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Doppel gene polymorphisms in Portuguese sheep breeds: Insights on ram fertility

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ABSTRACT

Transgenic knockout of the gene encoding the prion-like protein Doppel leads to male infertility in mice. The precise role of Doppel in male fertility is still unclear, but sperm from Doppel-deficient mice appear to be unable to undergo the normal acrosome reaction necessary to penetrate the zona pellucida of the oocyte. The objective of this study was to characterize Doppel (*Prnd*) gene polymorphisms in eight Portuguese sheep breeds and to determine a possible relationship between these polymorphisms and ram fertility. Ovine genomic DNA of 364 animals of different breeds (Bordaleira entre Douro e Minho, Churra Badana, Churra Galega Mirandesa, Churra Mondegueira, Merino da Beira Baixa, Merino Branco, Saloia and Serra da Estrela) were analysed by multiple restriction fragment-single-strand conformation polymorphism (MRF-SSCP). This analysis revealed a synonymous substitution G → A in codon 26 of *Prnd* gene. Churra Galega Mirandesa and Saloia breeds were more polymorphic ($P=0.005$ and $P=0.04$, respectively) than the overall population, while Serra da Estrela and Merino Branco animals were less polymorphic ($P=0.007$ and $P=0.04$). No polymorphism was found in Churra Mondegueira

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breed. Semen from 11 rams of Churra Galega Mirandesa breed (7 homozygous wildtype GG and 4 heterozygous GA) routinely used in the Portuguese Animal Germoplasm Bank was collected and frozen for fertility tests. A classification function was estimated, using data from post-swim-up semen motility and concentration and Day 6 embryo production rate, allowing the identification of the Doppel homozygous GG genotype with 86.7% of accuracy. This preliminary study detected the presence of only one polymorphism in codon 26 of *Prnd* gene in the Portuguese sheep breeds. In the polymorphic Churra Galega Mirandesa breed, GG genotype could be characterized through a model using three fertility traits, suggesting a relationship with male reproduction. Any future research should investigate not only AA genotype and its influence on ram fertility but also the possible consequences of the European Community selection program to eradicate Scrapie on the *Prnd* genotypes and indirectly on sheep breed's viability and preservation.

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