

ABSTRACT

Insights into the role of prion-like genes and proteins on scrapie susceptibility and ram fertility

The established association between polymorphisms of *prnp* prion gene and susceptibility to *scrapie* disease in sheep prompted the development of breeding programmes aimed at increasing the natural resistance to *scrapie* in the European Union. In order to study the possible undesirable consequences from the widespread selection for the *prnp* genotype on ovine genetic diversity and reproduction, we primarily focused our investigation in the ovine *prnd* gene which encodes a prion-like protein designated as Doppel that maps to the same chromosomal region as *prnp*, but is mainly expressed in testis. When *prnd* is overexpressed in the nervous tissue, Doppel is neurotoxic and causes neurodegenerative disease. We genotyped 460 animals (207 female and 253 male) from 8 Portuguese sheep breeds (Bordaleira entre Douro e Minho, Churra Badana, C. Galega Mirandesa, C. Mondegueira, Merino da Beira-Baixa, M. Branco, Saloia e Serra da Estrela), for *prnp* and *prnd* and established a parallel between a polymorphism in the *prnd* gene (78G>A) and scrapie susceptibility. We also identified an association between *prnd* polymorphisms and fertility traits. Upholding the emphasis on ovine reproduction, recombinant Doppel (rDpl) was supplemented in different concentrations to ram spermatozoa during the capacitation process. Regardless of dosage, rDpl improved sperm individual motility and vigour, and enhanced *in vitro* spermatozoa fertilizing ability. In addition, we determined by Nuclear Magnetic Resonance (NMR) spectroscopy the three-dimensional structure of the N-terminal (1-30) ovine Doppel (OvDpl) peptide, encompassing the 78G>A polymorphism and the entire signal peptide sequence. The new solved three-dimensional structure was subsequently used to construct molecular models with the M-domain of the signal recognition particle subunit (SRP54M). Simultaneously, we were able to obtain soluble mature Dpl protein through the co-expression with the SUMO chaperone. Finally, we developed a new polyclonal antibody (APPA) demonstrating for the first time that the recently discovered ovine *prnt* gene is a translated protein-coding gene and not a pseudogene, and identified through immunofluorescence and immunohistochemistry the location pattern of the encoded protein Prt in ram testis along spermatogenesis and in ejaculated spermatozoa. These results guided us to our last work, where data obtained through Prt blockage pointed to a physiological role for Prt in ovine fertilization (possibly through an interaction with zona pellucida proteins), reinforcing the importance of prion-like genes and proteins in the ovine reproductive physiology.

Key-words: antibody, Doppel, polymorphisms, prion, *prnd*, Prt, ram, reproduction, RMN, spermatogenesis.