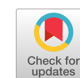
Electronic Journal of Biotechnology 39 (2019) 74–81

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Expression of *CPEB1* gene affects the cycle of ovarian granulosa cells from adult and young goats



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ARTICLE INFO

Article history:
Received 8 June 2018
Accepted 8 March 2019
Available online 14 March 2019

Keywords:
CPEB1
Follicular granulosa cell
Gene expression
Goat production
Goat
In vitro embryo transfer
Livestock improvement
Maturation of oocytes
Oocyte
Ovarian granulosa cell
RNA-binding protein

ABSTRACT

Background: CPEB is considered as an RNA-binding protein first identified in *Xenopus* oocytes. Although *CPEB*1 was involved in the growth of oocyte, its role in goat follicular granulosa cell has not been fully elucidated. To clarify the functions of this gene in goat follicular granulosa cells, *CPEB1*-overexpressing vector and interference vector were structured and transfected into follicular granulosa cells from Jiangsu native white goats of Nantong city, Jiangsu Province, China. The expression levels of differentiation-related genes including *CDK1*, *Cyclin B1*, and *C-mos* were determined 24 h after administration of *CPEB1* by quantitative real-time polymerase chain reaction and Western blotting methods.
Results: The expression levels of *CDK1*, *Cyclin B1*, and *C-mos* were significantly upregulated after overexpression and significantly downregulated after interference with *CPEB1*.
Conclusions: The *CPEB1* gene expression could affect the transcription of genes related to early cleavage divisions, which provided a reference for further research on its role in the growth and maturation of oocytes.
How to cite: Qu J, Guo H, Li Y, et al. Expression of *CPEB1* gene affects the cycle of ovarian granulosa cells from adult and young goats. Electron J Biotechnol 2019;39. https://doi.org/10.1016/j.ejbt.2019.03.002.

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1. Introduction

With the development of embryo transfer in vitro, increasing attention has been focused on the use of this technique for biotechnology applications and goat production. The use of such technology can shorten the breeding interval, make the most use of the reproductive potential of good genetic traits, and improve the genetic improvement process of livestock [1]. The quality of oocyte is a key factor that affects the success of juvenile in vitro embryo transfer (JIVET), and maintaining a certain number of granulosa cells around oocytes is a necessity for the success of juvenile in vitro fertilization (JIVF) [2]. However, studies in domestic animals have indicated that the abnormal fertilization of oocytes seems to be caused by the immaturity or malformation of oocytes [3]. Ovarian granulosa cells are distributed on the outside of the zona pellucida of the follicle, which are linked to the oocyte by gap junctions, thereby providing nutrients for the growth of oocytes. A variety of related hormones and growth factors, such as cytoplasmic polyadenylation element-binding protein *1* (*CPEB1*), cyclin-dependent kinase 1 (*CDK1*), cell cycle protein *B 1* (*cyclin B1*), and proto-oncogene protein c-mos (*C-mos*), secreted by granulosa cells affect their cell cycle and subsequently influence the growth and maturation of oocytes [4]. Previous studies have shown that *CPEB1* may be a key gene associated with the maturation of goat oocytes. *CPEB* was originally discovered in *Xenopus* oocytes [5], and subsequent studies have manifested that *CPEBs* act as an important determinant in cell development [6,7], especially in cell cycle regulation [8], cell decline [9], synaptic plasticity, memory, tumorigenesis, and progression [10]. *CPEB* is a highly conserved, sequence-specific RNA-binding protein [11] that promotes translation by inducing polyadenylation of cytoplasmic mRNAs [12,13]. Sunderland et al. [14] showed that *CPEB1* regulated cell meiosis by participating in the formation of spindle and synaptic complexes and also had a catalytic effect on the formation process.

CDK is a member of the serine/threonine kinase family, and its encoded protein is a highly conserved protein kinase complex catalyzed by subunits; this complex binds to *Cyclin B1* and subsequently forms *cyclin B1*/*CDK1* protein complexes, thereby promoting cell mitosis [15]. Thus, *CDK1* is important in regulating the cell cycle, especially in mitosis. CyclinB1 is an important cell cycle protein involved in the regulation of the G2/M phase; its content is low during the G1 phase, synthesis occurs at the S phase, activity peaks in the G2 phase, and level falls rapidly at the anaphase. CyclinB1 considered as regulatory factor combining with the catalytic subunit *CDK1* form mitosis-promoting factor (MPF), which promotes the

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Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2019.03.002
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transformation of G2 phase to M phase of cell cycle [16]. *C-mos* is a kind of proto-oncogene and a member of the serine/threonine kinase family of the germ cell-specific expression [17]. *C-mos* is also known as MAPKKK, and the kinase pathway of *C-mos* is mainly involved in the process of oocyte meiosis by activating the MOS/EKR/MAPK/p90RSK pathway [4]. The synthesis of MOS begins not long after progesterone stimulation and ceases near the end of oocyte maturation [18,19]. In this study, we used overexpression and RNA interference technologies to upregulate and downregulate the *CPEB1* gene, respectively, and we measured expression of genes closely related to regulation and control, such as *CDK1*, *Cyclin B1,* and *C-mos,* in goat follicular granulosa cells by RT-qPCR (quantitative reverse transcription PCR) and western blotting methods to untangle the role of *CPEB1* in oocyte growth.

2. Materials and methods

2.1. Collection of sample

The ovary samples used in this study were obtained from a goat market in Hai'an county of Nantong City, Jiangsu Province. Two to 8 ovarian tissues were collected from several Jiangsu native white goats that were in healthy condition; each group had 1- to 2-year-old adult ewes and 1- to 2-month-old lambs. The obtained fresh ovaries were soaked in 75% ethanol for 30 s and rinsed with 1× PBS thrice, and then the tissues were immersed in precooled 1× PBS and transported to the laboratory within 2–4 h in a 4°C cold chamber. The study protocol was approved by the Ethics Committee of Yangzhou University, with the approval number 20160510.

2.2. Separation and cultivation of goat ovarian granulosa cells

According to the procedure reported in Caloni et al. [20], we used a disposable syringe to pierce the follicles and extract the follicular fluid and paid attention to avoid blood vessels to separate and culture goat granulosa cells. The collected follicular fluid was centrifuged at 1000 rpm for 10 min at room temperature; then, the supernatant was discarded, and the precipitate was resuspended in 5% fetal bovine serum (FBS)-dissolved DMEM/F12 medium. Cells were inoculated in a culture plate at a density of 5×10^5 cells/ml and cultured under humidified environment and 5% CO_2 at 37°C in an incubator for 1–2 days and evaluated the extent of cell adherence to the plate wall and growth phases with time. The medium was changed after 24 h.

2.3. Construction of the overexpression vector

2.3.1. Total RNA extraction and reverse transcription

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA according to the method of Kingston et al. [21]. The concentration and purity of RNA were assessed with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Shanghai, China), and then the RNA was preserved at -80°C. The first cDNA was synthesized using PrimeScript II Reverse Transcriptase (RTase; Takara, Japan) following the manufacturer's instructions [Tiangen Biotech (Beijing) Co., Ltd.].

2.3.2. Primer design and synthesis

To clone the *CPEB1* gene, we designed pairs of degenerate primers according to the published *CPEB1* cDNA sequence in the NCBI database (GeneID: 102176273). The primer sequence and product length of the *CPEB1* gene listed in Table 1 were synthesized by Sangon Biotech (Shanghai, China).

2.3.3. PCR amplification and recycle of target gene

The synthesized cDNA as a template was used for PCR amplification according to the instruction of Vazyme Biotech Co., Ltd. The amplification reaction system was composed of 1 μg of the cDNA template, 1 μL of the forward primer, 1 μL of the reverse primer, 10 μL of 2× Taq PCR Master Mix, and RNase-free ddH_2O added up to 20 μL. Amplification was carried out under the following reaction conditions: 94°C for 3 min; 35 cycles of 30 s at 94°C, 60 s at 58°C, and 120 s at 72°C; and a 10 min extension at 72°C. The purity of PCR amplification products of the target gene was measured by agarose gel electrophoresis and recycled using the TIANgel Midi Purification Kit (Tiangen Biotech).

2.3.4. Construction of pcDNA3.1-CPEB1

The purified *CPEB1* PCR product was cloned into a pGEM-T vector using pGEM-T Vector Carrier Kit (Promega Biological Technology Co., Ltd.); this vector was subjected to double digestion with *Xba*I and *Hind*III, and the pGEM-T-*CPEB1* vector was transformed into *E. coli* DH5α competent cells. The recombinant plasmid with *CPEB1* products was obtained from a transformant using the HighPure Maxi Plasmid Kit (Tiangen Biotech), and the insertion was verified by agarose gel electrophoresis. It is necessary to sequence the target fragments in the recombinant plasmid with primers obtained from Shanghai Sangon Biological Co., Ltd. to confirm the validity. Furthermore, pcDNA3.1 and pGEM-T-*CPEB1* vectors were digested with *Xba*I and *Hind*III, respectively, at the same time, and fragments from both enzymes were purified by agarose gel electrophoresis, subsequently ligated with T4 ligase, and transformed into DH5α competent cells. pcDNA3.1-*CPEB1* clones were verified by PCR, plasmid DNA extraction, and sequencing. The no pcDNA3.1 vector-transfected goats were used as blank control groups, and the empty pcDNA3.1 vector-transfected goats were used as negative control groups.

2.4. Synthesis of siRNA-CPEB1

Starting from the initial code of the mRNA sequence of the target gene *CPEB1* published in the NCBI database, we searched for "AA" sequence and recorded the 19 base sequences of its 3′ end as potential siRNA target points. The justice chain and antisense chains were designed with these 19 bases (not including AA repetition). Then, the selected sequences were compared with those in a public database to ensure that the destination sequence is not homologous with other genes in the BLAST database. The no si*CPEB1* vector-transfected goats were used as blank control groups. A negative control should be used in a complete siRNA experiment, and the siRNA as a negative control should have the same composition as that of the selected siRNA sequence, but there was no evident homology with mRNA. The usual practice is to disrupt the selected siRNA sequence, as well as to check whether it is homologous with other genes. siRNAs were synthesized by Shanghai GenePharma Co., Ltd. (Table 2). After transfection of ovarian granulosa cells at 37°C for 24 h, total RNA was extracted and reverse transcribed into cDNA. *CPEB1* gene expression was evaluated by quantitative real-time PCR.

Table 1
Primer sequences of *CPEB1* gene.

Gene	Gene ID	Primer sequence (5′-3′)	Product size	Annealing temperature (°C)
CPEB1	102,176,273	*XbaI CPEB1*- F: CGTTTAAACGGCCCTCTAGAATGTCCAGACGGGAGCTGACC *HindIII CPEB1*-R: CAGCGGTTTAAACTTAAGCTTCTAGCTGGAGTCGCGGTTCTT	1824	58

Note: F represents forward primer; R represents reverse primer.

Table 2
siRNA sequences of *CPEB1*.

Group	S/A	Sequences (5′–3′)
si*CPEB1*-NC	S	5′-UUCUCCGAACGUGUCACGUTT-3′
	A	5′-ACGUGACACGUUCGGAGAATT-3′
si*CPEB1*-1	S	5′-CCACGUGUAGAAAUGCCAATT-3′
	A	5′-UUGGCAUUUCUACACGUGGTT-3′
si*CPEB1*-2	S	5′-GCAAGCUGCAGCUGUGAAUTT-3′
	A	5′-AUUCACAGCUGCAGCUUGCTT-3′
si*CPEB1*-3	S	5′-GCUGGGAUUGACACAGAUATT-3′
	A	5′-UAUCUGUGUCAAUCCCAGCTT-3′

Note: S represents sense strand; A represents anti-sense strand.

2.5. Cell transfection

When ovarian granulosa cells were cultured for 24 h with the density up to approximately 60%, transient transfections were carried out using FuGENE® HD Transfection Reagent (Invitrogen, Shanghai, China) according to the manufacturer's instructions. The ratio of pcDNA3.1-*CPEB1* and FuGENE® HD Transfection Reagent was 3:1. After 4 h of incubation, the media were substituted with growth media before cells were cultured for 48 h under humidification and 5% CO_2 at 37°C to determine the expression of related genes.

2.6. Quantitative Real-Time PCR (RT-qPCR) analysis

Total RNA was extracted from granulosa cells and treated with DNase, and then 1 μg of DNase-treated RNA was reverse-transcribed into cDNA using PrimeScript RT Reagent Kit mix (Takara, Japan) according to the manufacturer's instructions. The cDNA was then used for real-time PCR quantification of mRNAs using gene-specific forward and reverse primers (Table 3). The expression levels of *CPEB1*, *CDK1*, C*yclin B1,* and *C-mos* were assessed by quantitative fluorescence PCR using the primers synthesized by Sangon Biotech Co. Ltd. (Shanghai, China), as shown in Table 1. β-Actin was used as the internal standard. SuperReal PreMix Plus (Tiangen Biotech, Beijing, China) was used according to the manufacturer's instructions. The mRNA expression level of each gene was evaluated thrice, and the data were assessed using ABI 7500 quantitative fluorescence PCR software, v2.0.6 (Applied Biosystems) and relative gene expression levels computed by the $2^{-\Delta\Delta Ct}$ method.

2.7. Western blotting to detect related protein expression

Transient transfections used a Total Protein Extraction Kit (Solarbio, Shanghai, China) to extract total protein according to the manufacturer's instructions, and we used a standard protocol for western blotting. These cells with transient transfected pcDNA3.1-*CPEB*1/pcDNA3.1 vector were harvested and lysed by lysis buffer (250 mM sucrose, 1% Triton X-100, 2 mM EDTA, 10 mM EGTA, 50 mM Tris–HCl, pH 7.4, and 200 μg/mL leupeptin) [22]. The concentration of cell lysate was measured by the Lowry method [23]. For each line, 70 μg of protein was isolated by SDS-PAGE and electrophoresis, and then, the product of protein was transferred to a PVDF membrane according to the method given by Schägger [24]. After blocking the binding sites in the PVDF membrane with 5% nonfat milk, primary antibody was added to the membrane at room temperature and incubated for 1 h. Each immunoblot was used only once, and primary antibody dilutions were as follows: mouse anti-*CPEB1* antibody (1:400), mouse anti-*cyclin B1* antibody (1:1000), mouse anti-*CDK1* antibody (1:1000), and mouse antiactin antibody (1:1000). After washing the membrane four times with PBST, the blot was incubated with goat antimouse IgG (H + L)-HRP (1:5000) at room temperature for 1–2 h, and immunoreactive bands were visualized using a SuperSignal maximum sensitivity kit (Thermo Fisher Scientific).

2.8. Statistical analysis

One-way ANOVA was used to assess gene expression levels and the protein expression levels in adult and young goat groups. Data were presented as mean ± standard deviation, and the differences between groups were determined using Student's t test. SPSS 22.0 (SPSS, Inc., Chicago, IL, USA) software was used for data analysis, and differences were considered statistically significant when P values were < 0.05.

3. Results

3.1. Construction of pcDNA3.1-CPEB1

To investigate whether upregulated CPEB1 affects the growth of granulosa cells, we proceeded with the construction of an overexpression vector, pcDNA3.1-CPEB1. The total concentration of the pcDANA3.1-CPEB1 plasmid DNA that corresponds to each experimental component is 2 μg. As shown in Fig. 1, the purity and validity of PCR products were verified by 1% agarose gel electrophoresis. Sequencing analysis indicated that the coding sequence of CPEB1 in pcDNA3.1-CPEB1 matched with the coding sequence of *CPEB1* in the NCBI database, and sequencing verified that vector construction was successful.

3.2. Synthesis of siCPEB1

To further explore whether downregulated *CPEB1* affects the growth of granulosa cells, we proceeded with the synthesis of interference vectors, si*CPEB1*-1, si*CPEB1*-2, and si*CPEB1*-3. The interference efficiency of *CPEB1* gene expression was detected by real-time fluorescence-based quantitative PCR in each group. According to the results shown in Fig. 2, there was no significant difference in the relative expression of *CPEB1* in granulosa cells between the empty-transfected goat group and the no interference-transfected goat group ($P > 0.05$) in adult and young goats. In the adult goat group, the quantity of *CPEB1* gene expression in si*CPEB1*-1, si*CPEB1*-2, and si*CPEB1*-3 compared with that of the blank group (1.00 ± 0.06) was

Table 3
Quantitative real-time PCR primer pairs.

Genes	Gene ID	Primer sequence (5′-3′)	Product size	Annealing temperature (°C)
β-Actin	102179831	F: AGGCTGTGCTGTCCCTGTA R: CTTGATGTCACGGACGATTT	290	58
CPEB1	102176273	F: ATGCCGAAAGGGTATGTG R: CGAACACCGTCCTACTGG	232	58
CDK1	100861361	F: AGGGGTTCCTAGTACTGCA R: ATGAACTGACCAGGAGGG	174	58
Cyclin B1	102188170	F: CCCTCCAGAAATCGGTGAC R: CCCTCCAGAAATCGGTGAC	214	59
C-mos	102187061	F: TCTATTCCTTCGCCATCACCC R: CGAGTCGGTGAAGACGGGTG	137	58

Note: F represents forward primer; R represents reverse primer.

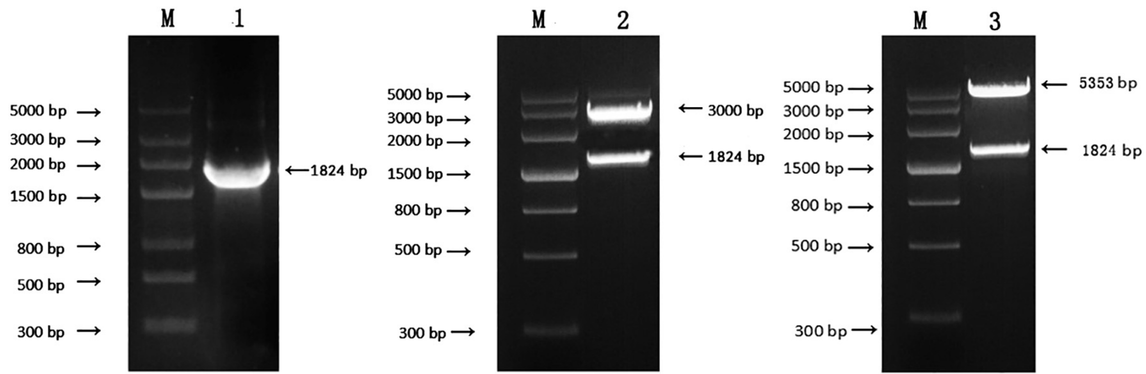

Fig. 1. PCR amplification of *CPBE1*. Lane M shows the molecular weight markers; Lane 1 represents the 1824 bp fragment of the amplification of the coding sequence of *CPEB1*; Lane 2 represents two fragments of approximately 3000 bp and 1824 bp of the pGEM-T-*CPEB1* vector yielded after digestion with restriction enzyme; Lane 3 represents two fragments of approximately 5353 bp and 1824 bp of the eukaryotic expression vector yielded, pcDNA3.1-*CPEB1, after* digestion with restriction enzyme.

reduced by 54.19% (0.45 ± 0.05 vs. 1.00 ± 0.06), 86.83% (0.13 ± 0.07 vs. 1.00 ± 0.06), and 82.55% (0.17 ± 0.05 vs. 1.00 ± 0.06), respectively. In the young goat group, the quantity of *CPEB1* gene expression in si*CPEB1*-1, si*CPEB1*-2, and si*CPEB1*-3 compared with that of the blank group (1.00 ± 0.05) was reduced by 48.30% (0.52 ± 0.07 vs. 1.00 ± 0.05), 64.48% (0.35 ± 0.05 vs. 1.00 ± 0.05), and 86.87% (0.52 ± 0.06 vs. 1.00 ± 0.05), respectively. The statistics above indicates that interference of the activity of si*CPEB1*–3 was up to 80% or more, which satisfies the requirement for subsequent study.

3.3. Real-time fluorescence-based quantitative PCR

Real-time fluorescence-based quantitative PCR was performed to investigate the expression level of related genes after changing the concentration of *CPEB1* in granulosa cells; these cells were assessed for the mRNA expression of *CPEB1*, *CDK1*, *Cyclin B1,* and *C-mos* genes, which are related to cell growth.

The result shown in Fig. 3 manifested that the mRNA expression level of *CPEB1*, *CDK1*, *Cyclin B1,* and *C-mos* genes in granulosa cells regardless of whether from the pcDNA3.1-*CPEB1*-transfected adult goat group or young goat group was significantly higher than that of the blank control group and the negative control group ($P < 0.01$), and no significant difference ($P > 0.01$) was observed between the blank control group and the negative control group. The relative gene

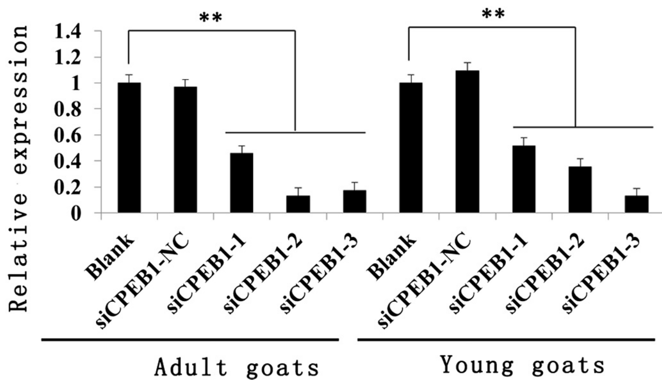

Fig. 2. *CPEB1* gene expression transfected with siRNA by real-time fluorescence-based quantitative PCR. *CPEB1* gene expression was measured after transfection with siRNA control (si*CPEB1*-NC), si*CPEB1*-1, si*CPEB1*-2, and si*CPEB1*-3. The statistics indicated that the interference efficiency of si*CPEB1*-2 is slightly lower than that in adult goat group transfected with si*CPEB1*-3, and compared with si*CPEB1*-2, *CPEB1* gene expression significantly decreased in young goat group transfected with si*CPEB1*-3. Therefore, taking a comprehensive consideration, si*CPEB1*-3 was selected to carry out the next experiment. The results are shown as the mean ± SD of three independent replicates. One-way ANOVA and t-tests were used for statistical analysis. Asterisks indicate significant differences. $^{**}P < 0.01$.

expression of *CPEB1*, *CDK1*, *Cyclin B1,* and *C-mos* in granulosa cells from the pcDNA3.1-*CPEB1*-transfected adult goat group compared to those of the blank control group increased by approximately 20-fold (19.97 ± 0.18 vs. 1.00 ± 0.49), 17-fold (17.08 ± 0.84 vs. 1.00 ± 0.58), 10-fold (9.99 ± 0.42 vs. 1.00 ± 0.39), and 10-fold (10.61 ± 0.44 vs. 1.00 ± 0.25), respectively. Furthermore, the relative gene expression of *CPEB1*, *CDK1*, *Cyclin B1,* and *C-mos* in granulosa cells in the pcDNA3.1-*CPEB1*-transfected young goat group compared to that in the blank control group increased by approximately 16-fold (15.27 ± 0.13 vs. 1.00 ± 0.49), 12-fold (12.44 ± 0.78 vs. 1.00 ± 0.58), 11-fold (10.99 ± 0.40 vs. 1.00 ± 0.39), and 7-fold (6.87 ± 0.44 vs. 1.00 ± 0.25), respectively.

In addition, as shown in Fig. 4, the mRNA expression level of *CPEB1*, *CDK1*, *Cyclin B1,* and *C-mos* genes in granulosa cells regardless of whether from the si*CPEB1*-3-transfected adult goat group or young goat group was significantly lower than that of the blank control group and the negative control group ($P < 0.01$), no significant difference was found between the blank control group and the negative control group ($P > 0.01$). The relative gene expression level of *CPEB1*, *CDK1*, *Cyclin B1,* and *C-mos* in granulosa cells from the si*CPEB1*–3-transfected adult goat group compared to that of the blank control group decreased by approximately 80% (0.17 ± 0.03 vs. 1.00 ± 0.04), 50% (0.47 ± 0.03 vs. 1.00 ± 0.03), 50% (0.53 ± 0.02 vs. 1.00 ± 0.03), and 65% (0.34 ± 0.03 vs. 1.00 ± 0.02), respectively. Additionally, the relative gene expression of *CPEB1*, *CDK1*, *Cyclin B1,* and *C-mos* in granulosa cells in the si*CPEB1*-transfected young goat group compared to that of the blank control group decreased by approximately 80% (0.13 ± 0.03 vs. 1.00 ± 0.04), 80% (0.19 ± 0.03 vs. 1.00 ± 0.03), 40% (0.64 ± 0.02 vs. 1.00 ± 0.03), and 40% (0.59 ± 0.02 vs. 1.00 ± 0.02), respectively.

3.4. Western blotting analysis of CPEB1, CDK1, and Cyclin B1 protein expression

Western blotting was performed to explore the relevant protein expression level after changing the concentration of *CPEB1* in granulosa cells, which were measured for protein expression of *CPEB1*, *CDK1*, *Cyclin B1,* and *C-mos* genes related to their growth.

The consequence shown in Fig. 5(A) suggests that the relative protein expression level of *CPEB1*, *CDK1,* and *Cyclin B1* in granulosa cells regardless of whether from the pcDNA3.1-*CPEB1*-transfected adult goat group or young goat group was significantly higher than that of the blank control group and the negative control group, and there was no significant difference between the blank control group and the negative control group. As shown in Fig. 6(A), the protein expression of *CPEB1*, *CDK1,* and *Cyclin B1* in ovarian granulosa cells from the pcDNA3.1-*CPEB1*-transfected adult goat group compared to that in the blank control group increased probably by 1.7-fold, 1.6-fold, and 1.7-fold, respectively. As seen in the Fig. 6(B), the protein

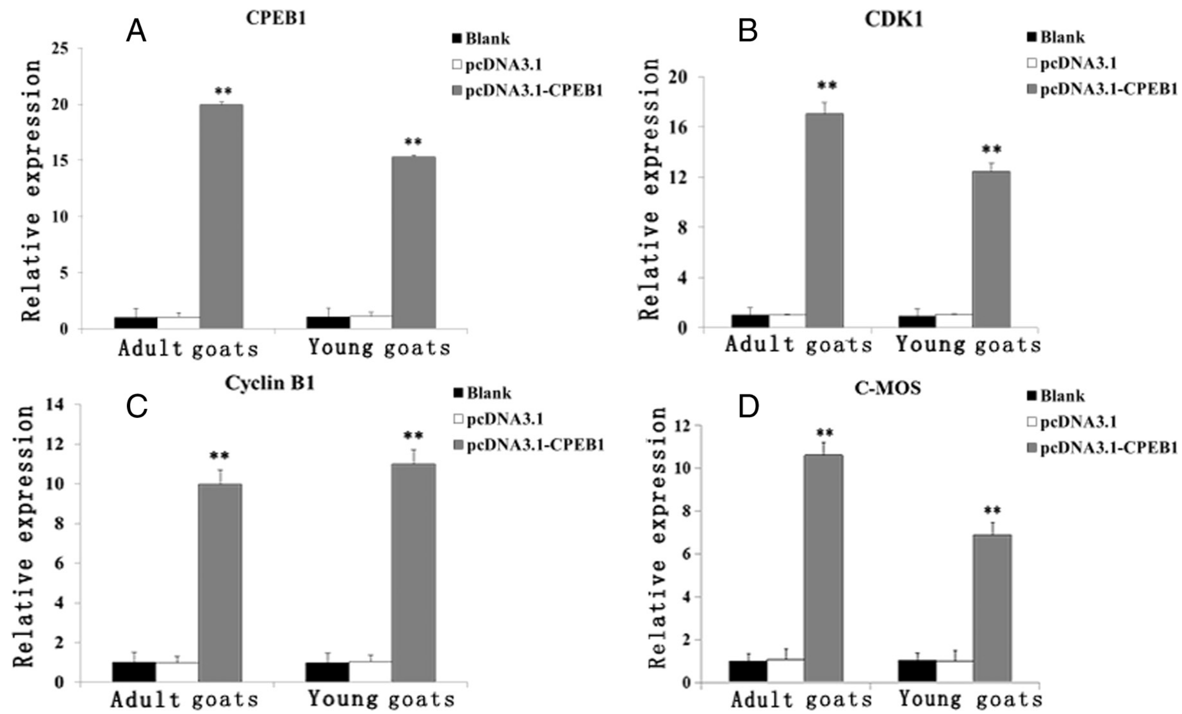

Fig. 3. Relative expression of *CPEB1*, *CDK1*, *cyclin B1*, and *C-mos* in overexpression groups. (A) *CPEB1* expression levels were significantly higher in the adult and young goat granulosa cells that were transfected with the *CPEB1* (pcDNA3.1-*CPEB1*) than those in the blank and the empty pcDNA3.1 vector-transfected group (pcDNA3.1). (B) The *CDK1* expression levels were significantly higher in the adult and young goat granulosa cells that were transfected with the *CPEB1* (pcDNA3.1-*CPEB1*) than that in the blank and the empty pcDNA3.1 vector-transfected group (pcDNA3.1). (C) *Cyclin B1* expression levels were significantly higher in the adult and young goat granulosa cells that were transfected with the *CPEB1* (pcDNA3.1-*CPEB1*) than that in the blank the empty pcDNA3.1 vector-transfected group (pcDNA3.1). (D) The *C-mos* expression levels were significantly higher in the adult and young goat granulosa cells that were transfected with the *CPEB1* (pcDNA3.1-*CPEB1*) than that in the empty pcDNA3.1 vector-transfected group (pcDNA3.1). The results are shown as the mean ± SD of three independent replicates. One-way ANOVA and t-tests were used for statistical analysis. Asterisks indicate significant differences. $^{**}P < 0.01$.

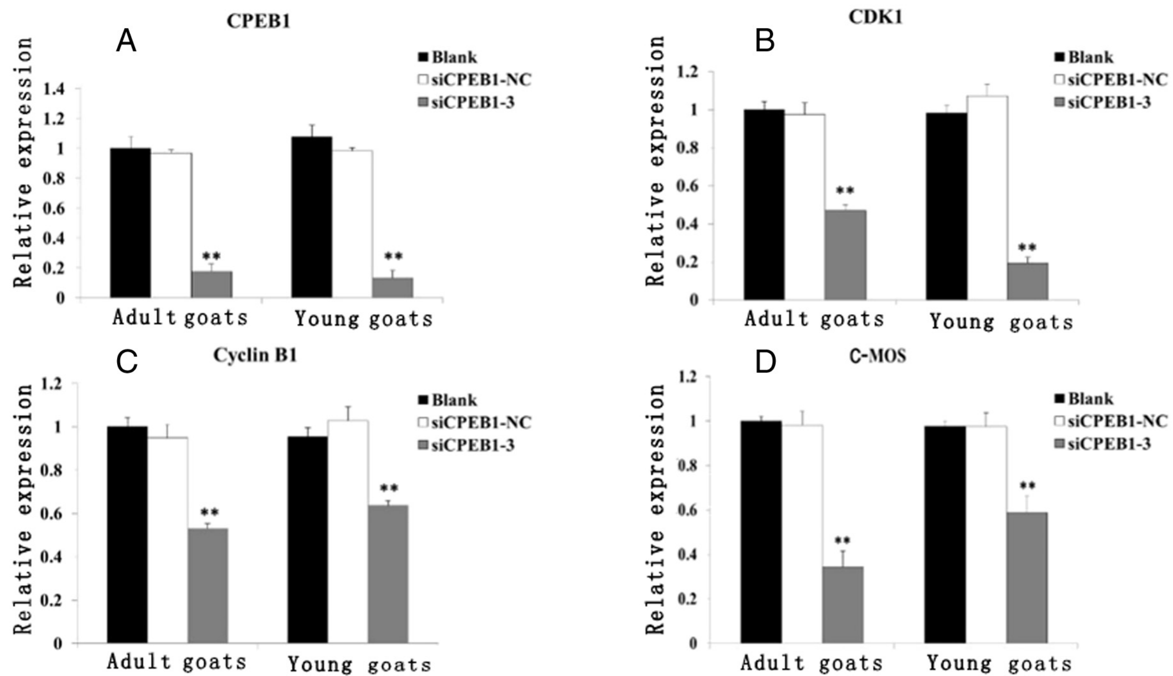

Fig. 4. Relative expression of *CPEB1*, *CDK1*, C*yclin B1*, and *C-mos* in interference groups. (A) The expression of *CPEB1* in adult and young goat granulosa cells transfected with *CPEB1*-specific siRNAs was lower than that in the blank and negative control groups. (B) The expression of *CDK1* in adult and young goat granulosa cells transfected with *CPEB1*-specific siRNAs was lower than that in the blank and negative control groups. (C) The expression of C*yclin B1* in adult and young goat granulosa cells transfected with *CPEB1*-specific siRNAs was lower than that in the blank and negative control groups. (D) The expression of *C-mos* in adult and young goat granulosa cells transfected with *CPEB1*-specific siRNAs was lower than that in the blank and negative control groups. The results are presented as the mean ± SD of three independent replicates. One-way ANOVA and t-tests were used for statistical analysis. Asterisks indicate significant differences: $^{**}P < 0.01$.

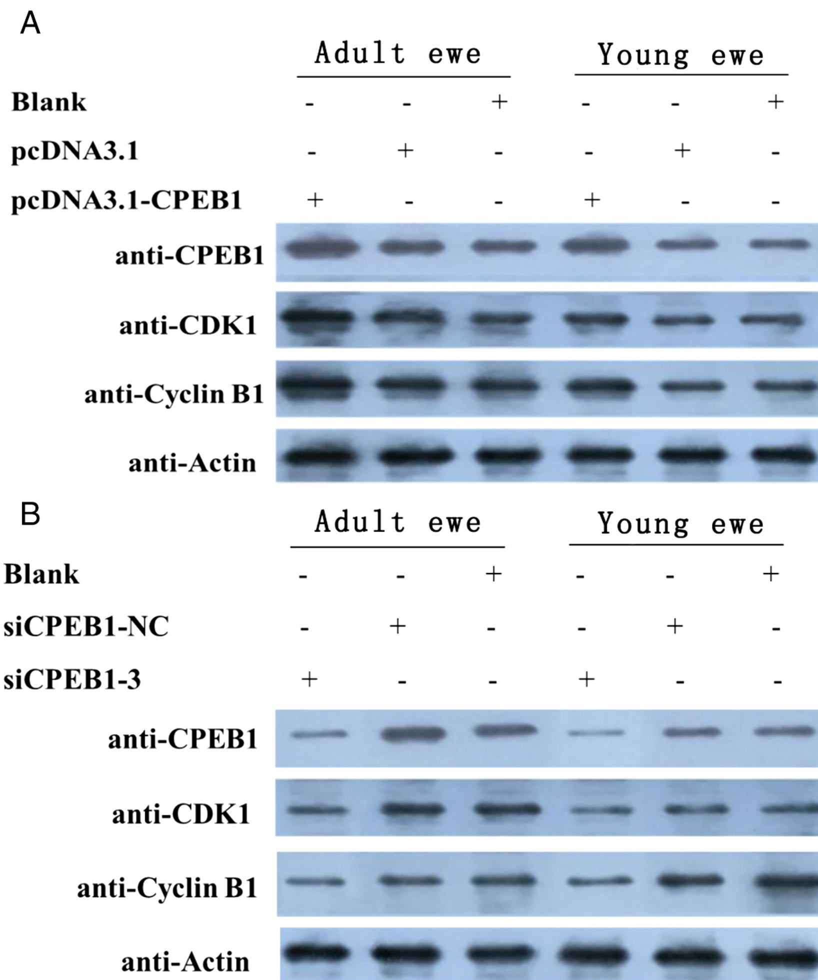

Fig. 5. Effect of overexpression or knockdown of *CPEB1* on the expression of related protein. (A) shows western blots of *CPEB1*, *CDK1*, and *cyclin B1* after transfection of ovarian granulosa cells with pcDNA3.1(+)-*CPEB1*. (B) shows western blots of *CPEB1*, *CDK1*, and *cyclin B1* after transfection of ovarian granulosa cells with siCPEB1–3.

expression of *CPEB1*, *CDK1*, and *Cyclin B1* in ovarian granulosa cells from the pcDNA3.1-*CPEB1*-transfected young goat group compared to that in the blank control group increased probably by 2-fold, 1.5-fold, and 2-fold, respectively.

The result shown in Fig. 5(B) manifested that the relative protein expression level of *CPEB1*, *CDK1*, and *Cyclin B1* in granulosa cells regardless of whether from the si*CPEB1*-3-transfected adult goat group or young goat group was significantly lower than that of the blank control group and the negative control group, and there was no significant difference between the blank control group and the negative control group. As shown in Fig. 6(C), the protein expression of *CPEB1*, *CDK1*, and *Cyclin B1* in ovarian granulosa cells from the si*CPEB1*-3-transfected adult goat group compared to the no vector-transfection adult goat group decreased probably by 55%, 40%, and 35%, respectively. As observed in Fig. 6(D), the protein expression of *CPEB1*, *CDK1*, and *Cyclin B1* in ovarian granulosa cells from the si*CPEB1*–3-*CPEB1*-transfected young goat group compared to that in the blank control group decreased probably by 65%, 35%, and 60%, respectively.

4. Discussion

To investigate the role of *CPEB1* in goat follicular granulosa cells, we upregulated and downregulated the *CPEB1* gene and assessed not only its expression but also that of *CDK1*, *Cyclin B1*, and *C-mos*. The results of this study manifested that the *CPEB1* gene was overexpressed in ovarian granulosa cells of the pcDNA3.1-*CPEB1*-transfected goats, and the relative expression level of *CDK1*, *Cyclin B1*, and *C-mos* increased significantly in ovarian granulosa cells from both adult and young goats, whereas the *CPEB1* gene was underexpressed in ovarian granulosa cells of the si*CPEB1*-3-transfected goats, and the relative expression level of *CDK1*, *Cyclin B1*, and *C-mos* decreased significantly in ovarian granulosa cells from both adult and young goats. Therefore, it is speculated that *CPEB1* is involved in the synthesis of *Cyclin B1* and upregulation of the expression level of *Cyclin B1*, which combines with *CDK1* to form a complex to accelerate the formation of spindle. The c-mos kinase pathway is mainly involved in the two meiosis processes of oocytes by activating the MOS/p90rsk/EKR/MAPK pathway. This is consistent with the research of Wilczynska et al. [25]. The cell cycle is composed of G1, S, G2, and M phases. G1 is the phase of cell growth preparation. The S phase is the period of DNA synthesis, while G2/M checkpoint is the accuracy of the genetic material after the replication. The M phase is when cells divide their genetic material and transfer it to the next generation of cells, followed by complete cell division [26]. Cell cycle of follicular granulosa cells is a complex process regulated by a variety of hormones and growth factors.

CPEB1 is an important regulator in animal cell cycle and plays an important regulatory role at the G2/M checkpoint [27]. In most cases, RNA is transcribed into mRNA and then poly(A) extension can be translated into the corresponding protein [18,28]. The role of *CEPB1* is to promote the translation process of mRNA, such as *cyclin B1* and *C-mos* cytoplasmic polyadenylation, induced by the combination of the specific sequence of *CPEB* elements of mRNA3' UTR, which is essential for the maturation of oocytes and may play an important role in the

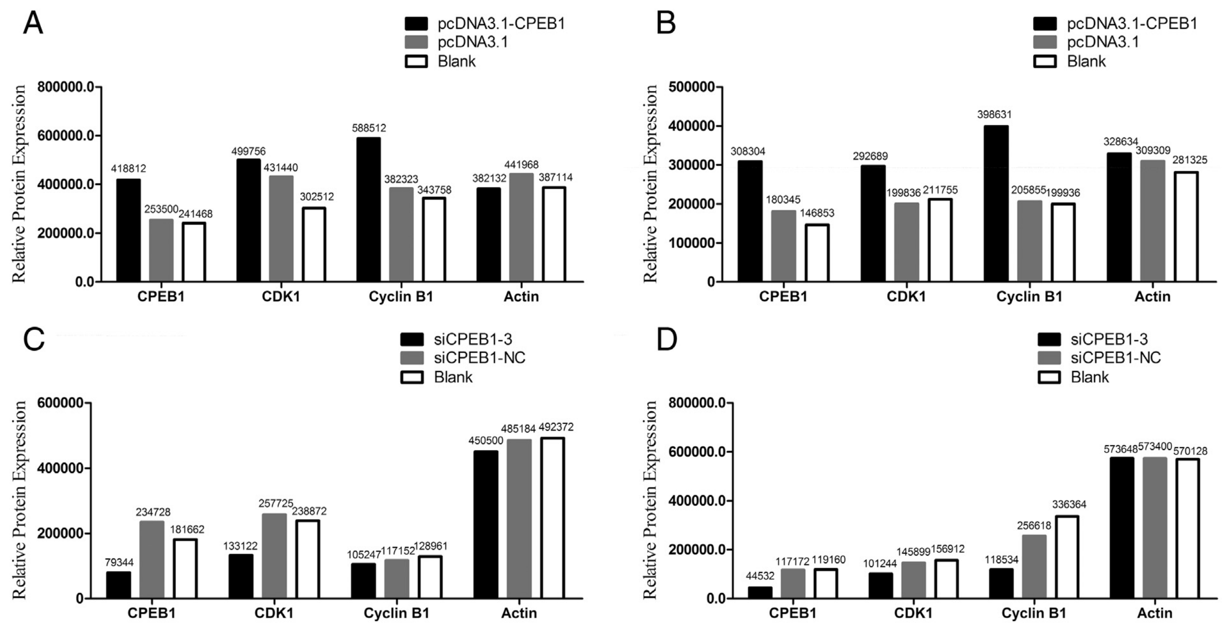

Fig. 6. Western blot grayscale values in adult and young ewes goat overexpressed/downregulated *CPEB1* group. The western blot grayscale values of *CPEB1*, *CDK1*, and C*yclin B1* proteins expressed in granulosa cells from adult goats are shown in Fig. 6-A, which indicate that the expression quantity of *CPEB1*, *CDK1*, and C*yclin B1* proteins of the cells that were transfected with the *CPEB1* overexpression vector was higher than that in negative control and blank groups. The western blot grayscale values of *CPEB1*, *CDK1*, and C*yclin B1* protein expressed in granulosa cells from young goats are shown in Fig. 6-B, which indicate that the expression level of *CPEB1*, *CDK1*, and *cyclin B1* proteins of the cells that were transfected with the *CPEB1* overexpression vector were higher than that in negative control and blank groups. The western blot grayscale values of *CPEB1*, *CDK1*, and *Cyclin B1* proteins expressed in granulosa cells from adult goats are shown in Fig. 6-C, which indicate that the expression level of *CPEB1*, *CDK1*, and *Cyclin B1* proteins of the cells that were transfected with si*CPEB1*–3 was lower than that in negative control and blank groups. The Western blot gray scale values of *CPEB1*, *CDK1*, and c*yclin B1* protein expressed in granulosa cells from young goats shown in Fig. 6-D, which indicate that the expression quantity of *CPEB1*, *CDK1*, and c*yclin B1* protein of the cells that were transfected with si*CPEB1*–3 was lower than that in negative control and blank groups.

early division and differentiation [11,13,29]. Nishimura et al. [27] found that before ovulation, the *CPEB1* gene in oocyte was overexpressed and the expression of *cyclin B1* was increased. Furthermore, the combination of *cyclin B1* and *CDK1* forms MPF, which promoted the cell to shift from the G2 phase to the M phase, and the higher the expression level of *cyclin B1* during meiosis, the stronger is the cell differentiation promotion ability. Over the years, it was reported that *CPEB4* activity is regulated by *ERK2*- and *CDK1*-mediated hyperphosphorylation, which additively activate *CPEB4* in the M phase by maintaining it in its monomeric state, and the regulation of *CPEB4* is coordinated with that of *CPEB1* through *CDK1*, which inactivates *CPEB1* further activating *CPEB4*, to integrate phase-specific signal transduction pathways and regulate cell cycle progression [30].

CDK1 regulates cell cycle, especially in the process of mitosis. In the S phase before mitosis, the protein kinases Wee1 and Myt1 catalyze the phosphorylation of, respectively, the Thr14 and Tyr15 of *CDK1* in the *cyclin B1-CDK1* complex, and hence, the function of the complex is inactivated [31]. During the G2/M phase transformation, the two sites Thr14 and Tyr15 are dephosphorylated to activate *CDK1*, which combined with cyclinB1 forms the *cyclinB1*/*CDK1* protein complex. The complex also known as MPF promotes the cells to enter into the M phase.

CyclinB1 is an important cycle protein for the regulation of the G2/M phase; its content is low in the G1 phase, synthesis occurs in the S phase, activity peaks in the G2 phase, level declines rapidly at the anaphase. When the cells enter the G2 stage, the two loci at Thr14 and Tyr15 of *CDK1* are dephosphorylated; *cyclinB1* combines with *CDK1* and forms MPF, and the cells enter into the M phase [16,31,32].

C-mos is a type of proto-oncogene, and its protein product MOS in Moroni transformation was originally recovered in the cells translated by the *Moroni mouse* sarcoma virus [17]. In the ovary, gene transcription is limited to oocytes, and the mRNA is accumulated in the growth of the GV-stage oocytes of maternal transcription. *C-mos* in GV oocytes has certain expression in germinal vesicle, and before germinal vesicle (GVBD) rupture, the expression quantity increased and began to disperse to oocyte cytoplasm. In the stimulation of progesterone (PG), *C-mos* mRNA 3′-UTR polyadenosine components (CPE) were identified and combined with *CPEB*, and the cytoplasmic polyadenylation was initiated and then the *MOS* protein synthesis was initiated within 30 min [4,33]. The study manifested that *C-mos* can prevent the degradation of *Cyclin B* and contribute to the activation of MPF [34,35]. In addition, Tachibana et al. [36] indicated that MOS is conservative in the spine and the vertebrates, and its function is not to support stagnation but, after the first meiotic division, to prevent meiosis before fertilization mitotic conversion to ensure the mother cell ploidy in half. A relevant study revealed that *CPEB* specifically combined with the CPEs-contained mRNA, which are crucial for the regulation of meiotic maturation and cell cycle transitions and translation of the mRNA, such as those encoding Mos, cyclin B proteins [27]. This result is consistent with the results of the research.

Animal superovulation, oocyte in vitro maturation (IVM), in vitro fertilization (IVF), and embryo culture in vitro (IVC) are the four important components for JIVET, which is a high-tech biological system [3,37]. Researchers have shown that using JIVET on a field scale in China was evaluated from 2006 to 2007. Their study showed that each donor lamb after stimulation produced an average of 48.6 transferable embryos that resulted in 4.04 viable and healthy progeny [38]. These results indicate that JIVET is a cost-effective method of multiplying desirable sheep genotypes in China. In addition, the quality of oocyte is a key factor affecting the success of JIVF, and maintaining a certain number of granulosa cells around oocytes is a necessity for the success of JIVF. However, problems that IVET has a low survival rate, stunted growth, and high malformation rate still exist. This may be related to some related regulatory hormones that cannot reach certain concentration in the immature oocytes. In previous studies, *CPEB1* was found to regulate cell meiosis by promoting the formation of spindle and synaptic complexes [27]. Thus, we evaluated the *CPEB1* influence on the expression of related genes in ovarian granulosa cells through adjustment of *CPEB1*

expression to increase the rate of mature oocytes for the improvement and application of JIVET technology to provide theoretical reference.

In our investigation, we found that expression of *CPEB1*, *CDK1*, *cyclin B1*, and *C-mos* in goat follicular granulosa cells transfected with pcDNA3.1-*CPEB1* or *CPEB1*-specific siRNAs was significantly altered. In our future study, we will focus on the expression of *CPEB1* that affects cell cycle of the oocyte maturation of goat ovary.

Financial support

This work was supported by funding from the Key Natural Science Program of Jiangsu Higher Education Institutions (13KJA230001) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD2011-137).

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