# Characterization of the functional domains of boar acrosin involved in nonenzymatic binding to homologous zona pellucida glycoproteins

**J.A. Crosby**, **R. Jones**, **C. Barros**, **P. Carvallo**.

## Abstract

During the first steps of the gamete interaction, the proacrosin/acrosin system seems to play a crucial role in the secondary binding, holding acrosome-reacted spermatozoa during their passage through the zona pellucida. To analyze the functional domains of acrosin, we decided to express recombinant boar acrosin proteins in bacteria and to study their binding capacities to zona pellucida glycoproteins (ZPGPs). The expressed proteins were immunodetected by Western blot with a polyclonal antiacrosin antibody. The recombinant truncated β-acrosin has a typical hyperbolic curve of a zymogen enzymatic activation. Three of the five recombinant forms (truncated β-acrosin, Ser/Ala$^{222}$-truncated β-acrosin, and truncated β-acrosin “heavy chain”) had the ability to bind ZPGPs. The two shorter forms (the amino and carboxy termini of truncated β-acrosin) failed to bind. The catalytic site mutant (Ser/Ala$^{222}$) of truncated β-acrosin does not differ from the recombinant truncated β-acrosin in its mechanism of interaction to ZPGPs, indicating that this secondary binding is done by a nonenzymatic process. Our results show that binding between acrosin and ZPGPs depends on the secondary and tertiary structures of acrosin and does not depend on an active catalytic site.