Studies on sperm capacitation, sperm-egg binding, and induction of acrosomal exocytosis in the marine shrimp *Sicyonia ingentis*.

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Abstract

During copulation in the marine shrimp *Sicyonia ingentis*, a male transfers sperm to the female's seminal receptacles. During spawning female *S. ingentis* simultaneously release ova and stored sperm and mix them externally to initiate gamete interaction. Sperm bind to a thin vitelline envelope (VE) via the tip of the sperm anterior appendage and within seconds are induced to undergo acrosomal exocytosis. Following acrosomal exocytosis sperm penetrate the VE and become secondarily bound to the surface coat (SC), a glycocalyx on the oocyte surface. After a period of a few minutes, exocytosed sperm form an acrosomal filament. Sperm removed from males will bind to ova, but do not undergo any phase of the acrosome reaction indicating that sperm undergo capacitation. Experimental analysis of capacitation indicated that sperm acquire the ability to undergo acrosomal exocytosis within 24 hr and acquire the ability to form acrosomal filaments after approximately 145 hr incubation in the seminal

receptacles. Microscopic examination of sperm revealed dramatic morphological changes during capacitation.

VEs and SCs were isolated from *S. ingentis* ova. Isolated VEs only mediated primary sperm binding (i.e. before the AR), while the isolated SCs only mediated secondary sperm binding (i.e. after the AR). Isolated *S. ingentis* VEs were used to characterize primary sperm binding activity. Solubilized VEs inhibited primary sperm binding in a concentration dependent manner, and immunolocalization demonstrated highly localized VE binding sites at the tip of the anterior appendage where sperm bind eggs. Biochemical dissection of solubilized VEs indicated that sperm binding activity was contained in carbohydrate ligands in VEs.

When *S. ingentis* sperm were incubated with the lectin *Con-A*, localization was seen at the tip of the sperm anterior appendage. *Con-A* inhibited sperm-egg binding and induction of EW-induced acrosomal exocytosis, suggesting a localized receptor on the tip of the anterior appendage for both these functions. A sperm membrane fraction was generated using phase separation of a Triton X-114 extract of sperm membranes, and separation of this fraction on a mannose specific lectin affinity column. A competitive assay indicated that this sperm membrane fraction had receptor activity for EW-induced acrosomal exocytosis.

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