

membrane dynamics (Sandvig and Deurs, 1990). This finding also supports our theory of participation of  $\alpha$ -tubulin in the process of the AR. On the other hand, Fath and Burgess (1993) suggest that microtubules are normally prevented from interacting with the plasma membrane and this interaction could be generated by disruption of the actin cytoskeleton, which is similar to the conclusion of van Deurs et al. (1996). A new point of view on the AR might issue from these reports. First of all, at the beginning of the AR the actin network could be disrupted and, consecutively, the interaction of tubulin cytoskeleton with the acrosomal membrane could lead to the AR.

By our results we clearly demonstrate the presence of  $\gamma$ -tubulin in boar spermatozoa. This is the first evidence of the presence of  $\gamma$ -tubulin as a participant of the AR in boar spermatozoa, because only one report about the presence of  $\gamma$ -tubulin in the incorporated mouse sperm basal body complex during fertilization has been published (Palacios et al., 1993). This result is completely new and the participation of  $\gamma$ -tubulin in the mechanism of the AR has not been known yet. Anyway, the reaction of  $\gamma$ -tubulin is much stronger than it was possible to see in the case of  $\alpha$ -tubulin. It is clear enough that  $\gamma$ -tubulin must play some role in the process of the AR, which might be different from the role of the other cytoskeletal proteins mentioned in our work. This problem is going to be the next field of our investigation.

Positive labeling was also observed in spermatozoa after its incubation with anti-spectrin antibody. These findings are in agreement with experiments on various mammalian spermatozoa (Virtanen et al., 1984a,b; Fouquet et al., 1991; Paleček et al., 1999). In this work we present specific changes in the localization of spectrin after the AR. They are detectable most of all in the outer acrosomal membrane and in the area of acrosome, particularly the interface between the nucleus and the inner acrosomal membrane, both in the control and in the samples treated with specific inhibitors (vinblastine and colcemide). Changeover of spectrin localization in the outer acrosomal membrane in boar spermatozoa during the AR and capacitation has been already observed by Paleček et al. (1999). In this work we document new occurrence of spectrin and its relocalization after incubation with specific inhibitors either before or after the AR. On the other hand, no detectable changes were found during the AR in hamster spermatozoa in the investigation by Fouquet et al. (1991).

In our opinion, there are two different points of view on the AR. First of all, an individual protein is responsible for the stability of the acrosome vesicle; if it is blocked, the AR starts. Second, one protein of the whole complex of cytoskeletal proteins is blocked, and that is why the AR does not take place. These events are not clear enough yet, but they are going to be the subject of our next investigation.

## Acknowledgement

We thank Dr. Hozák for electron microscopy results, Martin Couf, M.Sc., for photos from confocal microscopy, Dr. Dráber for his kind gift of anti- $\gamma$ -tubulin antibodies and others for their help.

## References

- Baccetti, B., Burrini, A. G., Collodel, G., Piomboni, P., Reniere, T. (1989) Localization of acrosomal enzymes in Arthropoda, Echinodermata and Vertebrata. *J. Submicrosc. Cytol. Pathol.* **21**, 385-389.
- Berger, T., Horton, M. B. (1988) Evaluation of assay conditions for the zona-free hamster bioassay of boar sperm fertility. *Gamete Res.* **19**, 101-111.
- Bourne, H., Liu, D. Y., Clarke, G. N., Baker, H. W. (1995) Normal fertilization and embryo development by intracytoplasmic sperm injection of round-headed acrosomeless sperm. *Fertil. Steril.* **63**, 1329-1332.
- Breitbart, H., Spungin, B. (1997) The biochemistry of the acrosome reaction. *Mol. Hum. Reprod.* **3**, 195-202.
- Breitbart, F., Lax, J., Rotem, R., Naor, Z. (1992) Role of protein kinase C in the acrosome reaction of mammalian spermatozoa. *Biochem. J.* **281**, 473-476.
- Brucker, C., Lipford, G. B. (1995) The human sperm acrosome reaction: physiology and regulatory mechanisms. An update. *Hum. Reprod. Update* **1**, 51-62.
- Burgoyne, R. D., Handel, S. E., Morgan, A. (1991) Calcium, the cytoskeleton and clypactin (annexin II) in exocytotic secretion from adrenal chromaffin and mammary epithelial cells. *Biochem. Soc. Trans.* **19**, 1085-1090.
- Dudani, A. K., Ganz, P. R. (1996) Endothelial cell surface actin serves as a binding site for plasminogen, tissue plasminogen activator and lipoprotein. *Br. J. Haematol.* **95**, 168-178.
- Dustin, P. (1984) *Microtubules*. Sec. Ed., Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.
- Fath, K. R., Burgess, D. R. (1993) Golgi-derived vesicles from developing epithelial cells bind actin filaments and possess myosin-I as a cytoplasmically oriented peripheral membrane protein. *J. Cell Biol.* **120**, 117-127.
- Fouquet, J. P., Fraile, B., Kann, M. L. (1991) Sperm actin and calmodulin during fertilization in the hamster: an immune electron microscopic study. *Anat. Rec.* **231**, 316-323.
- Kaláb P., Pěkníková J., Geussová G., Moos J. (1998) Regulation of protein tyrosine phosphorylation in boar sperm through a cAMP-dependent pathway. *Mol. Reprod. Dev.* **51**, 304-314.
- Koffer, A., Tatham, P. E. R., Gompert, D. (1990) Changes in the state of actin during the exocytolytic reaction of permeabilized rat mast cells. *J. Cell Biol.* **111**, 919-927.
- Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227**, 680-685.
- Liang, A., Ruiz, F., Heckmann, K., Klotz, C., Tollon, Z., Beisson, J., Wright, M. (1996) Gamma-tubulin is permanently associated with basal bodies in ciliates. *Eur. J. Cell Biol.* **70**, 331-338.
- Liu, D. Y., Martic, M., Clarke, G. N., Dunlop, M. E., Baker, H. W. G. (1999) An important role of actin polymerization in the human zona pellucida-induced acrosome reaction. *Mol. Hum. Reprod.* **5**, 941-949.

- Palacios, M. J., Joshi, H. C., Simerlz, C., Shatten, G. (1993)  $\gamma$ -tubulin reorganization during mouse fertilization and early development. *J. Cell Sci.* **104**, 383-389.
- Paleček, J., Ubbels, G. A. (1997) Dynamic changes in the tubulin cytoskeleton during oogenesis and early development in the anuran amphibian *Xenopus laevis* (Daudin). *Folia Histol. Cytobiol.* **35**, 3-18.
- Paleček, J., Pěkníková, J., Vítů, M. (1999) Changes in immunochemical localization of cytoskeletal proteins in human and boar spermatozoa before and after acrosome reaction. *Folia Biol. (Praha)* **45**, 13-20.
- Pěkníková, J., Moos, J. (1990) Monoclonal antibodies against boar acrosomal antigens labeling undamaged acrosomes of spermatozoa in immunofluorescence test. *Androl.* **22**, 427-435.
- Pěkníková, J., Moos, J., Mollova, M., Sršň, V., Čapková, J. (1994) Changes in immunochemical localization of acrosomal and sperm proteins in boar spermatozoa during capacitation and induced acrosome reaction. *Anim. Reprod. Sci.* **35**, 255-271.
- Sandvig, K., van Deurs, B. (1990) Selective modulation of the endocytic uptake of ricin and fluid phase marked without alteration in transferrin endocytosis. *J. Biol. Chem.* **265**, 6382-6388.
- Peterson, C. A., Gordon, H., Hall, Z. W., Paterson, B. M., Blau, H. M. (1990) Negative control of the helix-loop-helix family of myogenic regulators in the NFB mutant. *Cell* **10**, 493-502.
- Spungin, B., Margalit, I., Breitbart, H. (1995) Sperm exocytosis reconstructed in a cell-free system: evidence for the involvement of phospholipase C and actin filaments in membrane fusion. *J. Cell Sci.* **108**, 2525-2535.
- van Deurs, B., von Bülow, F., Vilhardt, F., Holm P. K., Sandvig, K. (1996) Destabilization of plasma membrane structure by prevention of actin polymerization. *J. Cell Sci.* **109**, 1655-1665.
- Towbin, H., Staehelin, T., Gordon, G. (1979) Electrophoresis transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350-4354.
- Virtanen, I., Badley, R. A., Paasivuo, V. P., Lehto, V. P. (1984a) Distinct cytoskeletal domains revealed in sperm cells. *J. Cell Biol.* **99**, 1083-1091.
- Virtanen, I., Lehto, V. P., Kallajoki, M., Blose, S. H. (1984b) Differential localization of alpha- and beta-tubulin in human sperm cells. *J. Cell Biol.* **99**, 41-45.
- Vogl, A. W. (1989) Distribution and function of organized concentration of actin filaments of mammalian spermatogenic cells and Sertoli cells. *Int. Rev. Cytol.* **119**, 1-56.
- von Bernhardt, R., de Ioannes, A. E., Blanco, L. P. (1990) Round-headed spermatozoa: a model to study the role of the acrosome in early events of gamete interaction. *Andrologia*, **22**, 12-20.
- Yanagimachi, R. (1994) Mammalian fertilization. In: *The Physiology of Reproduction*, eds. Knobil, E., Neil, J., pp. 189-317, Raven Press, New York.
- Zheng, Y., Wong, M. L., Alberts, B., Mitchison, T. (1995) Nucleation of microtubule assembly by a  $\gamma$ -tubulin-containing ring complex. *Nature* **378**, 578-583.