

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.

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 cyokeratin 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% OsO₄ 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-

Received August 28, 2001. Accepted December 5, 2001.

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

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



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



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

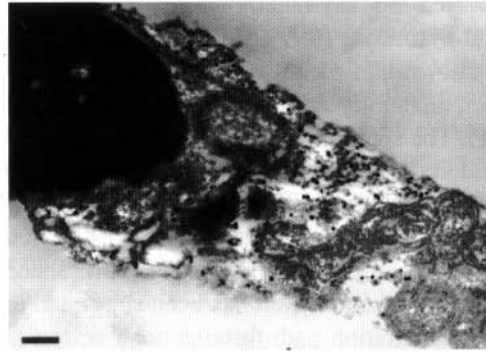


Fig. 3. Immunoelectron microscopic detection of vimentin in an abnormal middle piece. Longitudinal section, bar = 0.25 μ m.

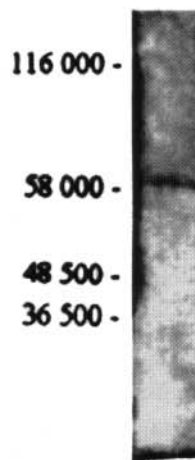


Fig. 4. Immunoblot detection of vimentin in normozoospermic human spermatozoa. On the left, positions of molecular-weight standards are indicated.

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).