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Construction and Functional Verification of siRNA Eukaryotic Expression Vector Directed at the Follicular Inhibin Alpha Gene in Ye Mule Sheep

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ZH and WH conceived and designed the experiments. ZH, BB, KR, OK, JZ, GU and WH contributed reagents/ materials/analysis tools, prepared and approved the final draft. ZH and NU performed the experiments. WH, NU, JZ and BB analyzed the data.

Key words
Ye Mule Aries (YM) sheep, INHα, siRNA, Q-PCR, pGenesil 10-3p-siRNA eukaryotic expression vector, Granulosa cells

ABSTRACT

The Ye Mule Aries (YM) Sheep is a valuable local sheep breed from Xinjiang, China. This breed is well adapted to harsh environmental conditions and displays strong disease resistance, fast growth, and high cold tolerance. The effect of small interfering RNA (siRNA) on the expression of follistatin inhibin alpha gene (INHα) in YM sheep was analyzed, and the INHα gene was amplified from total RNA of the ovary tissue of YM sheep and was successfully cloned. The eukaryotic siRNA expression vector was constructed with the cloned gene as the target and validated at the cellular level. Three RNA interference fragments at different sites were designed based on obtained cDNA of the target gene and the siRNA expression vector pGenesil 10-3p-siRNA was constructed. The siRNA expression vector was digested, identified and sequenced, and then transfected into YM sheep granulosa cells. The sequence of YM sheep INHα gene was obtained and uploaded on the NCBI database (accession number KP-113678.1). The INHα gene from YM sheep is 1109 bp long and translates to approximately 40 KDa protein. Q-PCR analysis showed that the plasmid pGenesil 10-3p-siRNA could interfere with the expression of INHα in granulosa cells to an efficiency of 83%, which was also confirmed through western Blot assay. This study successfully constructed the eukaryotic expression plasmid pGenesil 10-3p-siRNA, and confirmed that the plasmid interfered significantly with the expression of INHα gene in YM sheep. Our current findings can serve as a model for studying the effect of INHα on the breeding performance of YM sheep, providing a novel strategy to improve their reproductive capacities.

INTRODUCTION

Inhibin (INH) is a hormone secreted by testicular Sertoli cells and ovarian granulosa cells. It is a heterodimeric glycoprotein, consisting of an alpha and a beta subunit connected by a disulfide bond (Medan *et al*., 2007; Young and McNeilly, 2012) and exists in two forms, INHα and INHβ (Meldi *et al*., 2012). INH inhibits the secretion of follicle-stimulating hormone (FSH) by the anterior pituitary gland (Findlay *et al*., 1990; Roser *et al*., 1994; Kaneko *et al*., 1995; Donadeu and Ginther, 2002) and is the primary negative feedback regulator of FSH secretion in mammals. Levels of INH can influence the growth of the number of follicles, act as key determinant that regulates ovulation in animals (Kogo *et al*., 1993; Goldammer *et al*., 1995; Nambo *et al*., 1998; Shi *et al*., 2000), and also directly inhibit functions of gonads such as follicle maturation and development of the local regulation (Vale *et al*., 1988; Goldammer *et al*., 1995; Knight and Glister, 2001; Li *et al*., 2009). Studies have proven that immunization of females with INH preparations, increase the number of eggs through ovulation, improving fertility (Souza *et al*., 1998; Hiendleder *et al*., 2000; Ishigame *et al*., 2004; Medan *et al*., 2004). The immunosuppressant technique has achieved good results in improving ovulation rate, fertility, and superovulation in livestock. However, there are too many factors influencing the immune effects resulting in a low level of control. Often, the same vaccine and the same immune pathway, are likely to produce different immune effects in different cases. The ability to reduce follistatin through immunological

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methods is limited because of the ability of the antibody to bind to the antigen that neutralizes the follistatin. The RNA interference (RNAi)-dependent gene inhibition is a new method which acts rapidly, especially in revealing gene functions and is widely used in gene therapy. RNAi technology has been used to inhibit the expression of INH inhibitors in animals to reduce the level of inhibin in animals, and would be a significant improvement in gene immunization processes. RNAi technology uses 21~23 bp-sized siRNA fragments that specifically bind and target homologous mRNA sequences to degrade, and thereby inhibit gene expression. Compared with conventional gene knockout methods, RNAi has the advantages of high efficiency and strong specificity and is widely used in inhibiting expression of a specific gene. Zhao (2011) used RNAi technology to inhibit the expression of INH gene in bovine granulosa cells, and the results showed that the inhibition rate could be as high as 93%, showing an effective inhibitory effect. Likewise, Xie *et al*. (2011) constructed an RNAi expression vector that effectively suppressed the INH gene of buffalo, and reported the highest inhibition efficiency 48 h post-transfection.

Ye Mule Aries sheep (formerly known as Kalamu Mule) is a flock of sheep located in the western margin of the Junggar Basin in Emin County, Tacheng District, Xinjiang, China (Anivash *et al*., 2006). The Ye Mule Aries sheep, characterized by a small fat hip and fat body, were created in the 19th century through the long-term breeding of Kazakh sheep in a unique geographical location with a specific climate (Jahan and Arnivash, 2010). The YM sheep has significant potential for the development of the mutton industry and could play an important role in mutton production in Xinjiang. In recent years, due to various reasons, not much attention has been paid to the breeding of the YM sheep in Xinjiang. Therefore, the number of purebred YM sheep has fallen sharply and the species is even in danger of becoming extinct. Our research seeks to promote rapid development of the sheep industry in Xinjiang. We selected the YM sheep as the research object and constructed a tandem RNAi eukaryotic expression vector aimed at repressing the inhibin gene. This was expected to promote follicle growth and therefore lead to an increase in the reproductive potential of the YM sheep.

MATERIALS AND METHODS

Ethics statement

All animal care and experimental procedures were approved by the Animal Protection and Use Committee of Ningxia University and Shihezi University. All research work was carried out in strict accordance with experimental animal welfare and ethical guidelines of Ningxia University and Shi he zi University.

Experiment material

Purebred and YM sheep (healthy ewes that had earlier produced 2 to 3 normal fetuses) were obtained from the pastoral area of Halamumul Township, Emin County, Xinjiang. The PGenesil10-3p vector kit was purchased from Wuhan Jinsai Biotechnology Co., Ltd. p MD18-T vector and *E. coli* DH5α were provided by the Oasis Ecological Laboratory of Xinjiang Production and Construction Corps of China. Dulbecco's Modified Eagle Medium (DMEM)-12 medium and calf serum were purchased from GIBCO. Anti-inhibin antibody, IgGHRP were procured from Sigma. Sheep egg collection fluid, oocyte maturation culture medium, granulosa cell culture medium, and other reagents were also issued according to the actual needs of the experiment. Interference results were estimated according to the changes of different Ct values, $2^{-\Delta\Delta Ct}$ and data statistics in the same period.

Collection of YM sheep ovarian tissue

The ovaries were collected from purebred YM sheep (healthy ewes that normally produced 3 fetuses) from the pastoral area of Halamumul Township, Emin County, Xinjiang. The collected ovaries were placed in a 1.5 mL centrifuge tube in a gauze bag, quickly frozen in liquid nitrogen, and then placed at -80 °C until use.

Extraction of total RNA and cloning expression of INHα gene

After thoroughly grinding the collected ovarian tissue in liquid nitrogen, total RNA was extracted as specified in the TRizol total RNA extraction kit (TIANGEN) and stored at -80 °C . Primers were designed based on an already available sequence of full-length INHα gene (GenBank, XM 004.004955.1); the primers are listed in Table I.

Reverse transcription was carried out using a reagent kit (Takara) according to the manufacturer's instructions. The reaction mixture of 20 µL, consisted of 5x mix (4 µL), RNA (2 µg), and RNase-free water (16 µL). The reaction was performed at 37 °C for 15 min followed by 85 °C for 5 sec; the PCR product was stored at 4 °C. The target gene was amplified by PCR using the cDNA according to the TaKaRa kit instructions. The 25 µL reaction mixture, consisted of 2x mix (12.5 µL), upstream and downstream primers (100 µmol/L, 0.4 µL each), double-distilled water (9.7 µL), and cDNA (2 µL of 1.9 ng/µL). The PCR program consisted of pre-denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 40 s, 68 °C for 40 s, and 72 °C for 1 min 30 s, followed by a final extension at 72 °C for 7 min. The amplified product was observed by electrophoresis on a 1.0 % agarose gel.

Table I. List of all primers: According to the specificity of the inh gene sequence, PCR amplification primers, specific interference sequence primers and q-pcr primers were designed, as shown in Table I.

Primer name	Primer site	Synthetic fragment
Amplification of INHα gene primers	Upstream primer	5'-*ATG TGG CTT CAG CTG CTC CTC TT*C-3'
	Downstream primer	5-' *GAT GCA AGC ACA GTG CTG GGT G*-3'
Interfering fragment primers	QHX 5 78+778+836-A	TTTGGTCTCCGTGGCTTCACTAAGCAGGAACAGAGAGGATAAC TCGCTCACTGTCAAC
	QHX 578+778+836-B	TTTGGTCTCCCTGCAGGGGAAAGAGATATTGAGGGCGGTGT-TTCGTCCTTT
	QHX 578+778+836-C	TTTGGTCTCCCCACAGATGTAAGCAGGAACAGAGAGGA-TAACCTGCCTACTGCCTCG
	QHX 578+778+836-D3	TTTGGTCTCCTACCGCAGTAGTAGAAGATGAAACGGGAAA-GAGTGATCT
	QHX 578+778+836-E	GCAGGGAAAGAGATATTGAGGGCTTTTTCAAAAAGTTTCATCT-TCTACTACTG
	QHX 5 78+778+836-F	GGTACAGTAGTAGAAGATGAAACTTTTTGAAAAAGCCCT-CAATATCTCTTTCC
Internal reference gene GAPDH Q-PCR primers	Upstream primer	5'-*TTCTGCTGACGCTCCCA*-3'
	Downstream primer	5'-*CCTCCACGATGCCAAAG*-3'
INHα gene Q-PCR primers	Upstream primer	5'-*GTGACTGGGGAAGGTGGAGAT*-3'
	Downstream primer	5'-*GATGGCCGGAACACATACGT*-3'

Note: all primers are synthesized and purified by Shanghai sangong Biotech company.

The electrophoresed DNA was recovered and purified according to the instructions of the QuickGel Extraction Kit (Kangwei Co.). Subsequently, the amplified sequence was ligated into the pMD18-T vector at 16 °C, overnight. *E. coli* DH5α competent cells were transformed with this ligation product and grown overnight on LB plates containing 50 μg/mL ampicillin. Five putative positive clones were selected, and the positive recombinant plasmid was initially confirmed by PCR and double enzyme digestion.

Construction and identification of siRNA expression vector

Based on the mRNA sequence of the cloned INHα target gene from YM sheep, and as per standard siRNA design principles, three interfering sites of the YM INHα subunit gene were screened. The primer sequences of siRNA on the target gene are detailed in Table I. The fragment primers targeting interference were simultaneously divided into two groups of expanded PCR, the first round was carried out using set of primers A + B, and the second set of primers was C + D. The purified gene fragment was mixed with the digested pGenesil10 -3 p vector and the ligation reaction was carried out at 16 °C for 12 h after which, the ligation product was transformed into DH5α and plated overnight on LB agar containing antibiotics. A single, appropriately sized colony was screened by PCR, restriction enzyme digestion, and finally by DNA sequencing. After the final confirmation, the recombinant plasmid (pGenesil10 -3 p-siRNA) was extracted in large quantities according to the kit instructions. The 260/280 nm ratio of the recombinant plasmid obtained by NanoDrop 2000 ultra-micro spectrophotometer was 1.8, and it was stored at -20 °C. The interference recombinant plasmid construction method was used to construct the negative control plasmid (pGenesil10 -3 p-HK).

Isolation and culture of follicular granulocytes from YM sheep

The ovaries of YM sheep (healthy ewes that had earlier given 2 to 3 births) from the pastoral area of Halamumulu Township, Emin County, Xinjiang, were collected at the estrus phase, and immediately placed at 38 °C. In the left and right physiological saline thermos, the double-antibody was added and transported to the laboratory within 3~4 h. The ovaries were kept in a 38 °C water bath, and washed 2 to 3 times with saline, twice with absolute ethanol, finally again with physiological saline. Next, the adipose tissue was cut out in a beaker placed constant temperature water bath at 38°C for cutting eggs. The ovaries were placed in a petri dish containing the egg-washing solution, and the follicles on the surface of the ovary were gently cut with a blade. The dish was

then placed under a microscope and the complete oocyte was pried out using a sterile spatula. The culturing was carried out in a maturation solution, and the mature oocyte was digested with hyaluronidase. After digestion, the shed cumulus cells were collected into a centrifuge tube, and DMEM medium containing 10 % fetal bovine serum (FBS) was added and then centrifuged. The supernatant was removed, and the cells were washed with saline. The sediment at the bottom was blown out of the tube with the tip of the pipette nozzle, suspended, evenly dispersed, then centrifuged at 1000 r/min for 5 min. This washing step was repeated twice, and the cells were then resuspended. The suspensions were inoculated separately in culture flasks for cultivation and passage.

Transfection of granulosa cells

Briefly, the main experimental steps were: (1) the fine cells which were located in the logarithmic stage were digested with 0.25 % trypsin, then the complete medium was added to the ultra-clean table to suspend it. The suspended cells were then quantitatively divided into 6-well plate for culture after blowing evenly, and when the degree of confluence reached 80 ~ 90 %, it could be transfected, (2) after lightly washing twice with serum-free medium, 2 mL serum-free medium was added to the culture dish, (3) plasmid pGenesil10-3p-siRNA and pGenesil10-3p-NK were diluted into 150 µL serum-free medium by shaking gently; 12 µL Lip 2000 was diluted in 150 µL serum-free medium, while gently blowing and mixing with micro-liquid shifters, and then incubated at room temperature for 5 min; (4) 150 µL diluted Lip 2000 was added to the diluted plasmid, then incubated at room temperature for 20 min; each tube of 300 µL mixture added to the 6-well plate respectively and appropriately marked, (5) this transfected mixture was cultured in 5 % CO_2 37 °C incubator, and 5 h later, replaced with complete culture medium. After culturing further for 24 h, the fluorescence expression of the transferred plasmid was observed every 12 h.

Detection of interference effect of the siRNA plasmid

The primers were designed based on the target gene of INHα (GenBank: KP-113678), primers were designed using Primer 5.0.1) primers were designed for mRNA sequence and GAPDH (GenBank: NM_ 001190390.1) for real-time fluorescence quantitative PCR. Primers were synthesized by Shanghai Bioengineering Technology Co., Ltd and their sequences are listed in Table I.

After the transfection, the morphology of the cells and the expression of red fluorescent protein in the cells were observed under the fluorescence microscope at 12 h intervals. The interference effect of pGenesil10-3p-sirnasiRNA plasmid was predicted indirectly by assessing the expression level of the fluorescent protein. Further, the Q-PCR method was used to detect the interference of recombinant plasmid (PGenesil10-3p-siRNA) on the expression of INHα gene mRNA in granulosa cells. The mRNA expression of housekeeping gene GAPDH is relatively constant in sheep at any growth stage, the expression of GAPDH mRNA was selected as the standard of mRNA expression in each plate of granulosa cells. Then, the expression of INHα in blank control, negative control group, and experimental groups was calculated quantitatively.

Protein was extracted from cells of the blank control group, negative control group, and experimental group and pre-stained marker were resolved by SDS-PAGE and transferred to PVDF membrane using Bio-Rad electric transfer device. The transferred membrane was cut appropriately and made into two pieces and then incubated overnight with TBST containing 5 % skimmed milk powder at 4 °C. The next day, the membrane was washed thrice with TBST, and the milk powder was completely washed out. Then, an anti-His monoclonal antibody was added and incubated at room temperature for 1 h, and the TTBS membrane was washed thrice, each time for 10 min. Hrp-sheep anti-mouse IgG polyclonal antibody was added at 1:500 ratio, incubated for l h at room temperature, and the membrane was washed in TTBS buffer thrice, each time for 10 min. The remaining solution was absorbed using a filter paper, and the membrane was laid flat on a clean plastic wrap, followed by the addition of 1.4 mL western chemiluminescence substrate reaction liquid (A: B = 1:1). The film was fully immersed in the reaction liquid, taken out after 2 to 3 min, and excess liquid was absorbed using filter paper, and placed on a piece of tin foil. The film was then wrapped with plastic, placed inside the cassette for X-ray photography, and then developed in the darkroom.

RESULT

Total RNA isolation

The total RNA extracted from the ovary tissue of YM sheep was resolved by agarose gel electrophoresis. Two clear bands of 18 S and 28 S ribosomal RNA were observed (Fig. 1), indicating that the total RNA of ovarian tissue was intact and no degradation had occurred during the extraction process.

Cloning of the coding region of the YM sheep INHα gene

Total RNA from the ovary tissue of YM sheep from Xinjiang was reverse transcribed using Oligo dT primers, followed by PCR amplification using INHα-specific PCR primers. A specific target band of 1109 bp length was amplified (Fig. 2), which was preliminarily identified as

the fragment encoding INHα subunit region of YM sheep. The RT-PCR product was inserted into pMD18-T and transformed into *E. coli* DH5α cells. Plasmid DNA was isolated from randomly selected bacterial colonies and verified by DNA sequencing.

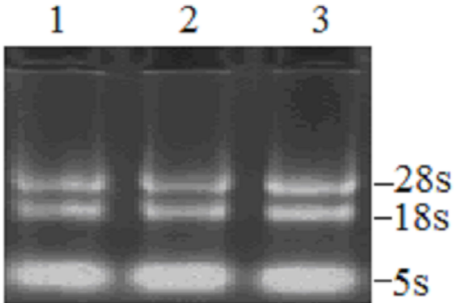

Fig. 1. Agarose electrophoresis of total RNA.
Lanes 1, 2, 3 total RNA from three representative YM sheep ovarian tissue extracts. Migration of 5S, 18S and 28S ribosomal RNA bands is indicated. The ratio of A260/A280 of 1.8.

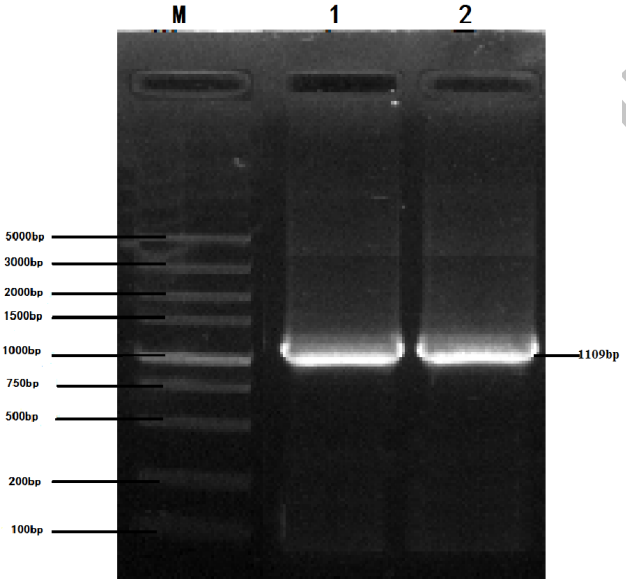

Fig. 2. Amplification of a cDNA fragment encoding the INh α gene from YM sheep.
M, DL5000 DNA Marker; Lanes 1 and 2, The amplification product of INH a gene.

Restriction enzyme digestion of recombinant plasmid (pGenesil10-3 p- siRNA)

The recombinant plasmid (pGenesil10-3p-siRNA) was digested with the enzyme *Pst*I Having only site on the vector, and a *Pst*I restriction site was added to the DNA template primer of the siRNA fragment, and, *Pst*I digestion led to the release of the cDNA fragment during agarose electrophoresis (Fig. 3).

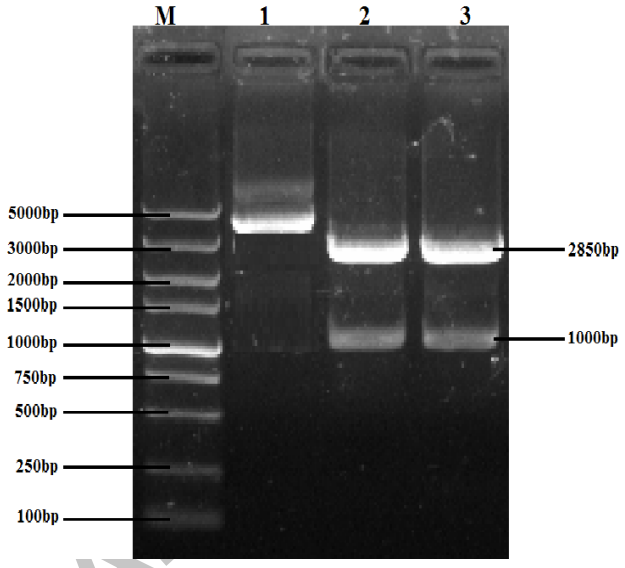

Fig. 3. The recombinant plasmid pGenesil10-3 p – siRNA was identified by enzyme digestion.
M, DL5000 DNA Marker; Lanes 1, pGenesil10-3 p–siRNA plasmid not digested by enzyme; Lanes 2 and 3, Identification of recombinant plasmid pGenesil10-3 p–siRNA by PstI enzyme digestion.

Isolation and culture of granular cells

The granulosa cells isolated from the follicles of YM sheep were isolated, cultured, and were found to grew well, with a good degree of confluence before the cells were cultured for 72 h. However, in this test, it was found that the wire drawing phenomenon occurred after the cells were cultured for 96 h, and (Fig. 4) the results were apparent when the granulosa cells at different stages were observed under an inverted microscope.

Transfected granulosa cells

The granulosa cells were transfected with pGenesil10 -3 p-siRNA or pGenesil10 -3 p-HK (control) plasmid and the expression of red fluorescence in the culture dish was observed every 12 h. Finally, red fluorescence expression was observed 48 h after transfection of the plasmid (Fig. 5).

Detection of INHα in granulosa cells

Two control groups were separated, and the total RNA from the granule cells of the interference blank group (ie, the same volume of DMEM-12 medium in the interference group), the negative control (pGenesil10-3p-HK), and the test group (pGenesil10-3p-siRNA) were extracted. The

corresponding cDNA was obtained by reverse transcription and used as a template. Fluorescent quantitative PCR primers were used for quantitative PCR amplification of the INHα gene and the internal reference gene GAPDH. The Ct values were between 16~24 cycles, when the Ct value reached about 26, the expression of INHα and the internal reference gene reached the plateau stage, and the curve inflection point of all the samples was clear. Overall, the parallelism was good, and the amplification baseline was straight, which met the requirements of the amplification curve index. In the control group and the experimental group, the INHα and the internal reference gene GAPDH dissolution profiles, the peak of the dissolution curve of each sample was single, and there were no non-specific amplification bands and primer dimers (Fig. 6A). The results showed that compared with the control group, the constructed siRNA plasmid targeting the INHα gene of the YM had a significant inhibitory effect on the INHα gene expression in granulosa cells, with a silencing efficiency of 83%.

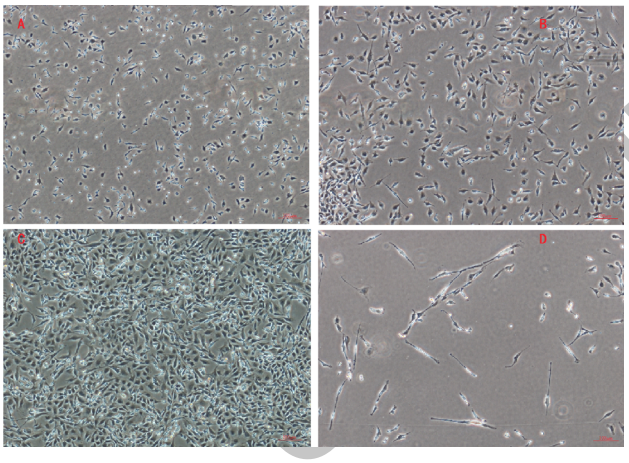

Fig. 4. Granulosa cells growing for different times.
A, after 24 h of granulosa cell culture, adherent growth entered into hysteresis period; B, granulosa cell culture for 48 h and entered the logarithmic phase; C, granulosa cell entered a stable phase after 72 h of culture; D, granulosa cell were culture for 96 h and entered the decline period.

Expression of INHα protein in granulosa cells

The total protein was extracted from granulosa cells. The results showed that there was no significant difference in protein expression between the blank control group and the negative control group. However, in agreement with the interference effect, the INHα expression in the cells expressing pGenesil10-3p-siRNA was significantly reduced compared with that in the blank and the negative control (pGenesil10-3p-HK) groups. The expression level of INHα fluorescent protein was significantly decreased in the cells (Fig. 6B and 6C).

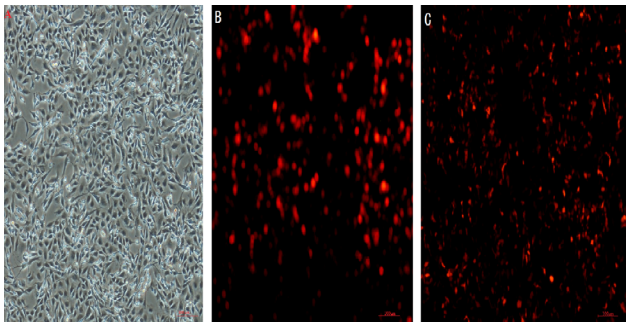

Fig. 5. Experimental diagram of plasmid transfection granule cells.
A, granulosa cell not transfected with plasmids in logarithmic phase; B, results of pgenesil 10-3p-HK plasmid transfected granulosa cells at logarithmic growth stage; C, results of pgenesil 10-3p-HK plasmid transfected granular cells at logarithmic growth stage.

DISCUSSION

Gene silencing by RNAi is a ubiquitously conserved mechanism in eukaryotes that is closely related to many biological processes in eukaryotic cells and has high sequence-specificity and heritable properties. Cai (2011) used RNAi-mediated transgenic animals in the study of mouse INHα and achieved an inhibition rate is as high as 60%. The experiment was carried out in strict accordance with the principle of RNAi eukaryotic expression vector construction, and according to the method used by Jiang *et al*. (2002). The effects of tandem INH gene immunization on mouse reproductive hormone and the sequence of INHα gene of YM white sheep were analyzed repeatedly and screened out for interference sites for RNA. Also, many scholars found that reducing the levels of INHα by gene immunization improved sheep twinning rates and fertility (Campbell *et al*., 1998; Foulds *et al*., 1998; Campbell and Baird, 2001; Evans *et al*., 2002; Fabre *et al*., 2003; Safwat *et al*., 2005). Here, we used the RNAi technique to lower INHα expression in granular cells of YM sheep. We identified three RNAi sites in different regions (between 500-900 bp of INHα gene) that could be useful in reducing INHα levels in sheep. An interference fragment was inserted into the red fluorescent pGenesil10-3p eukaryotic expression RNAi vector by PCR, gene tandem and gene-splicing techniques. The interference caused by a single interfering RNA fragment is not necessarily significant, the uncontrollable factors are too large, and the interference fragment itself is too small to be detrimental

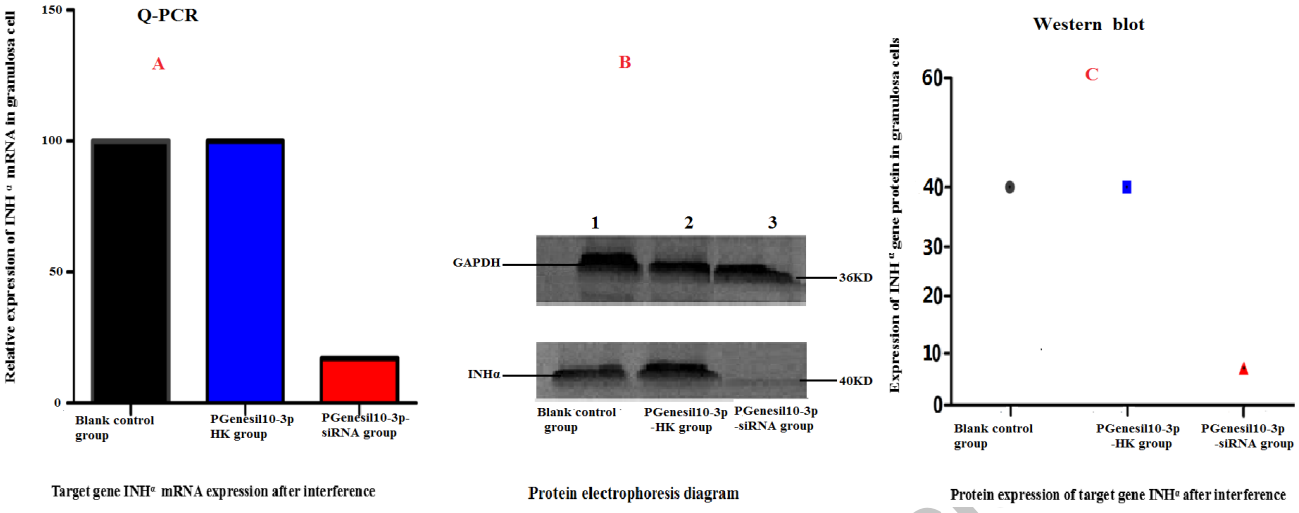

Fig. 6. Three picture show the expression of INHα gene in granulosa cells after interference.
A, the interference results were identified by Q-PCR; B, protein expression results of INHα gene in granulosa cells; C, interference results of western bolt identification.

to the later detection, therefore, the RNA fragments of different interfering sites in series were used. The tandem interference eukaryotic expression plasmid (pGenesil10-3p-siRNA) was transfected into ovarian granulosa cells and the expression of the siRNA was found to have a significant silencing effect, at an efficiency of nearly 83%.

INHα selectively inhibits synthesis and secretion of FSH, which is crucial for follicular maturation, ovarian development, and ovulation. Thus, FSH is one of the primary hormones regulating animal reproductive function. In this study, we selected relevant genes for the inhibition of FSH expression regulation to have a deeper understanding of the mechanism of the follistatin gene. We used a relatively new technology of RNAi tandem expression vector that effectively silenced the follistatin gene of YM Sheep, and provided a new way to improve the expression of target genes inhibited by interference phenomenon. This study imparts a theoretical framework that will help improve the quality of livestock and bring new opportunities in the sheep breeding work in Xinjiang. Importantly, these basic studies on the INH gene will also be of great importance for the study on reproductive disorders such as female ovarian cysts and insufficient sperm motility in males.

Overall, our work contributes to a new understanding of the regulation of the reproductive function of the sheep follistatin gene. The cloning and sequence analysis of the follicle INH gene of YM Sheep, as well as the tandem interference eukaryotic expression vector, was accomplished. This study is of great significance in improving the animal's reproductive performance, especially the fertility of single-fetal animals; it also established a cell model with a target gene interference fragment. The research lays a foundation, guides the normal maturation of follicles in female animals, and provides insights into the normal development of ovaries and smooth ovulation. Moreover, basic studies on inhibin genes will have significant reference especially in women with ovarian cysts and male sperm motility and other reproductive disorders.

CONCLUSION

The present study shows that inhibiting the YM sheep follicular inhibin gene by interfering expression fragment in an efficient and stable siRNA eukaryotic expression system can produce bioactive proteins by down-regulating the expression of target genes. Also, the inhibin gene is the regulator responsible for reproductive gene function which can improve the secretion of the follicular stimulating hormone by interfering with the expression of inhibitory genes, and thus, improve the fertility and reproductive performance of animals.

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Statement of conflict of interest

The authors have declared no conflict of interests.

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