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

Acta morphologica et anthropologica, 17
Sofia • 2011

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

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Ultrastructure studies of sperm of patients with various diseases of the reproductive system showed a wide range of distortions in the morphology of the sperm, leading to severe reduction of the fertilizing capacity of germ cells. Morphological studies of sperm of 664 patients (mean age 32.6 ± 3.59 years) with congenital, vascular, specific and unspecific inflammatory diseases of the male reproductive system are carried out according to the WHO criteria. The results of the morphological study on the ultrastructure changes in sperm cell can be combined into three major groups: deviations in the sperm head (a form of chromatin state and acrosome), deviations in the structure of the neck and middle piece and deviations in the tail of the spermatozoa.

Data from these studies contributes to better understanding of the etiology of male infertility, the selection of appropriate therapy and techniques for in vitro fertilization.

Key words: abnormalities spermatozoa, transmission electron microscopy, mail infertility.

Introduction

Light microscopic (LM) studies show different changes in the morphology of spermatozoa of ejaculate of men with diseases of the reproductive system. These changes can affect every part of the structure of the sperm cell – head, tail, and combinations of alterations. As a result of abnormalities, changes in the functional properties of the germ cells occur – e.g. their mobility and fertility. Better understanding and insight into the subcellular organization of sperm and organelle complex system can be achieved by transmission electron microscopy (TEM). An in-depth evaluation of semen quality by TEM provides substantial information about motility and the fertilizing competence of spermatozoa [10, 15, 12].

The *aim* of this study has been to determine characteristic malformations of sperm ultrastructure 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 unspecific 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:

– *Anamnesis and local andrologic status*

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

Table 1. Distribution of the surveyed patients

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

Results and Discussion

The results of the morphological study on the ultrastructure changes sperm cell can be combined into three major groups: deviations in the sperm head (a form of chromatin state and acrosome), deviations in the structure of the neck and middle piece and deviations in the tail of the spermatozoa.

I. Sperm head morphological deviations. Abnormal cells with irregular shaped head – "amorphous head" was observed by TEM (Fig. 1) Diversity of this anomalies was found and they were related to irregular surface in equatorial region of head or with abnormal location of acrosome. Expanded subacrosomale layer at different places was observed (Fig. 1-G, H) which defected snug fit of acrosome toward nuclear envelope. Often typical teratological spherical and elongated sperm head were seen in ejaculate of patients with nonspecific inflammatory and vascular diseases (Fig.1-B, C, E).

Elongated head revealed sharpening of posterior nuclear end and decreased transversal diameter. The head shape changed of oval to lake lance form (Fig. 2-C). The spherical head was illustrated with uncompleted acrosome development or absence of it (see Fig. 1). Morphological assessment of nuclear substance was presented by coarse granular appearance of inhomogeneous chromatin and presence of vacuoles with larger then normal dimensions. The heads with such chromatin are more often large with spherical shape (see Fig. 1-B).

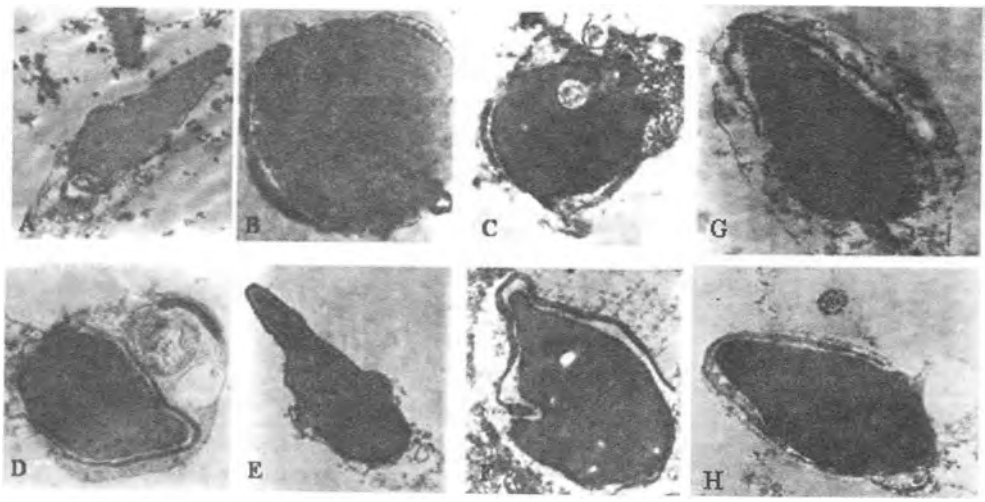

Fig. 1. Sperm heads: (A) normal sperm head, (B)-(H) different sperm head deviation – (B) Round sperm head with incomplete chromatin condensation, (C) round sperm head with vacuole and lost acrosome, (D)-(F) amorphous sperm heads – (D) with cytoplasmic droplet, (F) with invagination of the acrosome into nucleus, (G) with detached acrosome, (H) spear-like sperm head. TEM, × 20 000

In fact, morphological deviations of the spermatozoa head seems to be the frequent cause of the male infertility [8]. The structural elements that conferred upon spermatozoa the ability both to penetrate the oocyte's vestments and to fuse with the oolemma reside in the head.. They are the acrosome and the sperm plasma membrane, which covers the equatorial segment of the acrosome and the post-acrosomal region of the head.

Defects in the sperm head itself may concern size and form of the nucleus or chromatin condensation. Giant and dwarf heads, deformed heads and double heads are already to be identified in LM. Giant heads are often diploid or even triad- or tetraploid [5]. Another abnormality is the round-head-syndrome (globozoospermia) known in man [6, 11], when the sperm head is untypically rounded. Globozoospermia correlates with defective acrosome biogenesesis and tail defects that have a disruption in the intramanchette [7] and intraflagellar transport systems of molecules to the centrosome and the developing tail [13].

Abnormalities of the acrosome are often associated with abnormal spermiogenesis, sub- or even infertility in man [14]. Detached acrosomes, partially or totally lost acrosomes are identified regularly. In infertile patients, Yu and Xu [14] found acrosomes covering a bigger proportion of the sperm head and an acrosome less smooth and less intact. Asymmetric thickening of the acrosome cap is described by Holstein [6]. Droplets attached to the acrosome membrane were also shown [14]. Zamboni [15] and Latini et al. [8] mentioned "miniacrosomes". Vacuoles in the acrosome and in the nucleus reported by us have also been described in man [6]. Cavities filled with amorphous material and surrounded by two membranes are also known [15] and are similar to cytoplasmic droplets. The vesicles are formed by separation and expansion of the plasma membrane away from the underlying structure. Differential diagnoses to these vesicles are proximal cytoplasmic droplets with electron-dense material and organelles similar to the Golgi apparatus. Furthermore, swollen acrosomes, sometimes together with swollen mitochondria at the midpiece and a coiled axonema in a cytoplasmic droplet are thought to be indicative of apoptosis [1].

Local defects in chromatin condensation are observed regularly as small vacuoles, but sometimes the vacuoles are big or chromatin condensation is incomplete what results in an inhomogeneous structure. Nuclear vacuoles have been reported in spermatozoa from individuals with seminal infections, varicocele, fever, testicular tumors and inflammatory bowel disease [15, 9]. Incomplete condensation is a sign of immaturity what was already discussed by Fawcett [5]. Furthermore, it is associated with low chromatin stability and teratozoospermia of the sperm head. Some authors suggest that the spermatozoa with incomplete chromatin condensation apparently more often display single-stranded rather than double-stranded DNA or possess chromosomal abnormalities [11] than evidence suggests that living spermatozoa with abnormal chromatin have a sharply reduced capacity to fertilize oocytes or may be responsible for defective early development. This might be caused by chromatin fragmentation or defects in histone–protamine exchange [3].

Ultrastructure studies of sperm of patients with diseases of the reproductive system showed a wide range of various distortions in the morphology of the sperm. Of most importance on the fertility of the sperm cells are disabilities in a head, were observed by us predominantly in infertile men. Data from these studies contribute to better understanding of the etiology of male infertility, the selection of appropriate therapy and techniques for *in vitro* fertilization.

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