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Effect of chronic Epididymitis inflammation on maturity spermatozoa and male fertilizing ability

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According to the clinical data chronic epididymitis leads to impaired fertility.

The aim of the present study is to determine specific changes in sperm structure and the level of male infertility with chronic epididymitis.

Spermatological studies on ejaculates of 94 patients with Epididymitis chr. and 20 healthy men were carried out according to the WHO criteria.

Changes in the quality and quantity of ejaculate are expressed mainly in higher percentage of gametes with microcephal and round heads, cells without tails and a high percentage of immotile sperm in conditions of Normo- and Oligozoospermia (Gr. I-III).

Integrated application of different methods for qualitative and quantitative assessment of sperm appears appropriate for better monitoring of patients with infertility.

Key words: sperm, morphology, Epididymitis, fertility

Introduction

The most common disorders of the reproductive system are chronic non-specific inflammation processes – orchitis, epididymitis, prostatitis, urethritis. Based on the clinical data, chronic epididymitis leads to impaired fertility in 42% of cases [Kerr, 1998].

The inflammatory process in epididymis can be located in the head, tail, or affects the whole organ. It causes infiltration of the epithelium, forming lymph-epithelium complexes, fibroses changes (with or without the presence of sperm) and epididymal duct obstruction [Jones, 1999]. Morphological and functional damages influence on epididymis role to serve as a sperm depot and thus on spermatozoa post-testicular maturation and motility. There are changes in the quality and quantity of ejaculate [Ilieva et al., 2009]. They are expressed in deviation from the state of Normospermia to the state of Oligoastenozoospermia (Gr. I-III) and to obstructive azoospermia or even aspermia [Bedford, 1994; Jones, 1999].

The **aim** of the present study is to determine specific changes in sperm structure and the level of male infertility in chronic Epididymitis.

Material and Methods

Spermatological studies on ejaculates of 94 patients (mean age 34.8 ± 4.56 years) with Epididymitis chr. were carried out according to the WHO criteria (2010). The results were compared with those of 20 healthy men (mean age 30.12 ± 1.22 years old).

The following methods were used:

- *Medical history and physical examination;*
- *Light microscopic studies of spermatozoa after routine staining with Yashkovski, Papanicolaou, hematoxyline-eosin, and*
- Cytochemical study according to the method of E. Zvetkova [2000];
- *The following criteria for evaluation of the fertilizing ability in Epididymitis chr. patients were used:* Preserved fertilizing ability, Relatively Preserved fertilizing ability, Poor fertilizing ability, Missing fertilizing ability

Results were reported as *mean values ± SD* and as relative part in percentage, and statistically analyzed by *Student's t-test* using statistical package SPSS. Difference was considered significant at $p<0.001$.

Results

Spermatological parameters

In 46.81% of patients with Epididymitis chr we found reduced ***concentration*** *of the mature germ cells* in the semen plasma – various grades of Oligozoospermia (Gr. I-III) with mean number of gametes 19.64 million/ml (1 – 39 million/ml) (Table 1). The reduced number of spermatozoa in Oligozoospermia (Gr. I-III) was above four times lower in comparison to the control group and the cases with Normospermia.

The relationship between the number of spermatozoa and the percentage of gametes with normal and abnormal morphology in the ejaculate samples from Epididymitis chr. patients is shown in Table 1. The results demonstrated a significant increase in the number of the abnormal germ cells in the Epididymitis chr. groups with Normo- and Oligozoospermia.

The pathology of Epididymitis chr. was characterized by a combination of a low cellular concentration and an increased content of abnormal spermatozoa in the semen. However, in 6.38% of patients with Oligozoospermia we found relatively low quantity of metamorphic gametes and, at the same time, the number of morphologically normal and vital germ cells predominated. These differences are statistically significant ($p<0.001$).

Besides, the comparison between the *number of spermatozoa and sperm* ***motility*** *in the semen plasma* made possible to draw a conclusion on the degree of the functional disturbances in this disease, and the degree of man infertility (Table 2).

In most patients with *Normospermia*, the sperm motility is recognized as progressive (25.60%) and non-progressive (24.20%) with velocity values between 5 and 6 μm/sec, and immotility – 50.20%. In 17 cases of *Oligozoospermia*, there were no spermatozoa with high motility, the percentage of poorly motile gametes was between 10% and 30% with velocity >5 μm/sec, and the akinetic germ cells were 70% and 90%. There were 100% immotile cells in six patients with *Normospermia* and in seven cases with *Oligozoospermia*. In all other cases, there were no substantial changes in sperm motility in comparison to *Normospermia*.

The results were statistically significant ($p<0.001$).

Table 1. Number and morphology of spermatozoa in cases with Epididymitis chr.

Epididymitis chr. (n=94)	Concentration (million/ml)	Gametes with normal morphology *(%)*	Gametes with impaired morphology *(%)*
Normospermia (53.19 %)	*63.24 ± 20.59*	*50.22*	*49.78*
Oligozoospermia Gr. I-III (46.81 %)	*19.64 ± 11.71*	*36.05*	*63.95*
Control group	**78.73 ± 18.45**	**78.63**	**21.37**

Table 2. Motility, number and velocity of spermatozoa in cases with Epididymitis chr.

Epididymitis chr. (n=94)	Motility (%)			Velocity
Number gametes *(%)*	**Progressive motile**	**Non-progressive**	**Immotility**	**(µ/sec)**
Normospermia (53.19)	**25.60 ± 2.51**	**24.20 ± 1.54**	**50.20 ± 2.86**	**6**
Oligozoospermia (46.81)	**20.75 ± 2.18**	**17.22 ± 1.81**	**62.03 ± 3.23**	**4.47 ± 0.32**
Control group *(n=20)*	**64.17 ± 5.07**	**15.90 ± 5.13**	**19.93 ± 4.04**	**16.52 ± 2.93**

Table 3. Morphological changes in germ cells

I. Anomalies in head spermatozoa	*37%*
II. Anomalies in tail spermatozoa	*11%*
III. Cytoplasmic droplet	*4%*
IV. Mixed anomalies	*5%*
Total percentage of ♂ with impaired morphology	*57%*

Morphological changes in germ cells.

Changes in the shape of the spermatozoa head

The morphological studies in Epididymitis chr. showed two predominant types of *abnormal head*: microcephalic, and round (Fig. 1A, Fig. 2). The first configuration had the highest percentage (19%), and the round-headed gametes represented 9% of the abnormal shapes. Macrocephalic (6%) and elongate-headed (3%) spermatozoa were also observed. The other atypical forms (amorphous and double head) were rare.

Another morphological alterations in shape of the spermatozoa

Other anomalies in the sperm morphology such as cytoplasmic residue or flagellum malformation were rarely registered in comparison to the head abnormalities.

Gametes with no tail (6%) or coiled tail (3%) were most commonly observed, the short and broken tail could rarely be seen (Figure 1B and Figure 2C).

The cytoplasmic droplet as a particular deformation was most frequently found around the neck, embracing the postacrosomal section of the head or the anterior part of the mitochondrial sheath (Figure 2D).

The results showed the highest percentage of sperm head anomalies (37%), less commonly observed tail anomalies (11%) and rarely seen cytoplasmic droplet (Table 3).

Besides spermatozoa with single defects, a small percentage of gametes with two or more nomalies in the sperm head and tail (5%) accompanied by cytoplasmic droplet was observed in Epididymitis chr. (Fig. 2C).

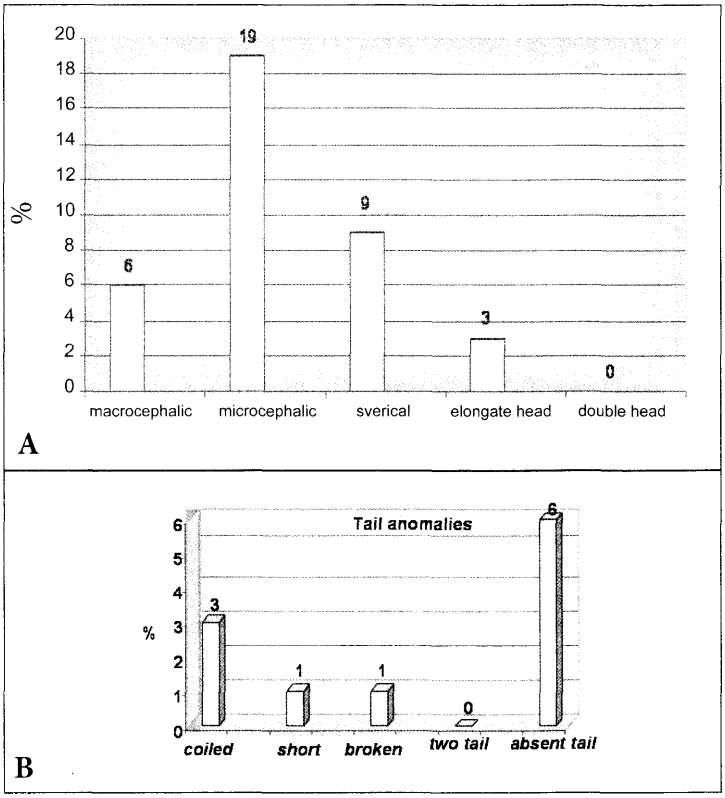

Fig. 1 Anomalies (%) in spermatozoa's head (A) and tail (B)

Cytochemical research of semen

We carried out a cytochemical research on semen plasma for meanwhile proving both nucleoproteins (DNP, RNP) and basic proteins in germ cells in order to follow up the maturity/immaturity of chromatin and basic proteins-containing (mainly protamine) cytoplasm.

In the control group of healthy men we found a large number of mature differentiated sperm intensively stained for basic proteins (Fig. 3A). The acrosome area, the nucleus and to a less extent, the area of the tail, were well visible. Basic proteins were stained in green by fast-gryin. The seminal plasma showed slight staining, and the nuclear chromatin – blue-purple, depending on the degree of decondensation (activation).

In semen samples from cases with Epididymitis, significantly reduced number of germ cells, immature sperm and dominated spermatids were observed (Fig. 3B) with a reduced amount of the basic proteins in the nucleus and chromatin decondensation.

Another sign indicating insufficient differentiation of gametes was the presence of so-called "oversize multinuclear cells" in the ejaculate which could be interpreted as similar to clusters, microcolony of undifferentiated spermatid cells, respectively. The nuclei of these cells probable are likely to be left in a common cytoplasm, "during the incomplete cell differentiation (Fig. 3D).

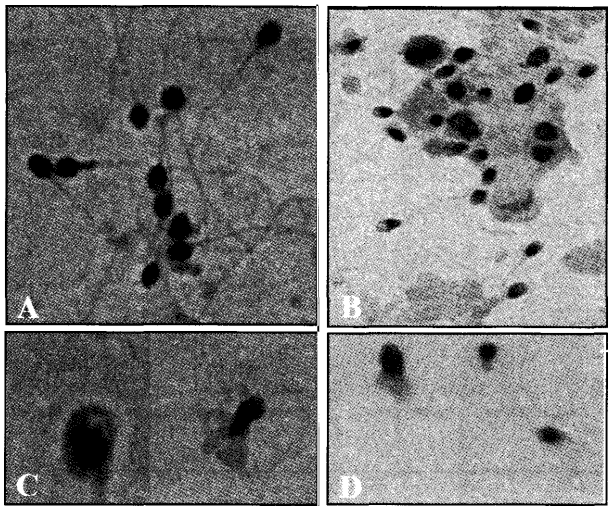

Fig. 2. Morphological changes in sperm in cases with Epididymitis chr. A-B – spermatozoa with normal, small, round and amorphous head and spermatids – (B); C – coiled tail and mixed anomalies; D – cytoplasmic droplet . Papanicolau, XE. X 450

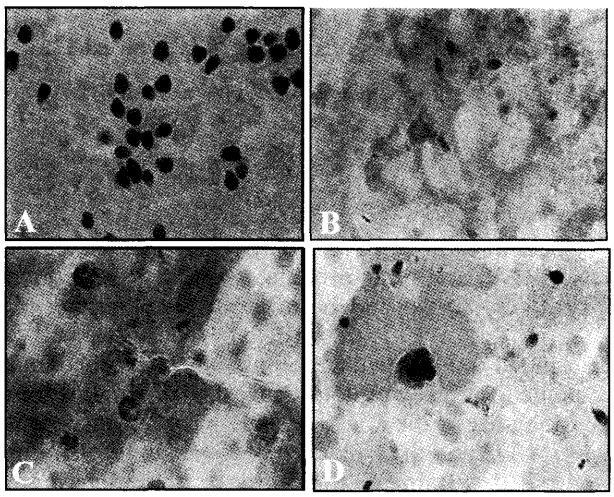

Fig. 3. Cytochemical research of semen plasma. A – normal spermatozoa, B-C – immature sperm, D – immature sperm and clusters of multinuclear cells. X 250; X 450; Immersio

Based on the above mentioned criteria for the degree of male infertility we can assess the fertilizing ability of patients with Epididymitis chr. We estimated it preserved in 46.67% of the cases, relatively preserved – in 38.09% and poor – in 14.28% fertilizing ability.

Discussion

Studies on the motility of gametes give a reason to some researchers to perceive this quality as a marker for good prognosis of male fertility – in vivo and especially in vitro fertilization [Auger et al., 1994; Elzanaty et al., 2002]. According to other authors [Lin et al., 1990] motility of sperm is the first visible indicator that changes with disturbance of the physico-chemical conditions of sperm regardless of etiologies factors. The studies in this area indicate that the epididymis and accessory sex glands play an important role in the formation of the functional status of the male gametes [Elzanaty et al., 2002; Gonzales et al., 1992].

Another major factor affecting the motility and fertility of germ cells appears their concentration and morphology. It is reported that in diseases of the male reproductive system changes occur in the morphology of germ cells, eg. increasing the number of immature gametes with abnormal shape [Fukuda et al., 1989].

Our results show a large number of Epididymitis patients with low concentration of sperm in the ejaculate. In different stages of Oligospermia (Gr. I-III), the number of spermatozoa decreased by about 3 times compared to the cases of Normospermia and about 4 times compared to the control group. It is notable the large proportion of gametes with abnormal morphology, both in Normospermia and Oligospermia. These data correlate with the results for the motility of the germ cells, demonstrating a lasting trend in the two groups (Normospermia and Oligozoospermia) to a very low number of normokinetic and hypokinetic gametes. Explanation of Oligo- and astenozoospermia could be the disadvantages that occur as a result of the presence of microorganisms (and their toxins) in the affected tissue, the entire range of elements of inflammation (high temperature etc.) and duration the disease process leading to the suppression and/ or inhibited sperm maturation [Viviani et al., 1991].

In our study on patients with Epididymitis chr. we found an increased number of morphologically damaged sperm. The most common changes in the structure of gametes concern the shape and size of the head and rarely the flagella.

Our results show a tendency to cells with small head and combination between small and round head, suggesting that the disease process affects the final stage of spermiogenesis. The presence of high percentage of small heads in inflammatory processes in the epididymis is usually the result of acrosome anomaly [Holstein et al., 2003] in the late spermatid stage. We can assume that these changes are dependent on the severity and duration of the disease and have individual character [Ilieva et al., 2009].

The cytochemical study according to the method of Zvetkova (2000) allows, on the one hand, a good differentiation of the nuclei with highly condensed chromatin from those with destructive DNA, and on the other, visualization of insufficient differentiated cells (spermatitds and prespermatids) and multicore claster similar forms with intense violet staining. Data for the seminal plasma quality are also obtained, which is strongly positive (green color by fast – green) for basic proteins with a probable origin from apoptotic or damaged spermatids or spermatogenic line cells. The results show a close correlation between the etiological factor of the infectious process and the extent of damage to the morpho-functional properties of gametes.

Moreover (even by electron microscopy), isolated large cytoplasmic residues are often seen, dispersed in semen. The reason for their appearance could be the presence of other cell types (lymphocytes, granulocytes and macrophages) and the influence of various biologically active substances, products of chronic inflammation [Giamarellon et al., 1984] and altered physico-chemical state of the ejaculate.

The injuries of the head and especially of the various segments of flagella decreased sperm motility and fertility, respectively [Weidner & Krause, 1999; Rives, 2005] and represent a common cause of male infertility.

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