Enzyme-linked binding assay (ELBA) for soluble proteins

One μg of protein in 100 μl 0.05 M $NaHCO_3$, pH 9.6, was applied per well and incubated overnight at 4°C. After rinsing with washing buffer (0.01 M Tris-HCl, pH 7.4, containing 0.85% NaCl, 3 mM $CaCl_2$, 0.02% Tween), the wells were deactivated with 200 μl of 3% BSA in washing buffer (2 h at 4°C). After washing, a solution of either biotinylated polyacrylamide derivatives of heparin, chondroitin sulphate, phosphorylcholine and cholesterol (100 μg/ml in 0.01 M Tris-HCl, pH 7.4) or biotinylated solubilized zona pellucida (10 μg/100 μl in 0.01 M Tris-HCl, pH 7.4) was applied to each well and incubated for 2 h at 37°C. After washing, 25 ng of avidin-peroxidase in 100 μl of 0.1 M Tris-HCl, pH 8.0, containing 1% BSA, were added to each well and incubated for 1 h at 37°C. After washing, the enzyme reaction was initiated by adding 100 μl of 0.09% H_2O_2, 0.01% ABTS in 0.1 M phosphate-citrate buffer, pH 4.6, to each well. After 15 min incubation at 37°C, absorbance at 405 nm was measured using a microplate reader (SLT-Spectra, SLT-Labinstruments, Vienna, Austria).

ELBA for epididymal and ejaculated spermatozoa

Ejaculated or epididymal sperm suspension (1 mg/ml) in 100 μl of coating buffer (0.05 M $NaHCO_3$, pH 9.6) were immobilized on plates overnight at 4°C. After washing with PBS (containing 0.02% Tween), the wells were deactivated using 200 μl of BSA solution (3% in PBS) for 1 h at room temperature. The solution of biotinylated aggregates I–V and their monomeric forms (100 μg/ml in PBS) was applied to each well (100 μl) and incubated for 2 h at 37°C. After washing with PBS, 100 μl of avidin-peroxidase solution (0.25 μg/ml) in 0.1 M Tris-HCl, pH 8.0, was added to each well and incubated at 37°C for 1 h. After washing, peroxidase was incubated with 100 μl substrate ABTS solution (0.1 mg/ml in 0.1 M phosphate-citrate buffer, pH 4.6, containing 0.09% H_2O_2). After 10 min incubation at 37°C, absorbance at 405 nm was measured using a microplate reader (SLT-Spectra, SLT-Labinstruments, Vienna, Austria).

Fluorescence labeling assay

One drop of sperm suspension (20 μl of 10^6 sperm/ml) and one drop of 20 μl of tested biotinylated fractions II–V (1 mg/1 ml PBS) were mixed, smeared on the slide and dried. Fixation of the slide was done by 5% buffered formaldehyde solution for 30 min. The slides were incubated in FITC-avidin solution (100 μg/ml PBS) and then washed three times by distilled water, dried and observed in a flurescence microscope with excitation by UV light at 360 nm. A biotinylated polyacrylamide derivative (Liberda et al., 1997) and FITC-avidin were used for control experiments.

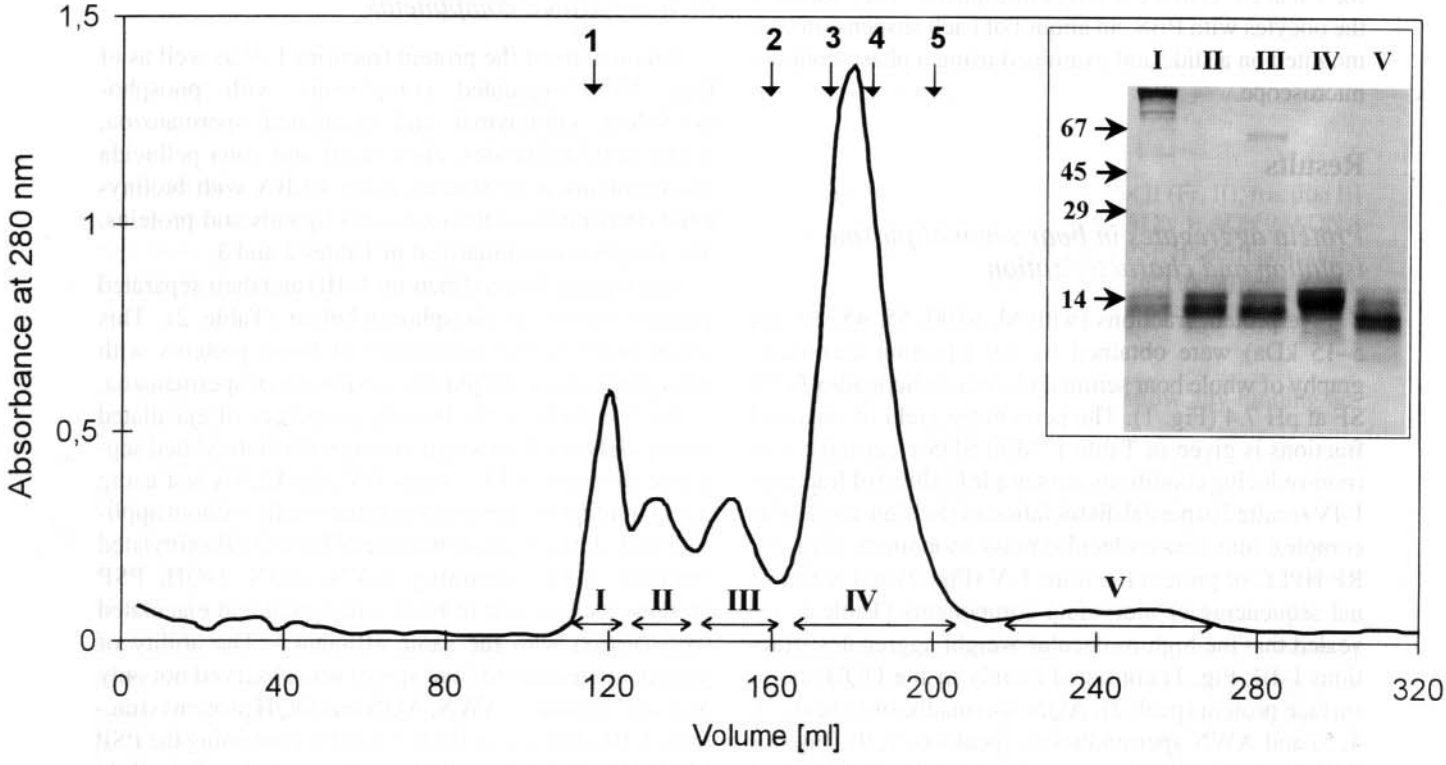

Fig. 1. Gel chromatography of boar seminal plasma on Sephadex G-75 SF. Molecular mass markers: 1 - Blue Dextran, 2 - egg albumin, 3 - carbonic anhydrase, 4 - chymotrypsinogen A, 5 - ribonuclease. The insert shows SDS polyacrylamide gel electrophoresis of the fractions I–V under mild conditions (non-reducing, no sample boiling). Standard molecular mass markers in kDa (bovine serum albumin, egg albumin, carbonic anhydrase, ribonuclease).