



UNIVERSITI PUTRA MALAYSIA

**ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS OF
SPAG11 GENE WITH SEMEN QUALITY AND TESTICULAR TRAITS OF
BOER GOATS**

MOHAMMADFAZEL HARIGHI

IPTSM 2019 8



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By

MOHAMMADFAZEL HARIGHI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfillment of the Requirements for the Degree of
Doctor of Philosophy**

May 2019

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DEDICATION

This dissertation is dedicated

To the fond memory of my late parents

*To my beloved siblings especially my kind brothers **Dr. Abdol Hosein Harighi** and **Dr. Mohamadalireza Harighi** for their constant love and generous support*

With love, respect and a bunch of memories
I Love You Forever



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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By

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May 2019

Chairman : Professor Abd Wahid Haron, PhD

Faculty : Institute of Tropical Agriculture and Food Security

Goat is a resistant species to Malaysian tropical climate. Boer is a goat's breed with a large body size that has been imported to Malaysia to improve the small body size of indigenous Katjang population. Since Boer population has become one of the important goat flocks in Malaysia, knowing about its characteristics, especially in connection with reproductive performance is an essential step for goat breeding programs. Bucks reproduction performance plays a key role in flock profitability affecting the number of pregnant does. Moreover, commercial sector is highly interested in identification of the buck fertility variation based on semen quality traits for artificial insemination (AI) purpose. Therefore, applying a proper selection method that is accurate in the selection of high fertile bucks would be a great approach.

Conventional selection methods are mostly time-consuming and costly. Besides, they are not proper for early selection purpose. Thus, using the single nucleotide polymorphism (SNP) of particular candidate genes as molecular marker might be an appropriate alternative. Sperm-associated antigen 11 (*SPAG11*) is known as an important candidate gene that affects the male reproductive performance. The objectives of this study were to find single nucleotide polymorphisms in a fragment of the *SPAG11* gene and to determine their associations with semen (both fresh and post-thaw) quality traits including testicular weight (TW), testes dimensions (length, width, and thickness), and testicular volume (TVm) in 52 heads Boer bucks. Semen was collected from each animal using electro-ejaculation (EE). Semen quality traits including sperm viability, morphology, motility, and density were analyzed using both Hemocytometer and light microscope. Semen volume and testes dimensions were measured using a graduated test tube and a dial

caliper, respectively. Levelled container was invented as an alternative method to measure TV. Genomic DNA was isolated from blood of each buck. Primers were designed to amplify two exons of the given gene. The SNPs were detected using single-base extension (SBE) method along with molecular cloning technique.

Four SNPs were detected in the coding region of exon 1 and 2 of the *SPAG11* gene, two of which were synonymous (1264A>G and 1333A>G) and other two namely 1278C>T and 1335G>A were non-synonymous SNPs caused changes in the amino acids Thr>Ile and Arg>Gln, respectively. The results indicated that the levelled container is a valid utility for measuring the testicular volume. There was a negative association between 1335G>A mutation with testicular volume ($p = 0.002$). Moreover, neither the studied testes dimensions (length, width, and thickness) nor TW were associated with detected SNPs. Although association between 1333A>G SNP and both fresh and post-thaw semen traits were insignificant, still negative effects on studied traits were observed. The *SPAG11* gene showed more association with sperm morphological traits rather than semen volume, sperm viability, and sperm density in fresh semen. The 1264A>G loci was negatively associated with sperm abnormality of head (%) trait in fresh ($p = 0.042$) and post thaw ($p = 0.018$) semen. The 1335G>A mutation was positively associated with sperm abnormality of head (%) ($p = 0.002$), mid piece (%) ($p = 0.008$), tail (%) ($p = 0.007$), and live sperm (%) ($p = 0.001$) traits in fresh semen.

In conclusion, this study elucidated significant association of *SPAG11* gene with sperm quality traits and TV in Boer goat. The detected polymorphisms of this candidate gene can be used as a marker assisted selection (MAS) for improving semen quality traits in the Boer goat populations. The detected SNPs (1264A>G and 1333A>G) that showed a negative association with semen quality traits, especially with both fresh and post thaw, can be considered as a marker for artificial negative selection. The significant association between this candidate gene and TV indicates that TV can be used as a proper trait for pre-selection of fertile Boer goats.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

HUBUNGAN POLIMORFISME NUKLEOTIDA TUNGGAL GEN SPAG11 DENGAN KUALITI AIR MANI DAN CIRI-CIRI ZAKAR KAMBING BOER

Oleh

MOHAMMAD FAZEL HARIGHI

Mei 2019

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Kambing adalah sepsis yang tahan terhadap iklim tropika di Malaysia. Kambing Boer merupakan sepsis kambing bersaiz besar yang telah diimport ke Malaysia untuk meningkatkan populasi saiz kecil badan kambing asli baka Katjang. Sejak populasi Kambing Boer menjadi salah satu kawanan kambing yang penting di Malaysia berkaitan tentang ciri-cirinya bagi prestasi pembiakan maka ia menjadi penting bagi program pembiakan kambing. Prestasi pembiakan kambing jantan memainkan peranan penting dalam keuntungan sektor peladangan yang memberi kesan positif dalam menambah bilangan ternakan. Selain itu, sektor perdagangan sangat berminat untuk mengenal pasti variasi kesuburan kambing jantan berdasarkan sifat kualiti air mani untuk tujuan inseminasi buatan (AI). Oleh itu, menggunakan kaedah pemilihan yang tepat dalam mengukur tahap kesuburan yang tinggi bagi kambing jantan akan menjadi pendekatan yang hebat.

Primer direka untuk menguatkan dua ekor gen yang diberikan. Serpihan yang disasarkan dihasilkan oleh teknik pengklonan molekul bersama dengan strategi penjujukan klon tunggal dan kemudian disempurnakan plasmid disusun menggunakan Kaedah Penjujukan Sanger. SNP dikesan menggunakan Kaedah Lanjutan Tunggal (SBE). Perisian *Geneious* digunakan untuk panggilan SNP. *Genotyping* SNP dijalankan berdasarkan urutan electropherograms untuk setiap individual. Empat SNP dikesan di kawasan pengekodan exon 1 dan 2 dari gen SPAG11, dua daripadanya adalah sinonim (1264A> G dan 1333A> G) dan dua lagi iaitu 1278C> T dan 1335G> A adalah SNPs tanpa identik menyebabkan perubahan dalam asid amino Thr> Ile dan Arg> Gln, masing-masing. Hasilnya menunjukkan bahawa "levelled container" adalah satu utiliti yang sah untuk mengukur isipadu testis. Terdapat persamaan negatif antara 1335G> Mutasi dengan jumlah testis ($p =$

0.002). Tambahan pula, dimensi ujian yang dikaji (panjang, lebar, dan ketebalan) atau TW dikaitkan dengan SNP yang dikesan. Walaupun hubungan antara 1333A> G SNP dan kedua-dua ciri-ciri semen segar dan pasca pencairan tidak begitu penting, namun kesan negatif terhadap ciri-ciri yang dikaji diperhatikan.

Gen SPAG11 menunjukkan lebih banyak perkaitan dengan ciri morfologi sperma berbanding dengan jumlah air mani, daya maju sperma, dan kepadatan sperma dalam air mani segar. 1264A> G loci dikaitkan secara negatif dengan kelainan sperma kepala (%) sifat segar ($p = 0.042$) dan selepas air cair ($p = 0.018$) air mani. 1335G> Mutasi positif dikaitkan dengan kelainan sperma kepala (%) ($p = 0.002$), bahagian tengah (%) ($p = 0.008$), ekor (%) ($p = 0.007$) ($p = 0.001$) sifat dalam air mani segar. Kesimpulannya, kajian ini membuktikan perkaitan gen SPAG11 yang signifikan dengan ciri kualiti sperma dan jumlah testicular dalam kambing Boer. Polimorfisme yang dikesan dari gen SPAG11 ini boleh digunakan sebagai pemilihan bantuan penanda (MAS) untuk meningkatkan sifat kualiti air mani dalam populasi kambing Boer. SNP yang dikesan (1264A> G dan 1333A> G) yang menunjukkan persamaan negatif dengan ciri-ciri kualiti air mani, terutamanya dengan pencairan segar dan pasca, boleh dianggap sebagai penanda untuk pemilihan negatif buatan. Hubungan ketara antara gen SPAG11 dan jumlah testicular ini menunjukkan bahawa jumlah testicular boleh digunakan sebagai sifat yang tepat untuk pra-pemilihan kambing Boer yang subur.

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Declaration by graduate student

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LIST OF ABBREVIATIONS

A	Adenine
μM	Micromole
$^{\circ}\text{C}$	Degree celsius
1X	Once
ADG	Average daily gain
AFLP	Amplified fragment length polymerase
AMOVA	Analyses of molecular variance
BLUP	<i>Best linear unbiased prediction</i>
bp	Base pair
BW	Birth weight
C	Cytosine
DF-REML	Derivative-free restricted maximum likelihood
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
DVS	Department of veterinary services
EBV	Estimated breeding value
EDTA	Ethylene diamine tera acetic acid
EPA	Extreme preferential amplification
EtBr	Ethidium bromide
FAO	<i>Food and agriculture organization</i>
H_o	Observed heterozygosity
h^2	Heritability
H_e	Expected heterozygosity
HMG	High mobility group

HWE	Hardy-weinberg equilibrium
LD	Linkage disequilibrium
MAS	Marker assisted selection
MgCl ₂	Magnesium chloride
ml	Millilitre
mM	Mill mole
mRNA	Messenger ribonucleic acid
MSA	Microsatellite analyzer
mtDNA	Mitochondrial dna
Na	Observed number of alleles
Ne	Effective number of alleles
ng	Nanogram
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RAPD	Random amplification of polymorphic dna
RFLP	Restriction fragment length polymorphism
rpm	Rotation per minute
SCD	Stearoyl-coa desaturase
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
T _m	Melting temperature
U	Unit
UPGMA	Un-weighted pair group method with arithmetic mean
UPM	Universiti putra malaysia
UV	Ultraviolet
μl	Micoliter

CHAPTER 1

GENERAL INTRODUCTION

Small ruminants (goats and sheep) are an important source of proteins raising in both rural and commercial systems (Smith et al., 2013). Goat as a hardy animal is an adapted livestock to Malaysian tropical climate. In 2017, goat (n \approx 450,000) comprised 75% of the total small ruminants' population in Malaysia (DVS, 2016).

Boer doe is highly fertile with a rate of 180% kidding (1.8 kid per doe), 90% conception, and 210% fecundity (fertility). The Boer goat can conceive at 6 months of age but the best age for gestation is between 10 to 14 months when it weighs approximately 40 kg. Since the reproduction of this goat breed is not seasonal and its gestation length is 148-155 days (about 5 months), it can kid every 8 months under excellent farm management (Malan, 2000).

Based on Boer doe reproductive traits, one young pregnant Boer doe can reproduce up to 22 heads in 5 years or to 451 heads in 10 years with the assumption that conception rate is 100% and mortality rate is 0%. However, in reality a Boer doe can only breed for 8 to 12 years and, on average, for 10 years thus, can reproduce 20-35 kids overall (Furstenburg, 2015).

High reproductive capacity of buck plays a key role in enhancing the conception rate of doe which eventually increases reproductive efficiency in goats (Mahgoub, et al., 2012). Reproductive efficiency also called reproductive performance is one of the selection criteria for goat meat production. It is considered as an important factor affecting profitability and productivity of goats' flock (Flores et al., 2000; Pardos et al., 2003; Martin et al., 2004).

Reproductive efficiency has been considered as an important factor because the increasing trend in red-meat consumption caused by world population growth definitely demands the optimal improvement in reproductive performance (Thornton, 2010).

Moreover, fertility status and genetic variation in reproductive potential of males are of substantial commercial interests (Notter, 2012). Particularly, semen quality traits are great criteria for selection of male animals to use in AI centres (Robinson and Buhr, 2005; Gonzalez-Pena, et al., 2016). Superior Boer bucks have a strategic role in AI technique to control kidding dates and optimize selection schemes in goat breeding programs (Arrebola et al., 2012). However, judicious and efficient use of candidate genes to increase

reproductive efficiency can be a reasonable strategy in Boer bucks (Notter, 2012).

Moreover, in order to enhance goat production, implementation of advanced breeding programs such as using marker assisted selection (MAS) through molecular markers are necessary. In this way, the maximum reproductive capacity of goat including genetic variation between and within breeds can be used properly.

1.1 Research Problem

Traditional selection methods fail to account the effects of genetic diversity on estimating the genetic merit of an animal (Sonstegard et al., 2001). This weakness is more critical in selection of traits with low heritability such as reproduction traits with heritability between 0.19 and 0.39 and semen quality (Druet et al., 2009). Moreover, conventional methods are based on genetic merit which is calculated from pedigree and phenotypic information that is often lacking regarding semen quality traits in Malaysia.

1.2 Research Hypothesis

More recently, selection techniques have been developed based on a combination of marker-assisted selection (MAS) and phenotypic information, known as genomic estimated breeding value (GEBV) (Hu & Yang, 2014). Detecting candidate genes polymorphisms and studying their association with semen quality traits would enable animal breeders to use the potential of molecular markers for accurate prediction of the semen quality traits values. This in turn might improve semen quality traits genetically in Boer goats.

1.3 Objectives

1.3.1 General Objective

The general objective of present study was to detect and analyse the single-nucleotide polymorphisms on semen quality and testicular traits to select the fertile Boer bucks.

1.3.2 Specific Objectives

The specific objectives of present study were:

- 1) To distinguish the characteristic of both fresh and thawed frozen semen in Boer goats.
- 2) To determine the characteristic of testicular traits in Boer bucks.
- 3) To investigate polymorphisms of *SPAG11* gene associated with testicular biometric and quality traits of both fresh and thawed frozen semen in Boer bucks.



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BIODATA OF STUDENT

Mohammad Fazel Harighi was born in Kermanshah, Iran. He obtained his MSc degree of Genetic and Animal Breeding in 2004. He has worked as a lecturer in Islamic Azad University of Kermanshah since 2004 to 2011 and he has selected as superior researcher during the years 2005-8 at the corresponding university. He has registered five inventions which were mostly in animal science area. During the study and teaching, he has followed training, courses and symposiums in the respective field of specialization.



LIST OF PUBLICATIONS

Journals

Harighi, M. F., Wahid, H., Rafii, M. Y., & Jesse, F. F. A. (2017). Novel SNPs in the SPAG11 gene and their association with testicular biometric parameters in Boer goats and application of the levelled-container technique. *Animal Reproduction science*. 2019.

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NAME OF STUDENT: MOHAMMADFAZEL HARIGHI

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