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Identification of bovine doppel protein in testis, ovary and ejaculated spermatozoa

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Abstract

Doppel (Dpl) protein is a recently identified prion-like protein. Although Dpl might be expressed in the brain after prion gene deletion, in both human and mice Dpl is normally expressed only in testis and spermatozoa, where it appears to be involved in male fertility. Little information is available so far about the expression pattern of Dpl in bovines, thus, hampering possible research on the role of this protein in bovine infertility. We have thus, designed, produced and validated through Western blotting a polyclonal antibody against bovine Dpl. With this antibody we then screened bovine tissues for Dpl expression by immunohistochemistry. Ejaculated spermatozoa were screened by flow cytometry and immunocytochemistry.

Bovine Dpl was expressed in all the developing stages of germinal cells, from spermatogones to ejaculated spermatozoa, in Sertoli cells and in ovarian follicles (granulosa cells and follicular fluid). Dpl immunoreactivity was also found on other tissues, where endothelial cells, peripheral nerves and scattered lymphocytes stained positive. This distribution pattern suggests that Dpl might be involved in sperm maturation/capacitation in bovines, like it might be in mice. This hypothesis needs to be verified by widespread application of the flow cytometric protocol established in this paper on spermatozoa from animals with reduced fertility.

Keywords: Doppel protein; Fertility; Western blotting; Immunohistochemistry; Flow cytometry

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1. Introduction

Doppel (Dpl) is an acronym for downstream prion protein-like [1]. *Prnd* is the gene encoding for Dpl protein and is located 16–20 kb (depending on the species) downstream from *Prnp*, the gene encoding for prion proteins (PrP) [2,3]. *Prnd* contributes, together with *Prnp* and with the recently discovered *Prnt*, to the so called ''prion gene complex'', *Prn*. Dpl and PrP present a 25% sequence homology [4,5]. Nevertheless, some structural differences and the lack of Dpl overexpression in patients with PrP-induced diseases [6] suggests that Dpl is not involved in prion pathology. Dpl expression induces neurological signs only in PrP knockout mice [1,5,7,8] and except for transient expression in the brain of neonatal mice [9] *Prnd* mRNA and/or Dpl protein are not expressed in the central nervous system (CNS) of healthy animals. On the other hand, intense expression has been recorded in both testis and heart [1,7,10]. Furthermore, the localization of human Dpl on Sertoli cells and spermatozoa [10] and the lack of expression observed in cases of male sterility in mice [11] suggests that the main function of this protein is male fertility regulation. The demonstration of a similar role in cattle could assist in the study, diagnosis and prevention of bovine infertility. Studies concerning Dpl function, however, require a precise knowledge of cellular types expressing the protein. Unfortunately, Dpl distribution in bovine tissues was studied only at the mRNA level [12]: *Prnd* mRNA was mainly detected in testis, ovary and spleen. The tissue distribution of the protein and its localization within each tissue have not yet been investigated in cattle.

Aims of the present study were: to produce a specific polyclonal antibody for bovine Dpl and to validate it's specificity by Western blotting against recombinant bovine Dpl; to characterize bovine Dpl in tissues by a panel of Western blotting approaches; to establish an immunohistochemical protocol and to verify whether Dpl distribution in bovine tissues is consistent with that recorded in other species; to identify bovine Dpl in ejaculated spermatozoa by immunocytochemistry and flow cytometry techniques.

2. Materials and methods

2.1. Production and validation of a polyclonal anti-bovine Dpl antibody

Polyclonal antibody anti-bovine Dpl (boDpl67–81) was raised in rabbits following standard immunization procedures and using the synthetic peptide DIDFGVEGNRYYEAN, corresponding to the residues 67–81 of bovine Dpl.

Recombinant bovine Dpl (rBoDpl) was produced using Dpl cDNA kindly provided by Prof. L. Ferretti (University of Pavia, Italy). After amplification, cDNA gene was cloned into the plasmid pQE30 (Qiagen SpA, Milano, Italy) via the BamH1 and KPN1 restriction enzyme sites and the plasmid was then overexpressed in *Escherichia coli* cultures. Recombinant BoDpl was then purified by chromatography, using MC-POROS column, 100 mm × 4.6 mm i.d. (Applied Biosystems, Milano, Italy), charged with Cu^{2+} ions according to manufacturer's instructions, and equilibrated in 10 mM Tris pH 7.5, 6 M Urea and 0.5 mM imidazole. rBoDpl was then eluted with 100 mM imidazole in equilibrating buffer. Urea was removed by dialysis.