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Characterization of boar sperm plasma membranes by two-dimensional PAGE and isolation of specific groups of polypeptides by anion exchange chromatography and lectin affinity chromatography

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High resolution, two-dimensional PAGE was used to characterize purified plasma membranes (PM) from boar spermatozoa. In addition to the abundance of polypeptides resolved, unusual features of PM discerned in gel patterns include a large number of basic polypeptides and the presence of at least three polypeptides with molecular weights in excess of 200 K daltons. In order to reduce these large numbers of polypeptides into smaller fractions, an important step in developing a surface map of these membranes, detergent-solubilized membranes were fractionated by the techniques of ion exchange chromatography and lectin affinity chromatography. PM polypeptides, solubilized in the nonionic detergents, were fractionated on DEAE Sephadex using phosphate ion gradients containing EDTA. Most of the major acidic polypeptides were eluted as a group at high phosphate ion concentrations. Membrane polypeptides can be separated into smaller populations by affinity chromatography on wheat germ agglutinin-agarose (WGA) or by Concanavalin A-agarose. Concanavalin A-agarose binds most PM glycoproteins, but partial purification of an important group of very basic proteins was obtained by repeated chromatography of polypeptides that failed to bind to this lectin. Since biologic activity (including immunologic potency) is often retained in the detergents used, the ability to fractionate the major polypeptide of the boar sperm PM into different and much smaller populations in large quantity and to characterize these proteins by electrophoresis provides a starting point for determining the functional significance and location of the major surface proteins of the boar sperm PM.

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