

# Morphological and functional evaluation of spermatozoa from patients with asthenoteratozoospermia

Małgorzata Piasecka<sup>1</sup>, Maria Laszczyńska<sup>1</sup>, Dariusz Gączarzewicz<sup>2</sup>

<sup>1</sup>Department of Histology and Embryology, Pomeranian Medical University, Szczecin, Poland

<sup>2</sup>Department of Animal Reproduction, University of Agriculture, Szczecin, Poland

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*In several cases of asthenoteratozoospermia, electron microscopic investigation displayed immature sperm forms, morphological apoptotic patterns of spermatozoa and many cytoplasmic conglomerates with fragments of the sperm. In these patients, TUNEL assay showed a high percentage of spermatozoa with nuclear DNA fragmentation. Moreover, thickened and deformed midpieces were observed which contained supernumerary and redundant mitochondria with normal oxidoreductive capability and normal membrane potential. In these cases a high percentage of spermatozoa with normal  $\Delta\Psi_m$  was detected. Nevertheless, a subpopulation of patients was found with an abnormal ultrastructure of sperm mitochondria and with a low percentage of spermatozoa with normal  $\Delta\Psi_m$ . These findings indicate that low motility of spermatozoa may be related to abnormal morphogenesis of the midpiece containing functional mitochondria and that this may be a possible consequence of an apoptotic mechanism. Furthermore, our results show that asthenoteratozoospermia may result from dysfunction of sperm mitochondria and/or with alternations of the structures involved in sperm motility, i.e. the dense outer fibres, the fibrous sheath and the axoneme.*

**key words:** spermatozoa, morphology, mitochondria, DNA integrity, apoptosis

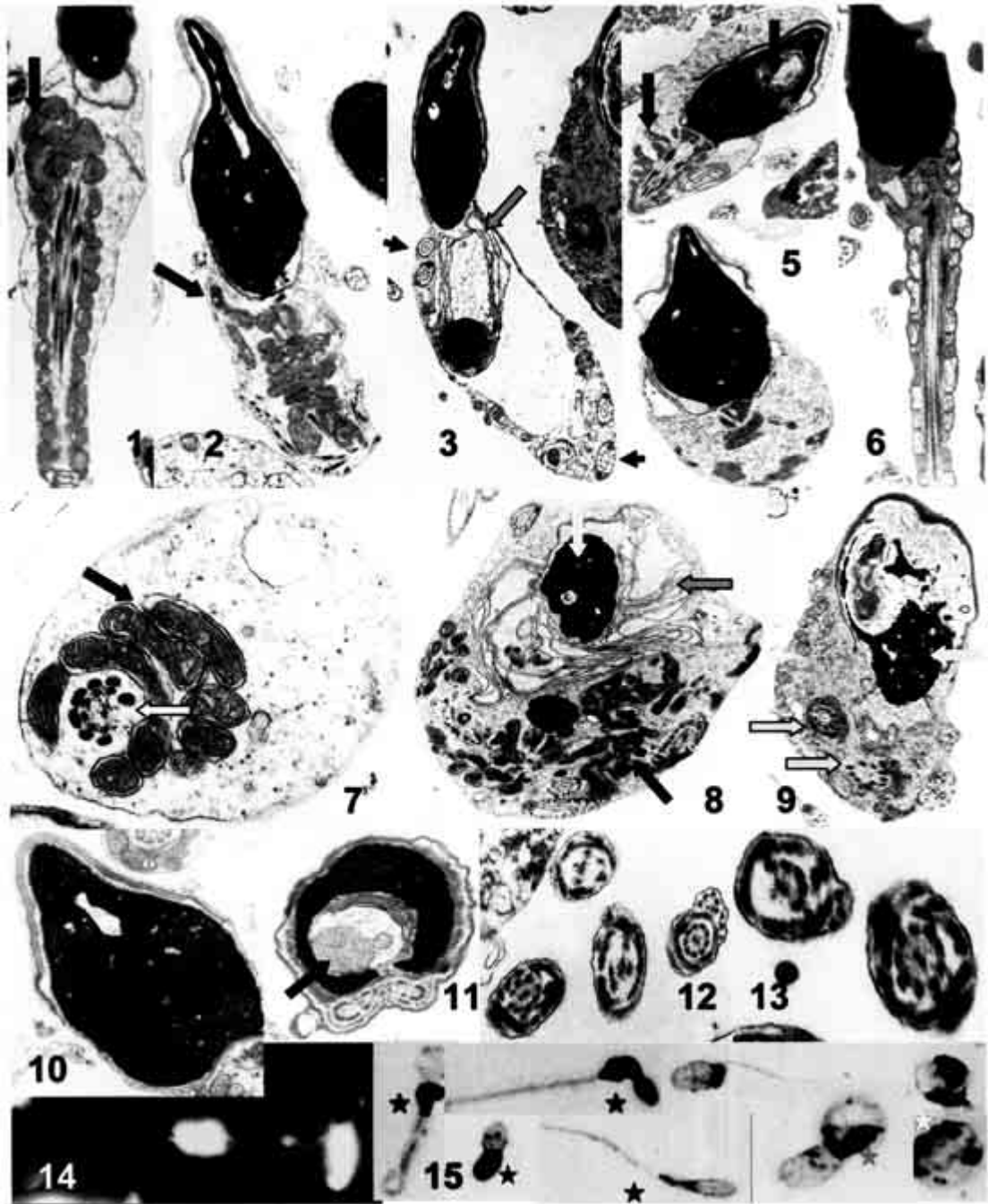
## INTRODUCTION

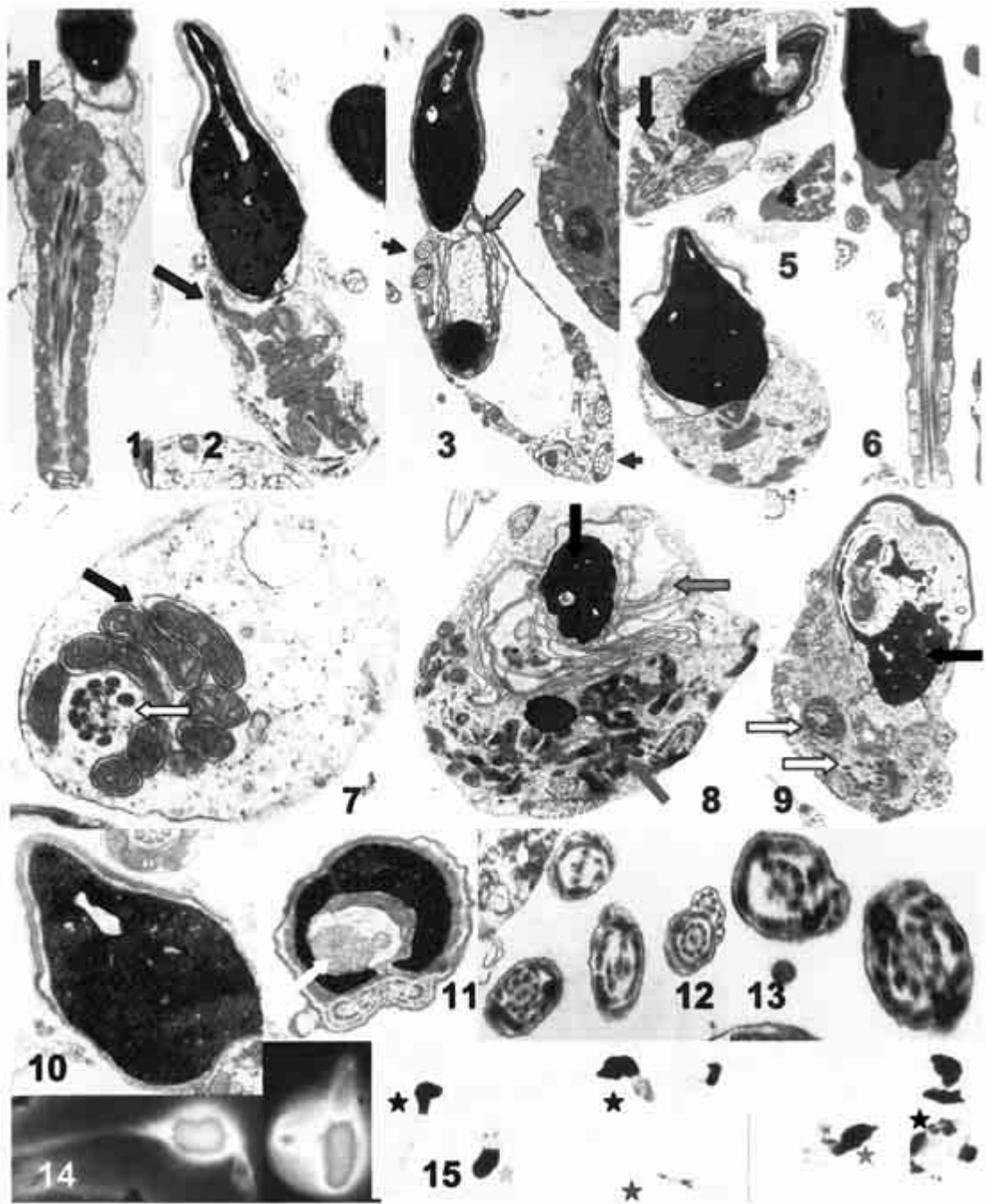
The development of molecular biology and genetics has allowed a deeper insight into the molecular basis of many forms of male infertility. Sperm abnormalities are manifested primarily as an inability to fertilise egg cells and contribute to low fertility or infertility [1, 2]. The objective of our study was to reveal morphological and functional abnormalities of spermatozoa in patients with asthenoteratozoospermia.

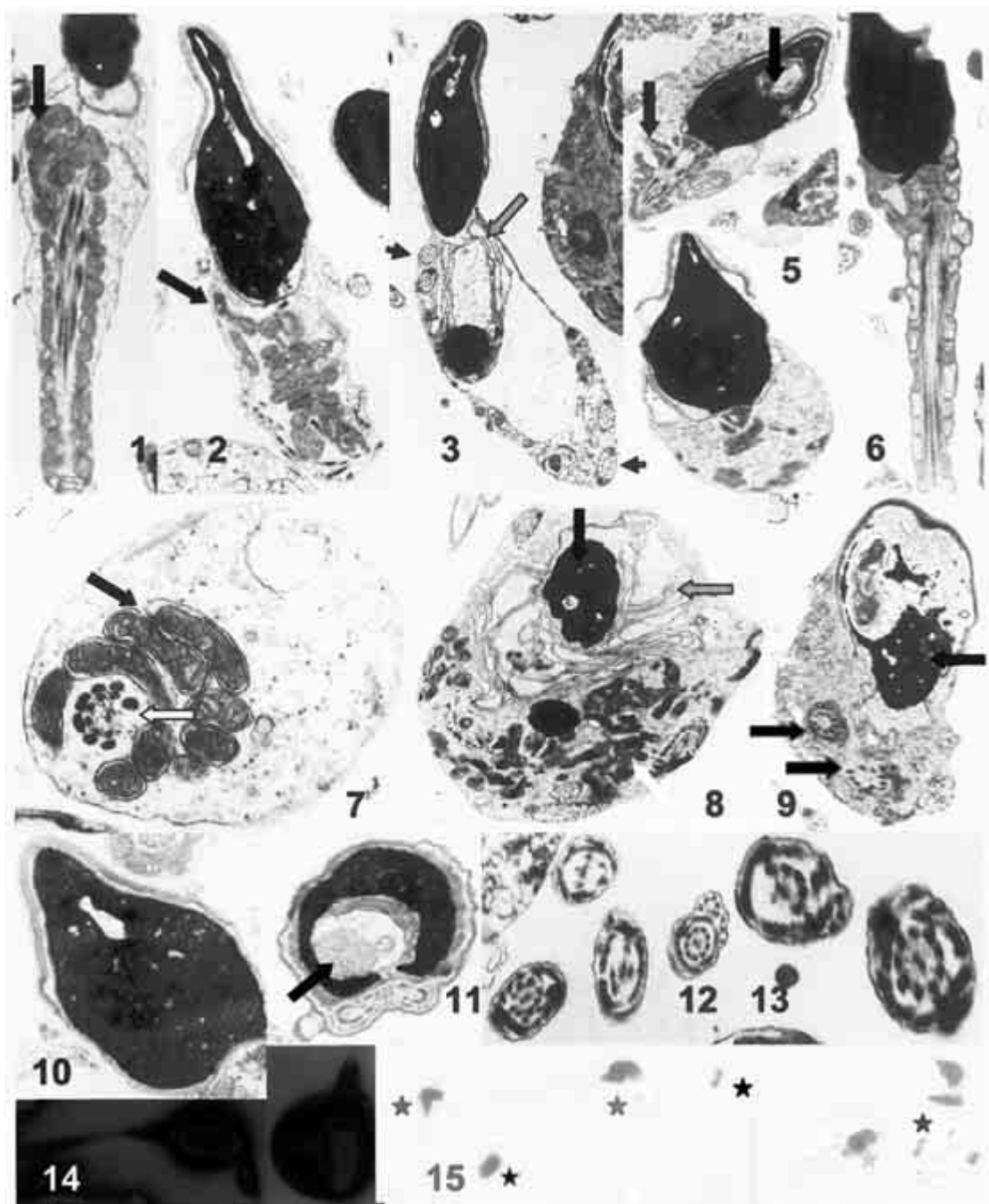
## MATERIAL AND METHODS

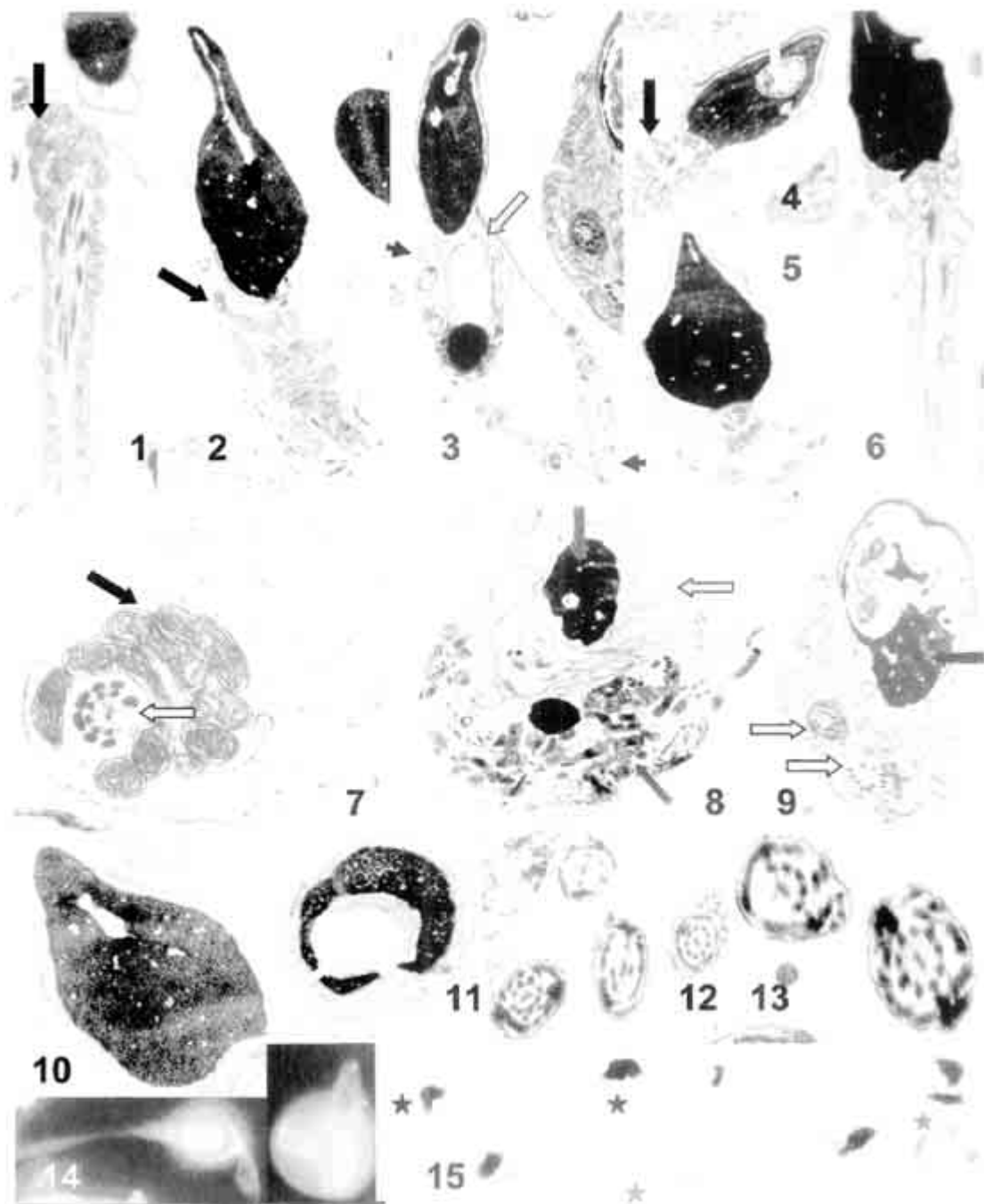
Studies were performed on ejaculated spermatozoa from patients of the Assisted Reproductive Technique Laboratory at the Reproduction and Gy-

naecology Clinic [22 subjects]. The routine parameters of semen were determined by standard methods recommended by the WHO [8] and Kruger et al. [3]. Spermatozoa for electron microscopic evaluation were prepared according to the routine method describe by Piasecka et al. [6]. Functional evaluation of sperm mitochondria was performed using: (1) a screening test — cytochemical reaction for mitochondrial NADH – dependent dehydrogenases (diphosphorase) [6] and (2) a JC-1 test to display changes in mitochondrial transmembrane potential ( $\Delta\Psi_m$ ) [6]. Nuclear DNA fragmentation was detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL assay) and monoclonal anti-









**Figure 1–15.** Morphological defects of human ejaculated spermatozoa from asthenoteratozoospermic patients. The abnormal shape of the head and the abnormalities of the acrosome (Fig. 2–6, 10, 11) and disorders of chromatin condensation (Fig. 4, 10, 11) and membranes in the sperm nucleus (green arrow); abnormal formed midpieces (Fig. 1–7); supernumerary, redundant mitochondria with normal ultrastructure in remnants of persistent cytoplasmic droplets (black arrow); spermatozoa have cytoplasmic “sacks” (Fig. 2–5), contain a disorderly arrangement of normal mitochondria, many sections through the principal piece (small black arrow), remnants of the nuclear envelope (grey arrow) and granular and lamellar material; spermatozoon with deformed midpieces containing mitochondria with abnormal ultrastructure (Fig. 6); cytoplasmic conglomerates (Fig. 8, 9) related to apoptotic bodies with partly-digested sperm chromatin (red arrow) and midpieces (yellow arrow), mitochondria with normal ultrastructure (blue arrow) and remnants of nuclear envelope (grey arrow); abnormalities of the dense outer fibres, axoneme and fibrous sheath (Fig. 7, 12, 13), and absence of the dense outer fibres (only 7 instead 9, Fig. 7, white arrow); JC-1 stained spermatozoa (Fig. 14), deformed midpieces occasionally with cytoplasmic droplets exhibit yellow-orange fluorescence, indicating that the deformed midpieces contain supernumerary, redundant polarised mitochondria with high  $\Delta\Psi_m$ ; cytochemical reaction in sperm midpieces and round cells (Fig. 15), the deformed midpieces revealing normal oxidoreductive capability as a filled formazans product of the reaction (black asterisk, compare to Fig. 1), (note) abnormal position of midpiece (yellow asterisk), spermatozoon with deformed midpiece and cytoplasmic droplet (grey asterisk), immature form of spermatozoon (green asterisk), absence of formazans in the midpiece (blue asterisk, compare to Fig. 6), round cells related to cytoplasmic conglomerates (compare to Fig. 8, 9) contain functional mitochondria (red asterisk). Fig. 1–13: transmission electron microscopy, JOEL, JEM-1200 EX; Fig. 14, 15: fluorescence (14) and light (15) microscopy Axioscop, Carl Zeiss; 1  $\times$  6000, 2  $\times$  8800, 3  $\times$  4900, 4  $\times$  6700, 5  $\times$  10,000, 6  $\times$  9700, 7  $\times$  36,000, 8  $\times$  7800, 9  $\times$  6400, 10  $\times$  12,400, 11  $\times$  12,000, 12  $\times$  24,000, 13  $\times$  33,400, 14  $\times$  14,400, 15  $\times$  2000.

body-FITC (fluorescein) labelling of single-stranded DNA (Kit APO-BRDU, BioSource International, Inc.). The percentage of cells with polarised mitochondria (spermatozoa with high  $\Delta\Psi_m$ ) and the percentage of TUNEL-positive cells was established by flow cytometry measurement using a FACSCalibur cytometer (Becton Dickinson, San Jose, CA, USA). The cytofluorometric results were analysed statistically. The conformity of variables with the normal distribution (Gaussian distribution) was examined using the Shapiro-Wilk W test. The unpaired Student t test (for Gaussian distribution) and the Mann-Whitney U test (for non-Gaussian distribution) were employed to determine statistical differences between the groups.

## RESULTS AND DISCUSSION

Electron microscopic observations displayed many spermatozoa with morphological defects (Fig. 1–13). In these cases, many cytoplasmic conglomerates were observed (Fig. 7, 8) and a high percentage of TUNEL-positive cells was found (> 4%). Moreover, the midpieces of many spermatozoa were abnormal with redundant and supernumerary mitochondria localised very often in a remnant of a persistent cytoplasmic droplet (Fig. 1). In many cases, spermatozoa had cytoplasmic “sacks” instead of a normal midpiece (Fig. 2–5). The supernumerary mitochondria had normal ultrastructures (Fig. 1, 2, 4, 7), a high  $\Delta\Psi_m$  (Fig. 14) and complete oxidoreductive capability (Fig. 15). It should be emphasised that spermatozoa with deformed midpieces containing supernumerary functional mitochondria did not exhibit rapid progressive movement. Instead they displayed non-progressive movement or were immotile. In these cases (11 out of 22 of the investigated subjects) a high percentage of spermatozoa with polarised mitochondria ( $75 \pm 12$ ) was noted. In the other cases studied (11 subjects), the percentage was significantly lower ( $30 \pm 14$ ,  $p < 0.001$ ) [6]. This finding depended on (1) the occurrence in the semen of a large number of spermatozoa with normal or deformed midpieces containing dysfunctional mitochondria with an abnormal ultrastructure (8 out of 11 subjects) (Fig. 6), (2) the occurrence of a large number of spermatozoa with a segmental or absent mitochondrial

sheath (3 subjects). Moreover, ultrastructural defects of the fibrous sheath, topographical alternations of the dense outer fibres and axoneme microtubules or partial absence of them accompanied the mitochondrial sheath (Fig. 7, 12, 13).

Electron microscopic findings give support to the interpretation that asthenoteratozoospermia may result from disturbances in spermatogenesis, particularly in an impaired formation of the midpiece (many immature sperm form in the semen) [4, 5]. This may be linked to genetic disorders [1, 7]. However, the large number of semen cytoplasmic conglomerates related to apoptotic bodies, morphological apoptotic patterns of spermatozoa and the high percentage of sperm cells with DNA strand breaks point to the apoptosis of germinal cells, mostly spermatids [2]. Additionally, the morphological and cytofluorometric results revealed that functional disorders of sperm mitochondria do not necessarily occur in low sperm motility [6].

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