...odies against boar sperm zona pellucida-binding protein AWN-1. Characterization of a continuous antigenic determinant and immunolocalization of AWN epitopes in inseminated sows

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Abstract:

Boar spermadhesin AWN-1 is a sperm surface-associated 14.7-kDa lectin and a major protein of porcine seminal plasma. AWN-1 binds to beta-galactosides and to porcine zona pellucida glycoproteins, suggesting that this protein might play a role in the primary binding of spermatozoa to the egg's external glycoprotein matrix. We have produced a collection of murine monoclonal antibodies against purified AWN-1. Five monoclonal antibodies recognized sequential antigenic determinants. All these epitopes were located at the C-terminal region of AWN-1 (residues 109-123) by competitive ELISA using overlapping synthetic peptides that cover the complete 133 amino acid sequence of the lectin. In a structural model of spermadhesin AWN-1, the polypeptide stretch 109-123 is fully solvent-exposed, providing a reasonable explanation for its high immunogenicity. In addition to epitope mapping, we have employed anti-AWN monoclonal antibodies for immunolocalization of the protein in the genital tract of inseminated sows. Clusters of AWN epitopes were occasionally found attached to the epithelium of the uterotubal junction and the adjacent lower isthmus. However, neither AWN-1 nor other seminal plasma proteins were found in the isthmic fluid collected 10-26 h after insemination. These results suggest that the whole amount of seminal plasma proteins are absorbed by the epithelium of the female genital tract, supporting the claim that removal of seminal plasma components from spermatozoa might be a major event in both in vitro and in vivo sperm capacitation.