



Fig. 4. Sperm-oocyte binding inhibition assays. Freshly isolated porcine oocytes were preincubated for 1 h with or without the AQN 1 protein and then co-incubated with capacitated spermatozoa. After washing, the oocytes were examined under a phase microscope. (Top) control without antigen. (Bottom) oocytes preincubated with 0.6 mg antigen/ml. Magnification 40x.

acidic polysaccharides but also with glycoproteins of solubilized zona pellucida (Table 3). AWN 1 in monomeric or aggregated forms might thus participate both in capacitation and in primary binding with zona pellucida.

Our results suggest the role of aggregated forms of DQH sperm surface protein, AQN, AWN, PSP II spermadhesins (fractions I–III) present in seminal plasma under physiological conditions in coating the sperm surface, in sperm capacitation and in primary binding of spermatozoa to zona pellucida of the ovum. The role of fraction IV (heterodimer PSP I/PSP II) bound to the sperm surface is subject of our future work.

References

- Bezouška, K., Sklenář, J., Novák, P., Halada, P., Havlíček, V., Kraus, M., Tichá, M., Jonáková, V. (1999) Determination of the complete covalent structure of the major glycoform of DQH sperm surface protein, a novel trypsin resistant boar seminal plasma O-glycoprotein related to pB1 protein. *Protein Sci.* **8**, 1551-1556.
- Bork, P., Beckmann, G. (1993) The CUB domain. A widespread module in developmentally regulated proteins. *J. Mol. Biol.* **231**, 539-545.
- Calvete, J. J., Sanz, L., Töpfer-Petersen, E. (1994) Spermadhesins: structure-functions relationships. *Ass. Reprod. Tech. Androl.* **6**, 316-330.
- Calvete, J. J., Mann, K., Schäfer, W., Raida, M., Sanz, L., Töpfer-Petersen, E. (1995) Boar spermadhesins PSPII: location of posttranslation modifications, heterodimer formation with PSP I glycoforms and effect of dimerization on the ligand binding capabilities of the subunits. *FEBS Lett.* **365**, 179-182.
- Calvete, J. J., Raida, M., Gentzel, M., Urbanke, C., Sanz, L., Töpfer-Petersen, E. (1997) Isolation and characterization of heparin and phosphorylcholine binding proteins from boar and stallion seminal plasma. *FEBS Lett.* **407**, 201-206.
- Chandonnet, L., Roberts, K. D., Chapdelaine, A., Manjunath, P. (1990) Identification of heparin-binding proteins in bovine seminal plasma. *Mol. Reprod. Dev.* **26**, 313-318.
- Dostálová, Z., Calvete, J. J., Töpfer-Petersen, E. (1995a) Interaction of non-aggregated boar AWN1 and AQN3 with phospholipid matrices. A model for coating of spermadhesins to the sperm surface. *Biol. Chem. Hoppe Seyler* **376**, 237-242.
- Dostálová, Z., Calvete, J. J., Sanz, L., Töpfer-Petersen, E. (1995b) Boar spermadhesin AWN-1. Oligosaccharide and zona pellucida binding characteristic. *Eur. J. Biochem.* **230**, 329-336.
- Gasset, M., Saiz, J. L., Laynez, J., Sanz, L., Gentzel, M., Töpfer-Petersen, E. (1997) Conformational features and thermal stability of bovine seminal plasma protein PDC-109 oligomers and phosphatidylcholine-bound complexes. *Eur. J. Biochem.* **250**, 735-744.
- Jonáková, V., Sanz, L., Calvete, J. J., Henschen, A., Čechová, D., Töpfer-Petersen, E. (1991) Isolation and biochemical characterization of a zona pellucida-binding glycoprotein of boar spermatozoa. *FEBS Lett.* **280**, 183-186.
- Jonáková, V., Tichá, M., Kraus, M., Čechová, D. (1995) Multifunctional sperm protein in gametic interaction. *Fertilität* **11**, 115-118.
- Jonáková, V., Kraus, M., Veselský, L., Čechová, D., Bezouška, K., Tichá, M. (1998) Spermadhesins of the AQN and AWN families, DQH sperm surface protein and HNK protein in the heparin-binding fraction of boar seminal plasma. *J. Reprod. Fertil.* **114**, 25-34.
- Kwok, S. C. M., Soares, M. J., McMurtry, J. P., Yurewicz, E. C. (1993) Binding characteristics and immunolocalization of porcine seminal protein, PSP-I. *Mol. Reprod. Dev.* **35**, 244-250.
- Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.
- Lane, M.-E., Thérien, I., Moreau, R., Manjunath, P. (1999) Heparin and high-density lipoprotein mediate bovine sperm capacitation by different mechanisms. *Biol. Reprod.* **60**, 169-175.

- Liberda, J., Tichá, M., Jonáková, V. (1997) Preparation of fluorescein-labelled and biotinylated derivatives of polysaccharides for lectin-saccharide binding studies. *Biotechnol. Techniques* **11**, 265-267.
- Liberda, J., Tichá, M., Zralý, Z., Švecová, D., Věžník, Z. (1998) Interaction of bull, stallion and boar seminal plasma proteins and sperms with acidic polysaccharides. *Folia Biol. (Praha)* **44**, 177-183.
- Liberda, J., Trnka, T., Sejbal, J., Jonáková, V., Kraus, M., Tichá, M. (1999) Preparation of biotinylated and FITC-labelled phosphorylcholine poly(acrylamide) derivatives and their application for protein ligand-binding studies. *Chimia* **53**, 528-532.
- Novotná, V., Mikeš, L., Horák, P., Jonáková, V., Tichá, M. (1996) Preparation of water-soluble and water-insoluble poly(acrylamide-allylamine) derivatives of polysaccharides. *Int. J. Biochromatogr.* **2**, 37-47.
- Sanz, L., Calvete, J. J., Mann, K., Gabius, H. J., Töpfer-Petersen, E. (1993) Isolation and biochemical characterization of heparin-binding proteins from boar seminal plasma: a dual role for spermadhesins in fertilization. *Mol. Reprod. Dev.* **35**, 37-43.
- Solís, D., Romero, A., Jiménez, M., Díaz-Mauriño, T., Calvete, J.J. (1998) Binding of mannose-6-phosphate and heparin by boar seminal plasma PSP-II, a member of spermadhesin protein family. *FEBS Lett.* **431**, 273-278.
- Thérien, I., Moreau, R., Manjunath, P. (1998) Major proteins of bovine seminal plasma and high-density lipoprotein induce cholesterol efflux from epididymal sperm. *Biol. Reprod.* **59**, 768-776.
- Tichá, M., Kraus, M., Čechová, D., Jonáková, V. (1998) Saccharide-binding properties of boar AQN spermadhesins and DQH sperm surface protein. *Folia Biol. (Praha)* **44**, 15-21.
- Tsunasawa, S., Kondo, J., Sakiyama, F. (1985) Isocratic separation of PTH-amino acids at picomole level by reverse-phase HPLC in the presence of sodium dodecyl sulfate. *J. Biochem.* **97**, 701-704.
- Varela, P. F., Romero, A., Sanz, L., Romao, M. J., Töpfer-Petersen, E. (1997) The 2.4 Å resolution crystal structure of boar seminal plasma PSPI/PSPII: a zona pellucida-binding glycoprotein heterodimer of the spermadhesin family built by CUB domain architecture. *J. Mol. Biol.* **274**, 635-649.
- Veselský, L., Pěkníková, J., Čechová, D., Kraus, M., Geussová, G., Jonáková, V. (1999) Characterization of boar spermadhesins by monoclonal and polyclonal antibodies and their role in binding to oocytes. *Am. J. Reprod. Immunol.* **42**, 187-197.
- Yanagimachi, R. (1994) Mammalian fertilization. In: *The Physiology of Reproduction*, eds. Knobil, E., Neil, J. D., pp. 189-317, Raven Press, New York.