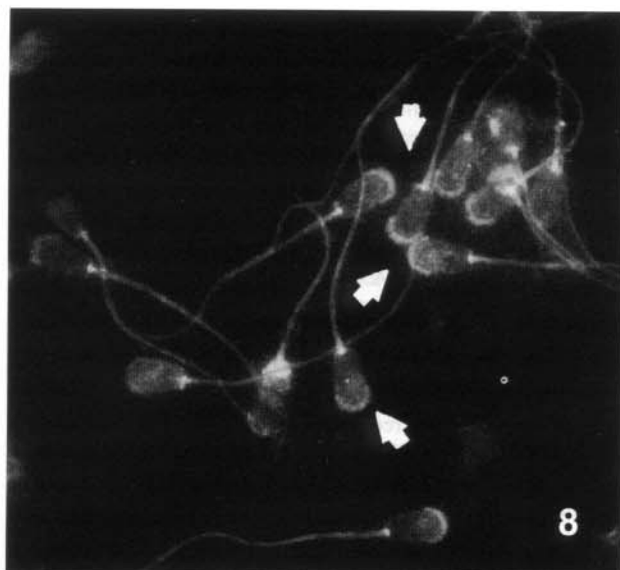

Fig. 8. Immunochemical localization of spectrin, indirect immunofluorescence, incubation of spermatozoa with colcemide, fluorescent microscopy. Magnification 250x.

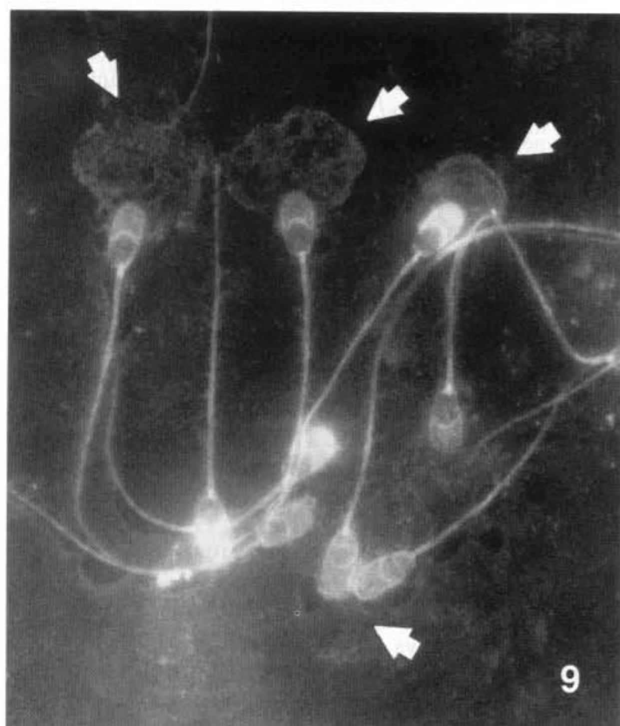

Fig. 9. Immunochemical localization of spectrin, indirect immunofluorescence, incubation of spermatozoa with nocodazole 100 μg/ml in TBS. Arrows show the outer acrosomal membrane and visible changes after the AR, boar spermatozoa, fluorescent microscopy. Magnification 250x.

cytoskeleton, such as cytochalasine B, vinblastine, colcemide and nocodazole. In the preceding studies, participation of cytoskeleton was found in the process of capacitation and in the AR of mammalian spermatozoa (Paleček et al., 1999), as well as in relocation of specific acrosomal proteins during capacitation and induced AR (Pěknicová et al., 1994).

The results of our experiments provide sharp evidence that actin is involved in the process of AR in boar spermatozoa. The presence of actin has been demonstrated in many mammalian spermatozoa, including boar and human (Paleček et al., 1999). It is also known that the subcortical actin network has been implicated in the regulation of exocytosis in some cells (Koffer et al., 1990; Burgoyne et al., 1991; Dudani and Ganz, 1996), and the AR is thus considered to be an exocytotic process and should therefore have mechanisms common with exocytosis in other cells (Liu et al., 1999). We incubated different samples of spermatozoa with cytochalasine B either with or without addition of calcium ionophore A23187. It has been shown that the AR started under the influence of cytochalasine B also without the presence of calcium ionophore. It is obvious that the AR might be provoked by the influence of cytochalasine B on the actin cytoskeleton. The question is why? It is known that cytochalasine B is a strong inhibitor of actin polymerization. It has been reported that inhibition of actin polymerization by cytochalasine B and D blocks penetration of spermatozoa into ZP in the case of human and guinea-pig fertilization *in vitro* (Liu et al., 1999). Peterson et al. (1990) have also demonstrated the presence of polymeric or oligomeric actin in the plasma and outer acrosomal membranes of boar spermatozoa. In our opinion cytochalasine B in our experiments blocks actin polymerization, but it can lead to the collapse of the microfilament cytoskeleton and afterwards to the AR caused by disruption of the actin cytoskeletal network. On the other hand, actin polymerization appears to be critical for the AR during the human fertilization process (Liu et al., 1999). There are also the presentations of Breitbart et al., 1992; Spungin et al., 1995; Breitbart and Spungin, 1997, who reported that depolymerization of the F-actin (polymeric filamentous form of actin) network between two membranes is essential for the AR in guinea-pig spermatozoa. Whether polymerization or depolymerization of actin leads to the AR in every species is not known yet. Nevertheless, our experiments clearly show that actin is present in the acrosomal area of boar spermatozoa and plays an important role in the process of the AR.

In the case of α-tubulin, it is apparent that there are also visible changes in its localization. Our presented results show bright immunofluorescence in the outer acrosomal membrane and a weaker signal in the postacrosomal segment and in the area of acrosome in the control of boar spermatozoa. After the AR caused either by calcium ionophore or by influence of drugs, it is possible to see both disruption of the outer acrosomal membrane and spilling of the acrosomal contents. This is in agreement with previous work of Paleček et al. (1999), and these new experiments support the idea that during the AR tubulin could polymerize and then be redistributed within the acrosome (Paleček et al., 1999). It is known that microtubules may be involved in plasma