Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 18
Sofia • 2012

Ultrastructure Studies of Abnormal Sperm in the Pathology of the Male Reproductive System. Deviations in Sperm Tail

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The presence and localization of the structural disturbances in the human sperm were determined by transmission electron microscopic (TEM) method in seminal plasma from patients with diseases of the reproductive system. Morphological studies of sperm of 664 patients (mean age 32.6 ± 3.59 years) with congenital, vascular, specific and nonspecific inflammatory diseases of the male reproductive system were carried out according to the WHO criteria. The results in present study, demonstrate the ultrastructural anomalies in the neck and middle piece and deviations in the tail of the spermatozoa.

Key words: abnormal spermatozoa, transmission electron microscopy, male infertility

Introduction

Many studies showed that pathomorphology of spermatozoa is highly correlated with fertility in man [7, 9]. In fact, morphological deviations of the spermatozoa head seems to be the frequent cause of the male infertility [8]. Conventional light microscopic assessment of sperm morphology for routine semen analysis allows visualization of the entire germ cells but no details of the subcellular entities. The fine structure of the spermatozoa may, however, be evaluated by electron microscopy. In the previous our study [5] was illustrated morphological changes in the sperm head (a form of chromatin state and acrosome) of the germ cells as well as the integrity of the cell envelope. Therefore, the *aim* of the present study is to determine ultrastructural anomalies in the tail of the spermatozoa in patients with pathology of the male germ system.

Material and Methods

Morphological studies of ejaculates of 664 patients (mean age 32.6 ± 3.59 years) with congenital, vascular, specific and nonspecific inflammatory diseases of the male

reproductive system are carried out according to the WHO criteria (1996). The results are compared with those of 20 healthy men (mean age 30.6 ± 3.59 years) (Table 1).

The following methods are used:

√ Medical history and physical examination

√ Transmission electron microscopy / "Opton" EM 109/ for evaluation of ultrastructure changes in the sperm cells.

T a b l e 1. Distribution of the surveyed patients

Patients with:	**Number**	**Patients with:**	**Number**
Congenital diseases of male sexual system	**118**	Epididymitis chronica	94
Kryptorchism	148	**Sexually transmitted infections - STI**	55
Kysta epididymis	23	**Vascular diseases**	**60**
Inflammatory diseases of male sexual system	**431**	Varicocele	56
Specific inflammatory diseases	152	Torsio testis	4
Tuberculosis of epididymis – EPID. TBC	9		
Mumps orchitis – MO	143	**Total number of patients**	**664**
Nonspecific inflammatory diseases	379		
Prostatitis chronica	285	**Control group healthy men**	**20**

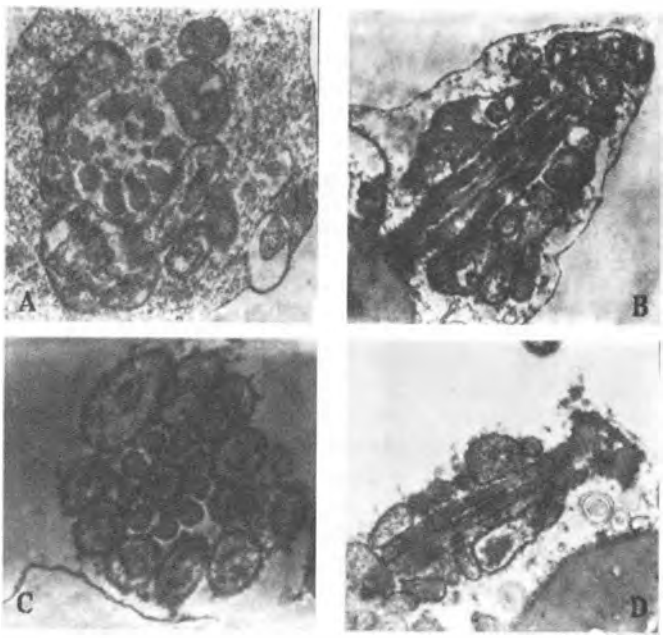

Figure 1. Neck and mid-piece anomalies in cross and longitudinal sections. (A) Disorganised of the mitochondria and disorganized of the axsoneme microtubules in mid-piece area, (B)-(D) increased number of mitochondria of different sizes and impaired helical arrangement around the axoneme. TEM, x 50 000

Results and Discission

The results of the morphological study on the ultrastructure changes sperm tail can be combined into two groups: deviations in the structure of the neck and mid-piece and deviations in the tail of the spermatozoa.

I. Sperm Neck and Midpiece Ultrastucture Changes. Infringements which most often occur in the neck of germ cells are normally associated with off-axis of articulation with the head. Longitudinal section showing "breaking" the neck region of the flagellum. Often there is unfulfilled connection in which the flagellum is completely separated from the head (Fig. 1-D). Longitudinal sections in the mid-piece showed disorganization of the mitochondria sheath or its complete absence (disorder ed spiral arrangement of mitochondria) around axonemal complex, as usually observed swelling and obligation of cristas in some of them. Other structural defects of the flagellum involve changes and alterations in the composition and numbers of the axonemal microtubules, particularly impairment of the dynein. The number of doublets was reduced (lack of 1, 2, 3 or 9, 8, 7 position) or impairment in their circular arrangement was established, as part of doublets entering the inner central area.

The nine dense fibrils also change its location around doublets. Some of them are *very thin* and adhere to each other, thus creating wide gaps between them or empty spaces due to their lack (Fig. 1-A, B, C).

Anomalies involving the neck and the mid-piece of the sperm are mostly in the presence of cytoplasmic droplets, disorganization of the mitochondria sheath and the so-called an "angular shapes". In most cases the cytoplasm droplet covers equatorial part of the head, neck and all the midpiece and contains multitude vesicles with electron dense granular contents, ribosomes, residual nuclear membrane and other cellular organelles. Disorganised midpiece ultrastructure, greater number of mitochondria or unformed of the mitochondrial sheath is established too [2]. The latter are dispersed groups in the cytoplasm without a specific sequence.

The angular shape of sperm cell is usually accompanied by thinning of the distal region of the nucleus and wrong detachable with neck. Saacke [12] describes the strong "inflexion" between the head and the connecting part of the flagellum with associated cytoplasmic droplet in the bull sperm. Bragina et al. [1] is observed similar defects in infertile men with astenozoospermia. The flexion including the head and area neck with displacement of the head from the axis of symmetry at an angle greater than 90°. In these cases the fault is due to disorganization in location of the centrioles [11], which does not come into contact between head and flagellum, or is a result from incorrect placement of the implantation fossa of the distal pole of the nucleus what results into the refraction in the neck.

In TEM, asymmetry in the mitochondrial size and distribution associated with disruption in the mid-piece [1, 3, 10]. These defects and combinations of teratogenic forms sperm is most often associated with hypokinesia or astenospermia.

II. Sperm Tail Ultrastucture Changes. Changes in the tail, as bent, coiled, type "loop", the presence of cytoplasmic droplets are common abnormalities in the morphology of spermatozoa. In longitudinal and cross sections thinning or even discontinuation of the fibrous sheath was observed in the principal piece of the tail (Fig. 2-H), ring turning or folding in an area of cytoplasmic remnant (Fig. 2-A) also was found.

Other structural defects of the principle piece involve changes and alterations in the composition and numbers of the axonemal microtubules, particularly impairment of the dynein arms. Cross-section of the distal part of the tail show various defects in the ultrastructure of axonemal complex consisting of the absence of one or more – three or four pairs of peripheral microtubules (the doublets are located in an arc) to complete their disorganization and random placement - translocation (Fig. 2-A, B, C, D).

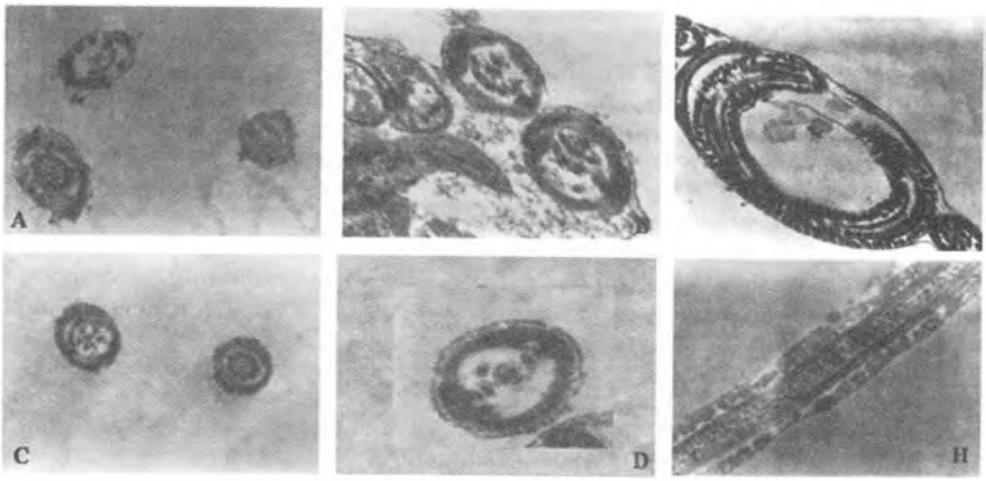

Figure 2. Different tail deviations in cross and longitudinal sections. (A)-(D) Cross sections of the principal piece with disorganized of the microtubular pattern and fibrous sheath, (G) coiled tail, (H) longitudinal section of principle piece with defects fibrous sheath. TEM, x 30 000

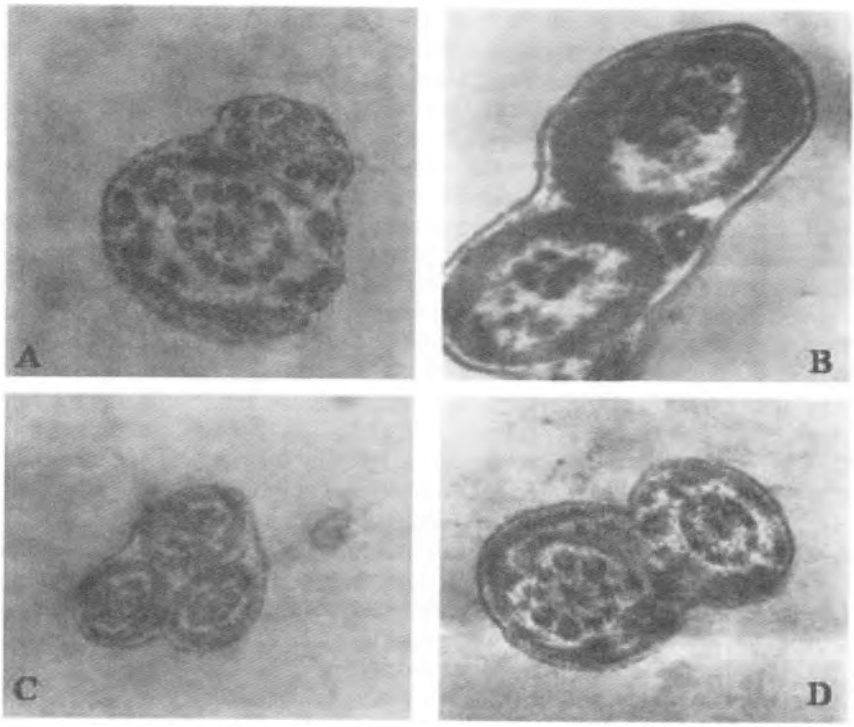

Figure 3. Double and triple tails. (A), (B) and (D) Double tails with defect axonems, (C) triple tail. TEM, x 30 000

The presence of double and even triple tails also show diversity in flagellum morphology on cross sections. There is a distinction in the diameters of two tails – the one is thicker than the other (Fig. 3-A, B). In some cases they are fused to each other, and in others there was no tightly between them (Fig. 3).

Besides single defects affecting primarily only one of the two tails meet and serious alterations in the structure of axonema - lack much of the peripheral doublets or crowding them into the center of the axonema, partially accompanied by outer dense fibrils (Fig. 3-B).

Very rarely the triple tails were obsirved and they were normally and equally developed (Fig. 3-C).

The defects in a tail structure show modification or disorganization of the axonema architecture. Most typical are deviations from the (9 x 2 + 2) configuration, some tails having only three or four microtubules at the distal part [7, 11]. The flagella sometimes lack the central pair microtubules, the "9+0" syndrome, but may possess dynein arms and radial spokes [6]. More specific and subtle malformation is a lack only of dynein arms. According Küpker et al. [8] this is one of the most common causes of immobility of spermatozoa.

Furthermore, reduced motility could be caused not only by deformed structure or incomplete arrangement of the axial filaments, but also by anomalies of the periaxonemal structures, for example abnormal size or position of outer dense fibres, hyperplasia and marked disorganization of the fibrous sheath [4].

Anomalies such as invagination or vacuolisation of the outer plasma membrane can be found at each part of the spermatozoon.

Conclusions

The different etiologic factors exert influence on the morphology of germ cells and hence on their fertility. Manifest of the specific deformation in the neck and tail leading to decrease sperm motility. The results of the TEM studies show that some of the flagellum anomalies are unimportant and can be overcome by gamete without interception of fertile process. Therefore, in selecting appropriate therapy and/or the application of *in vitro* technology is necessary ultrastructural analysis of ejaculated spermatozoa as routine investigation.

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