

Fig. 6. Immunolocalization of  $\gamma$ -tubulin (A, B – Tu30; C, D – Tu31): A) calcium ionophore, B) vinblastine C) control, D) vinblastine + calcium ionophore; confocal microscopy

membrane (Fig. 7A). The effect of the skull cap was also seen under the influence of colcemide (Fig. 8). After the AR in the head, spectrin was slightly visible in the acrosome region, but spilling of acrosomal contents was very well observed in all samples even with or without vinblastine (Fig. 7B, D). The AR was also induced by nocodazole (100  $\mu\text{g}/\text{ml}$  in TBS solution) (Fig. 9). There was no difference in the fluorescence of the postacrosomal segment and membrane (Fig. 7). All parts of the tail were without visible changes, too. No positive fluorescence was observed in the spermatozoa samples that had not been incubated with the tubulin antibody (Table 5).

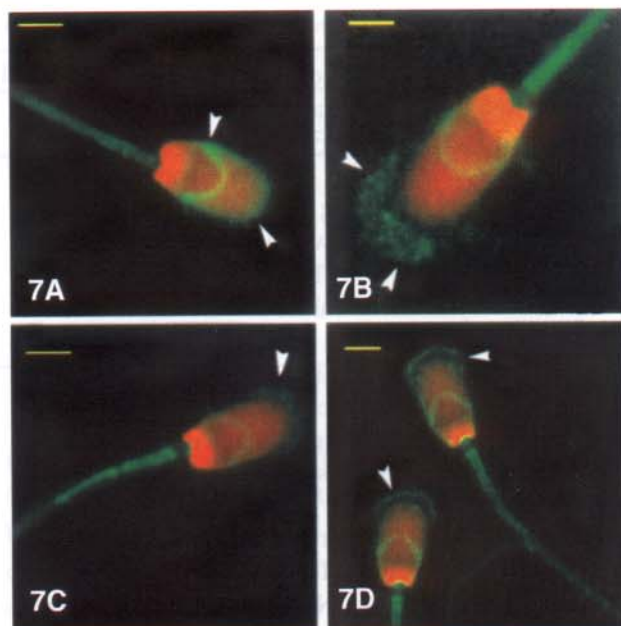


Fig. 7. Immunolocalization of spectrin: A) control, B) calcium ionophore, C) vinblastine, D) vinblastine + calcium ionophore; confocal microscopy. Magnification 630x, the bar 2  $\mu\text{m}$ ; arrows show the immunolocalization of cytoskeletal proteins localized in the head of boar spermatozoa and their different changes during and after the AR such as penetration of the outer acrosomal membrane and spilling of acrosomal contents.

## Discussion

This study demonstrates different changes of cytoskeletal proteins during and after the AR in boar spermatozoa. Whether individual proteins are involved in the process of the AR or not was the main question of our investigation. To find out these proteins and their possible relocation after the AR, we chose specific inhibitors of microfilamental and microtubular

Table 5. Localization of spectrin in boar spermatozoa under the influence of vinblastine and colcemide

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
vinblastine	++	+	+	–	+	–	–	–
vinblastine + ionoph.	±	+	–	–	+	–	–	spilling acrosome
colcemide	+	++	+	–	+	–	–	–
colcemide + ionoph.	–	++	–	–	+	–	–	spilling acrosome
vinblastine + colcemide	+	++	–	–	+	–	–	spilling acrosome
vinblastine + colcemide + ionophore	+	++	–	–	+	–	–	spilling acrosome

\*intensity of immunofluorescence labeling