table 4. The number of DEGs associated with reproduction in the top 15 GO terms was 309, and it accounted for 47.3% of all DEGs in the biological process category (653). It implied that body condition plays a profound role in the expression of genes related to reproduction.

Pathway enrichment analyses of DEGs

Pathway enrichment analyses of the DEGs using KEGG showed that DEGs were enriched in several pathways associated with reproduction and metabolism, such as cytokine-cytokine
receptor interaction, steroid hormone biosynthesis, fat digestion and absorption, caffeine metabolism, etc. The top 20 pathways enrichment of DEGs are shown in Figure 2. The main pathways enrichment related to reproduction are shown in Figure 3. The DEGs are mainly enriched in steroid hormone biosynthesis, the gonadotropin-releasing hormone (GnRH) signaling pathway, steroid biosynthesis, oocyte meiosis, progesterone-mediated oocyte maturation, etc. The main pathways enrichment of DEGs related to metabolism are shown in Figure 4. The DEGs are mainly enriched in metabolic pathways, Wnt signaling pathways, purine metabolism, insulin signaling pathways, mitogen-activated protein kinase (MAPK) signaling pathways, adipocytokine signaling pathways, etc.

**Gene expression validation by qRT-PCR**

Based on the results of GO and KEGG analysis, eight significant DEGs (PRLR, 3βHSD, cholesterol, APAF1, PDE5A, IGF-I, apolipoprotein A-I, Leptin R) related to reproduction and metabolism were selected for validation by qRT-PCR (Figure 5). The expression level of 3βHSD increased significantly in the obese group; however, the expression levels of PRLR and IGF-I were down-regulated. In general, the expression level of genes was similar to the expression pattern of RNA-Seq except that the expression of the apolipoprotein A-I gene was not significantly different between the two groups.

**Effects of BCS on intrafollicular concentration of hormones in estrous goats**

Follicular fluid was classified into two groups according to BCS. Reproductive hormones (E₂, P₄) and metabolic hormones (adiponectin and leptin) were determined by enzyme linked immunosorbent assay (ELISA). The relative ratios of E₂/P₄, leptin/adiponectin, adiponectin/E₂ and adiponectin/P₄ were calculated (Table 5). The results showed that P₄ and leptin in the obese group decreased significantly (0.91 ± 0.06 vs. 1.07 ± 0.03 ng/ml, 499.42 ± 23.29 vs. 543.42 ±
217 28.81 ng/ml), while the E₂/P₄ ratio increased significantly (10.41 ± 0.80 vs. 8.03 ± 0.40 ng/ml) in
the obese group. Other parameters were not significantly different.

DISCUSSION

The gene regulation of ovarian follicle development in goats has not yet been elucidated. Identifying new potential molecular indicators is important for evaluating the developmental competence of follicles and oocytes (Mamo et al., 2011). The construction of goat follicular transcriptome would advance the understanding of the molecular and cellular events underlying follicle development. The follicular globally sequenced genes covered a considerable proportion of the Capra hircus transcriptome. A total of 48,868,968 clean reads were assembled into 15,030 unigenes consisting of 16,147 known genes and 1,117 new genes. The functional annotation and comparative analysis of these unigenes would provide an invaluable resource for the functional genomics of goats.

Proper body condition is vital in the artificial control of reproductive efficiency. The increasing prevalence of obesity in women is of growing concern. Obese women required a significantly higher follicle stimulating hormone (FSH) start dose than did normal BMI women but obtained significantly fewer oocytes (p < 0.05) in superovulation. Morbidly obese class Π women had significantly reduced pregnancy rates compared to normal-BMI women (30.5 vs. 41.7%, respectively; p < 0.05. Deirdre et al., 2012). Zak et al. (1997) reported that maternal under-nutrition might reduce the follicle’s ability to support the maturation of oocytes and thus their quality. However, the effect of body condition on the ovarian follicle and oocyte is not well defined in ruminants. Compared with follicles from normal BCS, a number of genes and pathways involved in the regulating follicle development of obese goats were identified in this study. A total of 2,019 genes were differentially expressed, most of which (81.1%) were down-
Regulated. Gene cluster analysis showed that the follicular gene expression of 2.0-3.5 mm in diameter in the obese group was more similar to that of 1.0-1.9 mm in diameter of the normal BCS (data have not yet been published). It implied that follicles from obese goats were similar to the immature status or the initial stage of follicle development in the normal group.

GO is an international classification system for standardized gene functions and provides a comprehensive description of gene properties and their products. The top GO terms of DEGs enriched in obese group included growth, viral reproduction, reproduction, rhythmic process, etc. The total number of DEGs associated with reproduction was found to be 309 and accounted for 47.3% of all DEGs (653). It implied that body condition affect the expression of genes related to reproduction extensively. These results are consistent with the biological characteristic and function of the follicle.

KEGG analysis predicted that the expressed genes were involved in 216 pathways, of which “cytokine-cytokine receptor interaction” was the most enriched. Cytokines are soluble extracellular proteins or glycoproteins that are crucial intercellular regulators and mobilizers of cells engaged in innate as well as adaptive inflammatory host defenses, cell growth, differentiation, cell death, angiogenesis, development and repair processes aimed at the restoration of homeostasis. A number of important cytokines or their receptors (PRLR, IL-2 receptor, growth hormone receptor, etc.) belong to this pathway. The pathways related to the reproduction of DEGs are mainly enriched in steroid hormone biosynthesis, GnRH signaling pathways, steroid biosynthesis, oocyte meiosis, progesterone-mediated oocyte maturation, etc. The metabolism of steroid hormones is significantly important to the reproduction and reproductive processes. Animal endocrine pathways are maintained in a dynamic balance through the hypothalamus-pituitary-gonadal axis adjustment (Zhao et al., 2015).
GnRH secreted in the hypothalamus can stimulate the complex of gonadal cells in the adenohypophysis and the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which can promote the growth and development of ovarian follicles (Zhang et al., 2011). This indicates that these signaling pathways in obese goats were involved in the regulation of follicle development by body condition.

The expression levels of eight DEGs were verified by quantitative RT-PCR. 3βHSD is essential for steroid hormone production in porcine ovarian follicles (Rak-Mardyła et al., 2014). PRLR belongs to the type I cytokine receptor family and may function to modulate the endocrine and autocrine effects of prolactin in normal tissue and cancer. The expression of PRLR was detected in both oocytes and granulosa cells, and PRL effectively up-regulated PRLR expression in granulosa cells in the presence of FSH (Nakamura, et al., 2010). Cholesterol is the principal sterol synthesized by animals and also serves as a precursor for the biosynthesis of steroid hormones, bile acids and vitamin D. Leptin R (OB-receptor) belongs to the gp130 family of cytokine receptors known to stimulate gene transcription via the activation of cytosolic signal transducer and activator of transcription (STAT) proteins. This protein is a receptor for leptin (an adipocyte-specific hormone that regulates body weight) and is involved in the regulation of fat metabolism as well as in follicle growth. Mutations in this gene have been associated with obesity and pituitary dysfunction. Neonatal overfeeding reduced the number of ovarian follicles in adult rats and was associated with increased levels of ovarian leptin and its receptor (Ziko et al., 2016). APAF1 is a human homolog of C. elegans cell death protein 4 gene (CED-4). This gene encodes a cytoplasmic protein that forms one of the central hubs in the apoptosis regulatory network. The activation of Apaf-1 in granulosa cells leads to apoptosis (Robles et al., 1999). Guanosine 3',5'-cyclic monophosphate (cGMP), as a second messenger, plays a potential role in
ovarian functions. In addition, PDE5A specifically hydrolyzes cGMP to 5'-GMP and is involved in the regulation of intracellular concentrations of cyclic nucleotides. IGF promotes the proliferation and differentiation of granular cells. Meanwhile, apolipoprotein A-I is the major protein component of high-density lipoprotein (HDL) in plasma. The amounts of phospholipid and phospholipid/apoA-I ratio in FF were associated negatively to the percentage of oocyte fertilization. Therefore, the changes in the phospholipid and phospholipid/apoA-I ratios of FF might be regarded as indicators of female fertility (Fayezi et al., 2014). In general, the expression level of these genes exhibited similar trends to the expression pattern of RNA-Seq except that the expression of apolipoprotein A-I gene was not significantly different between the two groups.

Follicular fluid participates in transmitting nutrition signals to the oocytes and determining oocyte quality (Sinclair et al., 2008), especially reproductive hormones or metabolic hormones. During the collection of oocytes in IVM-IVF (in vitro maturation and in vitro fertilization) procedure or ART, without applying any invasive method more than the treatment process, we could obtain the corresponding one-to-one follicular fluid at the same time. Some components of follicular fluid are indicators of follicular development and are more accurate than parameters in serum. Leptin is an adipocyte hormone acting as a link between adipose tissue and the reproductive system (Zahra Kamyabi et al., 2015). It is also considered a type 1 cytokine due to its role in cell growth and maturation (Wertel et al., 2005). Previous studies showed a direct relationship between plasma leptin level and obesity. A significant relationship was found between leptin level and BMI as well as LH level among women with PCOS (p < 0.05, Jalilian N, et al., 2016). Leptin could be produced by both granulosa and cumulus cells of ovarian follicles (Wertel et al., 2005; Gogacz et al., 2001). Adiponectin is an adipokine secreted from the adipose tissue (Tsao et al., 2002) and affects the reproductive system through effects on the
hypothalamus and pituitary, peripheral effects on the ovaries and direct effects on the oocytes and embryos (Campos, Palin, Bordignon & Murphy, 2008). Previous research indicated that adiponectin inhibited the synthesis and secretion of GnRH through reducing the transcription of the Kiss-1 gene (Cheng et al., 2011). Concentrations of insulin, leptin, adiponectin and cytokines were highly correlated between plasma and FF (Sessions-Bresnahan et al., 2014). In this study, the concentrations of P₄ and leptin of 2.0-3.5 mm follicular fluid were lower in the obese group, and the ratio of E₂ to P₄ was higher in the obese group. It is well known that predominantly intrafollicular estrogenic environment is associated with good follicular growth and has anti-atresia effects. However, no significant difference was found in adiponectin and E₂ between the two groups.

In summary, our study constructed follicular transcriptome and identified a series of DEGs and pathways between obese and normal goats. A combined analysis of DEGs (3βHSD, Leptin R, apolipoprotein A-I, etc.), enriched pathways (progesterone-mediated oocyte maturation, steroid biosynthesis, etc.) and follicular fluid composition (P₄, leptin, E₂/P₄, etc.) implied that P₄ and leptin might be a potential biomarker of follicle development. These results will contribute to the nutritional regulation of body condition in breeding goats. The data filled a large gap in our knowledge about the effect of body condition on follicle transcriptome in goats and pave the way for future studies aimed at understanding the mechanisms of follicle development.

REFERENCES


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**Table 1** (on next page)

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Table 1. Sequence of Primers used for qRT-PCR analysis

Table 2. Summary of Reads and gene number of follicles from goats of different BCS

Table 3. Differentially regulated genes of follicles between obese and normal goats

Table 4. TOP 15 GO terms of DEGs enriched in biological process of follicles from goats of different BCS

Table 5. Effect of BCS on Intrafollicular Concentration of Hormones in Estrous Goats
Table 1. Sequence of Primers used for qRT-PCR analysis

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<th>Forward 5’-3’</th>
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**Table 2. Summary of Reads and gene number of follicles from goats of different BCS**

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<th>Clean Reads</th>
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<td>(2.02%)</td>
<td>(80.44%)</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Differentially regulated genes of follicles between obese and normal goats**

<table>
<thead>
<tr>
<th>Control</th>
<th>Total DEGs</th>
<th>DEGs cellular</th>
<th>DEGs molecular</th>
<th>DEGs biological</th>
</tr>
</thead>
</table>


<table>
<thead>
<tr>
<th>vs.</th>
<th>DEG (Up)</th>
<th>(Down)</th>
<th>components</th>
<th>functions</th>
<th>processes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese</td>
<td>2019</td>
<td>381</td>
<td>1638</td>
<td>674</td>
<td>638</td>
</tr>
<tr>
<td>NO. of</td>
<td>15919</td>
<td>—</td>
<td>—</td>
<td>5852</td>
<td>5574</td>
</tr>
</tbody>
</table>

* Screening criteria for DEGs: FDR (false discovery rate) < 0.05 and $|\log_{2}\text{FC}|>1$

Table 4. TOP 15 GO terms of DEGs enriched in biological process of follicles from goats of different BCS

<table>
<thead>
<tr>
<th>Gene Ontology term</th>
<th>No. of DEGs with GO annotation</th>
<th>Cluster frequency</th>
<th>Go Term ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>reproduction</td>
<td>41</td>
<td>6.28%</td>
<td>GO:0000003</td>
</tr>
<tr>
<td>embryo development</td>
<td>40</td>
<td>6.13%</td>
<td>GO:0009790</td>
</tr>
<tr>
<td>reproductive process</td>
<td>39</td>
<td>5.97%</td>
<td>GO:0022414</td>
</tr>
<tr>
<td>chordate embryonic development</td>
<td>26</td>
<td>3.98%</td>
<td>GO:0043009</td>
</tr>
<tr>
<td>embryo development ending in birth or egg hatching</td>
<td>26</td>
<td>3.98%</td>
<td>GO:0009792</td>
</tr>
<tr>
<td>Pathological Process</td>
<td>Count</td>
<td>Percentage</td>
<td>Gene Ontology ID</td>
</tr>
<tr>
<td>-----------------------------------------------------------</td>
<td>-------</td>
<td>------------</td>
<td>------------------</td>
</tr>
<tr>
<td>embryonic morphogenesis</td>
<td>23</td>
<td>3.52%</td>
<td>GO:0048598</td>
</tr>
<tr>
<td>developmental process involved in reproduction</td>
<td>19</td>
<td>2.91%</td>
<td>GO:0003006</td>
</tr>
<tr>
<td>viral reproduction</td>
<td>17</td>
<td>2.60%</td>
<td>GO:0016032</td>
</tr>
<tr>
<td>reproductive structure development</td>
<td>13</td>
<td>1.99%</td>
<td>GO:0048608</td>
</tr>
<tr>
<td>embryonic organ development</td>
<td>13</td>
<td>1.99%</td>
<td>GO:0048568</td>
</tr>
<tr>
<td>development of primary sexual characteristics</td>
<td>11</td>
<td>1.68%</td>
<td>GO:0045137</td>
</tr>
<tr>
<td>sex differentiation</td>
<td>11</td>
<td>1.68%</td>
<td>GO:0007548</td>
</tr>
<tr>
<td>multicellular organism reproduction</td>
<td>10</td>
<td>1.53%</td>
<td>GO:0032504</td>
</tr>
<tr>
<td>multicellular organismal reproductive process</td>
<td>10</td>
<td>1.53%</td>
<td>GO:0048609</td>
</tr>
<tr>
<td>sexual reproduction</td>
<td>10</td>
<td>1.53%</td>
<td>GO:0019953</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>309</td>
<td>47.30%</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Effect of BCS on Intrafollicular Concentration of Hormones in Estrous Goats

<table>
<thead>
<tr>
<th>Group</th>
<th>E₂</th>
<th>P₄</th>
<th>Adiponectin</th>
<th>Leptin</th>
<th>E₂/P₄</th>
<th>Leptin</th>
<th>Adiponectin</th>
<th>Adiponectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p ng/ml</td>
<td>ng/ml tin</td>
<td>ng/ml adiponectin</td>
<td>/adiponectin</td>
<td>in/ E₂</td>
<td>n/P₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>---------</td>
<td>-----------</td>
<td>-------------------</td>
<td>--------------</td>
<td>-------</td>
<td>------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obes</td>
<td>9.15 ±0.24</td>
<td>0.91 ±0.06</td>
<td>13.56 ±0.88</td>
<td>499.42 ±23.29b</td>
<td>10.41 ±0.80</td>
<td>26.59 ±1.61</td>
<td>1.49 ±0.09</td>
<td>15.64 ±1.03</td>
</tr>
<tr>
<td>Cont</td>
<td>8.55 ±0.35</td>
<td>1.07 ±0.03</td>
<td>13.47 ±0.76</td>
<td>543.42 ±28.81a</td>
<td>8.03 ±0.40</td>
<td>25.64 ±2.85</td>
<td>1.65 ±0.09</td>
<td>13.06 ±0.59</td>
</tr>
</tbody>
</table>
Figure 1

Top 20 pathways enrichment of DEGs by KEGG analysis
**Figure 2** (on next page)

Main pathways enrichment of DEGs related to reproduction
**Figure 3** (on next page)

Main pathways enrichment of DEGs related to metabolism
Pathway name

- Wnt signaling pathway
- Vitamin digestion and absorption
- Type II diabetes mellitus
- Type I diabetes mellitus
- Purine metabolism
- Protein digestion and absorption
- PPAR signaling pathway
- mTOR signaling pathway
- Metabolic pathways
- MAPK signaling pathway – fly
- MAPK signaling pathway
- Insulin signaling pathway
- Glycolysis / Gluconeogenesis
- Fructose and mannose metabolism
- Fatty acid metabolism
- Fat digestion and absorption
- Calcium signaling pathway
- Arginine and proline metabolism
- Adipocytokine signaling pathway

Gene number

- Rich factor

Pathway enrichment by -log10(Qvalue)
Figure 4

GO classifications of DEGs enriched of the obese and control goat

Left y-axis, percentage of genes; Right y-axis, number of genes. Red: up-regulated; Green: down-regulated.
Figure 5

qRT-PCR analysis of selected DEGs of follicles of different BCS

Control group (white bars) was compared to Obese group (black bars). All expression levels are relative to the level of expression in control follicles which has been arbitrarily one-fold.