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

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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 [5,6].

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
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'-TCTCTCCTCACCACAATTTC-3' and 5'-GTCGCCGTTTCAACTTCTATT-3'. The primers for β-actin were: 5'-TCCTCTGGAGAAGAATGCAGA-3' and 5'-TAGAGGTTCTTGGGATGTC-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 primer 1 μL, 5× Primer ScriptTM Buffer 4 μL and RNase Free ddH2O in a final volume of 20 μL. The RT temperature profile was 37°C for 15 min, 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 ddH2O 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 purified with a Gel Extraction Kit (OMEGA, Shanghai, China) according to the manufacturer’s protocol. The purified product was cloned into the pGM-T vector and then transformed into Escherichia coli DH5α (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 ddH2O. Real-time PCR cycle conditions were 1 cycle 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,
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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**REFERENCES**


