Diagnostic evaluation of oxidoreductive capability of sperm mitochondria

Piasecka M[1], Gączarzewicz D[2], Kurzawa R[4], Laszczyńska M[1], Kram A[3]

[1]Department of Histology and Embryology, [2]Department of Animal Reproduction University of Agriculture, [3]Department of Pathomorphology, [4]Clinic for Reproduction and Gynaecology, Pomeranian Medical University; Szczecin, Poland

Abstract

In the present paper, morphological and functional features of human sperm midpiece, contributing to the assessment of sperm fertility potential, have been described. The NADH-dependent NBT screening assay was used to identify and visualise: 1/ morphological defects of sperm midpiece, 2/ immature sperm forms with extensive cytoplasmic retention, reflecting developmental failure in spermatogenic remodelling process 3/ cytoplasmic sperm conglomerates, related to apoptotic bodies and 4/ sperm NADH-dependent oxidoreductase system at the mitochondrial level, related to the reaction intensity. The used assay is an adequate marker of sperm mitochondrial activity and sperm maturity. It can also help discover sperm defects that result in asthenozoospermia and can be used as an additional indicator in the evaluation of the sperm midpiece, as well as in routine morphological examination of spermatozoa, having a considerable predictive value for *in vivo* and *in vitro* fertilization.

Key words: spermatozoa, midpiece, mitochondria, cytochemistry, asthenozoospermia.

Introduction

Additional and complementary methods, enabling a precise and individual investigatation of spermatozoa, are needed to explain their pathophysiology, contributing to impaired fertility. Moreover, a precise diagnosis of spermatozoa is necessary, particularly to choose appropriate methods of assisted reproductive technique. The goal of our study was to determine morphological and functional features of sperm midpiece to discover defects leading to asthenozoospermia, very frequently associated with teratozoospermia.

ADDRESS FOR CORRESPONDENCE:
Małgorzata Piasecka
Department of Histology and Embryology
Pomeranian Medical University
Al. Powstańców Wielkopolskich 72; 70-131 Szczecin, Poland
tel/fax 0 91 4661677; e-mail: mpiasecka@ipartner.com.pl

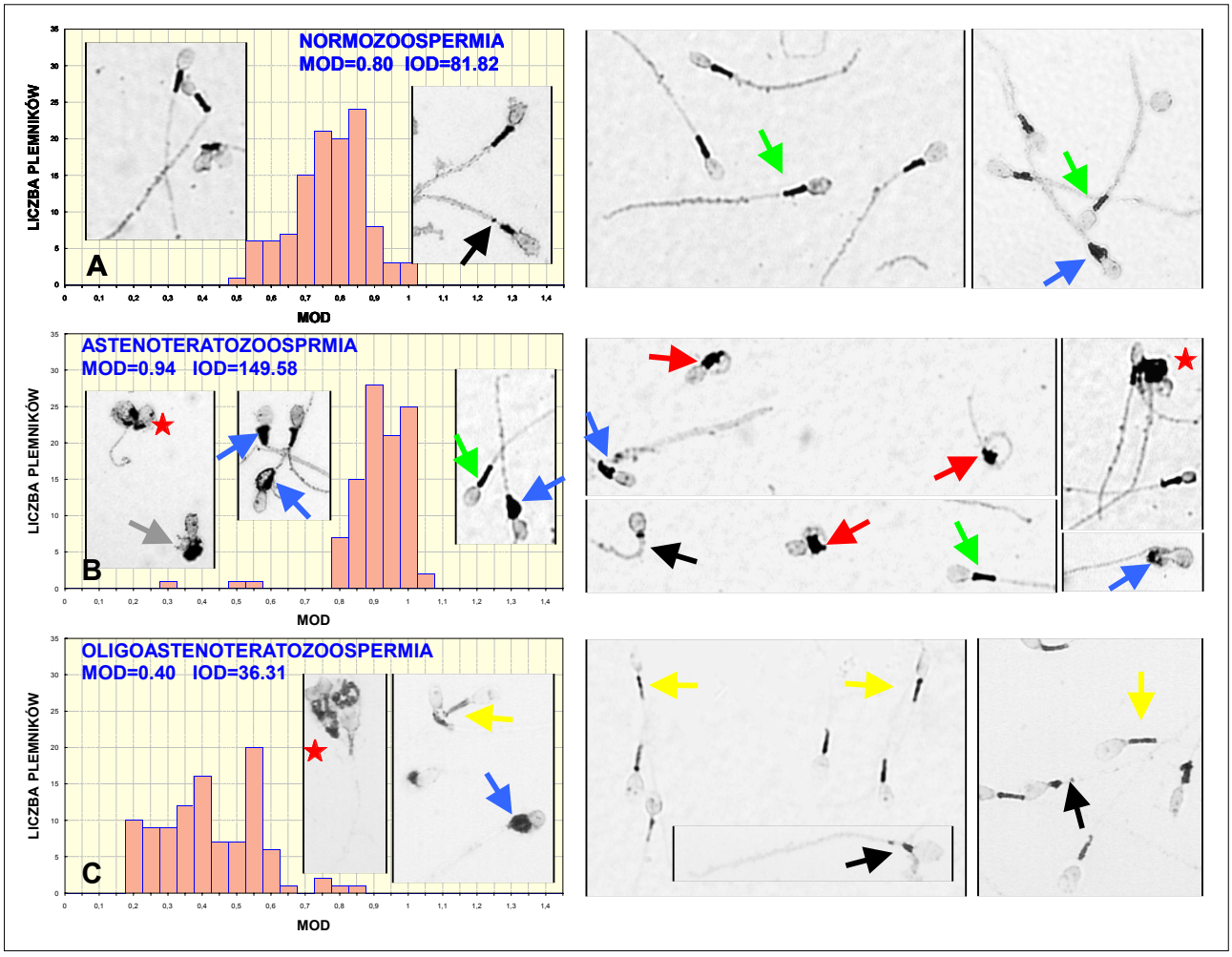
Material and Methods

Studies were performed on ejaculated spermatozoa from patients of the Assisted Reproductive Technique Laboratory (n=80). Routine semen parameters were determined, using standard criteria, recommended by WHO [1] and morphological strict criteria, according Kruger et al. [2]. The sperm midpieces were assessed by means of a screening cytochemical test [3, 4, 5, 6] for mitochondrial NADH-dependent oxidoreductases (diaphorase, related to flavoprotein), using NADH and NBT (nitroblue tetrazolium) as electron donor and an artificial acceptor of electron, respectively (NADH dependent NBT assay). Morphological evaluation of sperm smears with cytochemical reaction was based on elaborated own criteria and, partly, on the criteria given by Hrudka [3]. The intensity of the reaction (visualized morphologically as abundant blue deposits of formazans within the midpieces of spermatozoa) was assessed by a computer image analysing system (Quantimet 600S, Cambridge, UK) measuring the mean optical density (MOD, in each pixel of midpiece) and the integrated optical density (IOD, for the whole midpiece) of the reaction product-formazans. The densitometric measurements were expressed as arithmetic means, stated for 100 spermatozoa for each case and were referred to the activity of sperm mitochondria. The densitometric values depended on the formazan precipitation, due to the NBT reduction, determined by the oxidoreductase system of these organelles. Therefore, the oxidoreductive capability of the mitochondria could be related to the intensity of the reaction [5, 6].

Results

Different patterns of the cytochemical reaction could appear in normozoospermia and in patients with low standard

Figures 1 A, B, C. Histograms of the mean optical density (MOD) distribution in the population of spermatozoa (stated for 100 cells) and sperm smears with NADH-dependent NBT assay of patient with normal standard sperm parameters (A), low sperm motility and morphology (B) and with low sperm concentration, motility and morphology (C). Intense cytochemical reaction in: morphologically normal (green arrow), deformed (blue arrow), deformed and coiled sperm midpieces (red arrow), immature sperm form (grey arrow), sperm conglomerates (red asterisk), containing active mitochondria, filled with abundant formazan deposits (compact pattern); absence of formazan deposits (black arrow) in different segments of morphologically normal midpieces (focal pattern); weak cytochemical reaction (yellow arrow) in morphologically normal midpieces, containing dysfunctional mitochondria (diffused pattern); x1200.



sperm parameters. A positive-intense reaction (high values of MOD and IOD) was found in: 1/ morphologically normal midpieces (>50% in patients with normal sperm motility) (Fig. 1A), 2/, coiled, displaced, to long and deformed midpieces, occasionally with different size and shape of persistent cytoplasmic droplet (Fig. 1B), 3/ immature sperm forms with cytoplasmic retention, either failed or with cytoplasmic "sacks" (Fig. 1B) and 4/ sperm cytoplasmic conglomerates (Fig. 1 B, C). The deformed midpieces, immature sperm forms and cytoplasmic conglomerates occurred more frequently in terato- and asthenoteratozoospermic subjects (Fig. 1 B, C).

A weak cytochemical reaction (a reduced amount of formazan deposits) or the absence of formazans in various parts of the sperm midpiece (low values of MOD and IOD) were observed mainly in the morphologically normal midpiece (Fig. 1C). It suggested a functionally impaired mitochondrial sheath which contained, besides active, also dysfunctional mitochondria. Our morphologically and densitometric findings indicated that the decreased values of MOD and IOD, stated for 100 spermatozoa (for each case), resulted from the increased percentage of morphologically normal spermatozoa with a weak cytochemical reaction (Fig. 1C).

Discussion

Our previous [7, 8] and present studies indicated that morphologically normal and defected midpieces contained functional mitochondria with complete oxidoreductive capability and a high mitochondrial membrane potential. The deformations of midpieces resulted from supernumerary, functional mitochondria, located under plasma membrane or in a cytoplasmic droplet, placed at the level midpiece, the neck and even the head of spermatozoa (Fig. 1). Previous electron microscopic investigations demonstrated sperm conglomerates, containing sperm head, midpieces, principal pieces, supernumerary active mitochondria and a large amount of membranes and granular materials [8]. The sperm cytoplasmic conglomerates can be related to apoptotic bodies and they may reflect apoptosis of spermatids at a different stage of their differentiation [8, 9].

The densitometric studies demonstrated that high values of optical density (MOD and IOD) occurred not only in patients with normal sperm motility but also in some cases of asthenozoospermic patients (Fig. 1B). It was evident that low sperm motility in those subjects was not associated with energetic disorders of sperm mitochondria because the high values of optical density reflected high oxidoreductive capability of sperm mitochodria, which could generate energy-ATP for sperm movement. Those densitometric results confirmed our previous cytofluorometric study of JC-1 stained spermatozoa [7, 8]. In some cases of asthenozoospermia, a high percentage of spermatozoa with high mitochondrial membrane potential (polarized, functional mitochondria) was detected. Morphological examination of sperm smears from those asthenozoospermic patients revealed that the semen contained a large number of immature sperm forms with functional mitochondria and/or a large number of spermatozoa with morphological deformations of mitochondrial sheath, containing redundant and supernumerary functional mitochondria and, occasionally, it contained sperm cytoplasmic conglomerates with active mitochondria. But, on the other hand, our study has also shown low densitometric values in some cases of asthenozoospermic patients (Fig. 1C). Those densitometric and morphological results of the patients suggested energetic disorders of mitochondria, mainly in large number of morphologically normal sperm midpieces (Fig. 1C). The findings were consistent with our previous results [7, 8].

Based on the date of the presented study, the asthenozoospermia could result from 1/ disturbances in spermatogenic remodelling process, particularly, in sperm midpiece morphogenesis, 2/ failed cytoplasmic extrusion, 3/ apoptosis of spermatids and 4/ energetic disorders of sperm mitochondria.

It should be emphasized that subtle and, occasionally, drastic defects of sperm midpieces, particularly with remnants of non discarded cytoplasmic droplet, were very frequently invisible in routine morphological examinations of sperm smears (staining, according to Papanicolaou) [1]. Therefore, the proposed cytochemical test can be applied as an assay not only to show normal midpieces but mainly to display their diverse defects. Moreover, this test can be considered as an adequate marker of cytoplasmic droplet and, simultaneously, of sperm immaturity [4], as a valuable and comprehensive test and can be applied as an additional indicator in the evaluation of sperm. The proposed test can be added to the routine clinical andrology workshop and can be of considerable predictive value for either *in vivo* or *in vitro* fertilization [4, 7, 8].

Acknowledgements

The authors wish to thank Prof. Wenancjusz Domagała for his kind permission to use the computer image analyzing system, and Jeremi Waroch and Dorota Waroch for the valuable and critical discussion of the manuscript.

References

1. WHO Word Heath Organization 3rd ed. Cambridge: Cambridge University Press.; 1992.
2. Kruger TF, Acosta AA, Simons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril, 1988; 49: 112-7.
3. Hrudka F. Cytochemistry of oxidoreductases in spermatozoa: the technic revisited. Andrologia, 1979; 11: 337-52.
4. Gergely A, Kovanci E, Senturk L, Cosmi E, Vigue L, Huszar G. Morphometric assessment of mature and diminished-maturity human spermatozoa: sperm regions that reflect differences in maturity. 1999; 14: 2007-14.
5. Piasecka M, Wenda-Różewicka L, Kram A. Computerized analysis of cytochemical reaction of spermatozoa In rats chronically treated with lead acetate [Pb(II)]. Folia Histochem Cytobiol, 1997; 35: 129-31.
6. Piasecka M, Wenda-Różewicka L, Ogoński T. Computerized analysis of cytochemical reaction for dehydrogenases and oxygraphic studies as methods to evaluate the function of the mitochondrial sheath In rat spermatozoa. Andrologia, 2001; 33: 1-12.
7. Piasecka M, Kawiak J. Sperm mitochondria of patients with normal sperm motility and asthenozoospermia: morphological and functional study. Folia Histochem Cytobiol, 2003; 41: 125-39.
8. Piasecka M, Laszczyńska M, Gączarzewicz D. Morphological and functional evaluation of spermatozoa from patients with asthenoteratozoospermia. Folia Morphol, 2003; 479-81.
9. GandiniL, Lombardo F, Paoli D, Capobecchia L, Familiari G, Verlengia C, Dondero F, Lenzi A. Study of apoptotic DNA fragmentation in human spermatozoa. Hum Reprod, 2003; 62: 479-81.