MOLECULAR GENETIC STUDIES ON SOME FECUNDITY GENES IN SOME EGYPTIAN SHEEP BREEDS

By

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
D.W	Distilled water
FAO	Food and Agriculture Organization
Forced-RFLP	Forced Restriction fragment length polymorphysim
ICARDA	International Center for Agricultural Research in the Dry Areas
M	Marker
ms	Microsatellite
MS	Molecular Size
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction- Restriction fragment length polymorphysim
PCR-SSCP	Polymerase chain reaction – Single Strand Conformetional Polymorphism.
RACE	Rapid Amplification of CDNA Ends
RAPD	Random amplified polymorphic DNA
RF	Relative Front
SCAR	Sequencing characterization Amplified region
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats

ABSTRACT

Asmaa Mohammed Aly Abu Shady: Molecular Genetic Studies on Some Fecundity Genes in some Egyptian Sheep Breeds. Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2009.

The present study has been carried out in the Department of Genetics and Ain Shams Center for Genetic Engineering and Biotechnology Branch of Faculty of Agriculture, Ain shams university. (ACGEBFAG)

Two local breeds of Egyptian sheep (Ossimi and Barki) were studied to detect the fecundity genes (*FecB, FecX*^{I}) using biochemical and molecular genetic techniques. Protein banding patterns using native gel electrophoresis was carried out to identify the differences between each breed.

Meanwhile, a trial to identify molecular genetic markers of studied breeds has been also carried out. In this respect, twelve SSR primers were used to detect the fecundity gene (*FecB*). On the other hand a specific primer for a mutation that occur in *FecB* and *FecX*^{I} and its presence has been used Forced-RFLP technique.

On the other hand primers 300U, UNC5C (GC101), BMS2508 and LSCV043 could be used to detect differences between the heterozygote and homozygote genotypes forms of *FecB* gene. However, the Forced-RFLP primers gave almost similar results, which pointed that neither the high twining rate females nor low twining rate have a mutation in both *FecB* and *FecX*^{I} of the two breeds; Ossimi and Barki.

Key Words: Biochemical and molecular genetic markers, Egyptian sheep breeds, Fecundity genes, *FecB*, *FecX*, Forced-RFLP.

I. INTRODUCTION

Animal genetic resources are essential components of agriculture development. They have contributing to food and agriculture for more than 12000 years. Sheep is one of the small livestock of choice in Egypt, where sheep meat production is more important than fiber production. Egyptian sheep population increased by 66.7% from 1961 to 2005.

There are three major breeds in Egypt; Rahmani, Ossimi, Barki. Rahmani is spread mainly in north of the Nile delta, Ossimi in mid Egypt and Barki in western Mediterranean Coastal region. Minor breeds like Saidi, Sohagi, located in south Egypt, still need more investigation. **(ICARDA, 2006).**

Barki ewes are sexually early maturers compared with Rahmani and Ossimi ewe lambs. Ewes cycle year–round and lamb more than once a year, but are less active in late spring. The twining rate, {as average = 4.5 (n=937)} **Aboul-Naga 1976.** On the other hand, the twining rate in Ossimi breeds {as average =14.3} **El-Wishy *et al*., 1971** but there is no available data for Rahmani**.**

For increasing sheep meat production, the number of born lambs should be increased. This will be achieved through increasing both ovulation rate and litter size, these traits are genetically controlled. So studying genes affecting ovulation rate and litter size, which are known as fecundity genes, become a must.

Since 1980 there has been increasing interest in the identification and utilization of major genes for prolificacy in sheep. Mutations that increase ovulation rate have been discovered in the **BMPR-1B** (bone morphogenetic protein 1B receptor)**, BMP15 (**Oocyte-derived bone morphogenetic protein 15) and **GDF9** (growth differentiation factor 9) genes, and others are known to exist from the expressed inheritance patterns although the mutations have not been located.

The current knowledge of major genes for prolificacy in sheep falls into three categories: (1) genes where the mutation has been identified and DNA testing is available; (2) genes where the mode of inheritance

has been described but the mutation has not been identified; and (3) putative genes where there is evidence of apparent genetic segregation but there are insufficient records to ascertain the mode of inheritance.

Concerning of the types of Fecundity genes in sheep (*FecB, FecX and* $FecG^{H}$) it has been found in the case of *FecB* (Booroola Merino), is the result of a mutation in the BMPR-1B (bone morphogenetic protein 1B receptor). The mutation has been named FecB by the committee on Genetic Nomenclature of Sheep and Goats **(COGNOSAG, 1989)** and is considered to be single mutation, duplication, or deletion event **(Montgomery *et al*., 1992)** that is expresses in oocytes and granulose cells. Dominant single autosomal gene in chromosome 6. Additive effect of ovulation rate. One copy of FecB increases ovulation rate about 1.5 and two copies 3.0 These extra ovulations in turn increase litter size by 1.0 and 1.5. **(Davis, 2004)**. It is hypothesized that this mutation might be reduced the signaling through the receptors of granulosa cells **(Wilson et.al., 2001) .** The mutation that cause super prolificacy and the mutant sheep are characterized by precocious differentiation of ovarian follicles, leading to the production of large numbers of ovulatory follicles that are smaller in diameter than wild-type follicles.

The Booroola merino was the first breed of sheep where ovulation rate and litter size were shown to be affected by segregating major gene **(Piper *et al*., 1985).**

The inheritance pattern of the inverdale gene $FecX^{I}$ Romney sheep and Cambridge & Belclare breeds was discovered in 1990, that Inverdale sheep have a mutation in Oocyte-derived bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) are key regulators of follicular development. Here we show that these factors control cumulus cell metabolism, particularly glycolysis and cholesterol biosynthesis before the preovulatory surge of luteinizing hormone **(Su *et al*., 2008).** Transcripts encoding enzymes for cholesterol biosynthesis were down regulated in both Bmp15–/– and Bmp15–/– Gdf9+/– double

mutant cumulus cells, and in wild-type cumulus cells after removal of oocytes from cumulus-cell-oocyte complexes.

Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are two well-characterized oocyte-derived growth factors that play crucial roles in follicle growth and ovulation in all mammalian species studied, including rodents, domestic ruminants and humans. GDF9 and/or BMP15 are probably major players of the 'oocyte-granulosa cell regulatory loop'.

There are four different mutations have been discovered but each produced the same phenotype with four different alleles ($FecX^{I}$, $FecX^{H}$, $FecX^{G}$, $FecX^{B}$). X-linked, Increased ovulation rates in heterozygous and infertility due to streak ovaries in homozygous. **(Davis, 2004)**

Other prolific sheep breeds and genes affecting ovulation rate, such as Cambridge and Belclare breeds ($FecG^{H}$), Cambridge (*FecC*), Thoka (*FecI*), Javanese (*FecJ*), Olkuska, Belclare, Lacaune and Woodland ($FecX_2$) have been identified from various countries. All these mutations can be detected directly by a forced PCR restriction fragment length polymorphism RFLP **(Davis *et al*., 2002)**.

Thus it is highly recommended that biochemical and molecular genetic markers would act as good tools for detection of mutation in sheep breeds and will be helpful in the genetic breeding programs for the improvement of reproductive traits.

The objectives of this study were to obtain:

1. Molecular and Biochemical genetic analysis of the main two Egyptian sheep breeds (Ossimi and Barki) through protein and DNA studies.
2. Using both the Simple Sequence Repeats and Forced Restriction fragment length polymorphysim techniques for screening these two breeds for the presence of the Booroola fecundity genes.