PURIFICATION OF OVIDUCTIN FROM HAMSTER OVIDUCTS AND A NEW SCREENING METHOD FOR MONOCLONAL ANTIBODIES INHIBITING SPERM-OVIDUCTIN RECOGNITION

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In several mammalian species, glycoporteins called oviductin are secreted from the oviductal epithelial cells and coat the zonae pellucidae of oviductal eggs. Hamster oviductin was extracted from the oviducts with 0.3M lithium diiodosalicylate. An equal volume of 50% phenol was added to the extract to remove serum-derived proteins. After centrifugation, the crude oviductin fraction contained in the aqueous phase was precipitated by the addition of ethanol. Following this, oviductin was purified by affinity chromatography using a *Dolichos Biflorus* agglutinin-conjugated Sepharose column. The purified oviductin was composed of 20% protein and 80% carbohydrate components. Oviductin gave heterogeneous bands showing a molecular mass of about 200k daltons on SDS-PAGE. The treatment of ovarian eggs with oviductin enhanced the *in vitro* fertilization (IVF) rate from 20% to 80%.

An antiserum was prepared by immunizing a male rabbit with oviductin. This antiserum reacted most potently with oviductin at 80–fold dilution in ELISA and completely inhibited IVF at 50–fold dilution. On the other hand, a monoclonal antibody to hamster oviductin (AZPO–8, ascites) reacted with oviductin 20 times more strongly than the antiserum, but could not inhibit IVF.

In this study, we established a new ELISA procedure to screen monoclonal antibodies which can inhibit the sperm-oviductin recognition. After 360ng of oviductin was reacted with 5×10^7 spermatozoa at 37°C for 2 hours and then washed, the spermatozoa were attached to microtiter plate wells precoated with poly-L-lysine. The reacted oviductin was detected by 500–fold dilution of AZPO–8 and 1000–fold dilution of a peroxidase-conjugated second antibody. In this system, 85% of the oviductin that reacted with the spermatozoa was inhibited by preincubation of oviductin with 40–fold diluted anti-oviductin serum before being mixed with sperm. Screening of the monoclonal antibodies inhibiting sperm-oviductin recognition is now in progress using this new method.