Table 3. Localization of actin in boar spermatozoa under the influence of cytochalasine B

Treatment of sperms	Labeling of spermatozoa*							
	Acrosome	Postacros. segment	Outer acr. membrane	Neck	Middle piece	Principal piece	End piece	Special marks
control	+	+	+++	+	+	±	–	–
control + ionophore	±	+	+	+	+	±	–	spilling acrosome
cytochalasine B	+	+	+	+	+	±	–	spilling acrosome
cytochalasine B + ionophore	±	+	+	+	+	±	–	spilling acrosome

*intensity of immunofluorescence labeling

after the AR and the following effect of cytochalasine B it was possible to see disruption of the outer acrosomal membrane, and actin was also detectable on the top of spilling acrosomal contents. In all samples, the fluorescein-labeled actin antibody also labeled the neck and, slightly, the middle and principal pieces of the tail of all spermatozoa (Fig. 2). No positive fluorescence reaction was observed in the spermatozoa samples that had not been incubated with the anti-actin antibody (Table 3).

α-Tubulin

An immunofluorescence labeling pattern obtained with monoclonal antibody anti-α-tubulin was well perceptible in the outer acrosomal membrane, postacrosomal segment and acrosome region in all control samples (Fig. 3A). There was a special mark in the outer acrosomal membrane in the samples with added calcium ionophore followed by induction of the AR. It was clearly possible to see penetration into the acrosomal membrane and spilling out of the acrosomal contents (Fig. 3B, C). The same results were obtained when vinblastine and colcemide were added separately from the calcium ionophore (Fig. 3D). Similar results were observed in the case of colcemide and vinblastine added together (Fig. 4), but in this experiment the AR was provoked by simultaneus influence of these two specific inhibitors of tubulin without addition of calcium ionophore. A different labeling pattern was obtained by addition of different concentrations of nocodazole solution. Except for the post-acrosomal segment we have not seen any specific fluorescence. There were also changes in all parts of the tail in the case of nocodazole. However, in the other samples the tail showed strong immunofluorescence labeling (Fig. 3). No positive fluorescence was observed in the spermatozoa samples that had not been incubated with the tubulin antibody (Table 4).

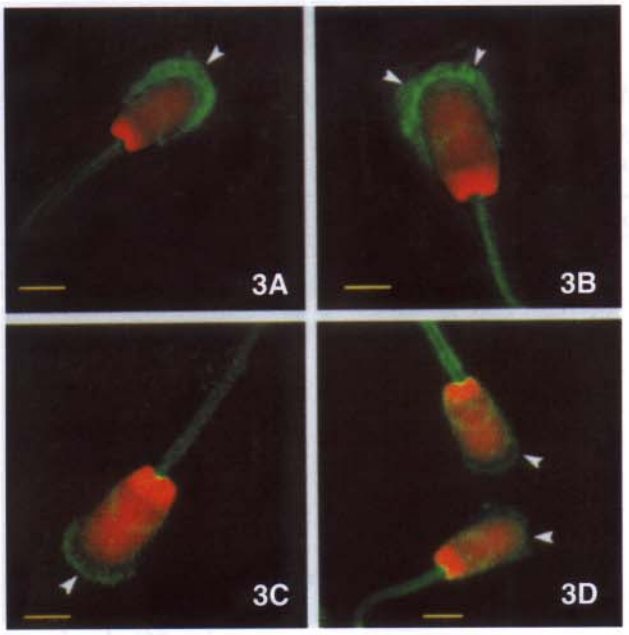

Fig. 3. Immunochemical localization of α-tubulin: A) control, B) calcium ionophore, C) vinblastine, D) vinblastine + calcium ionophore; confocal microscopy

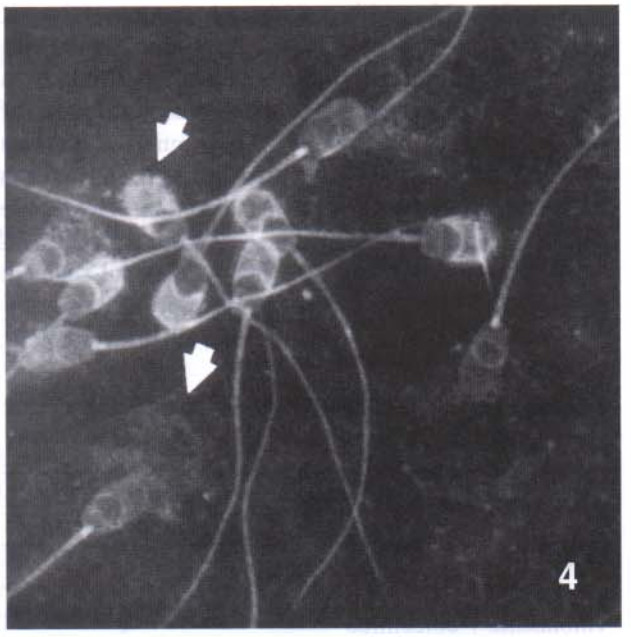

Fig. 4. Immunochemical localization of α-tubulin, indirect immunofluorescence, incubation of spermatozoa with vinblastine + colcemide, fluorescent microscopy. Magnification 250x.