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Spermatozoa Morphology Abnormalities in Men with Reproductive Problems Influenced by Various Environmental and Lifestyle Factors

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Abstract. Totally 1304 men were included in conventional analysis to determine their spermatozoa morphology. Deviations from the normal morphological status were found in 16.5% of them. The identified morphological abnormalities were analyzed by groups and their frequency of occurrence was determined, both individually and in various combinations. The established anomalies, in descending order according to their frequency, were as follows: tapered heads (95.2%); neck defects (88%); amorphous heads (87.2%); microcephalic – with small heads (79.2%); excess residual cytoplasm (48.8%); macrocephalic – with large heads (42.4%); tail defects (39.2%), double heads (25.6%), acephalic (<0.08%). In the studied individuals there were various complexes of morphological anomalies, which are discussed in detail. The information concerning environmental and lifestyle factors was collected on the base of voluntarily completed questionnaire. The results obtained showed that about 21% of the participants were undergoing occupational hazards, 22% were smokers, 42% alcohol consumers, 13% – drug users, 7% taking anabolic steroids, 31% – taking medications and 5% work or live under stress. Some of the men surveyed were influenced by multiple of the mentioned factors. Statistical analysis demonstrated differences in the clarity of the relationship between the established abnormal spermatozoa morphology and the studied environmental and lifestyle factors. The results of the study showed the presence of statistical significance in the relations "harmfulness – spermatozoa morphology" ($P<0.017$) and "occupations – spermatozoa morphology" ($P<0.003$).

Key words: spermatozoa morphology, environment and lifestyle, male reproductive health.

Introduction

Infertility in human populations is a worldwide problem affecting about 15% of couples of reproductive age. Approximately, half of these cases are related to the male factor (Yu et al., 2015). Studies show that at the root of this global puzzle is the deteriorating quality of sperm, and many researchers are turning their attention to the causes of increasingly global negative trends. There are evidences that male fertility is influenced by a variety of environmental factors, lifestyle and bad habits. Among the possible risk factors for male infertility are alcohol, cigarettes, androgenic anabolic steroids, different medications, occupational hazards, socio-psychological instability and stress (Martini et al., 2004; Brezina et al., 2012; Yu et al., 2015). Strict assessment of spermatozoa morphology correlates with fertilization capacity and has prognostic value in assisted reproduction. Spermatozoa pathology is related to their structural and functional defects. Its study helps to elucidate the mechanisms associated with the fertilization inefficiency and to identify the genetic causes for that. Abnormal morphology is associated with various changes in the organization and function of the sperm chromatin, the perinuclear cell wall, the acrosome, and the cytoskeleton.

Chemes & Alvarez Sedo (2012) pay attention to the fact that the correct identification of spermatozoa pathologies is associated with a more reliable determination of the fertility potential and with expected better results in the assisted reproduction. On this basis, it is possible to assess the genetic risk on a case-by-case basis.

The present study analyzes different abnormalities in spermatozoa morphology in men with reproductive problems who are influenced by various environmental and lifestyle factors, searching potential dependencies between spermatozoa morphology and these factors.

Material and Methods

This investigation was accepted by the Institutional Ethical Committee by a Certificate N 2/16.01.2019. Accordingly to the ethical principles, an informed consent was obtained from each patient. Men with varicocele, cryptorchidism, parotitis, azoospermia, genital trauma, infections and other genitourinary diseases were not included in this study. Totally 1304 individuals were involved in the conventional analysis to determine the spermatozoa morphology. The information concerning environmental (such as: hazardous chemicals, harmful fumes, Roentgen rays, high temperatures, radiation therapy, etc.) and lifestyle (such as: different professions - engineers, IT professionals and office workers, drivers, car mechanics and service workers, farmers, builders and carpenters, police officers, firefighters, military and sportsmen, cooks and kitchen workers; users of cigarettes, alcohol, drugs, anabolic steroids, medications; work or living under stress) factors was collected on the base of voluntarily completed questionnaire. All men, included in the study provided a standardized semen sample. The morphological abnormalities were calculated as % of all spermatozoa cells analyzed per individual (Stanislavov & Nikolova, 2013; WHO, 1999; 2010). For each individual, standard sperm smears were prepared on two slides. Totally 200 cells per individual were analyzed. Staining was performed with SpermBlue®.

The results obtained were characterized and compared by the usage of descriptive statistics. Differences between the groups compared were analyzed by χ^2 and t-test. Statistical significance was defined as $P<0.02$ and $P<0.005$.

Results

Spermatozoa morphology

The data from the conducted sperm analysis show that in 16.5% of the examined individuals there are abnormalities in the spermatozoa morphology. These are all cases in which cells with abnormal morphology are more than 14% (according to Stanislavov & Nikolova, 2013).

The identified morphological defects were analyzed in detail by groups and their frequency of occurrence was determined, both individually and in various combinations. The data on the types of morphological abnormalities in the spermatozoa and their frequency in the studied men are presented in Fig. 1 and in Table 1.

The established anomalies, in descending order according to their frequency, were as follows (Table 1): tapered heads (95.2%); neck defects (88%); amorphous heads (87.2%); microcephalic – with small heads (79.2%); excess residual cytoplasm (48.8%); macrocephalic – with large heads (42.4%); tail defects (39.2%), double heads (25.6%), acephalic (<0.08%). In the studied individuals there were various complexes of morphological anomalies, which are discussed in detail.

The analysis of the obtained data shows that in the individuals there are complexes of morphological anomalies (Table 1), as follows: in 32.8% - spermatozoa with tapered heads and macrocephalic spermatozoa in combination with neck defects; in 27.2% - sperm with amorphous and tapered heads in combination with neck defects; in 20.8% - spermatozoa with amorphous heads and macrocephalic spermatozoa in combination with neck defects and excess residual cytoplasm; in 14.4% - all reported defects except for neck defects; in 13.6% - spermatozoa with amorphous and tapered heads, microcephalic spermatozoa, tail defects and excess residual cytoplasm; in 11.2% - spermatozoa with amorphous and double heads, microcephalic spermatozoa and neck defects and in 7.2% - tapered heads, microcephalic and macrocephalic spermatozoa combined with neck defects (Table 1, Fig. 1).

Environmental and lifestyle factors

The results obtained show that about 21% of the participants in the present investigation were undergoing occupational hazards (presence of hazardous chemicals, harmful fumes, Roentgen rays, high temperatures, radiation therapy, etc.), 22% were smokers, 42% alcohol consumers, 13% – drug users, 7% using anabolic steroids, 31% – taking medications and 5% working or living under stress. Some of the men surveyed are influenced by multiple of the mentioned factors.

Table 1. Frequencies of different types of abnormal spermatozoa morphology and combinations of them in men with teratozoospermia: % – percentage of manifestation; (+) – presence. The percentage of men with morphological abnormalities in spermatozoa exceeds 100 due to the manifestation of different defects in the same individual

Types of abnormal spermatozoa morphology								**Frequency**
Amorphous heads	Tapered heads	Microcephalic	Macrocephalic	Double heads	Tail defects	Neck defects	Excess residual cytoplasm	**Total %**
+	+	+			+		+	13.6
	+	+				+		32.8
+		+		+	+			11.2
+			+			+	+	20.8
	+	+	+			+		7.2
+	+					+		27.2
+	+	+	+	+	+		+	14.4
87.2	**95.2**	**79.2**	**42.4**	**25.6**	**39.2**	**88**	**48.8**	**Total %**

Established statistical relationships

Based on the data from the investigation done, the effect of various environmental and lifestyle factors on the spermatozoa morphology was analyzed, and the found relationships between these factors and the spermatozoa morphologic features were characterized.

The results of the study show the presence of statistical significance in the dependences "harmfulness – spermatozoa morphology" ($P<0.017$) and "occupations – spermatozoa morphology" ($P<0.003$). Regarding the relationship between the other studied environmental and lifestyle factors and the available defects in spermatozoa morphology, the statistical data were not sufficiently definite ($P\geq0.1$).

Discussion

The results of the present study demonstrate that teratozoospermia is a heterogeneous condition involving a variety of changes in the morphology of different spermatozoa components.

Chemes & Alvarez Sedo (2012) emphasize the fact that the morphological abnormalities are combined with various functional disorders and genetic abnormalities in spermatozoa, which in a complex worsens male reproductive health. A number of researchers draw attention to the fact that the spermatozoa morphology features are related to the fertility capacity of the individual, and that their assessment has prognostic value in assisted reproduction (Chemes & Rawe, 2003, 2010; Chemes & Alvarez Sedo, 2012).

The results obtained in the present study show an alarming trend among the analyzed men - 16.5% of them have defects in sperm morphology. In parallel, among the analyzed sample of men with reproductive problems a significant part are exposed to various negative environmental and lifestyle influences.

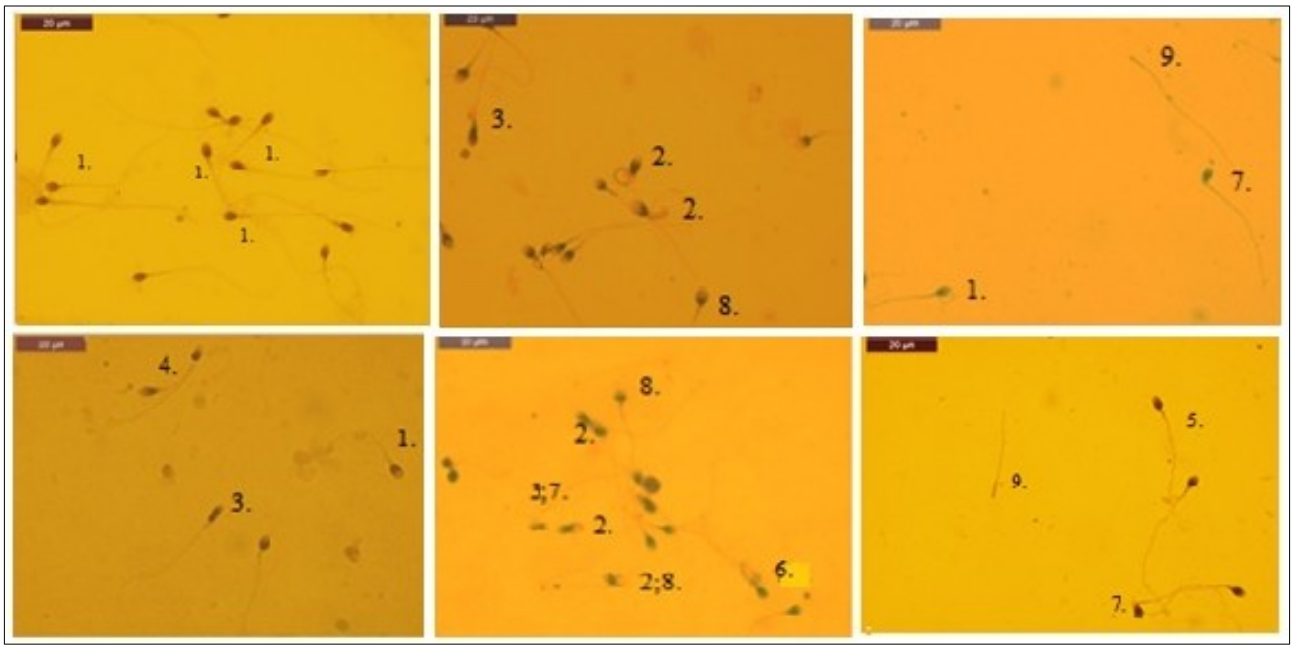

Fig. 1. Types of spermatozoa morphology anomalies: 1. normal morphology; 2. tail defects; 3. tapered heads; 4. amorphous heads; 5. microcephalic spermatozoa; 6. cytoplasmic residue; 7. defects in the neck and middle part; 8. macrocephalic spermatozoa; 9. acephalic sperm.

Statistical analysis on the collected data demonstrates differences in the clarity of the relationship between the established abnormalities of the spermatozoa morphology and the studied environmental and lifestyle factors.

The obtained results and their analysis lead to the conclusion that the harmful environment (presence of hazardous chemicals, harmful fumes, Roentgen rays, high temperatures, radiation therapy, etc.) and various professions (engineers, IT professionals and office workers, drivers, car mechanics and service workers, farmers, builders and carpenters, police officers, firefighters, military and sportsmen, cooks and kitchen workers), including unemployment (probably due to the stress impact), have high risk potential for the quality of sperm morphology.

The analyzes and conclusions of our study are similar to the findings of some other researchers (Hauser et al., 2007; 2015; de Souza et al., 2010; de Freitas et al., 2012), who note that toxic substances, affecting the reproductive processes include heavy metals, agricultural and industrial chemicals, petroleum products, different materials, separated from the light and the heavy

industry, such as dyes, varnishes, acetones, acids, alcohols, sulfur and nitrogen compounds, pesticides and others.

Janevic et al. (2014) and Nordkap et al. (2016) report the negative impact of stress from the work environment and lifestyle on spermatozoa morphology. This suggests that occupations with different harmful effects on sperm parameters are potentially risky for male reproductive health.

Although no statistically significant relationships were observed between spermatozoa morphology and alcohol, cigarette, androgenic anabolic steroid, drug, and medicamentation use in the present study, such dependences with high statistical significance were found in our earlier studies concerning other sperm parameters – ejaculate volume, spermatozoa concentrating and motility (Dzhoglov et al. 2021).

In addition, studies by a number of authors (Wong et al., 2000; Muthusami & Chinnaswamy, 2005; Hamad et al., 2014; Gundersen et al., 2015; Samplaski et al., 2015; Wdowiak et al., 2016; Christou et al., 2017; Jungwirth et al., 2018), prove the negative impact of these factors, both individually and in complex, on the male reproductive potential (including on sperm morphology). An exhaustive overview of the possible relationships between cigarette, alcohol and drug use and male reproductive health is also presented in the work of Sansone et al. (2018).

Knowledge about the spermatozoa morphology is essential in the detection (*in vitro* or *in vivo*) of abnormalities caused by various genotoxic factors. At the same time, the assessment of abnormalities in the spermatozoa head morphology is recommended by regulatory agencies as the main monitoring tool. In this aspect, the present study is pertinent and the described results and analysis could be useful when discussing measures for prevention and improvement of reproductive health in Bulgaria.

Conclusions

Different types of abnormalities in the spermatozoa morphology was found in 16.5% of the examined individuals. Dependencies in pairs "harmfulness – spermatozoa morphology" and "occupations – spermatozoa morphology" were found to be statistical significant which demonstrate that harmful environment and various professions have high risk potential for the quality of sperm morphology. The results obtained and conclusions done could be taken into consideration when discussing activities for prevention and improvement of male reproductive health in Bulgaria.

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