

γ -Tubulin

There is clear evidence of the occurrence of γ -tubulin (detected by monoclonal antibodies Tu-30 and Tu-31) in boar spermatozoa (Fig. 5). The outer acrosomal membrane reveals strong immunofluorescence after addition of vinblastine (Fig. 6B, C, D). The postacrosomal segment was stained weakly in the acrosome area and in the tail (Fig. 6). The reaction of spermatozoa with added cytoskeletal inhibitor vinblastine was different than it was observed in the case of α -tubulin, but changes were also visible in the outer acrosomal membrane as compared to the control. In this experiment it was possible to see spilling of the acrosomal contents both in the control and in samples with added vinblastine after induction of the AR by calcium ionophore. A strong and well-visible reaction was seen especially in the case of detection of γ -tubulin with monoclonal antibody Tu30 (Fig. 6A). No positive fluorescence was observed in the spermatozoa samples that had not been incubated with the anti- γ -tubulin antibodies.

Spectrin

In boar spermatozoa, spectrin was present in the acrosome domain of the head and in the middle piece; bright immunofluorescence (skull cap) was seen in the outer acrosomal membrane of control samples without addition of calcium ionophore and in the postacrosomal

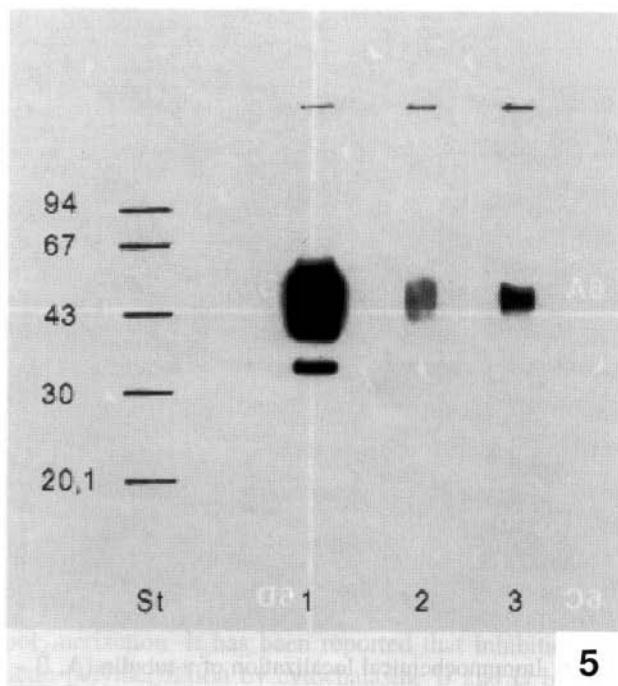


Fig. 5. Immunoblot analysis of boar spermatozoa extract with ACR.2 monoclonal antibody and monoclonal antibodies against γ -tubulin (Tu30, Tu31). Proteins were separated by 9% SDS PAGE. Lane 1: ACR.2, lane 2: Tu30, lane 3: Tu31, St: position of molecular-weight markers.

Table 4. Localization of α -tubulin in boar spermatozoa under the influence of vinblastine, colcemide and nocodazole

Treatment of sperms	Labeling of spermatozoa*							
	Acrosome	Postacros. segment	Outer acr. membrane	Neck	Middle piece	Principal piece	End piece	Special marks
control	+	+	++	++	+	+	+	compact membrane
control + ionophore	-	+	+	++	+	+	+	penetration of outer acr. m.
vinblastine	+	+	+	++	+	+	+	compact membrane
vinblastine + ionophore	±	+	+	++	+	+	+	spilling acrosome
colcemide	+	+	+	++	+	+	+	compact membrane
colcemide + ionophore	-	±	±	++	+	+	+	spilling acrosome
vinblastine + colcemide	-	+	+	++	+	+	+	spilling acrosome
vinblastine + colcemide + ionophore	-	+	+	++	+	+	+	spilling acrosome
nocodazole (100 μ g)	-	±	-	++	-	++	+	tail deformation
nocodazole (wash)	-	+	-	++	-	+	+	wavy tail
nocodazole (wash) + ionophore	-	±	-	++	-	-	-	tail deformation
nocodazole (50 μ g)	-	±	-	++	-	-	-	/
nocodazole (20 μ g)	-	+	-	++	+	±	-	/

*intensity of immunofluorescence labeling