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## JOURNAL ARTICLE

# Use of peanut agglutinin to assess the acrosomal status and the zona pellucida-induced acrosome reaction in stallion spermatozoa

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Peanut agglutinin (PNA) was used to assess the sperm acrosomal status and the acrosome reaction during gamete interaction in the equine species. PNA exclusively binds to the outer acrosomal membrane of stallion spermatozoa, as was established by transmission electron microscopy. Fluorescein isothiocyanate-PNA (FITC-PNA) labeling was used to monitor sperm acrosomal changes during a prolonged incubation period of 24 hours and during a 2-hours incubation in the presence of 5 micromM calcium ionophore A23187. In addition, after a 4-hours preincubation in SP-TALP medium, sperm samples were incubated with matching hemizona for 1 minute (onset binding) followed by a 60-minute incubation (1-hour binding) of the sperm-hemizona complexes in sperm-free medium to assess the acrosomal status of the bound spermatozoa. For acrosome assessment, spermatozoa and washed sperm-hemizona complexes were air dried onto microscope slides, fixed, permeabilized in ethanol, stained with FITC-PNA, and counterstained with the DNA dye ethidium homodimer. Both zona-bound and non-bound spermatozoa showed similar staining patterns. Acrosome-intact spermatozoa displayed intensively green fluorescence over the acrosomal cap, whereas reacting spermatozoa showed a patchy disrupted image of fluorescence. Sperm cells that completed the acrosome reaction were principally stained on the equatorial segment or not stained at all. During prolonged incubation and during the calcium ionophore treatment, the proportion of spermatozoa with an acrosomal modification (reacting) and a complete breakdown of the acrosome (reacted) increased noticeably. Significant induction of the acrosome reaction was observed within 60 minutes of sperm-zona contact ( $P < 0.001$ ). In conclusion, a rapid and reliable assessment of the acrosomal status and the incidence of the acrosome reaction of stallion spermatozoa at the zona surface were demonstrated in this study.

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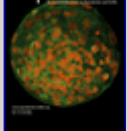
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Biol Reprod, October 1, 1998; 59(4): 733 - 742.

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