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Short Communication

An Investigation on the Relationship between the Azoospermia-Like (DAZL) Gene mRNA Expression and the Infertility in Male Cattle-Yak

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Abstract

This study was conducted to study the relationship between the infertility of male cattle-yak and the expression level of *DAZL* gene mRNA. The expression profiles were obtained by RT-PCR. The *DAZL* gene was specifically expressed in cattle, yak and cattle-yak testis tissues,which confirmed its important role in the progression of cell cycle. Real-time quantitative PCR analysis indicated that the expression levels of *DAZL* gene in cattle and yak testis were higher than its in cattle-yak, also cattle-yak and yak were significantly differet than cattle, respectively (P<0.05). Therefore, the low expression level of *DAZL* gene might result in male cattle-yak infertility.

Keywords: *Male infertility, DAZL, Real-time quantitative PCR, Phylogenetic relationship*

Sığır-Yak Melezlerinde Azoospermia-Benzeri (DAZL) Gen mRNA Ekspresyon Düzeyi ile İnfertilite Arasındaki İlişki Üzerine Bir Araştırma

Özet

Bu çalışma erkek sığır-yak melezlerinde Azoospermia-Benzeri (*DAZL*) gen mRNA ekspresyon düzeyi ile infertilite arasındaki ilişkiyi araştırmak amacıyla yapılmıştır. Ekspresyon seviyeleri RT-PCR ile belirlendi. *DAZL* geninin spesifik olarak sığır, yak ve sığır-yak melezi testis dokularında eksprese edilmesi hücre siklusunun ilerlemesinde önemli rolü olduğunu teyit etmekteydi. Gerçek Zamanlı kantitatif PCR analizi sığır ve yak testis dokularında *DAZL* gen ekspresyon düzeyinin sığır-yak melezlerindekinden daha fazla olduğunu ve sığır-yak melezi ve yaklardaki seviyelerin anlamlı derecede sığırlardan farklı olduğunu göstermekteydi (P<0.05). Sonuç olarak, düşük *DAZL* gen ekspresyonu erkek sığır-yaklarda infertiliteye neden olabilir.

Anahtar sözcükler: *Erkek infertilite, DAZL, Gerçek zamanlı kantitatif PCR, Filogenetik ilişki*

INTRODUCTION

Yak is the main livestock on the Qinghai-Tibetan Plateau, which belong the unique topographic features and the original bovine. It has high coarse, cold-resistant characteristics which adapts alpine hypoxia environment, but its milk, and meat production performances are lower. In order to improve the production performance of yak, yak is crossbred with cattle. Thus the production performance of hybrid cattle-yak which were in growth, fleshy, labor force and production performance were significantly better than the those of yak, but the infertility of male cattle-yak has been greatly limited in the production and breeding [1,2].

Over the past decades, the cattle-yak males sterile were significant studied by a lot of scholars about the complex phenomenon, but the main reason about the males sterile was had not found. Genes of the *DAZ* (Deleted in Azoospermia) gene famiv, *DAZ*, *DAZL* and *BOULE*, *DAZL* was originated from *BOULE* on Chromosome 2q, *BOULE* is the ancestral gene of *DAZ* gene family on Chromosome 3q [3]. The *DAZL* gene which is being studied in animal infertility at present is the research focus [4]. A lot of studies indicates that the absence of *DAZL* gene or the base mutation of *DAZL* gene brings on meiosis arrest and spermatogenic failure, which may lead to azoospermia and male infertility [5-8].

At present, the study of the relationship between the infertility of cattle-yak and the expression level of *DAZL* gene is rare. In this study, combined with cattle and yak, the *DAZL* gene from 3 representative Qinghai-Tibetan Plateau Bovine breeds were amplified, sequenced, Real-time PCR was amplified and data was analyzed to provide theoretic

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basis which finally revealed the infertility of cattle-yak mechanism.

MATERIAL and METHODS

Specimen Collection

The tissues samples of bovine breeds were collected from the Guomaying Town in Qinghai province. Male cattle-yaks (n=10), male cattle (n=10) and male yaks (n=10) which were adult and healthy were slaughtered. Testis, epididymis, hypothalamus, pituitary, heart, liver, spleen and pectoral muscle were removed and frozen in liquid nitrogen.

Design of Primers

The β-actin gene was used as an internal control. The primers were designed according to the cattle *DAZL* gene sequence (GenBank Accession Number: EF501823.2) and *β-actin* gene sequence (GenBank Accession Number: NM_173979) using primer 3.0 software and synthesized (Sangon, Shanghai, China). The primers for DAZL gene were: 5′-TCCTCCTCCACCACAATTTC-3′and 5′-GCTCCGGTG TCAACTTCATT-3′. The primers for β-actin were: 5′-TCCCTGG AGAAGAGCTACGA-3′and 5′-TAGAGGTCCTTGCGGATGTC-3.

Total RNA Isolation and cDNA Synthesis

Total RNA from the cattle, yak and cattle-yak tissues (testis, epididymis, hypothalamus, pituitary, heart, liver, spleen and muscle) were extracted using standard methods according to the manufacturer's protocol (RNA Extraction Kit, Fastagen, Shanghai, China). The cDNA was synthesized according to the manufacturer's protocol (Reverse Transcription Kit TaKaRa, Dalian, China). Operation procedure: 10 μg of purified total RNA, Prime ScriptTM RT Enzyme Mix1 1 μL, Oligo (dT) primer 1 μL, Random primer1 μL, 5×Primer ScriptTM Buffer 4 μL and RNase Free ddH_2O in a final volume of 20 μL.The RT temperature profile was 37°C for 15min, 85°C for 15 s, and final cooling to 4°C. The cDNA was stored at -20°C until use.

PCR Amplification, Molecular Cloning and Real-time PCR Amplification

PCR was carried out in a 25 μL reaction mixture containing 5 μL RT products, 12.5 μL 2×PCR buffer (Sangon, Shanghai, China), 0.6 μL of 10 mM of each oligonucleotide primer, and ddH_2O in a final volume of 25 μL. PCR was performed on a DNA amplification machine (ABI, USA) with an initial denaturation at 94°C for 4 min, 40 cycles of 94°C for 50 s, 57°C for 30 s and 72°C for 30 s and a final extension step of 7 min at 72°C. Reaction products were run on 2% agarose gels stained with ethidium bromide, and the target bands purifed with a Gel Extraction Kit (OMEGA, Shanghai, China) according to the manufacturer's protocol. The purifed product was cloned into the pGM-T vector and then transformed into Escherichia coli DH5a (TIANGEN, Beijing, China). The positive clone plasmid which was extracted according to the manufacturer's protocol (Sangon, Shanghai, China) was identified and sequenced (Sangon, Shanghai, China).

Real-time quantitative PCR which was performed on a DNA amplification machine (ABI, USA) was used to quantitatively determine the expression level of *Dazl* gene in various bovine testical tissues. Real-time PCR was performed in a 20 μL reaction mixture containing 2 μL RT products,10 μL SYBR Premix Ex TaqTM II (2×) (TaKaRa, Dalian, China), 0.4 μL Rox Reference Dye II (50×) (TaKaRa, Dalian, China), 0.8 μL of 10 μM of each oligonucleotide primer, and 6 μL ddH_2O. Real-time PCR cycle conditions were 1cycle of 95°C for 30 s, 40 cycles of 95°C for 5 s, 57°C for 20 s and 72°C for 34 s and 1 cycle of 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. The plasmid of positive clone fragment was standard after gradient dilution to make the standard curve. The quantitative results were performed using SPSS17.0 software for statistical analysis.

RESULTS

The Expression Profile of DAZL Gene

According to the DNA marker and the sequencing results, the size of *DAZL* and *β-actin* expected PCR products were 270 bp and 179 bp. The expression of *DAZL* gene in cattle, yak and cattle-yak tissues (testis, epididymis, hypothalamus, pituitary, heart, liver, spleen and muscle) was detected by RT-PCR. The results showed that *DAZL* gene was expressed specifically in cattle and yak testises, but not in other tissues.

The mRNA Expression Level of DAZL Gene

The mRNA expression level of *DAZL* gene was analyzed by Real-time PCR *(Fig. 1)*. The results showed *(Table 1)* that the mRNA expression level of *DAZL* gene in cattle (8.3980±2.26146) and yak (1.2020±0.70539) testical tissues were higher than its in cattle-yak (0.9810±0.25899). Cattle-yak crossbred and yak were significantly different than cattle, respectively ($P<0.05$).

DISCUSSION

Yaks are main breeds of Qinghai-Tibet Plateau, cattle yaks, the F1 hybrid between cattle and yaks, exhibit significant hybrid vigor. However, the males are sterile, which greatly restricts the utilization of this hybrid vigor.

The *DAZL* gene, a member of the *DAZ* gene family, which shows a specific expression in germ cells, is the key regulation factors during meiosis of human and animal spermatogenesis [9,10]. The absence of *DAZL* gene brings on meiosis to arrest and failure of spermatogenesis,

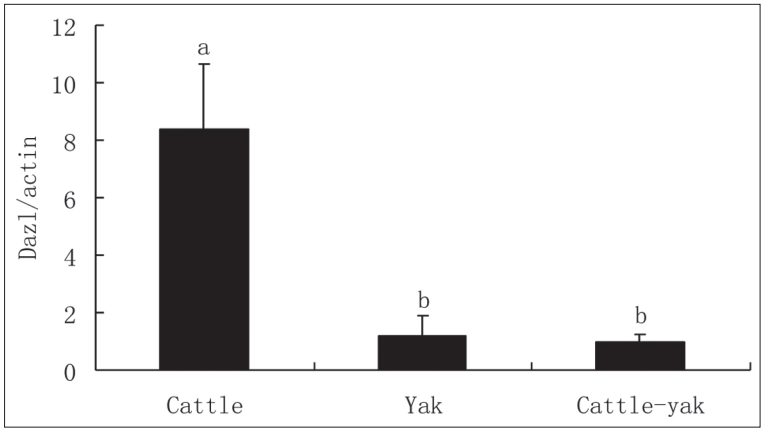

Fig 1. Developmental changes of expression level of *DAZL gene* mRNA expression in cattle, yak and cattle-yak's testises. Note: Different letters show significant differences at P<0.05

Şekil 1. Sığır, yak ve sığır-yak melezlerinin testislerindeki DAZL geninin mRNA ekspresyon seviyesindeki değişiklikler. Farklı harfler anlamlı farkın olduğunu göstermektedir (P<0.05)

Table 1. *The variance analysis of DAZL gene's mRNA expression level in cattle, yak and cattle-yak's testis tissues*
Tablo 1. *Sığır, yak ve sığır-yak melezlerinin testis dokusunda DAZL genine ait mRNA ifade seviyesinin varyans analizi*

The Source of Variation	DAZL gene			
	Sum of Squares	df	Mean Square	F
Between the species	356.144	2	178.072	94.071*
Within the category	51.110	27	1.893	
Total variation	407.253	29		

** means hows that differences significantly important*

which may lead to infertility of animal [11]. So presumably *DAZL* gene in also plays an important role in cattle-yak spermatogenesis.

In this study, to understand the function of the bovine *DAZ* gene family. The *DAZL* gene was highly expressed in bovine testis showing normal spermatogenesis but the case that mRNA level was low in testis possibly shows a defect in spermatogenesis, which suggest that *DAZL* gene might involve in spermatogenesis in the bovine testical tissue and arresting its transcription might result in infertility for male cattle-yak crossbred. Taken together with the report of *DAZL* gene [12], the result of low expression level of *DAZL* gene in cattle-yak testis suggests that *DAZL* gene might be associated with reproduction, which provide a theoretical basis of study the relationship between male sterility of cattle-yak and *DAZL* gene.

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