Short Communication

Asymmetric Vimentin Distribution in Human Spermatozoa

(asymmetry / vimentin / spermatozoa / cytoskeleton / ultrastructure / fertilization)

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Abstract. The subject of the study was vimentin distribution in spermatozoa from human ejaculates by immunofluorescence and immunogold electron microscopy. In accordance with earlier reports, vimentin was found in the sperm head and was localized mainly in the equatorial segment region. However, electron microscopic observations revealed an additional intriguing detail: vimentin-associated gold granules showed asymmetric distribution. This asymmetry tended to be more pronounced in heads with defects such as cytoplasmic droplets. Abnormal cells also had positive reaction for vimentin in the neck and the proximal midpiece, but in these domains gold granule distribution was apparently uniform. These findings seem to support the hypothesis that the surfaces of the mammalian sperm head are functionally non-equivalent, although morphological basis for such a phenomenon is evident only in rodents. It is also interesting that asymmetry in vimentin distribution correlates with distinct types of sperm structural defects.

Mammalian fertilization is a complex process requiring specific structural prerequisites and functional competence of the sperm cells (Vatev, 1981). Cytoskeletal components such as the intermediate filament protein vimentin in the sperm head have been suggested to be important in this process (Virtanen et al., 1984). Several authors have reported the presence of vimentin in the head of human spermatozoa (Virtanen et al., 1984; Baccetti et al., 1984, 1988; Ochs et al., 1986; Seppälä-Lehto and Lehto, 1993; Marinova et al., 1996; Paleček et al., 1999). However, its exact cellular localization and relation to sperm abnormalities are still controversial. Because cytoskeletal proteins are among the most important cell shape determinants (e.g. Aumüller et al., 1992), their influence on the sperm head shape also requires further study. In particular, the sperm head has been supposed to possess asymmetry with functional importance during zona binding and penetration (Bedford, 1998) which, to our knowledge, has not yet been linked to any particular cytoskeletal component. For this reason, we studied the vimentin distribution in human spermatozoa immunocytochemically with special attention to possible asymmetry.

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Abbreviations: BSA – bovine serum albumin, FITC – fluorescein isothiocyanate, PBS – phosphate-buffered saline, SDS – sodium dodecyl sulphate.

Material and Methods

Human spermatozoa from 7 normozoospermic and 18 teratozoospermic ejaculates after washing with PBS (10 mM phosphate buffer, pH 7.2, 150 mM NaCl) were investigated by immunofluorescence, immunogold electron microscopy and immunoblotting. Immunofluorescence followed the protocol of Yagi and Paranko (1995). Monoclonal anti-vimentin antibody (IgM isotype, clone VIM-13.2, product No V 5255, Sigma Co., St. Louis, MO) diluted 1 : 100 was used as a probe and Polyvalent anti-mouse Ig(G,A,M) FITC conjugate (Sigma Co., product No F 1010) diluted 1 : 50 as the second antibody. For immunogold electron microscopy, cells were soaked for 10 min in PBS with 1% BSA (Institute of Infectious and Parasitic Diseases, Sofia, Bulgaria) and permeabilized by 0.5% Triton X-100 (Koch-Light Laboratories, Coinbrook Bucks, England, UK) in PBS for 30 min at 4°C as recommended by Jassim and Chen (1994). Then they were fixed with 1% glutaraldehyde in PBS for 30 min at 4°C. The immunogold reaction was performed according to Baccetti et al. (1988). The spermatozoa were incubated for 1 h at room temperature in the same anti-vimentin antibody diluted 1 : 200 in PBS – 0.1% BSA. For some samples, monoclonal anti-vimentin antibody (IgM isotype, clone VI-01, Institute of Molecular Genetics, Prague, Czech Republic, kindly donated by Prof. V. Viklický) was also used as the first antibody. In negative controls the first antibody was omitted or replaced with monoclonal anti-desmosomal cytokeratin antibody (IgM isotype, clone DK80.20, product No C 1041, Sigma Co.). After that, cells were incubated for 1 h at room temperature in anti-mouse IgM 10 nm gold conjugate (Sigma Co., product No G 5652) diluted 1 : 20 in PBS – 0.1% BSA. Further preparation of samples included postfixation in 1% OsO_4 for 1 h at 4°C, dehydration in ethanol series and embedding.

Immunoblotting was done in order to check the specificity of antibody detection. Sperm cells were lysed in sample buffer and their proteins were separat-

ed by SDS electrophoresis in 10% polyacrylamide gel according to Laemmli (1970). After that, immunoblotting was performed as described by Towbin et al. (1979). Proteins were transferred onto a nitrocellulose membrane (Bio-Rad, Richmond, CA). After blocking in PBS with 2% BSA for 2 h, the membrane was left to react for 2 h with the VIM-13.2 anti-vimentin monoclonal antibody, diluted 1 : 50 in PBS with 1% BSA. After washing, it was immersed for 1 h in anti-mouse IgM 10 nm gold conjugate (Sigma Co.), diluted 1 : 100 in PBS with 1% BSA. The reaction was visualized by silver enhancement using a silver enhancer kit (Sigma Co., product No SE-100). All incubations of the blotted membrane were done at room temperature. Molecular weights were estimated using a standard mixture (Sigma Co., product No SDS-7B).

Results and Discussion

By immunofluorescence and immunoelectron microscopy vimentin was found in the sperm head. It was localized mainly in the equatorial segment region in both normal and abnormal cells, with broader and more intensive staining in the latter. At the light microscopic level, the reaction was not asymmetric, except for rare cells with asymmetrically localized large cytoplasmic droplets (data not shown). At the electron microscopic level, vimentin-associated gold granules in the prevailing majority of sperm heads showed asymmetric distribution, being localized mainly at one side (Fig. 1). This pattern was found in 34 apparently normal sperm heads documented by us versus only 4 with symmetric reaction. In heads with structural defects, this asymmetry was diminished or absent in some cases (32 cells), but distinct types of sperm structural defects such as cytoplasmic droplets, abnormal membraneous structures and double nuclei tended to correlate with more pronounced asymmetry than in apparently normal heads (46 cells; Fig. 2). Abnormal cells were often also characterized by positive reaction for vimentin in the neck and the initial segment of the middle piece, but in these domains gold granule distribution was apparently uniform – 67 cells (Fig. 3) versus 35 with visible asymmetry. In more distal tail regions vimentin was not detected. No binding was observed in the negative controls (data not shown).

Fig. 1. Immunoelectron microscopic detection of vimentin on a human sperm head by monoclonal anti-vimentin antibody (Sigma). Longitudinal section, bar = 0.25 μm.

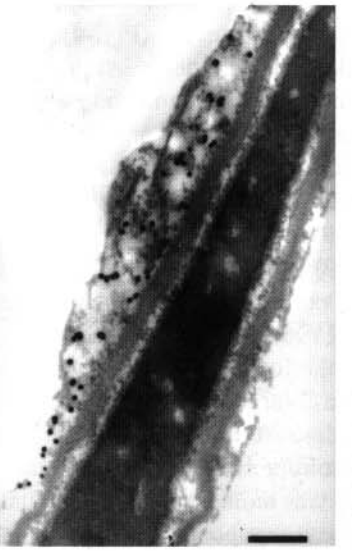

Fig. 2. Immunoelectron microscopic detection of vimentin on a sperm head with residual cytoplasm. Oblique section, bar = 0.25 μm.

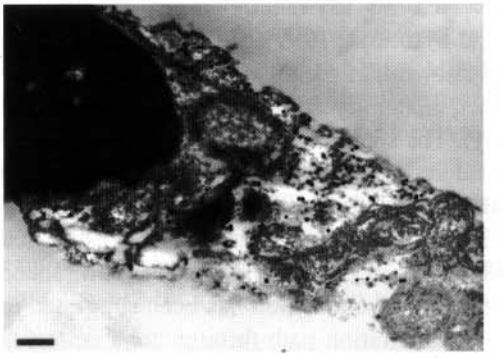

Fig. 3. Immunoelectron microscopic detection of vimentin in an abnormal middle piece. Longitudinal section, bar = 0.25 μm.

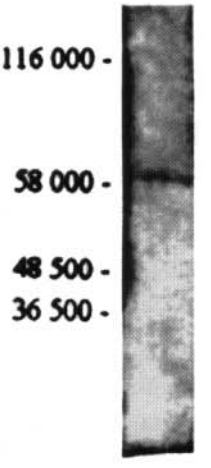

Fig. 4. Immunoblot detection of vimentin in normozoospermic human spermatozoa. On the left, positions of molecular-weight standards are indicated.

By immunoblotting, a single band with the apparent molecular weight 58 000 was detected (Fig. 4). This was interpreted as additional evidence supporting vimentin presence in human spermatozoa and its reaction with the antibody used as a specific probe.

Detection of vimentin in the equatorial segment of human sperm heads was in accordance with several reports (Virtanen et al., 1984; Seppälä-Lehto and Lehto, 1993; Paleček et al., 1999) and the observed changes in abnormal spermatozoa corresponded to our earlier results (Marinova et al., 1996). However, the asymmetric distribution that was the focus of this study has not been mentioned in the literature available to us. Because necks and midpieces in the same samples showed symmetric reaction for vimentin, the asymmetric labelling of the heads was unlikely to be artefactual. Another human sperm head component, the epidermal growth factor receptor, studied by us using the same method, revealed no asymmetry in its localization (Markova and Marinova, 1999).

It is well known that rodent sperm heads are asymmetric (in fact, bilaterally symmetric with a single plane of symmetry). The sperm heads of other eutherians are usually considered as "bi-radially" symmetric, with two perpendicular planes of symmetry and equivalent opposite sides. However, some studies have also detected sperm head asymmetry in the rabbit, pig, cow, sheep and horse, and this phenomenon has been suggested to determine the penetration path through zona pellucida (Bedford, 1998, and references therein).

At the electron microscopic level, we observed the asymmetry as a difference between the two broad sides of the spatulate human sperm head. By immunofluorescence these sides cannot be distinguished, because the cell is fixed on the slide lying on one of them. Therefore, the failure to detect asymmetry in most cells by immunocytochemistry at the light microscopic level should not be surprising.

Our findings seem to support the hypothesis that the surfaces of the mammalian sperm head are functionally non-equivalent. To our knowledge, they are the first structural data of that kind concerning human spermatozoa. It is also interesting that asymmetry in vimentin distribution correlates with distinct sperm structural defects.

References

Aumüller, G., Schulze, C., Viebahn, C. (1992) Intermediate filaments in Sertoli cells. *Micr. Res. Tech.* **20,** 50-72.

Baccetti, B., Bigliardi, E., Burrini, A. G., Gabbiani, G., Jockusch, B. M., Leoncini, P. (1984) Microfilaments and intermediate sized filaments in sperm tail. *J. Submicr. Cytol.* **16,** 79-84.

Baccetti, B., Burrini, A. G., Collodel, G., Magnano, A. R., Piomboni, P., Renieri, T. (1988) Immunocytochemistry and sperm pathology. *J. Submicr. Cytol. Pathol.* **20,** 209-214.

Bedford, J. M. (1998) Mammalian fertilization misread? Sperm penetration of the eutherian zona pellucida is unlikely to be a lytic event. *Biol. Reprod.* **59,** 1275-1287.

Jassim, A., Chen, Y.-L. (1994) AJ-FS1 monoclonal antibody detects a novel group of non-glycosylated antigens within the human sperm tail fibrous sheath. *Hum. Reprod.* **9,** 1459-1465.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680-685.

Marinova, Ts. Ts., Markova, M. D., Stanislavov, R. K. (1996) Distribution of vimentin in abnormal human spermatozoa. *Andrologia* **28,** 287-289.

Markova, M. D., Marinova, T. T. (1999) EGF receptor-like determinants on human spermatozoa and their possible cytoskeletal association. *Folia Biol. (Praha)* **45,** 143-145.

Ochs, D., Wolf, D. P., Ochs, R. L. (1986) Intermediate filament proteins in human sperm heads. *Exp. Cell Res.* **167,** 495-504.

Paleček, J., Pěknicová, 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.

Seppälä-Lehto, R., Lehto, V. P. (1993) Immunohistochemical detection of actin, vimentin, fodrin, tubulin and tyrosin phosphorylation in normal and asthenospermic human sperm. *Hum. Reprod.* **8,** (Suppl. 1), 90.

Towbin, H., Staehelin, T., Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76,** 4350-4354.

Vatev, I. (1981) Sperm capacitation in mammals and man. *Akush. Ginekol. (Sofiia)* **20,** 168-174.

Virtanen, I., Badley, R. A., Paasivuo, R., Lehto, V. P. (1984) Distinct cytoskeletal domains revealed in sperm cells. *J. Cell Biol.* **99,** 1083-1091.

Yagi, A., Paranko, J. (1995) Actin, α-actinin, and spectrin with specific associations with the postacrosomal and acrosomal domains of bovine spermatozoa. *Anat. Rec.* **241,** 77-87.