# Potentials of electron microscopic examination in the diagnosis of genetically determined disorders related to morphology and motility of human spermatozoa

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**Abstract.** Despite infertility is known as a serious medical and social issue, commercial panels for the determination of genetically caused disorders of morphology and motility of spermatozoa are not available in the territory of the Eurasian Economic Union. Electron microscopic examination of spermatozoa (EMIS) represents a promising method that allows to visualize and analyze structural anomalies in spermatozoa at a level which is not accessible by other methods. The study included transmission electron microscopy (TEM) of spermatozoa. Native sperm was diluted and fixed with 2.5% glutaraldehyde. Ultrathin sections were obtained on an UltraCut III microtome. Analysis was performed at magnifications of x4000, x25000. Spermatozoa were analyzed on a JEM-1011 electron microscope at different magnifications to identify the general appearance, axonemal anomalies, chromatin of the nucleus, and mitochondria. EMIS serves as a tool for detailed analysis of the morphology of spermatozoa in patients with asthenozoospermia (AZS) and teratozoospermia (TZS), and their combination. The obtained data provide a basis for more accurate diagnosis and personalized treatment approaches, contributing to increased effectiveness in overcoming infertility.

## 1 Introduction

Male factor infertility accounts for approximately 50% of infertile marriages [2]. Asthenoteratozoospermia, resulting from moderate to severe morphological defects of the sperm flagella, has a genetically determined nature [3]. Significant research into the genetic basis of multiple morphological anomalies of the flagella (MMAF) in sperm has been conducted over the last decade [4-6]. There are reports linking MMAF with mutations in the CFAP43 and CFAP44 genes, with the proportion of sperm with abnormal flagella in men with asthenoteratozoospermia carrying mutant alleles ranging from 79.5% to 99.5% [7].

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Mutations in the DNAH1 gene, which plays a role in the formation of the sperm axoneme, are associated with flagellar defects [8]. Variants in DNHD1 and CFAP65 may have a negative impact on both the axoneme structure and the mitochondrial membrane [9, 10].
Additionally, candidate genes characteristic of other types of anomalies have been identified. The most common morphological disorders include head defects, which can be caused by, for example, the AURKC gene, mutations of which lead to macrozoospermia – sperm with a large head, and mutations in DPY19L2, C2CD6, CCIN, GGN, PICK1, SPATA16, ZPBP1, noted for globozoospermia. Mutations in PICK1 lead to a phenotype similar to globozoospermia, but there is also evidence of its role in acrosome formation disorders, causing its fragmentation at the early stages of spermatogenesis [11]. Cases of severe teratozoospermia with predominant amorphous head anomalies, triggered by a missense variant of the endogenous meiotic inhibitor 2 gene FBXO43, have been described [12].
Currently, in the Eurasian Economic Union (EAEU), panels for determining genetically determined disorders of sperm morphology and motility are not commercially available, making it important to explore decision-making tools for patients with asthenoteratozoospermia. One such tool could be electron microscopic examination of sperm (EMIS). EMIS could provide a more complete picture of anomaly types in patients with asthenoteratozoospermia, determining the tactics for overcoming infertility (type of sperm selection for ICSI, use of donor material, further examination).

## 2 Materials and Methods

For transmission electron microscopy, native sperm was diluted with isotonic NaCl solution (Mosfarm, Russia) at a ratio of 1:10, and 0.1 ml of fixative – 2.5% glutaraldehyde solution (Ted Pella Inc., USA), prepared in 0.1M cacodylate buffer (pH 7.2) (Sigma, USA), was added. The mixture was centrifuged at 1500 rpm (Elmi, Latvia) for 15 minutes, the supernatant was removed, and the sediment was fixed again with the same fixative, post-fixed with a 1% solution of osmium tetroxide (Serva, Germany), and embedded in epoxy resin – Epon (Fluka, Germany). Ultrathin sections were cut on an UltraCut III microtome (Reichert Jung Optische Werke AG, Austria), stained with aqueous solutions of uranyl acetate and lead citrate, and examined under a JEM-1011 electron microscope (JEOL, Akishima, Japan), equipped with an Orius SC1000 W camera (Gatan Inc., Pleasanton CA, USA) [13, 14]. The overall appearance of spermatozoa was studied at a magnification of x4000, acrosomes, nuclear chromatin, and mitochondria at x25000, and axonemal anomalies on cross-sections of flagella at x25000. In each sample, at least 150 gametes were analyzed [15].

## 3 Results and Discussion

The obtained images illustrate defects in the morphology of spermatozoa, which can be the cause of impairments in their motility and fertilizing function. The presented cross-section of the flagellum (Fig. 1A) clearly demonstrates an axonemal anomaly: incorrect positioning of microtubules and a disruption in the number of axonemal microtubules. Disruptions in the structure of the axoneme lead to a decrease in the motility index in ejaculate, which in turn results in reduced fertility. Heterogeneous anomalies in the morphology of the axoneme of the flagellum in cases of asthenozoospermia are typically the result of functional disturbances. The identification of homogeneous anomalies of the axoneme (absence of axonemal dynein arms, absence of the central pair of microtubules) indicates the possibility of a genetically determined form of asthenozoospermia (Fig. 1B). Structural changes affecting the neck region of a spermatozoon and mitochondria can be the cause of ATZS

development. Changes in the shape of mitochondria, their sizes, and internal structure indicate potential dysfunctions in operation and interaction with other cellular components (Fig. 1C), affecting the spermatozoon's movement and its energy supply. A spermatozoon with a disrupted structure of the mitochondrial sheath – the number of mitochondria is significantly reduced, the mitochondria have an electron-transparent matrix and lack cristae, and the acrosome is destroyed. These changes are accompanied by an abnormal structure of the peri-axonemal fibrous sheath of the flagellum, indicating genetically determined asthenozoospermia – dysplasia of the fibrous sheath of the flagellum.

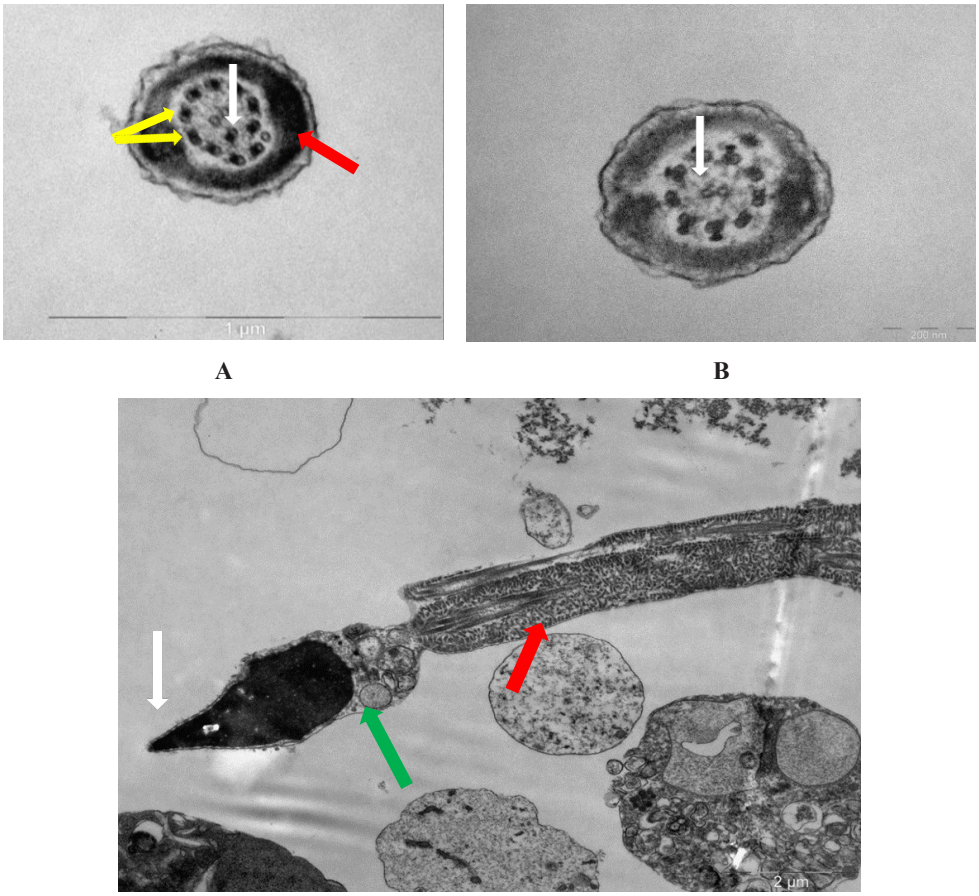


**Fig. 1.** Defects in the morphology of spermatozoa. Note: (A) cross-section through the flagellum of a spermatozoon from the ejaculate of a patient with asthenozoospermia. Abnormal arrangement of axonemal microtubules. The ninth pair of microtubules is shifted towards the center of the axoneme, with two additional doublets and an extra single microtubule present (white arrow), fibrous sheath of the flagellum (red arrow), axonemal doublets (yellow arrow). (B) cross-section through the flagellum of a spermatozoon from the ejaculate of a patient with absolute asthenozoospermia. Absence of dynein arms on the peripheral doublets of axonemal microtubules (white arrow). (C) longitudinal section through a spermatozoon from the ejaculate of a patient with absolute asthenoteratozoospermia. Isolated mitochondria (green arrow) with an electron-transparent matrix and absence of cristae, acrosome is destroyed (white arrow). The peri-axonemal fibrous sheath of the flagellum (red arrow) consists of fibers oriented in a disordered manner.

The use of TEM (Transmission Electron Microscopy) for spermatozoon research provides high resolution for detailed study of the morphology of male gametes. The images

obtained offer a more comprehensive characterization of morphological anomalies, providing information to identify the causes of teratozoospermia and asthenozoospermia development and to devise personalized treatment strategies. Some types of anomalies in teratozoospermia can be overcome with the ICSI (Intracytoplasmic Sperm Injection) procedure, however, severe pathological changes in spermatozoa may necessitate the use of donor material only. Detailed and complete diagnostics of abnormal morphology are essential for an accurate evaluation of ICSI effectiveness and the selection of spermatozoon selection type or donor services recommendations. TEM can also support genetic studies, helping to identify genetically determined anomalies that affect the morphology and function of spermatozoa.

## Conclusion

The data obtained lay the foundation for more detailed diagnostics and a personalized approach to infertility treatment. TEM is a crucial tool for the detailed analysis of sperm morphology in patients with teratozoospermia and asthenozoospermia and should be incorporated into the practice of ART (Assisted Reproductive Technology) departments as a tool to enhance the effectiveness of IVF (In Vitro Fertilization) programs. It should also be noted that often, the causes of idiopathic male infertility are hidden behind the diagnosis of "normozoospermia," and TEM represents a reserve that can and should be utilized, in our view, in cases of repeated unsuccessful IVF attempts.

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