



# The Effect of miR-1468 on Growth Hormone in Pituitary Cells of Yanbian Yellow Cattle

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## ABSTRACT

At present, the relationship between miRNAs and *GH* has been reported. However, the effect of miR-1468 on growth hormone (*GH*) is unknown. The objective of this study was to evaluate effects of miR-1468 on *GH* in pituitary cells of Yanbian yellow cattle in this study. Real-time quantitative PCR (qPCR), Western blot, Targetscan and RNAhybrid analysis software and Dual-Luciferase reporter gene system was used to analyze the regulation mechanism between miR-1468 and *GH*. The results showed that the *GH* mRNA and protein level in pituitary cells of Yanbian yellow cattle could be significantly decreased by adding miR-1468 mimics ( $P < 0.01$ ), while these were significantly increased by adding miR-1468 inhibitor ( $P < 0.05$ ). The results of bioinformatics analysis and double luciferase reporter gene system validation showed that miR-1468 targeted 3'UTR of cAMP responsive element binding protein 1 (*CREB1*). miR-1468 mimics could significantly inhibit the *CREB1* mRNA and protein level ( $P < 0.01$ ), whereas miR-1468 inhibitor could significantly increase the *CREB1* mRNA and protein level ( $P < 0.01$ ). This study demonstrated that miR-1468 could regulate *GH* in pituitary cells by regulating the expression of *CREB1* gene. The results of this study will provide a theoretical basis for studying the mechanism of growth and development of animals regulated by miRNAs.

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### Authors' Contribution

L-zG and C-dC designed the experiments. T-hJ and L-zY wrote the manuscript. A-gL and J-xJ performed animal and *in vitro* experiments and most analysis with the help of co-authors.

### Key words

miR-1468, Yanbian yellow cattle, Pituitary cells, *GH*, *CREB1*

## INTRODUCTION

Yanbian yellow cattle are characterized by slow growth rate, long growth cycle, which lead to the waste of feed resources and increase of feeding costs and consequently negative effects on the Yanbian yellow cattle breeding industry (Han *et al.*, 2010; Tian *et al.*, 2011; Ji *et al.*, 2014). Therefore, how to improve the growth rate of Yanbian cattle is the key to accelerate the rapid development of Yanbian cattle breeding industry.

Some studies have shown that miRNAs in play an important role in regulating the growth and development of animals. For example, Melnik *et al.* (2013) found that miRNA-21 in milk could promote calf growth. Chen *et al.* (2014) used miR-PC-86 and miR-PC-263 derived from pig to treat C2C12 cells and found that miR-PC-86 and miR-PC-263 could regulate the expression of IGF-1R receptor on C2C12 cells and further regulate the growth of porcine muscle cells.

The growth axis plays a key role in the growth and development of animals (Vgontzas *et al.*, 2010;

Allen *et al.*, 2011; Bustin and Jenkins, 2001). The growth hormone (GH) secreted by the pituitary gland in the growth axis is one of the most important members in the regulation of animal growth (Piotrowska *et al.*, 2017; King *et al.*, 2005). GH can be transported by binding to growth hormone binding protein (GHBP) and to growth hormone receptor (GHR) in target organs, which promotes the production of insulin-like growth factors (IGFs). IGFs are then transported to whole body tissues and cells by binding to insulin-like growth factor binding protein (IGFBP) to stimulate the growth and differentiation of bone and chondrocyte and regulate the metabolism of protein, sugar and fat, etc. (Gesing, *et al.*, 2016; Bartke *et al.*, 2003). Some studies have shown that there is a relationship between miRNAs and *GH* gene. Qi *et al.* (2015) found that mi-141-3p might regulate *GH* by targeting SOCS7 (suppressor of cytokine signaling) and inhibiting CREB1 (cyclic AMP response element binding protein 1) expression and activity. In our previous study, the miRNAs related to *GH* regulation in the pituitary of Yanbian yellow cattle were analyzed by RNASEQ sequencing and bioinformatics techniques, and finally miR-1468 was selected out (Lou *et al.*, 2019).

However, the effect of miR-1468 on GH secretion in pituitary of Yanbian yellow cattle is still unclear.

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Therefore, the purpose of this study is to explore the regulation mechanism of miR-1468 on GH secretion in pituitary of Yanbian yellow cattle at the level of pituitary cells *in vitro*.

## MATERIALS AND METHODS

### *Primary culture of pituitary cells of Yanbian yellow cattle*

The pituitary tissues of three Yanbian yellow cattle were separated under aseptic conditions and cut into 1 mm×1 mm tissue mass, respectively and then were washed with PBS for three times at pH 7.0. The tissue precipitation was transferred to culture flask, and 0.5 mL 0.25% collagenase and trypsin (TaKaRa, Tokyo, Japan) were added to each pituitary tissues and incubated in shaking bed at 37 °C for 25 min. The pituitary cells were filtered with 100 meshes cell sieve. The filtrate was centrifuged at 4°C, 2 000 rpm for 10 min and the supernatant was discarded. Cells were suspended in DMEM/F12 medium (QIAGEN, Hilden, Germany) and cultured in 75 mL culture flask at 37 °C and 5% CO<sub>2</sub> incubator. When the cells grew up to 80% of convergence, they were digested with 0.25% collagenase (TaKaRa, Tokyo, Japan). The cells were isolated by differential adherence method and primary cultured pituitary cells were obtained.

### *Expression level of miR-1468 in pituitary cells*

After the primary culture of Yanbian yellow cattle pituitary cells was established, the pituitary cells were inoculated into 6-well culture plate according to the density of 2.0×10<sup>6</sup> cells/hole, and transfection test was carried out when the cell confluence was 60%-70%. The pituitary cells were washed with 2 mL PBS once before transfection, and 0.8 mL DMEM/F12 medium was added. Mimics (miR-1468-mi group), mimics reference substance (NC group), inhibitor (miR-1468-in group) and inhibitor reference substance (iNC group) of miR-1468 were diluted to 100 μL DMEM/F12 medium respectively. The miR-1468 mimic, inhibitor, NC and inhibitor were purchased from Sangon Biotech Co. (Shanghai, China). The sequences were as follows: miR-1468 mimic (5'-CUCCGUUUGCCUGUUUUGCUGA-3'), miR-1468 inhibitor (5'-UCAGCAAACAGGCAAACGGAG-3'), NC (5'-UCAAACGCCACCGUAG CAUUC -3'), and iNC (5'-GAGUAACGCCCUAGUUGACCU-3'). The 10 μL transfection reagent Lipofectamine™ 2000 (QIAGEN, Hilden, Germany) was diluted to 100 μL DMEM/F12 culture medium and then mixed with above diluents in a ratio of 1:1, respectively. After incubation at room temperature for 10 min, 200 μL mixture was added into the cell pore of the preconditioned cells, and the final concentration of mimics, mimics reference substances, inhibitor and inhibitor reference substances of miR-1468

was 200 pmol/L, with three replicates in each group. After culture for 48 h, the cells were collected and the total RNA was extracted. miR-1468 expression level was detected using fluorescence quantitative PCR (qPCR).

Total RNA was extracted from pituitary cells by Trizol reagent and digested with *DNase I* (TaKaRa, Tokyo, Japan) to remove trace DNA contamination. The cDNA synthesis was catalyzed by M-MLV reverse transcriptase (TaKaRa, Tokyo, Japan) using total RNA as a template and the specific neck loop primer (5'CTCAACTGGTGTCTGGAGTCGGCAATTAGTTGAGCACAAATT3') (Qi *et al.*, 2015). Primer Premier 6.0 software was used to design the forward and reverse primers for the PCR amplification of miRNA and *U6* genes. The forward and reverse primers of miR-1468 were 5'-CCTACCCTGTAGAACCG-3' and 5'-TGGTGTCTGGAGTCG -3', respectively; The forward and reverse primers of *U6* were 5'-CTCGCTTCGGCAGCAC-3' and AACGCTTCACGAATTTGCGT, respectively. PCR mixture contained 5 μL of SYBR Green Master Mix (TaKaRa, Tokyo, Japan), 0.5 μL of 10 mM each of primers, 1 μL of cDNA and 3 μL of PCR water. The PCR conditions were as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 56 °C for 20 s and 72 °C for 20 s, and the fluorescence signals were then collected. The expression level of miR-1468 was calculated by the 2<sup>-ΔΔCT</sup> method. The formula is as follows: ΔCt=miR-1468 Ct-U6 Ct; ΔΔCt=[(miR-1468 Ct-negative control Ct)-(U6 Ct-negative control Ct)]

### *Effect of miR-1468 on GH mRNA transcription level in Yanbian yellow cattle pituitary cells*

The cDNA synthesise was catalyzed by M-MLV reverse transcriptase (TaKaRa, Tokyo, Japan) using total RNA as template and OligodT-18 as primer. Primer Premier 6.0 software was used to design upstream and downstream primers of *GH* gene and *β-actin* gene in Yanbian yellow cattle. The forward and reverse primers of *β-actin* were: CCACGAAACTACCTTCAACTC and CCCACGAAACTACCTTCAACTC, respectively. The forward and reverse primers of *GH* were: CTCCAACCTGCTGGCTGCCGACAGCTA and CGATGTCTGCTGGGCTCGTCC, respectively. The PCR reaction system consisted of 10 μL of SYBR Green Master Mix, 0.3 μL of 10 mM each of primers, 1 μL of cDNA and 8.4 μL of sterile water. The PCR reaction procedure was: 95°C for 1 min, then 40 cycles of 95 °C for 15 s, 55 °C for 15 s and 72 °C for 40 s, and the fluorescence signals were collected at the end of the extension. The expression level of *GH* gene was calculated by 2<sup>-ΔΔCT</sup> method. The formula was: ΔCt=*GH* Ct-*β-actin* Ct. ΔΔCt=[(*GH* Ct-negative control Ct)-(β-actin Ct-negative control Ct)].

#### *Effect of miR-1468 on GH protein expression in pituitary cells of Yanbian yellow cattle*

According to the steps of the total protein extraction kit (Roche, Basel, Switzerland), the total protein was extracted from the above obtained cells, and the GH protein was detected by Western blot method. The specific steps were as follows: 10% polyacrylamide gel electrophoresis (PAGE) separating gel and 5% PAGE concentrating gel were prepared according to the conventional method and put into the vertical electrophoresis tank. After solidification, the wood comb was pulled out and the 1×glycine buffer was added into the comb hole, then 50 g prepared total protein sample was added into the comb hole for electrophoresis. After electrophoresis, the protein glue was transferred to polyvinylidene fluoride (PVDF) membrane (Roche, Basel, Switzerland) with a voltage of 110 V for 60-70 min. Then the PVDF membrane was taken out and stained with Ponceau red to test the effect of transmembrane. The PVDF membrane was washed for several times with 1×Tris-HCl and tween buffer (TBST) solution to remove the Ponceau dye solution, and then sealed for 2 h at room temperature with 5% skimmed milk powder. After that, the sealed PVDF membrane was placed in a 5 mL centrifugal tube and 2 mL first antibody (rabbit, monoclonal antibody) (Bioss, Beijing, China) of GH protein was added in to the centrifugal tube and incubated for 12 h at 4 °C. After incubation, PVDF membrane was washed 3 times with 1×TBST solution for 10 min each time. The washed PVDF membrane was placed in a new 5 mL centrifugal tube and incubated at room temperature for 2 h with 2 mL 1:5000 diluted second antibody (mouse monoclonal antibody) (Bioss, Beijing, China) of GH protein. After incubation, the PVDF membrane was washed 3 times with 1×TBST for 5 min each time. Then the PVDF membrane was placed in the luminescent liquid for 1 min and exposed in the gel imaging system. The relative expression of GH protein was calculated by comparing with the expression of  $\beta$ -actin as a reference gene.

#### *Target gene prediction of miR-1468*

Targetscan and RNA hybridization analysis software were used to predict the target relationship between miR-1468 and GH related genes including growth hormone releasing hormone receptor (*GHRHR*), somatostatin receptor 2 (*SSTR2*), lymphoid enhancer 1 (*LEF1*), pituitary specific transcription factor 1 (*POU1F1*), somatostatin receptor 5 (*SSTR5*) and cyclic adenosine phosphate effector binding protein 1 (*CREB1*).

#### *Verification of the target relationship between miRNA-1468 and CREB1*

The upstream and downstream sequence within 30 bp

of *CREB1* 3'UTR region matched with the seed sequence of miR-1468 was synthesized, and the 5 bases of the seed sequence of miR-1468 were mutated artificially as a control. The synthetic fragments and mutant fragments were digested by *XhoI* and *XbaI* (TaKaRa, Tokyo, Japan). Then the recombinant pirGLO-*CREB1*-3'UTR expression vector and mutant vector were constructed by linking the digested products with the luciferase gene report vector (pirGLO vector), respectively. Mimic (miR-1468-mimic group) and mimics reference substance (NC group) of miR-1468 and the normal or mutant vector of pirGLO-*CREB1*-3'UTR were diluted to 200 pmol/L respectively, and then co-transfected into Chinese hamster ovary cell (CHO) cells by transfection reagent Lipofectamine™ 2000. The specific transfection process was as described above. Cells were collected and lysed after incubating for 48 h and luciferase activity was detected by luciferase detection kit (Promega, Wisconsin, USA).

#### *Effects of miR-1468 on the transcriptional level and protein expression of CREB1 in pituitary cells of Yanbian yellow cattle*

The mRNA transcription level and protein expression of *CREB1* were evaluated by the detection methods for GH. *CREB1* fragment was amplified by forward (ACCCATGCTACTGTCCACTA) and reverse (GAAAGCCCTAGGCTGAATGA) primers, respectively. For Western blot test, the PVDF membrane containing *CREB1* protein was incubated with 2 mL 1:500 diluted first rabbit monoclonal antibody (Bioss, Beijing, China) against *CREB1* protein and 2 mL 1:5000 diluted second mouse monoclonal antibody (Bioss, Beijing, China).

#### *Statistical analysis*

All statistical analyses were performed using SPSS 17.0 statistical software (SPSS 17.0, Chicago, IL, USA). A t-test was performed to examine the significant difference among treatments.  $P < 0.05$  represented significant difference,  $P < 0.01$  represented extremely significant difference.

## RESULTS

#### *Expression level of miR-1468 in pituitary cells*

The pituitary cells were transfected with miR-1468 mimics and inhibitor, and qPCR was used to detect miR-1468 expression. Transfection of miR-1468 mimics significantly increased miR-1468 expression level, while transfection of miR-1468 inhibitor significantly decreased miR-1468 expression level ( $P < 0.01$ ) (Fig. 1A and 1B).

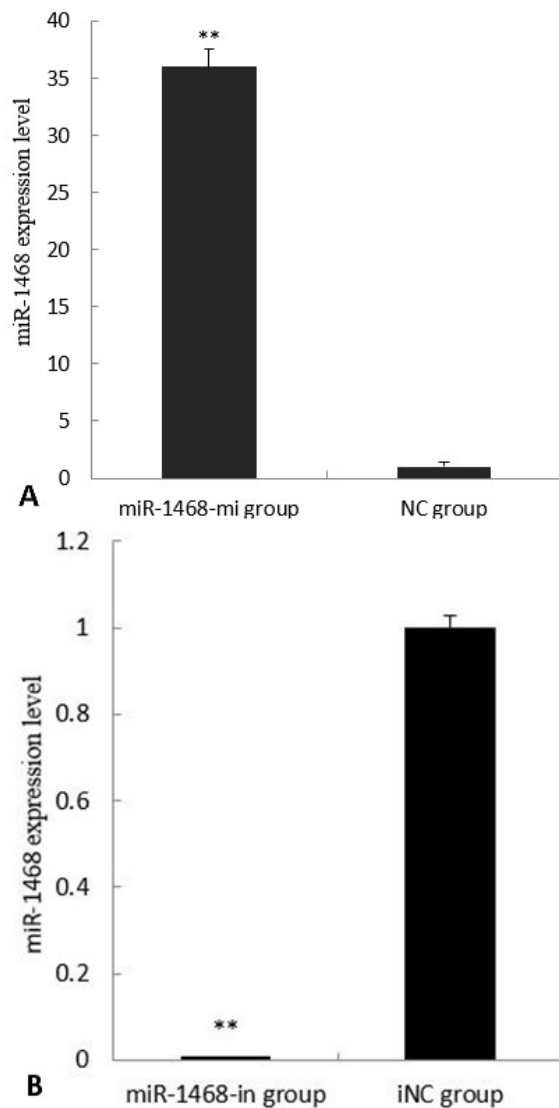


Fig. 1. Expression level of miR-1468 in pituitary cells of Yanbian yellow cattle. (A) The expression level of miR-1468 in pituitary cells transfected with the mimics (miR-1468-mi group) and mimics reference substance (NC group) of miR-1468. Compared with NC group, the column marked by \*\* showed significant difference ( $P<0.01$ ); (B) The expression level of miR-1468 in pituitary cells transfected with the inhibitor (miR-1468-in group) and inhibitor reference substance (iNC group) of miR-1468. *U6* was used as an internal reference.

#### Effect of miR-1468 on *GH* mRNA transcription level

The transcriptional level of *GH* gene in Yanbian yellow cattle was detected by qPCR. The results showed that the *GH* mRNA transcription level in pituitary cells of Yanbian yellow cattle in miR-1468-mi group was extremely significantly lower than that in NC group ( $P<0.01$ ) (Fig.

2A), while the *GH* mRNA transcription level in pituitary cells of Yanbian yellow cattle in miR-1468-in group was significantly higher than that in iNC group ( $P<0.05$ ) (Fig. 2B). These results suggested that miR-1468 could regulate *GH* transcription.

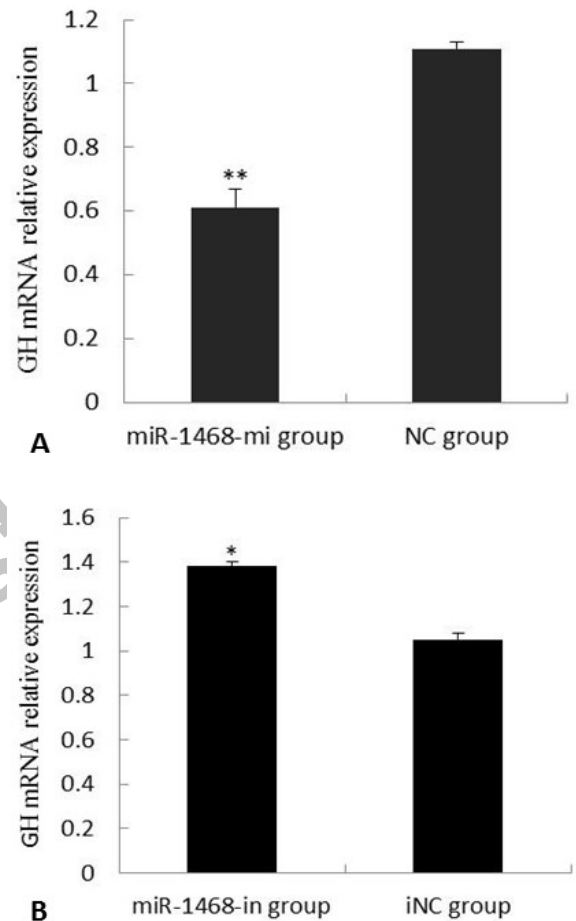


Fig. 2. Effect of miR-1468 on *GH* mRNA transcription level in pituitary cells of Yanbian yellow cattle. (A) The relative transcription level of *GH* mRNA in pituitary cells transfected with miR-1468 mimics. Mimics (miR-1468-mi group) and mimics reference substance (NC group) of miR-1468 were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with NC group, the column marking\*\* showed extremely significant difference ( $P<0.01$ ); (B) The relative transcription level of *GH* mRNA in pituitary cells transfected with miR-1468 inhibitor. Inhibitor (miR-1468-in group) and inhibitor reference substance (iNC group) of miR-1468 were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with iNC group, the column marking\* showed significant difference ( $P<0.05$ ).  $\beta$ -actin was used as an internal reference.

### Effect of miR-1468 on GH protein expression level

In order to further verify the effect of miR-1468 on GH, GH protein expression level in Yanbian yellow cattle was further detected by Western blot based on the transcription results of GH mRNA. The results showed that GH protein expression level in pituitary cells of Yanbian cattle in miR-1468-mi group was extremely significantly lower than that in NC group ( $P<0.01$ ) (Fig. 3A), while GH protein expression level in pituitary cells of Yanbian yellow cattle in miR-1468-in group was extremely significantly higher than that in iNC group ( $P<0.01$ ) (Fig. 3B). These results suggested that miR-1468 could regulate GH protein expression.

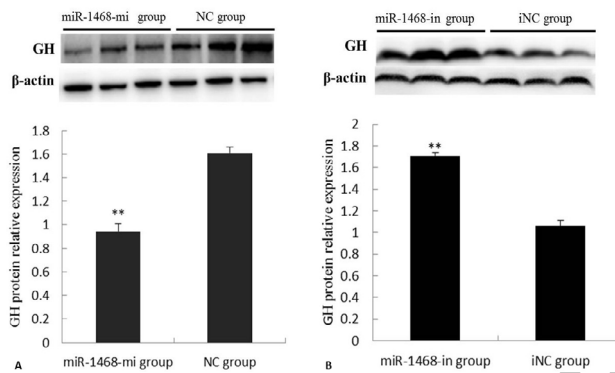


Fig. 3. Effect of miR-1468 on GH protein expression level in pituitary cells of Yanbian yellow cattle. (A) The relative expression level of GH protein in pituitary cells transfected with miR-1468 mimics. Mimics (miR-1468-mi group) and mimics reference substance (NC group) of miR-1468 were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with NC group, the column marking\*\* showed extremely significant difference ( $P<0.01$ ); (B) The relative expression level of GH protein in pituitary cells transfected with miR-1468 inhibitor. Inhibitor (miR-1468-in group) and inhibitor reference substance (iNC group) of miR-1468 were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with iNC group, the column marking\*\* showed significant difference ( $P<0.01$ ).  $\beta$ -actin was used as an internal reference. Electrophoresis of the GH and  $\beta$ -actin gene fragment was from two separate gels.

### Prediction of the target relationship between miR-1468 and GH secretion related genes

The targets can and RNA hybrids analysis software were used to analyze the target relationship between miR-1468 and GH secretion related genes (*GHRHR*, *SSTR2*, *LEF1*, *POU1F1*, *SSTR5* and *CREB1*). The results showed that the target gene of miR-1468 was *CREB1* (Fig. 4).



Fig. 4. Prediction of target relations for miR-1468.

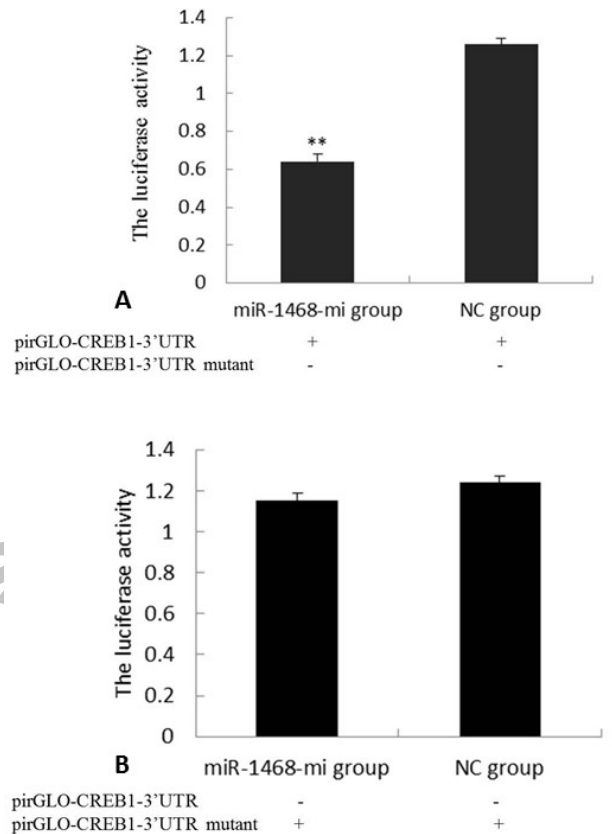


Fig. 5. Luciferase activity determination. (A) The changes of luciferase activity after PirGLO-*CREB1*-3'UTR normal plasmid co-transfecting with miR-1468 mimics (miR-1468-mi group) and mimics reference substance (NC group) for 48 h. Compared with NC group, the column marking\*\* showed extremely significant difference ( $P<0.01$ ); (B) The changes of luciferase activity after PirGLO-*CREB1*-3'UTR mutant plasmid plasmid co-transfecting with miR-1468 mimics (miR-1468-mi group) and mimics reference substance (NC group) for 48 h. Compared with NC group, the column without marking\*\* or\* showed no significant difference ( $P>0.05$ ).

### Verification of the target relationship between miR-1468 and CREB1

The change of luciferase activity was observed 48 h later, and the target relationship between miR-1468 and *CREB1* was analyzed by the changes of luciferase activity. The results showed that the luciferase activity in pirGLO-

*CREB1*-3'UTR normal plasmid was significantly decreased by adding miR-1468 mimics (Fig. 5A) ( $P<0.01$ ), but no inhibitory effect on that in the pirGLO-*CREB1*-3'UTR deletion vector ( $P>0.05$ ) (Fig. 5B). It could be concluded that miR-1468 could bind and act on the 3'UTR region of *CREB1*, and there is a target relationship between miR-1468 and *CREB1*.

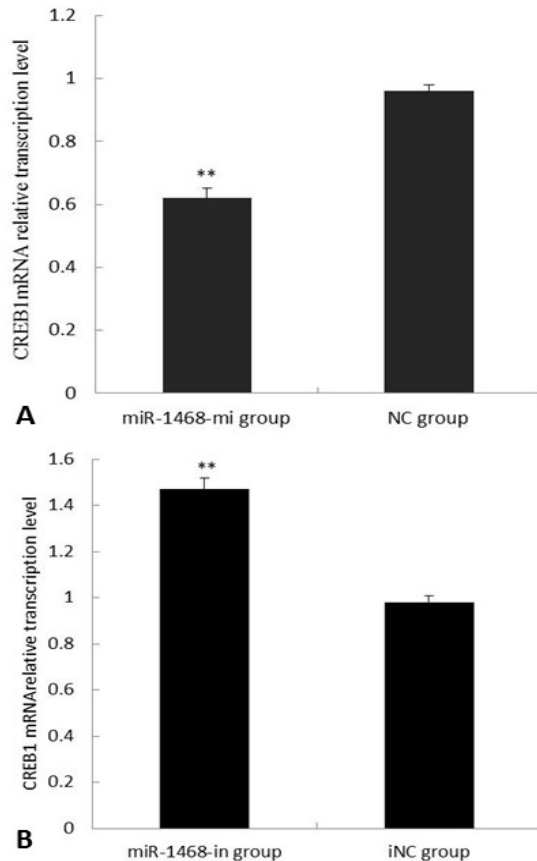


Fig. 6. Effect of miR-1468 on transcription level of *CREB1* mRNA in pituitary cells of Yanbian yellow cattle. (A) The relative transcription level of *CREB1* mRNA in pituitary cells transfected with miR-1468 mimics. Mimics (miR-1468-mi group) and mimics reference substance (NC group) of miR-1468 were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with NC group, the column marking\*\* showed extremely significant difference ( $P<0.01$ ); (B) The relative transcription level of *CREB1* mRNA in pituitary cells transfected with miR-1468 inhibitor. Inhibitor (miR-1468-in group) and inhibitor reference substance (iNC group) of miR-1468 were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with iNC group, the column marking\*\* showed extremely significant difference ( $P<0.01$ ).  $\beta$ -actin was used as an internal reference.

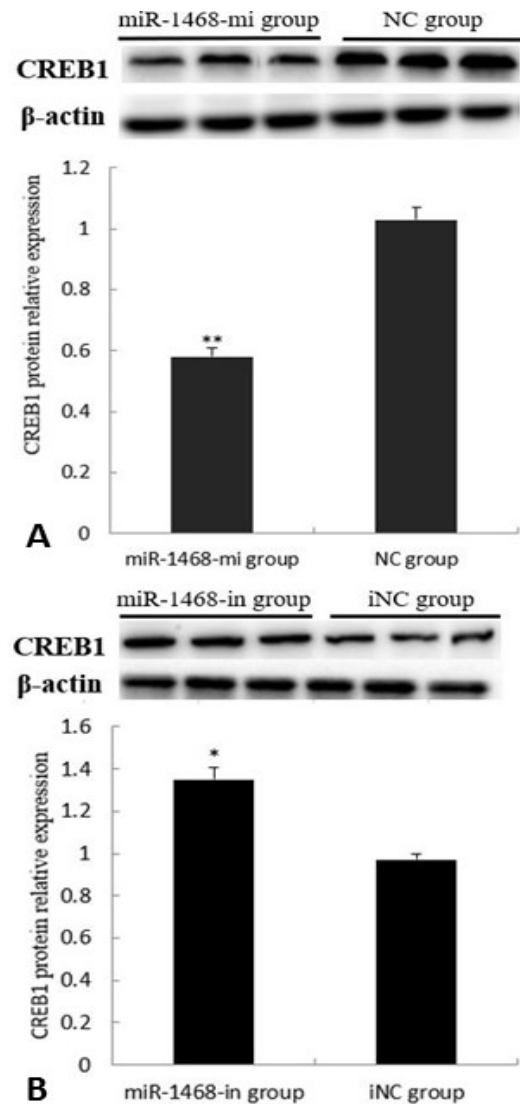


Fig. 7. Effect of miR-1468 on CREB1 protein expression level in pituitary cells of Yanbian yellow cattle. (A) The relative expression level of CREB1 protein in pituitary cells transfected with miR-1468 mimics. Mimics (miR-1468-mi group) and mimics reference substance (NC group) of miR-1468 were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with NC group, the column marking\*\* showed extremely significant difference ( $P<0.01$ ); (B) The relative expression level of CREB1 protein in pituitary cells transfected with miR-1468 inhibitor. Inhibitor (miR-1468-in group) and inhibitor reference substance (iNC group) of miR-1468 were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with iNC group, the column marking\* showed significant difference ( $P<0.05$ ).  $\beta$ -actin was used as an internal reference. Electrophoresis of the *CREB1* and  $\beta$ -actin gene fragment was from two separate gels.

*Effect of miR-1468 on transcription level of CREB1 mRNA*

In order to verify the regulatory function of miR-1468 on *CREB1* gene, the transcription level of *CREB1* mRNA was detected by qPCR. The results showed that the transcription level of *CREB1* mRNA in the miR-1468-mi group was significantly lower than that in the NC group (Fig. 6A) ( $P < 0.01$ ), while the transcription level of *CREB1* mRNA in the miR-1468-in group was significantly higher than that in the iNC group (Fig. 6B) ( $P < 0.01$ ). These results suggested that miR-1468 could regulate the transcription of *CREB1*.

*Effect of miR-1468 on CREB1 protein expression level*

In order to further verify the effect of miR-1468 on CREB1 protein expression level in pituitary of Yanbian cattle, CREB1 protein expression level was detected by Western-blot based on the transcription results of *CREB1* mRNA. The results showed that the CREB1 protein expression level in the miR-1468-mi group was significantly lower than that in the NC group (Fig. 7A) ( $P < 0.01$ ), while the CREB1 protein expression level in the miR-1468-in group was significantly higher than that in the iNC group (Fig. 7B) ( $P < 0.05$ ). These results suggested that miR-1468 could regulate the CREB1 protein expression.

## DISCUSSION

As a posttranscriptional regulatory factor, there are few studies on the regulation of miR-1468 on animal growth, especially on the relationship between miR-1468 and *GH*. Therefore, in order to determine whether miR-1468 is related to *GH*, we first observed the effect of miR-1468 on *GH* mRNA transcription and protein expression at the level of pituitary cells in vitro. The results showed that miR-1468 could promote *GH* mRNA transcription and protein expression, which indicated that there was a close relationship between miR-1468 and *GH*. However, previous researchers have demonstrated that the main function of miRNA is to negatively regulate the expression of target genes (Houbaviy, 2005). Therefore, we concluded that *GH* was not directly regulated by miR-1468, and the target gene of miR-1468 needed further verification.

In order to further determine the specific regulatory mechanism of miR-1468 on *GH*, the target relationship between miR-1468 and GH secretion related regulatory genes was analyzed through biological analysis software in this study. Research data showed that *GHRHR*, *SSTR2*, *LEF1*, *POU1F1*, *SSTR5* and *CREB1* were the main genes related to GH synthesis and secretion (Song *et al.*, 2007; Martari and Salvatori, 2009; Hiral *et al.*, 2014; Li *et al.*, 2012; Hagberg, 2010). Therefore, we observed the relationship between miR-1468 and the 3'UTR region of

the above genes using targetscan and RNA hybrids software in this study. The results showed that the seed sequence of miR-1468 only had a target relationship with *CREB1* gene. Some studies have shown that CREB1 protein is a transcription factor specifically expressed in animal pituitary gland, which can promote *GH* transcription and expression and play an important regulatory role in animal growth and development (Hagberg, 2010). The results of luciferase reporter gene validation showed that the activity of luciferase in the normal pirGLO-*CREB1*-3'UTR vector was significantly decreased by miR-1468, but the luciferase activity in the mutant pirGLO-*CREB1*-3'UTR vector was not affected. These results not only indicated that there was a target relationship between miR-1468 and *CREB1*, but also preliminarily demonstrated that miR-1468 could affect GH secretion by regulating the expression of *CREB1*.

After determining the relationship between miR-1468 and 3'UTR of *CREB1*, we observed the effects of miR-1468 on *CREB1* mRNA and protein of pituitary cells in vitro in this study. The pituitary cells of Yanbian cattle were transfected with miR-1468 mimics and inhibitor at the cellular level, and the changes of *CREB1* mRNA transcription and protein expression were detected by qPCR and Western blot. The results showed that *CREB1* mRNA transcription and protein expression was significantly inhibited by the transfection of miR-1468 mimics, but was significantly increased by the transfection of miR-1468 inhibitor. Therefore, it could be inferred that the regulation of miR-1468 on GH secretion was mainly achieved by targeting *CREB1*. The present study further revealed that the main role of miRNA was to negatively regulate the expression of target genes, which was consistent with the previous study (Houbaviy, 2005; Shi and Sun, 2016; Valencia *et al.*, 2014).

## CONCLUSIONS

These results demonstrated that miR-1468 could regulate *GH* by targeting *CREB1*. The result of this study will not only provide a theoretical basis for further study the mechanism of miRNA on regulating animal growth and development, but also provide a new target for the growth and development regulation of Yanbian yellow cattle.

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*Statement of conflict of interest*

The authors have declared no conflict of interest.

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