Table 2. Interaction of protein fractions I–V obtained by gel chromatography on Sephadex G-75 SF and separated components with a biotinylated polyacrylamide derivative of phosphorylcholine and of epididymal and ejaculated spermatozoa with biotinylated fractions I–V and biotinylated individual components

Protein	Phosphorylcholine	Epididymal spermatozoa (caput)	Epididymal spermatozoa (cauda)	Ejaculated spermatozoa
Fr. I	++	+++	+++	+++
Fr. II	++	+	+	+
Fr. III	++	+++	++++	+++
Fr. IV	–	++	++	++++
Fr. V	+	–	–	+
DQH	+	+	+	+++
AQN 1	+	++	++	+++
AQN 2	–	++	++	+++
AQN 3	++	+++	+++	+++
AWN 1	+++	++	++	++++
AWN 2	+++	+++	++++	++++
PSP I	–	++	++	++
PSP IIa	+	+++	++++	++++
PSP IIb	+	+++	++++	++++

(+) – positive reaction, number of (+) indicates the relative binding activity of proteins,
(–) – negative reaction

Table 3. Interaction of protein fractions I – V and their HPLC-separated components with biotinylated polyacrylamide derivatives of acid polysaccharides and cholesterol and biotinylated zona pellucida glycoproteins

Protein	Heparin	Chondroitin sulphate	Cholesterol	Zona pellucida
Fr. I	+++	+++	+	++
Fr. II	++	+++	+	+
Fr. III	++	+++	++	+
Fr. IV	–	–	–	–
Fr. V	+	++	+	–
DQH	+++	+++	n.t.	++
AQN 1	+	+	n.t.	++
AQN 2	+	+	n.t.	++
AQN 3	+	+	n.t.	++
AWN 1	++++	++++	n.t.	++++
AWN 2	++	++	n.t.	++
PSP I	–	–	n.t.	–
PSP IIa	++	(+)	n.t.	(+)
PSP IIb	++	(+)	n.t.	(+)

(+) – positive reaction, number of (+) indicates the relative binding activity of proteins,
(–) – negative reaction,
n.t. – not tested

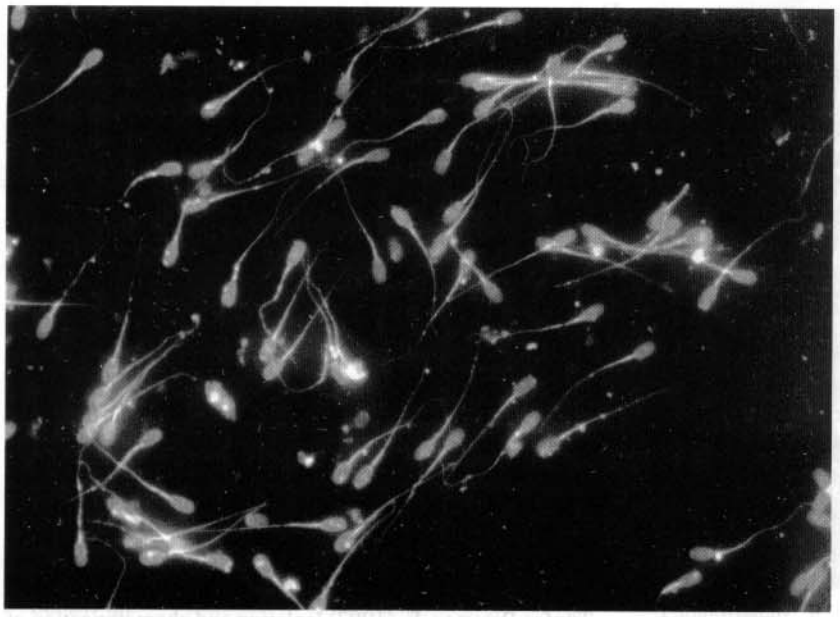

Fig. 3. Binding of biotinylated aggregate IV to ejaculated boar sperm (FITC-avidin was used). Magnification 800x.

The pretreatment of oocytes with AQN 1 resulted in reduced sperm binding to oocytes; 90% inhibition was reached with 0.6 mg of AQN 1 per ml (Fig. 4), approximately 60% and 30% inhibition was observed with 0.4 mg/ml and 0.2 mg/ml, respectively (results not shown).

Discussion

Seminal plasma proteins are known to interact with various types of ligands, such as saccharide moiety of glycoproteins, polysaccharides of glycosaminoglycan type, phospholipids, lipoproteins, collagen, protease inhibitors, sperm surface, and zona pellucida. Specific interactions between seminal plasma proteins leading to the formation of higher-molecular-weight aggregates cannot be excluded. The binding properties of seminal plasma components might be important in individual steps of fertilization. Although separated low-molecular-weight components of boar seminal plasma have been studied in detail regarding their binding ability, less attention has been paid to their native state in seminal plasma.

The interaction of seminal plasma proteins bound on the sperm surface with polysaccharides of glycosaminoglycans of oviductal epithelial cells is the most probable event leading to sperm capacitation in the female reproductive tract (Yanagimachi, 1994; Solís et al., 1998). Binding of boar seminal plasma proteins to different types of acidic polysaccharides (sulphated, non-sulphated, different polysaccharide chain) is not related only to the charge density of polysaccharides. The position and alignment of sulphate groups and conformation of polysaccharide backbone are important for binding (Liberda et al., 1998; Solís et al., 1998). The ability of protein aggregates containing AWN, AQN, DQH, PSP II proteins (fractions I–III) to interact with acidic polysaccharides correlated with the binding activities of their monomeric components, especially of spermadhesin AWN 1 and DQH protein. The binding properties of the fraction IV (PSP I/PSP II heterodimer) isolated by us from boar seminal plasma are in full agreement with the data obtained with re-aggregated heterodimer described by Solís et al. (1998). The same is true for separated components of this heterodimer (proteins PSP I and PSP II). This also indicates that the method used by us (ELBA) for characterization of the binding of seminal proteins and their aggregates to biotinylated polyacrylamide derivatives of ligands (Novotná et al., 1996; Liberda et al., 1997) yielded results comparable with those obtained by another method (affinity chromatography, Solís et al., 1998). ELBA was also successfully applied for the study of the ability of protein aggregates to bind to epididymal and ejaculated sperm. In the ELBA test using direct sperm binding to microtiter wells, the interaction of both types of sperm did not significantly differ. Binding of biotinylated aggregates containing AWN, AQN and DQH proteins to boar epididymal and ejaculated spermatozoa corresponded to the results obtained in studies with separated biotinylated monomeric components (Dostálová et al., 1995a; Jonáková et al., 1998). Both ELBA and fluorescence microscopy revealed that fraction IV (heterodimer PSP I/PSP II) was bound to epididymal or ejaculated spermatozoa (Table 2, Fig. 3) although earlier, a loose contact of the PSP I/PSP II dimer with sperm had been taken for granted (Calvete et al., 1995; Solís et al., 1998). Similarly as in the case of acidic polysaccharides, aggregation of these proteins did not affect their binding to spermatozoa and indicated their roles in particular stages of fertilization (Table 3). AQN 1, AWN 1, DQH proteins are distributed on the surface of the sperm plasma membrane covering the acrosome (Jonáková et al., 1998; Veselský et al., 1999). AQN 1 interacts most strongly with glycoproteins containing O-glycosidically linked oligosaccharides (Tichá et al., 1998). The interaction of AQN 1 with glycoproteins containing O-glycosidically bound oligosaccharide chains might be responsible for its interaction with glycoproteins of zona pellucida. AQN 1 blocked the sperm-oocyte binding. These findings make the AQN 1 spermadhesin a very likely mediator of the first contact of sperm with the ovum.

The AWN 1 spermadhesin binds to glycoproteins containing O- and N-linked saccharide chains (Dostálová et al., 1995b). AWN 1 as a monomer or in the aggregated forms (fractions I–III) interacts strongly not only with