studies have shown that D-fructose might participate in some steps of the fertilization process. D-fructose completely blocked the sperm penetration through the human zona pellucida (Mori et al., 1993). Human acrosin was inhibited by D-fructose, but trypsin was not (Anderson, et al., 1985).

Our results have shown that D-fructose inhibits the heparin-binding activity of bull seminal plasma proteins and epididymal sperm. Such inhibition was not observed in the presence of low-molecular-weight components of seminal plasma, i.e. in the case of complete seminal plasma (not shown) or ejaculated sperm. This fact suggests that D-fructose binds to seminal plasma proteins and thus modulates the binding properties of both the seminal plasma proteins and the sperm. The results of ELBA tests were confirmed by affinity chromatography on immobilized heparin followed by elution of adsorbed proteins with the D-fructose solution. A similar approach was used for the isolation of phosphorylcholine-binding proteins from bull or boar seminal plasma (Calvete et al., 1996a; Calvete et al., 1997; Jonáková et al., 1998). Some proteins identified in the D-fructose fraction (PDC-109 and RNAase dimer) were also found in the heparin-binding fraction eluted with 3 M NaCl. This fact might be explained by the presence of different aggregated forms of seminal plasma proteins, which differ in their binding properties; different binding properties of aggregated forms of boar seminal plasma proteins were described by Gasset et al. (1997) and Maňásková et al. (2000).

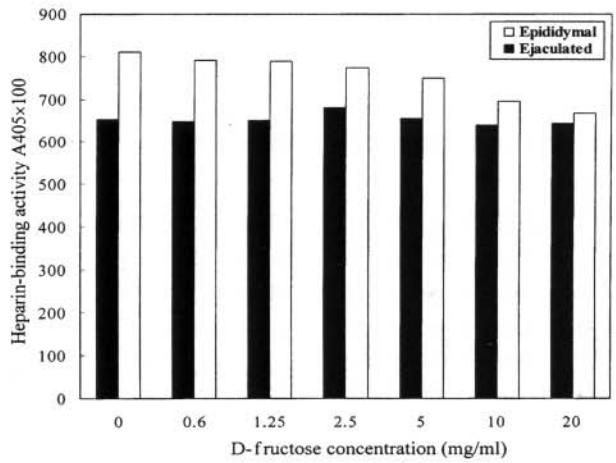

Fig. 2. Inhibition of the heparin-binding activity of bull ejaculated and epididymal sperm by saccharides
Suspension of sperm 10^8 cells/ml, the solutions used: 0–20 mg/ml D-fructose, 50 μg/ml biotinylated polyacrylamide derivative of heparin. Absorbance at 405 nm – heparin-binding activity.

The binding studies on the interaction of biotinylated protein fractions (fraction I–III) showed that the D-fructose-binding fraction was bound to sperm significantly less than the fraction III. This fact suggests that

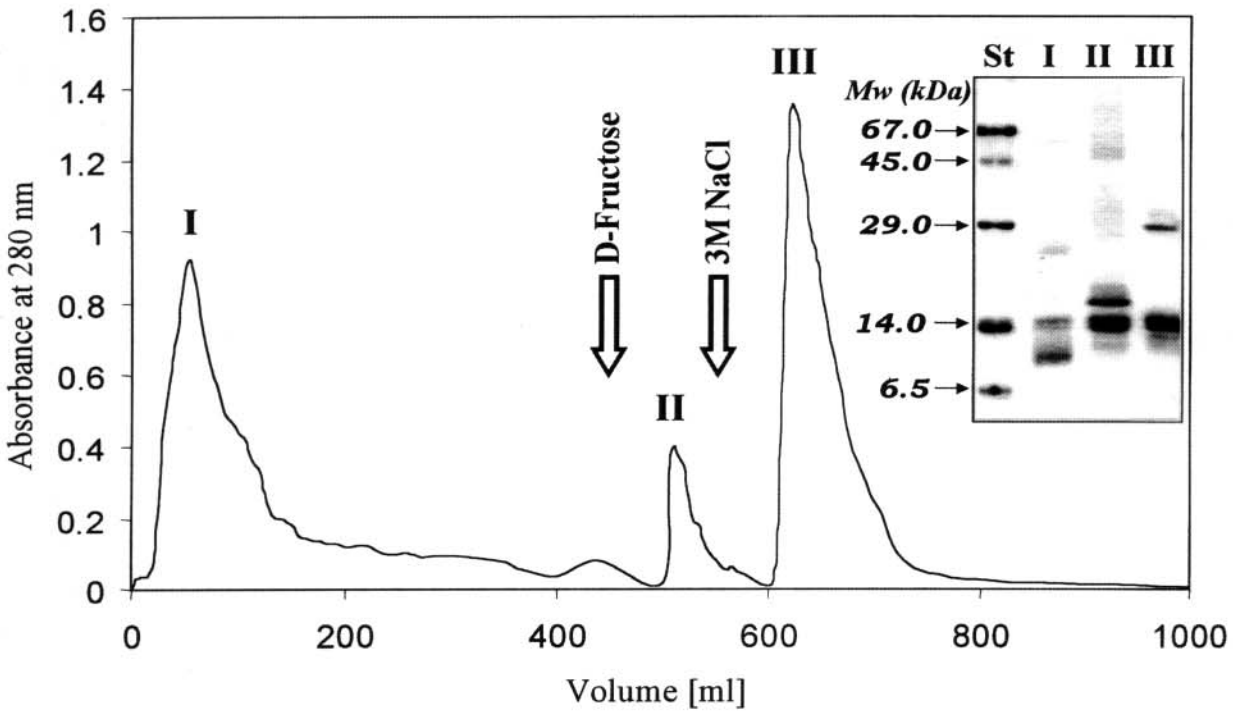

Fig. 3. Affinity chromatography of bull seminal plasma on a heparin-polyacrylamide column
Ammonium sulphate-precipitated proteins from 600 mg of lyophilized bull seminal plasma applied to a heparin-polyacrylamide column (3 x 15 cm); elution: PBS (fraction I), 2% D-fructose (fraction II) and 3 M NaCl (fraction III). The insert shows SDS-PAGE of the fractions I, II and III under non-reducing conditions. Standard molecular mass markers in kDa (BSA, egg albumin, carbonic anhydrase, ribonuclease, pancreatic trypsin inhibitor).

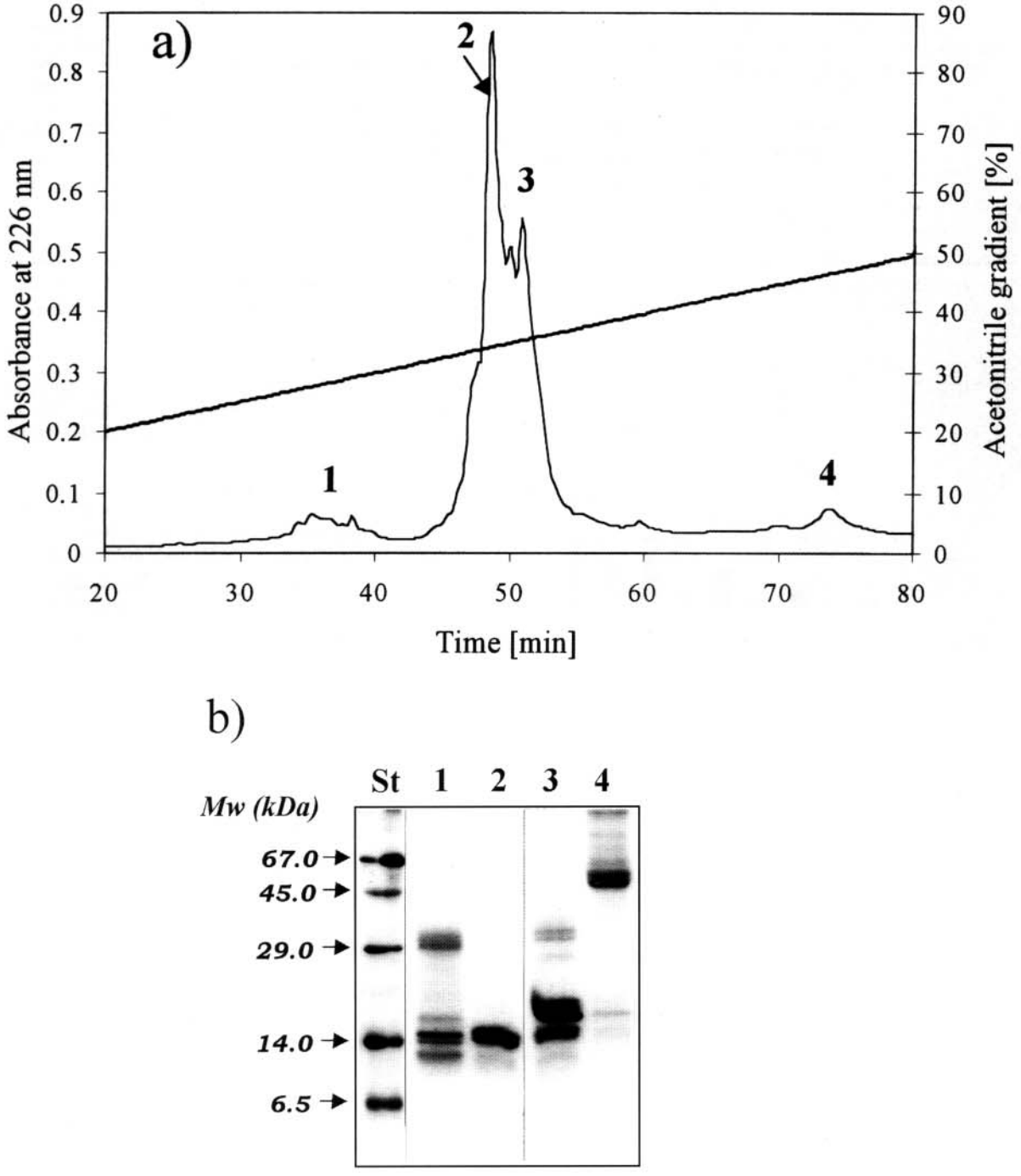

Fig. 4. Separation of the D-fructose-binding fraction (fraction II) by RP HPLC
Absorbance at 226 nm (—); acetonitrile gradient (▬); protein fractions 1–4 (a). SDS-PAGE of the fractions 1–4 under non-reducing conditions. Standard molecular mass markers in kDa (bovine serum albumin, egg albumin, carbonic anhydrase, ribonuclease, pancreatic trypsin inhibitor) (b).