A NEW ALLELE FOR THE OVINE PROLACTIN GENE

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INTRODUCTION

Prolactin (PRL), a protein hormone produced mainly in the anterior pituitary gland is responsible for more than 300 known physiological actions that were extensive reviewed (Bole-Feysot *et al*., 1998 ; Freeman *et al*., 2000). Prolactin is very complex at the structural and functional point of view and the variants can be a result of alternative splicing of the primary transcript, proteolytic cleavage and other post-translational modifications of the amino acid chain, like dimerization and polymerization, phosphorylation, glycosylation, sulfation and deamidation. The mechanism of action is also complex since PRL may act as a hormone, by the classic endocrine pathway, and as a growth factor, neurotransmitter, or immunoregulator, in an autocrine-paracrine fashion.

Prolactin, growth hormone and placental lactogens, constitute an hormone family that probably results from the duplication of an ancestral gene (Cooke *et al*., 1981). Duplicate genes arise frequently in eukaryotic genomes, either via local events that generate tandem duplications, larger-scale events that duplicate chromosomal regions or entire chromosomes, or genome wide events that result in complete genome duplication (polyploidization) (Lynch and Force, 2000).

Little is known about the sheep prolactin gene. The first cDNA sequence was obtained by Adams *et al.* (1989), followed by Varma *et al*. (1989) and the chromosomal location is unknown.

The aim of this work was obtain information about the *Hae*III and *Mse*I RFLP in some Portuguese breeds based on the work of Vincent and Rothschild (1997).

MATERIAL AND METHODS

Peripheral blood samples were collected from 50 unrelated animals from several flocks of Churra Mondegueira (CM) and Churra Terra da Quente (CTQ) Portuguese sheep breeds registered in each genealogical book. DNA was purified using a saline method (Montgomery and Sise, 1990).

Using a modified methodology and primers previously described by Vincent and Rothschild (1997) we performed a PCR amplification (10 µl final volume) using 50 ng of genomic DNA, 350 µM each dNTP, 0.3 µM each primer, 1.1 mM $MgCl_2$,1 x *Taq* Extender Buffer, 0.5 units *Taq* Extender, and 0.5 units *Taq* polymerase. The thermal cycler profile consisted in 92°C for 2 min ; 35 cycles of 92°C for 45 sec, 56°C for 45 sec, and 72°C for 3 min, followed by a final extension step at 72°C for 7 min.

The 10 microliters of the 2.5 kb product were digested with *Bsu*RI (*Hae*III) (MBI Fermentas) restriction enzyme according to producer recommendations to generate bands of approximately 1400, 530, 360 and 150 bp for the A allele and 1400, 510, 360 and 150 bp for the B allele, and with *Tru*1I (*Mse*I MBI Fermentas) restriction enzyme, according to producer recommendations

to produce bands of approximately 715, 510, 265, 245, 175, 120, 100 and 40 bp for the A allele and bands of approximately 715, 510, 265, 185, 175, 120, 100 and 40 bp for the allele B.
DNA fragments were separated by electrophoresis in 3 % agarose gels and visualised using UV light following ethidium bromide staining.

RESULTS AND DISCUSSION

The bands profiles obtained for *Hae*III PCR-RFLP are shown in figure 1. There are bands that are common to all genotypes 1400, 360 bp) and others that are specific for the genotypes (510, 530,150 and 680 bp). The last 6 lanes are the profiles described by Vincent and Rothschild (1997) for AA, AB, BB genotype respectively.

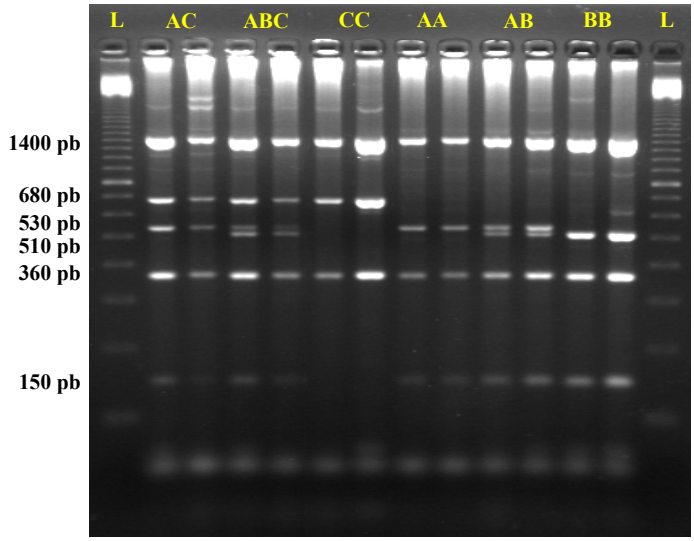

Figure 1. Patterns observed with *Hae*III PCR-RFLP. L – 100 bp Lader (Amersham Pharmacia Biotech)

The first 6 lanes are new genotypes found in Portugueses breeds, that differ from the previously reported patterns by the presence of an additional band with approximately 680 bp length assigned as C. The animals that are homozygous for C (lane 5 and 6) don't show the 530 bp (allele A) or 510 bp (allele B) and 150 bp bands, therefore, the band C could be due to the loss of the *Hae*III site restriction site responsible for generating the 530 and 150 bp bands. We observed genotypes AC and ABC but we have not observed the BC genotype (see table 1). The presence of animals with genotype ABC in these breeds could be due to incomplete digestion of the PCR product as the bands don't appear to be equimolar or possibly gene duplication. In fact abnormal digestion patterns of heterozygotes, particularly double heterozygotes can some times be observed, due for example to the formation of heteroduplex DNA molecules (Bradley *et al*., 1998). This could be the reason for the apparent absence of BC animals, and the presence of CC animals. We have excluded the possibility of contamination by repeating the

genotyping. We also observed the presence of the C allele and the genotype ABC in 12 other Portuguese sheep breeds (data not published). There are many examples of gene duplication, Valinsky *et al.* (1990) found a duplication in the sheep growth hormone gene using the RFLP technique. Using the isoelectric focus technique, Oliva *et al.* (2000) found three isoforms for the ovine pituitary prolactin.

Table 1. Genotypic frequencies for 50 CM and 58 CTQ sheep breeds

Genotype	CM	CTQ	CM (%)	CTQ (%)
AA	12	21	24	36.2
AA-C	8	7	16	12.1
AB	20	20	40	34.5
AB-C	2	5	4	8.6
BB	6	3	12	5.2
CC	2	2	4	3.4

To better describe the three allele resulting from the *Hae*III digestion, two animals were selected for each genotype found (AC, ABC, CC, AA, AB, BB) and the PCR products were digest with *Mse*I restriction enzyme. The results are shown in figure 2.

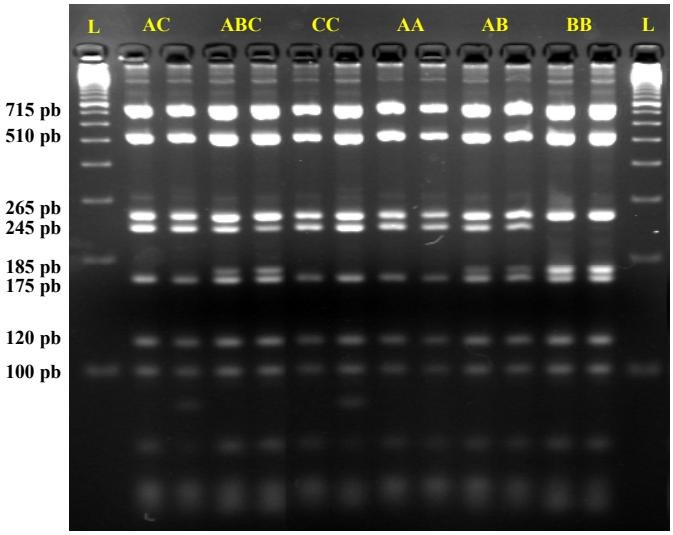

Figure 2. Patterns observed with *Mse*I PCR-RFLP. L - 100 bp Lader (Amersham Pharmacia Biotech)

The AC, CC, and AA genotypes from *Hae*III PCR products digestion showed the same pattern when digested with *Mse*I restriction enzyme(see figure 2).This suggest that the C allele was

derived from A allele by mutation on the *Hae*III restriction site. At this moment we are to proceed to the sequencing of the alleles and search for gene duplication by Southern blot analysis.

ACKNOWLEDGEMENTS

The study was supported by the FCT - Fundação para a Ciência e Tecnologia (project POCTI/34664/CVT/2000) and ICETA-UTAD. The authors are grateful to the breeders associations ANCOTEQ and COVICÔA for helping on collecting the blood samples. The authors wish to thank Allan Crawford for useful comments on the draft manuscript.

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