Transcriptome Analysis of the *Capra hircus* Ovary

Zhong Quan Zhao¹‡, Li Juan Wang¹‡, Xiao Wei Sun¹, Jiao Jiao Zhang², Yong Ju Zhao¹, Ri Su Na¹, Jia Hua Zhang¹*

¹ Chongqing Engineering Research Center for Herbivores Resource Protection and Utilisation, Southwest University, Chongqing, China, ² Genetic Engineering and Stem Cell Biology Laboratory, Department of Animal Biotechnology, Faculty of Biotechnology, Jeju National University, Jeju, South Korea

‡ These authors are co-first authors on this work.
* jhzhang007@aliyun.com

Abstract

Background

*Capra hircus* is an important economic livestock animal, and therefore, it is necessary to discover transcriptome information about their reproductive performance. In this study, we performed *de novo* transcriptome sequencing to produce the first transcriptome dataset for the goat ovary using high-throughput sequencing technologies. The result will contribute to research on goat reproductive performance.

Method and Results

RNA-seq analysis generated more than 38.8 million clean paired end (PE) reads, which were assembled into 80,069 unigenes (mean size = 619 bp). Based on sequence similarity searches, 64,824 (60.6%) genes were identified, among which 29,444 and 11,271 unigenes were assigned to Gene Ontology (GO) categories and Clusters of Orthologous Groups (COG), respectively. Searches in the Kyoto Encyclopedia of Genes and Genomes pathway database (KEGG) showed that 27,766 (63.4%) unigenes were mapped to 258 KEGG pathways. Furthermore, we investigated the transcriptome differences of goat ovaries at two different ages using a tag-based digital gene expression system. We obtained a sequencing depth of over 5.6 million and 5.8 million tags for the two ages and identified a large number of genes associated with reproductive hormones, ovulatory cycle and follicle. Moreover, many antisense transcripts and novel transcripts were found; clusters with similar differential expression patterns, enriched GO terms and metabolic pathways were revealed for the first time with regard to the differentially expressed genes.

Conclusions

The transcriptome provides invaluable new data for a functional genomic resource and future biological research in *Capra hircus*, and it is essential for the in-depth study of candidate genes in breeding programs.
Introduction

Capra hircus is one of the most important livestock animals and the oldest economic domesticated species. Goats have long been used for their milk, meat, hair and skins throughout the world [1–6]. The Dazu black goat, a protected national goat strain in China, has pure black hair and high reproductive performance. The average litter size of multiparous ewes is 2.72. The ovary is a dynamic organ that undergoes structural changes during the mammal reproductive cycle [7–9], which is tightly regulated by a multitude of genes and various endocrine hormones [10–13]. The ovaries play an important role during reproductive processes. There exist many significant differences in the endocrine characteristics and activities of the ovary between mature and immature ewes. In the immature ewe, there is no ovulation, whereas the endocrine pattern varies and ovulation is normal in mature ewe.

Hormone secretion and ovarian follicular development are complex [13,14] and will often change during different developmental stages and in different breeds. Ovarian function is tightly regulated by a large number of genes [13,15,16]. The identification and validation of mRNAs in the ovary at different developmental stages and different breeds [17], however, has been limited. It is needed to identify differentially expressed mRNAs in the ovaries during different developmental stages and different breeds.

In the present study, we investigated the differential expression of mRNAs in different development periods in the ovaries of immature and mature ewe using RNA-seq technology. We used transcriptome sequencing technology to analyse and identify the full repertoire of mRNAs expressed in the ovary during different developmental stages [18,19]. The data provide a large amount of useful information about mRNAs that are related in mammal reproductive biological processes [20–22]. The result will help researchers to further understand the importance of mRNAs in reproductive processes, including hormone secretion and follicular development, and may help to further studies of breeding practices and reproductive regulation in the future.

Methods and Materials

This study was carried out in strict accordance with the recommendations in the Guide for the International Cooperation Committee of Animal Welfare (ICCAW), which is responsible for animal care and use in China. The experimental conditions were approved by the Committee on the Ethics of Animal Experiments of Southwest University (No. [2007] 3) and the Animal Protection Law in China, and all efforts were made to minimise suffering.

In this study, we performed de novo transcriptome sequencing to produce the first transcriptome dataset for the goat ovary using high-throughput sequencing technologies. Our experimental animals were Dazu Black goats which were selected from the Dazu Black Goat Farm at Southwest University, Chongqing, China. We divided our experimental goats into two groups: group A contained sexually mature, barren ewes, and group B contained immature female lambs. Each group contained three goats. When these goats were killed, we collected the ovary immediately.

Ovary collection and RNA extraction

After pentobarbital sodium 100mg/kg was injected into jugular vein, the muscles were relaxed, the heart and respiratory activities were arrested, we dissect the goats and collected the ovaries immediately and the ovaries were frozen in liquid nitrogen. Total RNA was isolated by a TRIzol Plus RNA Purification Kit (Invitrogen). The concentration and quality of total RNA were determined by an Agilent Technologies 2100 Bioanalyzer.
cDNA library preparation and Illumina sequencing for transcriptome analysis

To obtain complete gene expression information, a pooled RNA sample that included different developmental stages was used for transcriptome analysis. According to the Illumina manufacturer’s instructions, poly (A)$^+$ RNA was purified from 20 μg of pooled total RNA using oligo (dT) magnetic beads and fragmented into short sequences in the presence of divalent cations at 94°C for 5 min. The cleaved poly (A)$^+$ RNA was transcribed, and second-strand cDNA synthesis was then performed. After the end repair and ligation of adaptors, the products were amplified by PCR and purified using the PureLink PCR Purification Kit (Invitrogen) to create a cDNA library.

De novo assembly and function annotation and classification

After removing the reads that contained adaptor contamination, low quality bases and undetermined bases from each of the datasets [23,24], the clean high-quality reads were de novo assembled by a Trinity RNA-Seq Assembler [25].

BLASTX was used to search the NR databases and KEGG database (E-value $10^{-5}$). Blast2go (http://www.blast2go.org/) and InterProScan software [26] were used for Gene Orthology (GO) and KEGG Orthology (KO) annotations of unigenes.

Digital gene expression (DGE) library sequencing and mapping of DGE tags

Total RNA was extracted from mature and immature ewes using a TRIzol Plus RNA Purification Kit (Invitrogen). Poly (A)$^+$ RNA was purified by using oligo (dT) magnetic beads, DGE libraries were prepared by using the Illumina gene expression sample prep kit.

To map the DGE tags, the sequenced raw data were filtered to remove low quality tags, empty tags and tags with only one copy number [27–30]. When annotated, the clean tags were designated as unambiguous clean tags that contained "CATG", and 21 bp tag sequences were mapped to our transcriptome sequences [31,32]. The number of clean tags for each gene were calculated and normalised to TPM (number of transcripts per million clean tags) for gene expression analysis.

Quantitative real-time PCR (qRT-PCR) validation

Quantitative real-time reverse transcription PCR (qRT-PCR) was performed to validate the mRNA sequencing data. Total RNA of the two samples corresponding to two growth phases of the Dazu black goat was extracted by TRIzol Plus RNA Purification Kit (Invitrogen). The concentration of each RNA sample was adjusted to 1 μg/μL with nuclease-free water, and 2 μg of total RNA was reverse transcribed in a 20 μl reaction system using the iScript cDNA Synthesis Kit (BIO-RAD). qRT-PCR was performed using the SsoAdvanced SYBR Green Supermix (BIO-RAD) on a CFX96 Real-Time System (BIO-RAD) according to the manufacturer’s protocol, and at least three technical replicates were performed for all genes in each pool. β-actin was used as an internal control gene. The sequences of the specific primers are listed in Table 1. Gene expression difference between two samples was calculated by the $2^{-ΔΔCt}$ method.
Results

Illumina sequencing and reads assembly

In this study, the Illumina sequencing method was used to analyse the transcriptome of Capra hircus. The transcriptome sequencing data from Dazu black goat have been deposited in the NCBI Sequence Read Archive database (accession number: SRR1556738). A total of 38,771,668 clean reads were generated through Illumina paired-end sequencing and assembled into 150,431 contigs and 80,069 unigenes of the Dazu black goat (Table 2). Those contigs comprised 45.8 Mb of transcriptome sequences with an average length of 305 bp and a N50 length of 479 bp. Those unigenes comprised 49,587,490 total length, with an average length of 619 bp and a N50 length of 1067 bp, including 12,589 distinct clusters and 67,480 distinct singletons.

Gene coverage statistics showed that the number of genes with transcript coverage between 90% and 100% was 73,820 (92%), and genes with coverage between 80% and 90% was 4,652 (6%) (Fig 1). Thus, genes with high coverage accounted for the largest proportion of the mapped genes, indicating that the alignment results were good.

The results of a statistical analysis of these new mapped transcripts are shown in Fig 2. The E-value distribution showed that matches with an E-value of 0 made up the largest portion

Table 1. Primers used in validation experiments.

| Unigene Number | Similarity                      | Abbreviation | qRT-PCR Primer Sequences       |
|----------------|---------------------------------|--------------|--------------------------------|
| Unigene13218   | cyclin-D1                       | CCND1        | GGCAGGAGAGAAGACAGCAG          |
|                |                                 |              | GTGTCAGGGGTGATAGGA            |
| Unigene17906   | nucleoporin 54kDa               | NUP54        | TCCAGAGGAGAAGTGGCTAT          |
|                |                                 |              | TTGGGACATCGTCTCTCTTA          |
| Unigene27274   | interferon alpha-inducible protein 27 | IFI27       | CCCACCTGTCCCTCTCTCC          |
|                |                                 |              | AATCAACGCTCTCTCTCTTA          |
| Unigene286     | cyclin-B1                       | CCNB1        | AATGACCGCAACTAAA             |
|                |                                 |              | AAGGCGACCCAGACTAAA           |
| Unigene30802   | nucleoprotein TPR               | TPR          | CAAATTCTTTTACGGCTTCA         |
|                |                                 |              | CGACGTCATCCCTCTCA           |
| Unigene7179    | intraflagellar transport protein 81 | IFT81      | GAGATGCAGAAAACAA             |
|                |                                 |              | TTCAATCACGAGACCT            |
| Unigene7507    | retinol dehydrogenase 10       | RDH10        | TGGCTTTGTTTCTTTTGA           |
|                |                                 |              | ATTTGGAGTCGTGGGTTT           |
| Unigene7616    | zona pellucida glycoprotein 4   | ZP4          | CCACTTAAGTCGAGACCTG         |
|                |                                 |              | GTAGAGGATAGCGCTTTT          |
| Unigene9759    | 26S proteasome subunit 11-like  | PSMD11       | GGTTCCTGAGGTCTCGGGAGT       |
|                |                                 |              | ATGGCCATTGTCTGTTGTTG        |

doi:10.1371/journal.pone.0121586.t001

Table 2. Summary of the transcriptome.

|                      |                         |
|----------------------|-------------------------|
| Total Number         | 80,069                  |
| Total Length (nt)    | 49,587,490              |
| Mean Length (nt)     | 619                     |
| N50                  | 1067                    |
| Total Consensus Sequences | 80,069              |
| Distinct Clusters    | 12,589                  |
| Distinct Singletons  | 67,480                  |

doi:10.1371/journal.pone.0121586.t002
(18.2%), followed by matches with an E-value of (0~1) ×10^{-100} (17.0%) and 1×10^{-100}~1×10^{-60} (10.4%) (Fig 2A). The similarity distribution showed that transcripts that shared 95%~100% similarity with known sequences accounted for the largest proportion (59.8%), followed by transcripts that shared 80%~95% similarity (21.7%) and transcripts with 60%~80% similarity (6.8%) (Fig 2B). These results indicate that the BLAST results were reliable. The species distribution showed that the majority of matches were with known Bos Taurus sequences (62.9%), followed by Sus scrofa (4.7%), Saimiri boliviensis boliviensis (3.8%), Homo sapiens (3.8%), Ovis aries (1.6%), Mus musculus (1.6%), and other species (19.9%) (Fig 2C). A considerable proportion of the potential unigenes were similar to other species, therefore, further studies are required to improve the goat genome annotations.

Functional annotation and classification of transcriptome sequences

We annotated the total 80,069 unigenes and then obtained 64,824 (80.96%) transcript-derived unigenes (Table 3). The sequence similarity search against the non-redundant (NR) protein database revealed that 39,266 out of 64,824 transcript-derived unigenes (60.57%) could be assigned a known function. Gene ontology (GO) annotation (Fig 3) was performed to functionally classify the unigenes that had hits in the NR database, and 29,444 (45.42%) unigenes were annotated with 2,000,299 GO IDs. In the biological process ontology, we found that the most abundant terms annotated to the unigenes were cellular process, metabolism process, and biological regulation, whereas in the cellular component ontology, the most abundant terms were cell, cell part, organelle, and organelle part, and in the molecular function ontology, the most abundant terms were binding and catalytic activity. By mapping the 64,824 unigenes to the Cluster of Orthologous Groups for eukaryotic complete genomes (COG) database [33–35], we found that general function prediction, translation, ribosomal structure and biogenesis were the most frequently represented functional clusters in our transcriptome (Fig 4). We also noted that the replication, recombination and repair cluster and cell cycle control, cell division, chromosome partitioning cluster was frequently represented, indicating the complicated reproductive and reproductive process and its regulation. We also mapped the unigenes to the Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway database [36] and found that 27,766 transcripts mapped to 258 pathways (Fig 5).
Fig 2. Alignment statistics of the transcriptomes against the nr, nt, and SwissProt databases. A. E-value distribution. B. Similarity distribution. C. Species distribution.

doi:10.1371/journal.pone.0121586.g002

Table 3. Annotation of unigenes.

| Database   | Number of Annotated Unigenes | Percentage of Annotated Unigenes |
|------------|------------------------------|----------------------------------|
| NR         | 39,266                       | 60.57%                           |
| NT         | 64,571                       | 99.61%                           |
| Swiss-Prot | 36,910                       | 56.94%                           |
| KEGG       | 27,766                       | 42.83%                           |
| COG        | 11,271                       | 38.28%                           |
| GO         | 29,444                       | 45.42%                           |

doi:10.1371/journal.pone.0121586.t003
Digital gene expression profiling (DGE) and qRT-PCR validation

Two DGE libraries of goat ovaries, including mature ewes and immature ewes, were sequenced, and 6,191,236 and 5,998,876 raw tags were then generated. After filtered the low quality tags, the total number of clean tags in mature library and immature library were 5,870,617 and 5,658,243, and the percentage of clean tags among the raw tags in mature library and immature library were 94.82% and 94.32%. The sequencing data from two libraries of Dazu black goat have been deposited in the NCBI Sequence Read Archive database (accession number: SRR1561954, SRR1561955). Among the clean tags, the number of sequences that could be mapped to gene tags were 4,818,689 (82.08%) and 4,622,465 (81.69%), and the unique map to gene tags were 3,576,456 (60.72%) and 3,430,932 (60.64%).

To identify the Differentially Expressed Genes (DEGs) associated with the transition from two phases in our DGE, we realigned the short reads that were generated from the two independent libraries related to the two different growth phases back to all transcripts. This process enabled us to evaluate the gene expression abundance by calculating the $|\log_2\text{Ratio}|$ and to estimate the significance of the DEGs between two samples. We observed that no less than 80% of the total clean reads in each library could be mapped uniquely to the all transcripts. In this study, when the False Discovery Rate (FDR) was less than $10^{-3}$ and the $|\log_2\text{Ratio}| \geq 1$ between two samples, then the transcripts were considered to be statistically significant DEG.

To validate the DEGs with a biological replicate between mature ewes and immature ewes, we selected 9 significant DEGs to perform real time quantitative PCR (qPCR) on the other goat ovary tissues that were previously sampled during the same phase. We found that gene expression profiling of these DEGs using qPCR revealed similar variation trends with RNA-Seq samples (Fig 6), indicating the high credibility of these genes in transcript abundance between mature ewes and immature ewes from different individuals.
Differentially expressed genes (DEGs) between mature and immature ewe

GO analysis of the transcripts showed that the category associated with reproduction and reproductive process accounted for a large proportion, with the majority of them being involved in the “reproduction” category and there were 1,759 unigenes related to reproduction. Analysing the mature and immature ovary digital gene expression profiling (DGE) data, we found 709 unigenes that were significant different expressed, of which 360 transcripts-derived unigenes from mature ewes were up-regulated compared with immature ewes, and 349 were down-regulated. GO cluster annotation showed that there were 42 transcripts response to reproduction, 36 transcripts response to single organism reproductive process, 23 transcripts response to steroid hormone stimulus, 15 transcripts response to estrogen stimulus, 11 transcripts response to female pregnancy, 9 transcripts response to estradiol stimulus, 6 transcripts response to progesterone stimulus (Table 4). We will continue our research into oocyte maturation. These results provide robust data for further annotation of genes that are related to goat reproduction.

Discussion

Traditionally, gene expression analysis of Capra hircus has relied mostly on cDNA microarrays, serial analysis of gene expression (SAGE) and expressed sequence tags (EST). However,
Fig 5. **KEGG classifications of unigenes.** Overall, 27,766 unigenes were assigned to 258 pathways. The pathways that were mapped by more than 500 unigenes are shown.

doi:10.1371/journal.pone.0121586.g005

Fig 6. **Transcriptome validation.** This figure shows that gene expression profiling of the DEGs using qPCR exhibited similar trends to the RNA-Seq samples.

doi:10.1371/journal.pone.0121586.g006
each of those approaches has some inherent limitations, such as cloning biases, a requirement for prior sequence knowledge, cost, and so on. Thus, the newly developed deep sequencing approaches have significant advantages for investigating the functional complexity of the transcriptome. RNA-Seq is a relatively efficient method for large-scale transcriptomic studies that is constantly being improved. RNA-Seq is used extensively in transcriptomic studies, including

| Gene Ontology term                                      | DEGs with GO annotation | GO Term ID   |
|----------------------------------------------------------|--------------------------|--------------|
| response to estrogen stimulus                            | 15                       | GO:0043627   |
| female pregnancy                                         | 11                       | GO:0007565   |
| single organism reproductive process                     | 36                       | GO:0044702   |
| embryonic morphogenesis                                  | 19                       | GO:0048598   |
| reproductive process                                     | 42                       | GO:0022414   |
| reproduction                                             | 42                       | GO:0000003   |
| multicellular organism reproduction                      | 21                       | GO:0032504   |
| multicellular organismal reproductive process            | 21                       | GO:0048609   |
| developmental process involved in reproduction           | 16                       | GO:0003006   |
| embryo implantation                                      | 3                        | GO:0007566   |
| embryonic organ morphogenesis                            | 8                        | GO:0048562   |
| regulation of reproductive process                       | 8                        | GO:2000241   |
| multi-organism reproductive process                      | 19                       | GO:0044703   |
| development of primary male sexual characteristics       | 5                        | GO:0046546   |
| development of primary sexual characteristics            | 9                        | GO:0045137   |
| reproductive structure development                      | 10                       | GO:0048608   |
| reproductive system development                         | 10                       | GO:0061458   |
| male sex differentiation                                 | 5                        | GO:0046661   |
| embryonic organ development                              | 11                       | GO:0048568   |
| sex differentiation                                      | 9                        | GO:0007548   |
| negative regulation of reproductive process              | 3                        | GO:2000242   |
| cellular process involved in reproduction                | 12                       | GO:0048610   |
| positive regulation of reproductive process              | 4                        | GO:2000243   |
| ovarian follicle development                             | 3                        | GO:0001541   |
| embryo development                                       | 23                       | GO:0009790   |
| single-organism reproductive behavior                    | 2                        | GO:0044704   |
| in utero embryonic development                           | 10                       | GO:0001701   |
| chordate embryonic development                           | 13                       | GO:0043009   |
| embryo development ending in birth or egg hatching       | 13                       | GO:0009792   |
| reproductive behavior                                   | 2                        | GO:0019098   |
| female gonad development                                 | 3                        | GO:0008585   |
| sexual reproduction                                      | 11                       | GO:0019953   |
| ovulation cycle process                                  | 3                        | GO:0022602   |
| ovulation cycle                                          | 3                        | GO:0042698   |
| positive regulation of viral reproduction                | 2                        | GO:0048524   |
| post-embryonic development                               | 2                        | GO:0009791   |
| development of primary female sexual characteristics     | 3                        | GO:0046545   |
| female sex differentiation                               | 3                        | GO:0046660   |
| regulation of viral reproduction                         | 2                        | GO:0050792   |
| viral reproductive process                               | 7                        | GO:0022415   |
| viral reproduction                                       | 8                        | GO:0016032   |

doi:10.1371/journal.pone.0121586.t004
gene expression, alternative splicing, determination of non-coding RNA function, and development of SNP or SSR markers. The use of RNA-Seq in studying gene transcriptome information has attracted considerable attention.

In this study, a Dazu black goat ovary pool was constructed by mixing three mature healthy female Dazu black goat ovary samples, to obtain an ovary sequencing library, which after sequencing, yielded 42,377,782 raw reads. After removing low-quality sequences, 38,771,668 clean reads were obtained. The base composition and quality analyses showed that the ratio of Q20 (i.e., the quality of base ≥20) was 98.14% and the GC content was 49.19%, which indicated successful library construction and good sequencing quality. Whole clean reads were assembled into 150,431 contigs and 80,069 unigenes of the Dazu black goat. Those contigs comprised 45.8 Mb of transcriptome sequences with an average length of 305 bp and a N50 length of 479 bp. Those unigenes comprised 49,587,490 total length, with an average length of 619 bp and a N50 length of 1067 bp, including 12,589 distinct clusters and 67,480 distinct singletons.

The function of the expressed genes were annotated by GO and KEGG analyses. A total of 25 GO categories under biological processes were assigned to the transcripts, of which the largest proportion was “cellular process”, followed by “metabolic process” and “biological regulation”. In addition, 1759 unigenes and 1738 unigenes were associated with “reproduction” and “reproductive process”. These results are consistent with the biological characteries and function of the ovary. A total of 18 GO categories under cellular component were assigned to the transcripts of which the largest proportion was “cell” (23873 unigenes). In addition, a significant proportion of the transcripts were associated with membrane components (10185 unigenes), which are important in the physiological activity of the Capra hircus ovary. As an example, endothelial cells of the ovarian capillary and lymphatic system form intracellular channels and a plasma membrane vesicle system, which could play an important role in the tissue fluid and the transport of macromolecular material [6, 9]. A total of 18 GO categories under molecular function were assigned to the transcripts, of which the largest proportion was “binding”. Previous microarray studies showed that the RNA-binding molecular function category accounted for a large proportion in the expressed genes in bovine oocytes [37–39]; thereby confirming the results of the present study that “binding” may plays an important role in the normal physiological activities of goat ovary.

KEGG analysis predicted that the expressed genes were involved in 258 pathways, of which “metabolic pathways” was the most enriched (Fig 5). The metabolism of steroid hormones was significantly important to reproduction and reproductive process. Animal endocrine pathways are maintained in a dynamic balance through the hypothalamus-pituitary-gonadal axis adjustment. Sex steroid hormones include estradiol, testosterone, progesterone and their derivatives [40]. Gonadotropin-releasing hormone (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 [6]. “Focal adhesion” was the next most highly enriched. Focal adhesion, a connection function mediated by cells and extracellular matrix (ECM), results in a dynamic cell anchor type of connection, in which the integrins anchor to the ECM [41]. The ECM has comprehensive influence on morphogenesis of embryonic development, including organ formation, or in the adult in maintaining the structure and functional (including immune response and wound repair, etc.), and generally in all life phenomenon. In addition to the “focal adhesion” pathway, the “ECM-receptor interaction” pathway was also enriched. The ECM is a complex matrix of biological macromolecules, such as collagen, fibronectin, laminin, glycosaminoglycans, proteoglycan, adhesive and elastin. The ECM has an important function in various aspects of cell physiological activities, including cell cell shape, structure, function, survival, proliferation, differentiation, migration and so on, by interactions with its surface receptor
(ECM receptor). For example, the ECM can send signals to the cells via the surface receptors, and the ECM can send signals to the cytoplasm and nucleus to influence cellular activities or gene expression [42]. In the present study, transcripts associated with the "ECM-receptor interaction" pathway were highly expressed, which suggested that they may be complementary to "focal adhesion", and both pathways have an important functions in promoting cell adhesion and connection. The enrichment of these two pathways in the transcriptome indicated that cell connections occur extensively in goat ovary. Besides, adherens junction and tight junction were both enriched in the transcriptome (Fig 5). An oocyte mainly communicates with its surrounding cells, such as granulosa and theca cells, through cell adhesion and connection. Numerous small molecular substances that contain metabolites, information, and nutrients that regulate oocyte growth and development are transported through this connection [43, 44]. The "regulation of actin cytoskeleton" pathway was also enriched in the goat ovary transcriptome. Recent studies have shown that actin cytoskeleton also has an important function in the early development of oocyte maturation [45], by assisting in the cytokinesis or the formation of cytoplasmic channels, as well as in the transport of oocyte-specific RNA and proteins. The high expression of transcripts involved in the "regulation of actin cytoskeleton" pathway suggested the existence of dynamic changes in the cytoskeleton structure of goat ovary [46].

Three signaling pathways, mitogen-activated protein kinase (MAPK), chemokine, and Wnt, were also enriched (Fig 5). MAPK is a serine/threonine protein kinase that is available in various signaling pathways, which acts as a common component of signaling transduction in the regulation of cell growth, cell differentiation and cell cycle. The MAPK signaling pathway is also important in eukaryotic signaling networks where it plays a role in passing the upstream signal to the downstream elements [47]. The MAPK pathway is activated when phosphorylation occurs during oocyte mature and metaphaseIIarrest [48]. Wnt is a secreted glycoprotein, which participates in autocrine or paracrine activity. The Wnt protein is important in the regulation of cell proliferation, differentiation, and migration during an organism’s growth and development, and it can determine cell polarity, fate, and proliferation of progenitor cells [49]. Recent studies have shown that the Wnt signaling pathway is necessary to regulate the normal development of the mammalian reproductive system. This pathway is involved mainly in the formation of Mueller’s pipe, control of follicular development, ovulation, and luteinization, and as well as in the establishment of normal pregnancy [50–52]. The enrichment of the MAPK and Wnt pathways in the yak ovary transcriptome is similar to previous studies on oocyte of different species [45, 48, 50, 52], thereby indicating that these two signaling pathways are important in maintaining physiological activity of the ovary. Chemokines play a role in the endometrium of the uterus prior to the implantation of the embryo. The chemokine–receptor signaling at the maternal–fetal interface enables human trophoblast (fetal) to migrate and move into the epithelial region of the endometrium. The bidirectional chemokine mediated signaling between the trophoblast and the maternal endometrium may enable the successful implantation of the embryo. Chemokines also play a functional role in embryogenesis and development of the central nervous system (CNS). The chemokine receptors CXCR4 and CXCR7 and their ligand stromal cell derived factor-1α (SDF-1α/ CXCL12) influence CNS development through the homing of the neuronal precursor cells to their respective target areas of the developing brain [53].

Some enriched pathways, namely, "pathways in cancer", "amoebiasis" and "Herpes simplex infection", have no obvious associations with the reproductive function of the ovary. Although these pathways were derived from their corresponding physiological processes, we speculate that some of the genes involved in these pathways may be related to the functions and activities of the ovary. For example, although "pathway in cancer" is related to cancer, an important feature of cancer is proliferation, which is related to the proliferation of germ cells. Thus, some
genes involved in the "pathway in cancer" may also be involved in the proliferation and development of ovary-related cells. However, further studies are needed to determine the exact functions of these pathways in the goat ovary.

Conclusion
In total, 150,431 contigs and 80,069 unigenes were detected in the ovaries of Dazu black goats using transcriptome sequencing technology. Of these, 29,444 and 11,271 unigenes were assigned to Gene Ontology (GO) categories and Clusters of Orthologous Groups (COG), respectively. Searches in the Kyoto Encyclopedia of Genes and Genomes Pathway database (KEGG) showed that 27,766 unigenes were mapped to 258 KEGG pathways. Furthermore, we obtained a sequencing depth of over 5.6 million and 5.8 million tags in the mature and immature ewe, respectively. The transcriptome provides invaluable new data for a functional genomic resource and future biological research in Capra hircus, and it is essential for the in-depth study of candidate genes in breeding programs.

Acknowledgments
The study was founded by the Chongqing Municipal Natural Science Foundation project cstc2012jiA80016 and the Fundamental Research Funds for the Central Universities (XDJK 2013B024). The authors gratefully thank Beijing Genomics Institute at Shenzhen (BGI Shenzhen) for assisting in sequencing.

Author Contributions
Conceived and designed the experiments: ZQZ LJW XWS JJZ YJZ RSN JHZ. Performed the experiments: ZQZ LJW. Analyzed the data: ZQZ LJW XWS JJZ YJZ RSN JHZ. Contributed reagents/materials/analysis tools: ZQZ LJW XWS JJZ YJZ RSN JHZ. Wrote the paper: ZQZ LJW XWS JJZ YJZ RSN JHZ.

References
1. Igwebuike UM, Ezeasor DN (2013) The morphology of placentomes and formation of chorionic vinos trees in West African Dwarf goats (Capra hircus). Veterinarski Arhiv 83: 313–321.
2. Vas J, Chojnacki R, Kjøren MF, Lyngwa C, Andersen IL (2013) Social interactions, cortisol and reproductive success of domestic goats (Capra hircus) subjected to different animal densities during pregnancy. Appl Anim Behav Sci 147: 117–126.
3. Xu T, Guo X, Wang H, Hao F, Du X, Gao X, et al. (2013) Differential gene expression analysis between anagen and telogen of Capra hircus skin based on the de novo assembled transcriptome sequence. Gene 520: 30–38. doi:10.1016/j.gene.2013.01.068 PMID: 23466980
4. Wang L, Fan J, Yu M, Zheng S, Zhao Y (2011) Association of goat (Capra hircus) CD4 gene exon 6 polymorphisms with ability of sperm internalizing exogenous DNA. Mol Biol Rep 38: 1621–1628. doi:10.1007/s11033-010-0272-2 PMID: 20927996
5. Wang PQ, Deng LM, Zhang BY, Chu MX, Hou JZ (2011) Polymorphisms of the cocaine-amphetamine-regulated transcript (CART) gene and their association with reproductive traits in Chinese goats. Genet Mol Res 10: 731–738. doi:10.4238/vol10-2gmr1091 PMID: 21523652
6. Zhang C, Luo YM, Zhang JH, He JJ, Jin L, Zhao QZ (2011) Variation of plasma INH B, ACT A and FSH concentrations during an estrus cycle in Dazu black goat and Samnen dairy goat. Yichuan 33: 607–612. PMID: 21684866
7. Peng JY, Xin HY, Han P, Zhao HB, Bai L, An XP, et al. (2013) Identification and gene expression analyses of natriuretic peptide system in the ovary of goat (Capra hircus). Gene 524: 105–113. doi:10.1016/j.gene.2013.04.054 PMID: 23644022
8. Sharma RK, Singh R, Bhardwaj JK, Saini S (2013) Topographic and Ultrastructural Variations in Isthmus Segment of Oviduct During Oestrus Cycle in Caprines. Scanning 35: 344–348. doi:10.1002/sca.21073 PMID: 23364947
9. Souza-Fabjan JM, Pereira AF, Melo CH, Sanchez DJ, Oba E, Mermillod P, et al. (2013) Assessment of the reproductive parameters, laparoscopic oocyte recovery and the first embryos produced in vitro from endangered Caninde goats (Capra hircus). Reprod Biol 13: 325–332. doi: 10.1016/j.repbio.2013.09.005 PMID: 24287041

10. Fournier A, Rychen G, Marchand P, Toussaint H, Le Bizec B, Feidt C (2013) Polychlorinated biphenyl (PCB) decontamination kinetics in lactating goats (Capra hircus) following a contaminated corn silage exposure. J Agric Food Chem 61: 7156–7164. doi: 10.1021/jf401048j PMID: 23822602

11. Ganguly A, Meur SK, Ganguly I (2013) Changes in circulatory FSH of Barbari goats following treatment with high molecular weight inhibitin isolated from buffalo follicular fluid. Res Vet Sci 95: 374–380. doi: 10.1016/j.rvsc.2013.03.013 PMID: 23602073

12. Garcia A, Masot J, Franco A, Gazquez A, Redondo E (2013) Histomorphometric and immunohistochemical study of the goat reticulum during prenatal development. Histol Histopathol 28: 1369–1381. PMID: 23843023

13. Ranjan R, Singh RK, Yasotha T, Kumar M, Puri G, Kumar M, et al. (2013) Effect of actin polymerization inhibitor during oocyte maturation on parthenogenetic embryo development and ploidy in Capra hircus. Biochem Genet 51: 944–953. doi: 10.1007/s10528-013-9619-4 PMID: 23846112

14. Ziy XD, Mu XK, Wang Y (2013) Variation in sequences and mRNA expression levels of growth hormone (GH), insulin-like growth factor I (IGF-I) and II (IGF-II) genes between prolific Lezhi black goat and non-prolific Tibetan goat (Capra hircus). Gen Comp Endocrinol 187: 1–5. doi: 10.1016/j.ygcen.2013.03.023 PMID: 23578901

15. Lin X, Luo J, Zhang L, Wang W, Gou D (2013) MiR-103 controls milk fat accumulation in goat (Capra hircus) mammary gland during lactation. PLoS One 8: e79258. doi: 10.1371/journal.pone.0079258 PMID: 24244462

16. Zhu B, Xu T, Yuan J, Guo X, Liu D (2013) Transcriptome sequencing reveals differences between primary and secondary hair follicle-derived dermal papilla cells of the Cashmere goat (Capra hircus). PLoS One 8: e76282. doi: 10.1371/journal.pone.0076282 PMID: 24069460

17. Metcalfe NB, Monaghan P (2013) Does reproduction cause oxidative stress? An open question. Trends Ecol Evol 28: 347–350. doi: 10.1016/j.tree.2013.01.015 PMID: 23485157

18. Bent ZW, Brazel DM, Tran-Gyamfi MB, Hamblin RY, VanderNoot VA, Branda SS (2013) Use of a capture-based pathogen transcript enrichment strategy for RNA-Seq analysis of the Franciscella tularensis LVS transcriptome during infection of murine macrophages. PLoS One 8: e77834. doi: 10.1371/journal.pone.0077834 PMID: 24155975

19. Lanes CF, Bizzuayehu TT, de Oliveira Fernandes JM, Kiron V, Babiak I (2013) Transcriptome of Atlantic cod (Gadus morhua L.) early embryos from farmed and wild broodstocks. Mar Biotechnol (NY) 15: 677–694. doi: 10.1007/s10126-013-9527-y PMID: 23867676

20. Chavan SS, Bauer MA, Peterson EA, Heuck CJ, Johann DJ Jr. (2013) Towards the integration, annotation and association of historical microarray experiments with RNA-seq. BMC Bioinformatics 14 Suppl 14: S4. doi: 10.1186/1471-2105-14-S14-S4 PMID: 24288045

21. Chitwood JL, Burrell VR, Meyers SA, Ross PJ (2014) RNA-seq transcriptome profiling of individual rhesus macaque oocytes and pre-implantation embryos. Reprod Fertil Dev 26: 179.

22. Faustino LR, Lima IMT, Carvalho AA, Silva CMG, Castro SV, Lobo CH, et al. (2013) Transcriptome and pathway analysis of the goat ovary. Small Rumin Res 114: 112–119.

23. Tong C, Wang X, Yu J, Wu J, Li W, Huang J, et al. (2013) Comprehensive analysis of RNA-seq data reveals the complexity of the transcriptome in Brassica rapa. BMC Genomics 14: 689. doi: 10.1186/1471-2164-14-689 PMID: 24098974

24. Xue S, Liu Y, Zhang Y, Sun Y, Geng X, Sun J (2013) Sequencing and analysis of the hemocytes transcriptome in Litopenaeus vannamei response to white spot syndrome virus infection. PLoS One 8: e76718. doi: 10.1371/journal.pone.0076718 PMID: 24204661

25. Zhao Z, Wu G, Wang J, Liu C, Qiu L (2013) Next-generation sequencing-based transcriptome analysis of Helicoverpa armigera Larvae immune-primed with Photobacterium luminescencis TT01. PLoS One 8: e80146. doi: 10.1371/journal.pone.0080146 PMID: 24302999

26. Ma H, Lu Z, Liu B, Qiu Q, Liu J (2013) Transcriptome analyses of a Chinese hazelnut species Corylus mandshurica. BMC Plant Biol 13: 152. doi: 10.1186/1471-2229-13-152 PMID: 24093758

27. Han XJ, Wang YD, Chen YC, Lin LY, Wu QK (2013) Transcriptome sequencing and expression analysis of terpenoid biosynthesis genes in Litsea cubeba. PLoS One 8: e76890. doi: 10.1371/journal.pone.0076890 PMID: 24130803

28. Harding LB, Schultz IR, Goetz GW, Luckenbach JA, Young G, Goetz FW, et al. (2013) High-throughput sequencing and pathway analysis reveal alteration of the pituitary transcriptome by...
29. He Q, Duan Z, Yu Y, Liu Z, Liang S (2013) The venom gland transcriptome of Latrodectus tredecimguttatus revealed by deep sequencing and cDNA library analysis. PLoS One 8: e81357. doi: 10.1371/journal.pone.0081357 PMID: 2412294

30. Huang YJ, Liu LL, Huang QJ, Wang ZJ, Chen FF, Zhang QX, et al. (2013) Use of transcriptome sequencing to understand the pistillate flowering in hickory (Carya cathayensis Sarg.). BMC Genomics 14: 691. doi: 10.1186/1471-2164-14-691 PMID: 24106755

31. Jorth P, Trivedi U, Rumbaugh K, Whiteley M (2013) Probing bacterial metabolism during infection using high-resolution transcriptomics. J Bacteriol 195: 4991–4998. doi: 10.1128/JB.00875-13 PMID: 23974023

32. Pashaj A, Yi X, Xia M, Canny S, Riethoven JJ, Moreau R (2013) Characterization of genome-wide transcriptional changes in liver and adipose tissues of ZDF (fa/fa) rats fed R-alpha-lipoic acid by next-generation sequencing. Physiol Genomics 45: 1136–1143. doi: 10.1152/physiolgenomics.00138.2013 PMID: 24102404

33. Ahn YK, Tripathi S, Kim JH, Cho YI, Lee HE, Kim DS, et al. (2014) Transcriptome analysis of Capsicum annum varieties Mandarin and Blackcluster: Assembly, annotation and molecular marker discovery. Gene 533: 494–499. doi: 10.1016/j.gene.2013.09.095 PMID: 24125952

34. Alkan N, Meng X, Friedlander G, Reuveni E, Sukno S, Sherman A, et al. (2013) Global aspects of pacC regulation of pathogenicity genes in Colletotrichum gloeosporioides as revealed by transcriptome analysis. Mol Plant Microbe Interact 26: 1345–1358. doi: 10.1094/MPMI-03-13-0080-R PMID: 23902260

35. Aprea J, Prenninger S, Dori M, Ghosh T, Monasor LS, Wessendorf E, et al. (2013) Transcriptome sequencing during mouse brain development identifies long non-coding RNAs functionally involved in neurogenic commitment. EMBO J 32: 3145–3160. doi: 10.1038/emboj.2013.245 PMID: 24240175

36. Fisch KM, Saito M, Akagi R, Duffy S, Su AI, Lotz MK (2013) Identification of aberrant pathways in osteoarthritis using RNA-Seq. Arthritis Rheum 65: S807–S808. doi: 10.1002/art.38216 PMID: 24085684

37. Regassa A, Rings F, Hoelker M, Cinar U, Tholen E, Looft C, et al. (2011) Transcriptome dynamics and molecular cross-talk between bovine oocyte and its companion cumulus cells. BMC Genomics 12: 57. doi: 10.1186/1471-2164-12-57 PMID: 21261964

38. Hammond Geoffrey L (2011) Diverse Roles for Sex Hormone-Binding Globulin in Reproduction. Biol Reprod 85:431–441. doi: 10.1095/biolreprod.111.092593 PMID: 21613632

39. Mamo S, Carter F, Lonergan P, Leal CL, Al Naib A, McGettigan P, et al. (2011) Sequential analysis of global gene expression profiles in immature and in vitro matured bovine oocytes: potential molecular markers of oocyte maturation. BMC Genomics 12: 151. doi: 10.1186/1471-2164-12-151 PMID: 21410957

40. Chow RW, Handelsman DJ, Ng MK (2010) Rapid Actions of Sex Steroids in the Endothelium. Endocrinology 151(6):2411–2422. doi: 10.1210/en.2009-1456 PMID: 20392826

41. Chen CS, Alonso JL, Ostuni E, Whitesides GM, Ingber DE. (2003) Cell shape provides global control of global gene expression profiles in immature and in vitro matured bovine oocytes: potential molecular markers of oocyte maturation. BMC Genomics 12: 151. doi: 10.1186/1471-2164-12-151 PMID: 21410957

42. Alldinger S, Groters S, Miao Q, Fonfara S, Kremmer E, Baumgärtner W (2006) Roles of an extracellular matrix (ECM) receptor and ecm processing enzymes in demyelinating canine distemper encephalitis. Dtsch Tierarztl Wochenschr 113: 151. doi: 10.1002/dvdy.21491 PMID: 18351675

43. Eppig JJ (1991) Intercommunication between mammalian oocytes and companion somatic cells. Bioessays 13: 569–574. PMID: 1772412

44. Gilchrist RB, Ritter LJ, Armstrong DT (2004) Oocyte-somatic cell interactions during follicle development in mammals. Anim Reprod Sci 82–83: 431–446.

45. Sun QY, Schatten H (2006) Regulation of dynamic events by microfilaments during meiotic maturation of the mouse oocyte: Integrating time and space. Reproduction 130: 801–811. PMID: 16322540

46. Brunet S, Maro B (2005) Cytoskeleton and cell cycle control during meiotic maturation of the mouse oocyte: integrating time and space. Reproduction 130: 801–811. PMID: 16322540

47. Seger R, Krebs EG (1995) The mapk signaling cascade. FASEB J 9:726–735. PMID: 7601337

48. Harrouk W, Clarke HJ (1995) Mitogen-activated protein (MAP) kinase during the acquisition of meiotic competence by growing oocytes of the mouse. Mol Reprod Dev 41: 29–36. PMID: 7619503

49. Huang H, He X (2008) Wnt/beta-catenin signaling: new (and old) players and new insights. Curr Opin Cell Biol 20: 119–125. doi: 10.1016/jceb.2008.01.009 PMID: 18339531

50. Harwood BN, Cross SK, Radford EE, Haec BE, De Vries WN (2008) Members of the Wnt signaling pathways are widely expressed in mouse ovaries, oocytes, and cleavage stage embryos. Dev Dyn 237: 1099–1111. doi: 10.1002/dvdy.21491 PMID: 18351675
51. Boyer A, Goff AK, Boerboom D (2010) Wnt signaling in ovarian follicle biology and tumorigenesis. Trends Endocrinol Metab 21:25–32. doi:10.1016/j.tem.2009.08.005 PMID: 19875303

52. Wang HX, Tekpetey FR, Kidder GM (2009) Identification of Wnt/beta-catenin signaling pathway components in human cumulus cells. Mol Hum Reprod 15:11–17. doi:10.1093/molehr/gan070 PMID: 19038973

53. Raman D, Sobolik-Delmaire T, Richmond A (2011) Chemokines in health and disease. Experimental Cell Research 317:575–589. doi:10.1016/j.yexcr.2011.01.005 PMID: 21223965