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The Correlation Between Goat *Cox II* Gene Single Nucleotide Polymorphism and Sperm Motility

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Abstract: This study is about the correlation between *COX II* gene genetic variation and adult buck sperm motility traits, to provide genetics reference for the goat breeding. In this study, 120 adult male goat semen samples were collected from four populations. *COX II* gene polymorphism information was rapid screened using DNA pool and sequencing method. Each individual polymorphism of these goats was detected by RFLP. Sequencing results of DNA pool showed a single nucleotide mutation in *COX II* gene (A→G). The mutation caused a change of Hind III restriction site. RFLP results showed that the mutation associated with goat sperm traits. The vitality of fresh sperm and frozen sperm from BB-type goats was significantly higher than AA-type. This study provided foundation for establishment relationship between *COX II* gene SNP and goat sperm motility traits. It could be considered as a reference marker-assisted selection for buck semen quality traits and goat breeding.

Key words: Goat, *COXII* gene, genetic variation, sperm motility, breeding, mutation

INTRODUCTION

Studies showed that sperm motility was closely related to genetic factors of male livestock itself. The variations of mammalian mitochondrial DNA will affect the energy supply of sperm motility (Mohammad *et al*., 2006; Montiel-Sosa *et al*., 2006). Cytochrome Oxidase (COX) located in the inner mitochondrial membrane is an oxidoreductase. It is the only cytochrome complex which could pass electron to oxygen molecules and its damage will affect mitochondrial function directly (Kadenbach *et al*., 2004). Cytochrome Oxidase II (COX II) which is one of subunits made up the catalytic center of the enzyme is encoded by mitochondrial DNA. It is the combination site of mitochondrial Cytochrome C. The changes of structure or function of COX II will destruct the mitochondrial respiratory chain directly (Piel *et al*., 2005). Mitochondrial sheath provided the energy for sperms movement and the sperm motility was affected by Adenosine Triphosphate (ATP). The changes of structure and function of COX II will influence sperms activity through ATP (Thangaraj *et al*., 2003; Venkatesh and Dada, 2011; Venkatesh *et al*., 2010; Palanichamy and Zhang, 2011). At present, studies about *COX II* gene are focused on the liver and heart tissue cell apoptosis (Zhang *et al*., 2006; Zickermann *et al*., 1995). However, the effects of *COX II* gene mutation on goat sperm motility traits have not been reported.

MATERIALS AND METHODS

Sample collection and DNA extraction: About 120 semen samples were collected from 4 population (30 from each population), Huanghuai goats, Anhui white goats, Boer goats and Boer x Huanghuai Hybrid goats which were 12 months old and provided by Hefei Bo Da Livestock Technology Development Co., Ltd. Genomic DNA samples were extracted from the semen samples according to Phenol-Chloroform Extraction Method (Sambrook and Russell, 2001). The concentration and purity of genomic DNA were determined by spectrophotometer. The concentration of all DNA samples were diluted to 50 ng μL^{-1}.

Construction of DNA pools: DNA sequencing pool is an effective method to scan base mutations, presenting the possibility of an incredibly fast and economical experiment (Bansal *et al*., 2002; Sham *et al*., 2002). The

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Table 1: Primer information

Primers name	DNA sequence (5'-3')	Size (bp)	Tm (°C)
P-1	F-AGCCAACACCATAACCACTA	867	54
	R-GTGGCATATCATCAAGGAGAGT		
P-2	F-CGGTCTGAACTATCTTAC	291	52
	R-GAACGTCTTCGGAAGAGA		

diluted genomic DNA was analyzed by electrophoresis in 1% agarose gel. About 5 μL DNA samples from 40 individuals without towing in electrophoresis were pipetted into a eppendorf tube and mixed gently. The quantity of each DNA sample in a DNA pool was equal.

PCR and sequencing: According to the goat mitochondrial DNA sequence (Accession No. GU229281), the primers P-1 (Table 1, Sangon Biological Engineering Technology and Services Co., Ltd. Shanghai, China) were designed to amplify the full length sequence of *COX II* gene. The PCR reactions were performed in a final volume of 50 μL with the DNA pool as a template. Amplified DNA segments were analyzed by electrophoresis in 2% agarose gel. The PCR products were purified using the gel extraction kit (TIANGEN, Beijing, China) and sequenced by ABI PRISM3730 Genetic Analyzer (Sangon Biological Engineering Technology Co., Ltd. Shanghai, China).

PCR-RFLP: Primers P2 (Table 1), for amplification of the 291 bp gene segment covered the cleavage site were designed according to *COX II* gene by Primer 5.0. The different genotypes of *COX II* gene were identified by the PCR-RFLP Method at 37°C for 12 h with the following reaction system: a final volume of 20 μL containing 5 μL PCR products, 0.5 μL 5U Hind III endonuclease, 1 μL 1×magnesium-free PCR buffer. The reaction products were analyzed by electrophoresis in 2% agarose gel and the genotype were recorded.

Determination of sperm motility and statistical analysis: About 20 μL fresh semen and thawing frozen semen were put on the loading platform slides whose temperature is 38°C constantly. The sperm motility was observed using the micro scope with a monitor screen 400 times. The fresh semen was diluted five times by 0.9% NaCl solution which was determined by sperm density analyzer. For each population, one-way ANOVA was employed using SAS Version 8.0 to compare the difference of sperm motility from different genotype buck and t-test was used to analyze statistical significance. The $p<0.05$ was deemed to be significant and $p<0.01$ highly significant level. Values were presented as mean±SD.

RESULTS AND DISCUSSION

PCR products of DNA pool to screen SNP: The agarose electrophoresis of the PCR products using DNA pool as template was clear and in the right range corresponding with expectation which indicated that the quality of the goat genomic DNA is fine and meet experiment requirement perfectly. SNP screening was analyzed by comparing the sequencing diagrams. The mutation $A^{421}\rightarrow G$ was detected in the amplified sequence with P1 primers (Fig. 1).

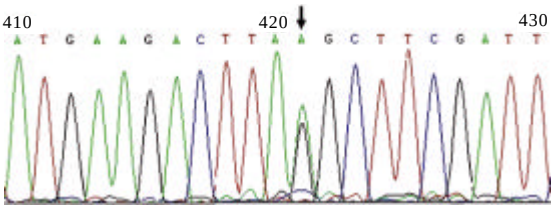

Fig. 1: *Cox II* gene sequencing result of goats pool DNA

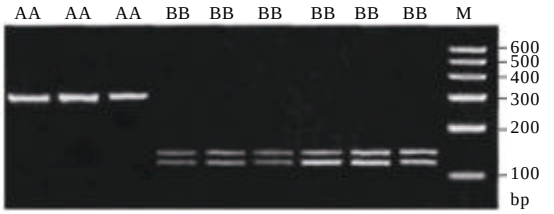

Fig. 2: RFLP agarose gel electrophoresis of *COX II* gene

Analysis of Hind III site in *COX II* gene of goat: The electrophoresis of products after Hind III restriction showed that there were two genotypes of goat *COX II* gene. The genotype which had one band (291 bp) was named AA and the other one which had two bands (161 and 130 bp) was named BB (Fig. 2).

The mutation $A^{162}\rightarrow G$ was identified in the amplified fragments of *COX II* gene using P2 as primers which destroyed the restriction recognized by endonuclease Hind III. The allele A with mutation G^{421} (AGGCTT) cannot by digested by Hind III. However, the allele B with A^{421} can be recognized by Hind III. The fragment was cut into two paragraph (161 and 130 bp) by endonuclease Hind III. There is not reorganization in mitochondria DNA which meant there were two genotypes AA and BB at this site.

The PCR-RFLP polymorphic loci genotyping results of goat *COX II* gene showed that there were two genotypes in four goat breeds. Among them, the genotype BB was the dominant genotype while the genotype AA was the mutant genotype (Fig. 3). The number of samples and individual of each haplotype were shown in Table 2.

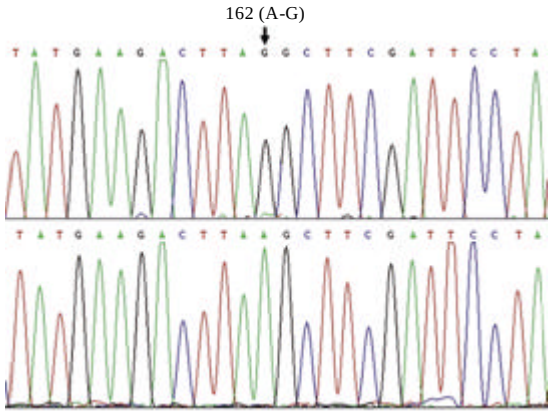

Fig. 3: Genotype AA and BB sequencing result of goats *COX II* gene

Table 2: Sample number and the number of individuals of each haplotype

Breed name	Sample size	Genotype AA	Genotype BB
Huanghuai goat	30	9	21
New grope of Anhui white goat	30	8	22
Boer goat	30	6	24
Boer x Huanghuai Hybrid goat	30	5	25

The relationship between the Hind III genetic variation of goat *COX II* gene and sperm motility: The buck DNA samples of Huanghuai goat, Anhui white goat, Boer goat and Boer x Huanghuai Hybrid goat were used to detect COX II genotype. The semen quality parameters of the corresponding samples were analyzed using mean±SD (Table 3).

The indicators, sperm density, vitality of original semen, frozen sperm motility, semen volume, acrosomal integrity and malformation rate were used to evaluate the semen quality. There was significant difference in the indicators of vitality of original fine and frozen sperm motility between the genotype AA and BB while there were no significant differences in the other four indicators.

The abnormalities of mammalian mitochondrial may affect the sperm motility and activities. The proteins encoded the mitochondrial DNA takes little part of mitochondrial proteins however, they constitute the important subunit of the mitochondrial respiratory chain enzymatic complexes and take part in mitochondrial respiratory chain and oxidative phosphorylation which play an important role in the energy production and maintenance of spermatozoa motility (Thangaraj *et al*., 2003; Carra *et al*., 2004). In recent years, people have focused on correlation between the defects of mtDNA and the obstacles of sperm activity in fertility study of human spermatozoa (Nakada *et al*., 2006; Folgero *et al*., 1993). Kao *et al*. (1998) investigated the deletions in 4977 and 7345 bp and mutation in 7599 bp of mtDNA were related with the obstacles of sperm motility.

Cytochrome Oxidase II (COX II) which is one of subunits made up the catalytic center of the enzyme is encoded by mitochondrial DNA. It is the combination site of mitochondrial cytochrome C. The changes of structure or function of COX II will destruct the mitochondrial respiratory chain directly (Piel *et al*., 2005). There is increasing evidence that mitochondrial DNA (mtDNA) anomalies in sperm may lead to infertility. Point mutations, deletions and depletion have been associated with decline of fertility and motility of human sperm. The association between occurrence of mtDNA 4977 bp deletion with diminished fertility and motility of human spermatozoa was studied. The results showed that the ratio of the deleted mtDNA in the spermatozoa with poor motility and diminished fertility were significantly higher than those in the spermatozoa with good motility and fertility. The finding suggested that mutation and deletion may play an important role in some pathophysiological conditions of human spermatozoa (Mohammad *et al*., 2006).

Since, male-specific phenotypes such as sperm have no fitness consequences for mitochondria due to maternal mitochondrial (mtDNA) inheritance, mtDNA mutations that are deleterious in males but which have negligible or no fitness effect in females can persist in populations. But in another study, to test whether there were associations between mtDNA variation and sperm performance, the researchers haplotyped 250 zebra finches (*Taeniopygia guttata*) from a large pedigreed-population and measured sperm velocity using computer-assisted sperm analysis (Mossman *et al*., 2010). Using quantitative genetic animal models, they found no effect of mtDNA haplotype on sperm velocity. The differences between these results and other findings may be due to the differences in genetic model between the mammals and birds. In the research, 200 idiopathic oligo-asthenozoospermic patients were classified on the basis of rapid progressive motility (a) and sperm concentration. Mitochondrial enzymatic activity was studied and correlated to the viability of sperm cells (Carra *et al*., 2004). Mitochondrial DNA purified from both motile and non-motile sperm of the same Individuals was amplificated using PCR. Results suggested that only motile sperm have organelles functional in oxygen consumption, unequivocally demonstrating that sperm motility depends on the mitochondrial activity. Mitochondrial DNA of oligo-asthenozoospermic patients seemed to present some defects that made DNA unavailable for amplification (Carra *et al*., 2004).

At present, the progeny test which was a long cycle and large investment method was mainly used to evaluate the valuation of livestock. The marker-assisted selection will be a shortcut for the evaluation. Sequencing results

Table 3: Goat semen quality traits of different COX II genotype

Indicators	Breeds							
	Huanghuai goat		Anhui white goat		Boer goat		Boer x Huanghuai Hybrid goat	
Genotype	AA	BB	AA	BB	AA	BB	AA	BB
Sperm density (hundred million mL^{-1})	12.41±0.020^{Aa}	12.28±0.010^{Bb}	12.00±0.010^{Aa}	12.00±0.010^{Aa}	11.98±0.010^{Aa}	12.09±0.020^{Aa}	12.00±0.020^{Aa}	11.98±0.020^{Aa}
Vitality of original semen (%)	0.71±0.024^{Bb}	0.81±0.033^{Aa}	0.78±0.039^{Bb}	0.85±0.038^{Aa}	0.75±0.036^{Bb}	0.83±0.043^{Aa}	0.73±0.044^{Bb}	0.82±0.043^{Aa}
Frozen sperm mtility (%)	0.35±0.020^{Bb}	0.41±0.017^{Aa}	0.39±0.021^{Bb}	0.43±0.021^{Aa}	0.38±0.020^{Bb}	0.44±0.015^{Aa}	0.38±0.015^{Bb}	0.42±0.021^{Aa}
Semen volume (mL)	1.28±0.074^{Aa}	1.16±0.059^{Aa}	1.00±0.059^{Aa}	0.96±0.044^{Aa}	1.07±0.039^{Aa}	0.89±0.048^{Aa}	1.27±0.055^{Aa}	1.23±0.055^{Aa}
Acrosomal integrity (%)	78.30±1.330^{Aa}	76.12±1.520^{Aa}	78.81±0.730^{Aa}	77.39±0.940^{Aa}	80.63±0.780^{Aa}	80.04±1.360^{Aa}	79.59±0.930^{Aa}	78.30±0.690^{Aa}
Malformation rate (%)	11.25±0.530^{Aa}	11.20±0.820^{Aa}	12.65±0.460^{Aa}	9.80±0.760^{Aa}	13.74±0.310^{Aa}	13.19±0.590^{Aa}	11.82±0.430^{Aa}	10.15±0.680^{Aa}

The values in the table are the mean±SD; the lowercase superscripts showed that genotype AA and BB in the same species had significant differences ($p<0.05$) while the values marked in the superscripts as uppercase were very significant difference ($p<0.01$)

of DNA pool showed a single nucleotide mutation in *COX II* gene (A→G), the mutation caused a change of Hind III restriction sites. RFLP results showed that the mutation associated with goat sperm traits, fresh sperm vitality and frozen sperm vitality of BB-type goats were significantly higher than AA-type. However, there is no significant difference in sperm density, semen volume, acrosomal integrity and malformation between the two genotypes. An analysis of mitochondrial genes that included Cytochrome Oxidase I (COX I), Cytochrome Oxidase II (COX II), Adenosine Triphosphate Synthase6 (ATPase6), ATPase8, transfer Ribonucleic Acid (tRNA) serine I, tRNA lysine and NADH Dehydrogenase3 (ND3) revealed, for the first time, 9 missense and 27 silent mutations in the sperm's mitochondrial DNA (mtDNA) but not in the DNA from the blood cells (Thangaraj *et al*., 2003). Among them, there was a 2-nucleotide deletion in the mitochondrial *COX II* genes, introducing a stop codon which might be responsible for low sperm motility. These results showed that the changes of *COX II* gene sequence were likely to affect sperm activity via the generation and transmission of ATP energy which could affect sperm motility.

At present, studies about *COX II* gene are focused on the liver, heart tissue cell apoptosis research but the effects of *COX II* gene mutation on goat sperm motility traits has not been reported. This study provided the establishment of the relationship between *COX II* gene SNP and goat sperm motility traits. It could be considered as a reference marker-assisted selection for goat buck semen quality traits and goat breeding.

CONCLUSION

The study showed that the mutation of *COX II* gene associated with goat sperm traits. The vitality of fresh sperm and frozen sperm from BB-type goats was significantly higher than AA-type. This study provided foundation for establishment relationship between *COX II* gene SNP and goat sperm motility traits.

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REFERENCES

Bansal, A., D. van den Boom, S. Kammerer, C. Honisch and G. Adam *et al*., 2002. Association testing by DNA pooling: An effective initial screen. PNAS., 99: 16871-16874.

Carra, E., D. Sangiorgi, F. Gattuccio and A.M. Rinaldi, 2004. Male infertility and mitochondrial DNA. Biochem. Bioph. Res. Commun., 322: 333-339.

Folgero, T., K. Bertheussen, S. Lindal, T. Torberqsen and P. Oian, 1993. Mitochondrial disease and reduced sperm motility. Hum. Reprod., 8: 1863-1868.

Kadenbach, B., S. Arnold, I. Lee and M. Hutteman, 2004. The possible role of cytochrome coxidase in stress-induced apoptosis and degenerative disease. Biochim. Biophys. Acta, 1655: 400-408.

Kao, S.H., H.T. Chao and Y.H. Wei, 1998. Multiple deletions of mitochondrial DNA are associated with the decline of motility and fertility of human spermatozoa. Mol. Hum. Reprod., 4: 657-666.

Mohammad, H.S., H. Massoud, B. Ali and S.S.P. Mehdi, 2006. Low sperm motility due to mitochondrial DNA multiple deletions. J. Chinese Clin. Med., 1: 181-187.

Montiel-Sosa, F., E. Ruiz-Pesini, J.A. Enriquez, A. Marcuello and C. Diez-Sanchez *et al*., 2006. Differences of sperm motility in mitochondrial DNA haplogroup U sublineages. Gene, 368: 21-27.

Mossman, J.A., J. Slate and T.R. Birkhead, 2010. Mitochondrial haplotype does not affect sperm velocity in the zebra finch *Taeniopygia guttata*. J. Evolut. Biol., 23: 422-432.

Nakada, K., A. Sato, K. Yoshida, K. Yoshida and T. Morita *et al*., 2006. Mitochondria-related male infertility. Proc. Natl. Acad. Sci., 103: 15148-15153.

Palanichamy, M.G. and Y.P. Zhang, 2011. Are ATPase6 polymorphisms associated with primary ovarian insufficiency? Arch. Gynecol. Obstet., 283: 671-672.

Piel, D.A., A.R. Khan, R. Waibel, M. Birbach and M.S. Cohen *et al*., 2005. Chronic hypoxemia increases myocardial cytochrome oxidase. J. Thorac. Cardiovasc. Surg., 130: 1101-1106.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual. 3rd Edn., Cold Spring Harbor Laboratory Press, New York, ISBN-13: 9780879695774, Pages: 99.

Sham, P., J.S. Bader, I. Craig, M. O'Donovan and M. Owen, 2002. DNA pooling: A tool for large-scale association studies. Nat. Rev. Genet., 3: 862-871.

Thangaraj, K., M.B. Joshi, A.G. Reddy, A.A. Rasalkar and L. Singh, 2003. Sperm mitochondrial mutations as a cause of low sperm motility. J. Androl., 24: 388-392.

Venkatesh, S. and R. Dada, 2011. An evolutionary insight into mutation of ATPase6 gene in primary ovarian insufficiency. Arch. Gynecol. Obstet., 284: 251-252.

Venkatesh, S., M. Kumar, A. Sharma, A. Kriplani and A.C. Ammini *et al*., 2010. Oxidative stress and atpase6 mutation is associated with primary ovarian insufficiency. Arch. Gynecol. Obstet., 282: 313-318.

Zhang, G.Q., X.L. Ling and Z.T. Cheng, 2006. Experimental study on expression of cytochrome oxidase II mitochondrial DNA in hepatocellular carcinoma cell lines and parental cell lines. Mod. Med. Heal., 22: 3709-3711.

Zickermann, V., M. Verkhovsky, J. Morgan, M. Wikstrom and S. Anemuller *et al*., 1995. Perturbation of the CuA site in cytochrome oxidase of Paracoccus denitrificans by replacement of Met227 with isoleucine. Eur. J. Biochem., 234: 686-693.