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## JOURNAL ARTICLE

# Immunocytochemical localization of acrosin on both acrosomal membranes and in the acrosomal matrix of porcine spermatozoa

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Immunocytochemical techniques were employed to determine, at the ultrastructural level, the location of acrosin in porcine spermatozoa. Antisera to highly purified porcine acrosin was produced in rabbits. The (Fab')<sub>2</sub> fragments of the immunoglobulins were purified and conjugated with horseradish peroxidase (HRP). Washed, formaldehyde-fixed spermatozoa were reacted with the labeled antiacrosin immunoglobulins, utilizing a direct staining technique. Electron microscopy revealed that the peroxidase reaction product of HRP-antiporcine acrosin was distributed evenly over the outer acrosomal membrane of spermatozoa with intact acrosomes. The labeled antibody was also distributed evenly over the inner acrosomal membrane of cells when the overlying acrosomal structures were absent. In some spermatozoa, labeling was noted throughout the acrosomal matrix. No significant labeling was observed in control specimens when spermatozoa were exposed to HRP-antiporcine acrosin immunoglobulins that had been adsorbed previously with excess purified acrosin or exposed to HRP-conjugated rabbit antiporcine immunoglobulins. This pattern of labeling is consistent with the hypothesis that acrosin may function as a zona lysin. The observation that the outer acrosomal membrane and acrosomal matrix are labeled suggests that acrosin is not exclusively located on the inner acrosomal membrane and, thus, could participate in physiologic events other than zona penetration.

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