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Supplementary Material

Identification of Differentially Expressed Long Noncoding RNAs and mRNAs Involved with Dominant Follicle Selection in Goats using RNA-seq

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DESCRIPTION OF ANIMAL AND SAMPLING

The experimental conditions of this study were approved by the Committee on the Ethics of Animal Experiments of the Southwest University (No. [2007] 3) and the Animal Protection Law of China.

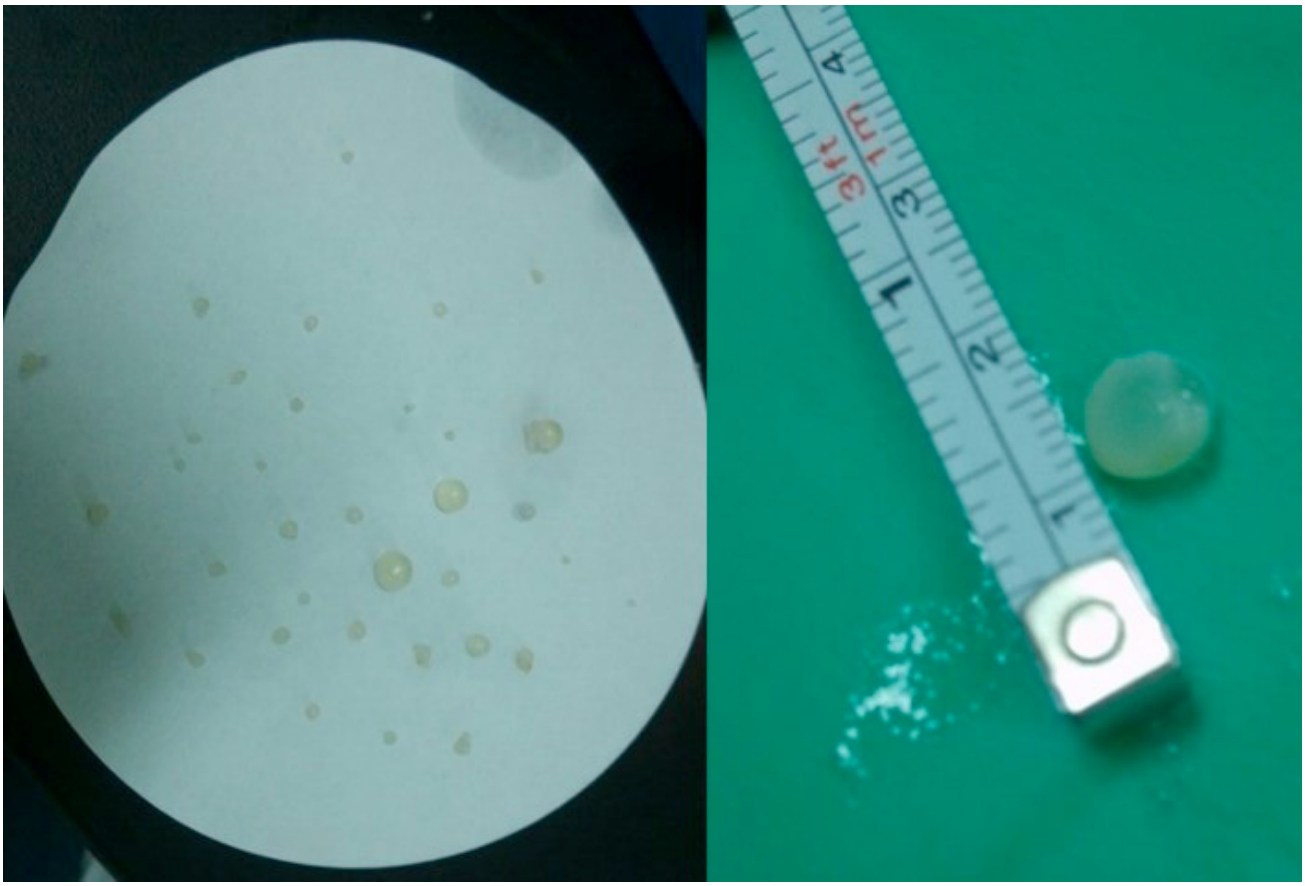
Ovaries of three samples of Dazu black goats 1.1-1.2 years old were collected on October 12, 2015 at the domestic animal conservation field of the Southwest University (Chongqing, China; E106°25′29.19″, N29°49′36.72″, 763 m), and detail information about individuals in this suty as Supplementary Table I.

The collected tissue was stored, and the follicles were stripped in 37°C saline immediately after removal.

The follicles of 3 female individuals were separated into dominant (diameter > 5 mm) and nondominant follicles (3mm < diameter < 5mm) using the standard protocol (Medan *et al*., 2005; Supplementary Fig. S1).

Supplementary Table I.- Information of experimental animal in this study.

Number	Breed	Sex	Coat Color	Body Weight (Kg)	Age (Year)
1	Dazu black goat	Female	Black	43.5	1.2
2	Dazu black goat	Female	Black	42.6	1.2
3	Dazu black goat	Female	Black	40.2	1.1



Supplementary Fig. S1. Schematic diagram of stripping follicles.

DETAIL INFORMATION OF OPERATION

RNA extraction

For the lncRNA and mRNA gene pools, total RNA was extracted from the follicle pools using TRIzol® Reagent according to the manufacturer's protocol (Invitrogen, USA), and genomic DNA was removed using DNase I (TaKara, Japan).

RNA quality was determined using a 2100 Bioanalyzer (Agilent), and RNA was quantified using the ND-2000 (NanoDrop Technologies).

Equal amounts of RNA from three different goats and the same follicle developmental phases (dominant and nondominant) were pooled.

Library preparation, genome-wide resequencing and analysis

The ribosomal RNA was removed by Epicentre Ribo-zero rRNA Removal Kits (Epicentre, Madison, WI, USA), and the rRNA-free residue was precipitated with ethanol.

In addition, high strand-specificity libraries were generated using the rRNA-depleted RNA of the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB, Ipswich, MA, USA). Briefly, the libraries were prepared as described by Ren et al. (2016).

After quantification by the Agilent Bioanalyzer 2100 system, paired-end libraries were sequenced on the Illumina HiSeq 2500 platform (2 × 125 bp read length) by Gene Denovo Technologies (Guangzhou, China).

The raw, paired-end reads were trimmed and quality controlled using SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) with the default parameters.

The clean reads of each pool were separately aligned to the C. hircus reference genome (CHIR_1.0) (Dong et al., 2012) in orientation mode using Bowtie v2.0.6 (Langmead et al., 2013) and TopHat v2.0.9 (Kim et al., 2013) software.

The coding potential and conservation of the lncRNAs were analyzed using CNCI v2 (Sun et al., 2013), iPfam (Finn et al., 2014), and PhyloCSF (Lin et al., 2011) to identify the final candidate lncRNAs for the next step in the analysis.

To identify the DEGs (differentially expressed genes) in the two different pools, the expression level for each transcript was calculated using the fragments per kilobase of exon per million mapped reads (FRKM) method. Cuffdiff (http://cufflinks.cbcb.umd.edu/) (Trapnell et al., 2012) was used for the differential expression analysis.

The DEGs of the two pools were selected using the following criteria: 1) a fold change greater than 2, and 2) a P-value FDR (false discovery rate) of less than 0.05.

To understand the functions of the differentially expressed genes, GO functional enrichment and KEGG pathway analysis were carried out using Goatools (https://github.com/tanghaibao/Goatools), KOBAS (http://kobas.cbi.pku.edu.cn/home.do) (Xie et al., 2011) and R package (clusterProfiler).

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