RESEARCH ON CERTAIN AGE MODIFICATIONS IN MORPHOLOGY AND PHYSIOLOGICAL INDICATORS OF SPERM IN PATIENTS WITH REPRODUCTIVE PROBLEMS

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Abstract:

In this study, data about of the main indicators in spermograms - volume, concentration, motility, and deviations in sperm morphology of patients with reproductive problems were observed and analyzed. The correlation of these indicators with the age of the patients was followed. For this purpose, 99 patients observed for one year are divided into three age groups: 20 to 29 years; from 30 to 39 years; 40 years and over. Semen analysis according to the WHO and assessment of sperm morphology according to Kruger criteria is applied. As a result, a statistically significant negative correlation between the age of the patients and the volume of the ejaculate was established. There is no age-related change in sperm concentration and motility. There are no significant differences in sperm morphology in individual age groups.

Key words: *assisted reproduction, spermatozoa, sperm morphology, semen analysis*

Introduction

Statistical analyzes indicate that 15% of couples of reproductive age are not successful in attempting to have a child in the first year. In about 35-50% of cases this is due to male infertility or sterility.

Explaining the relationship between fertility and age is of increasing importance as the number of men who decide to become adult parents is increasing. Spermatogenesis, in contrast to oogenesis, continues in older age, and allows this trend to grow. From 1980 until today in the US there are 24% more fathers between 35 and 54 years old. In Germany, the percentage of men who first became parents over 35 years has risen from 23.1% in 1990 to 38.5% in 2001.

A new study in Human Reproduction journal conducted by the Stanford University School of Medicine looked specifically at how first-time dads' ages have changed from 1972 to 2015 in the U.S., and to no one's surprise, they have risen a fair bit. According to the research, which analyzed more than 168,000,000 births, the mean paternal age has increased from 27.4 to 30.9 since four decades ago. The percentage of first-time fathers who are older than 40 years is now 9%, while almost 1% are older than 50 [1].

Functional and organic disorders of male sexual function can be a cause of reduced or lack of fertility. The most common cause of infertility in men is pathological spermatogenesis, for which the various processes in the development of the male sexual apparatus play a major role - hypospadias, epispadias, cryptorchism (unilateral or bilateral), testicular twisting, invasive hernia, varicocele. Common causes have been infections of the testis, prostate gland, bladder and kidneys. Immediately after a high fever inflammatory process, disturbances in spermatogenesis - oligozoospermia, asthenozoospermia and teratospermia have been reported [2].

Disturbed spermatogenesis may be a consequence of endocrine disorders - diabetes, adiposogenital dystrophy, obesity, Klinefelter syndrome. Also severe common diseases, acute and chronic infections - epidemic mumps with orchitis, tuberculosis, traumas, abdominal surgery and surgical interventions of sexual organs can affect the normal way of the spermatogenesis [3].

Exogenic factors affecting spermatogenesis (e.g. nicotine) play a role in the etiopathogenesis of male infertility, too. The germinal epithelium of the testis is very easily damaged by various physical and chemical compounds (pesticides, heat, medications, and ionizing radiation). Causes of disturbed spermatogenesis can also be traumas of the sexual system.

In the process of aging at all levels of organization of the organism, including the genital system, physiological changes occur. As age increases, there was a decrease in the number of Leydig cells, Sertoli cell, serum testosterone levels [4], [5]. Aging is associated with disturbances in both testicular function and hypothalamic regulation of gonadotropic secretion.

Oxidative stress also reduces sperm fertilization potential. There are changes in seminal vesicles. With age, in some men the prostate enlarges its size (hypertrophy), presses the urinary tract and causes benign prostatic hyperplasia. The duct system may lose its elasticity (a process called sclerosis). The epididymis, seminal vesicles and prostate gland lose some of the cells on their surface but continue to perform their functions. Infestations and inflammation of the prostate gland (prostatitis) occur. With age, there are more cases of prostate cancer. In the normal aging process, age-related accompanying diseases also increase - chronic kidney, liver, lung diseases, etc. [6]

Objectives:

The purpose of this study is to track age-related changes in four of the main indicators (volume, concentration, mobility and morphology) in spermograms of patients with reproductive problems.

In pursuance of the stated objective, studies were carried out on the following tasks:

1. Determine the volume of samples tested.
2. Count sperm counts according to the relevant requirements to determine their concentration in millions / ml.
3. Trace the sperm motility in a number of fields and calculate the overall mobility rate.
4. Evaluation of sperm morphology in samples according to strict Kruger criteria and determination of percentage of spermatozoa with deviation in morphology.

The study covered 101 unselected patients who had been consulted at the Vita University Hospital for infertility in 2012-2013. Each of them gave one sample.

Materials and Methods:

Semen analysis is performed according to the stages stipulated in the fifth edition of the WHO. Seed samples were collected in sterile containers by masturbation after sexual abstinence within 3-5 days. Samples are stored at room temperature (but not lower than 20 ° C and not higher than 37 ° C). The analysis is performed after liquefaction of the semen within 30 minutes to 1 hour. A light microscope (NUND) is used to investigate the indicators described below. To determine the concentration, a Makler count camera is used. The morphology of the sperm is evaluated according to Kruger's strict criteria by placing 5 μl of the sample on pre-stained cell morphology glass "Testsimplets". The study included 101 patients whose sperm counts the values of four indicators - volume (ml), concentration (million/ml), motility (%) and morphology abnormalities (%).The age of the youngest patient is 20 years and the oldest is 55 years. Patients are conventionally divided into three age groups as follows: 1) from 20 to 29 years; 2) 30 to 39 years; 3) 40 years and over.

Data analysis was done with a Student t-test using the capabilities of MS Excel to determine whether two samples could be considered to be derived from identical generic sets with the same averages. The analysis is carried out consecutively for each pair of age groups at a chosen level of significance for a two-tailed test of 0.05 p-value (Tables 1 ÷ 3).

Results and Discussion

For all observed parameters, the calculated t-value is less than values given in the table, which does not give reason to reject the null hypothesis, ie. with 90% certainty, it can be assumed that the samples are derived from the same generic sets with the same averages.

Regarding statistically significant differences in sample volume, it was found that when comparing the results of the first and second age group there was no difference in the mean volume (fig.1). A statistically significant difference in average volume exists between the first and third age groups at a level of significance of 0.159. There is also a statistically significant difference between the second and third age groups at a significance level of 0.033.

Data analysis revealed that the mean sperm concentration in semen decreased with age (Fig.2). Although the mean concentration in the age groups decreases the level of significance, it shows that there is no reason to reject the null hypothesis (i.e. to equalize the mean concentration). The result is mainly due to the sensitive variation in concentration within the appropriate age groups (visible from the minimum and maximum values). This gives reason to believe that there are many other factors that predict the concentration of sperm in semen, which are generally not age-dependent, but rather environmental, disease, etc.

The research on the correlation of sperm motility with age of the patients shows a tendency of decrease with age. Here again, the level of significance shows that there is no reason to reject the null hypothesis (i.e. for the equality of the average motility by age groups). The result is dictated by the sensitive variation in motility within the respective age groups (Fig. 3). This gives reason to believe that there are many other factors that predestine the mobility of sperm, such as environmental effects, diseases, etc.

Data on variance in sperm morphology by age showed that variance is the highest in the second age group (Fig. 4). The statistical analysis shows that there is no reason to reject the null hypothesis for equality of the mean deviations in morphology by age groups. The larger deviations in the second age group are due to the larger sample in this case. Data from other studies show that sperm morphology is strongly influenced by various environmental factors, as well as current or past illnesses.

As a result of the studies, we found a statistically significant negative correlation between the age of the patients and the volume of the ejaculate. This result is confirmed by a number of experiments and by other authors. Validation of our results is confirmed by Levitas et al. [7], where a decrease in volume is reported on average from 3.51 to 2.21 (respectively for the age group 30 to 34 years and over 55 years). According to various sources, the sperm volume decreases in the scope from 3% to 22% [8].

This result can be explained by age changes in the body of the man. The decrease in volume may be associated with changes in seminal vesicles that produce most of the semen. Prostate-related problems (e.g. smooth muscle atrophy) may also affect the volume of sperm. According to some studies, smoking has an impact on this indicator, with dependence being inversely proportional to the number of smoked cigarettes [7], [9], [10]. There are also opinions that the volume of seminal fluid is not influenced by the age of the male [11].

After the investigations we made, we found that the volume of the semen sample was the only indicator in which statistically significant differences with age increases were observed. The results of the analysis give reason to assert that the volume of the ejaculate decreases as patient age increases.

For the other three indicators (concentration, mobility and deviations in morphology), the statistical significance level of the result rejects their age-dependency. Our results are confirmed by studies of other authors, and in most cases their studies have different degrees of deviations from sperm morphology with increasing age of patients. [8], [12]. The lack of correlation between sperm morphology and the age of patients in our case can be explained by the relatively small sample we have studied. Different methods used could also influence the obtained results in comparison with results obtained by other authors.

In the course of the work and on the basis of the data obtained, we have raised the question of collecting additional information about the lifestyle of the persons surveyed, particularly those that have a critical attitude to the fertility qualities of the semen - eg smoking, regular alcohol use, sports and healthy diet etc. However, the difficult collection of such data is understandable, as some are of a personal nature and patients may refuse to share them. However, the collection and analysis of data related to the reproductive health of the population as well as their publication should actively continue not only because of the purely scientific necessity of such information but also in view of the complicated demographic situation in our country.

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Acknowledgements: We thank to Hospital "Vita" for the ability to use their equipment and facilities for carrying out the recent study. We express our gratitude to Ms Iliana Grigorova for her active participation in the process of working out and analyzing some of the results in this investigation.

Tables

Table 1. Observed t-value for 1st and 2nd group with bilateral distribution and two samples with different dispersions on the evaluation parameters.

	Volume [ml]	Concentration [million/ml]	Motility [%]	Deviation in morphology [%]
t-statistical	0,849092	0,679743	0,965959	0,598763
Number of observations in the 1st age group	30	30	30	23
Number of observations in the 2nd age group	48	47	47	38
Standard deviation of the 1st age group	2,008	28,837	0,093	0,046
Standard deviation of the 2nd age group	1,423	32,688	0,115	0,049
Leveling the degrees of freedom	48,38	69,60	73,08	50,74
t-table value	2,0106	1,9944	1,9930	2,0076

Table 2. Observed t-value in 1st and 3rd group in bilateral distribution and two samples with different dispersions according to the evaluation parameters.

	Volume [ml]	Concentration [million/ml]	Motility [%]	Deviation in morphology [%]
t-statistical	0,13226	0,59237	0,44717	0,70414
Number of observations in the 1st age group	30	30	30	23
Number of observations in the 3rd age	21	20	20	19
Standard deviation of the 1st age group	2,008	28,837	0,093	0,046
Standard deviation of the 3rd age group	1,341	34,903	0,141	0,044
Leveling the degrees of freedom	50,85	36,99	31,06	41,32
t-table value	2,0076	2,0262	2,0395	2,0195

Table 3. Observed t-value in 2nd and 3rd group with bilateral distribution and two samples with different dispersions according to the evaluation parameters.

	Volume [ml]	Concentration [million/ml]	Motility [%]	Deviation in morphology [%]
t statistical	0,03111	0,81626	0,42975	0,91857
Number of observations in the 2nd age group	48	47	47	38
Number of observations in the 3rd age group	21	20	20	19
Standard deviation of the 2nd age group	1,423	32,688	0,115	0,049
Standard deviation of the 3rd age group	1,341	34,903	0,141	0,044
Leveling the degrees of freedom	42,22	35,33	31,51	42,32
t-table value	2,0181	2,0301	2,0369	2,0181

Figures

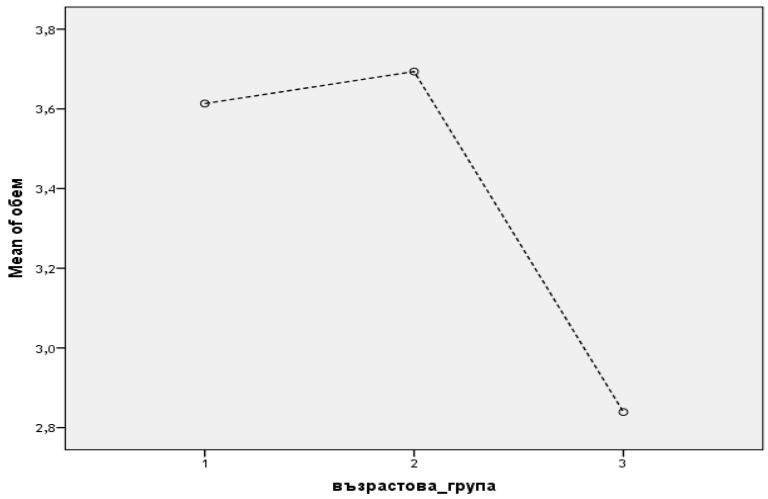

Fig. 1. Correlations between the mean values of semen volume and the age of patients.

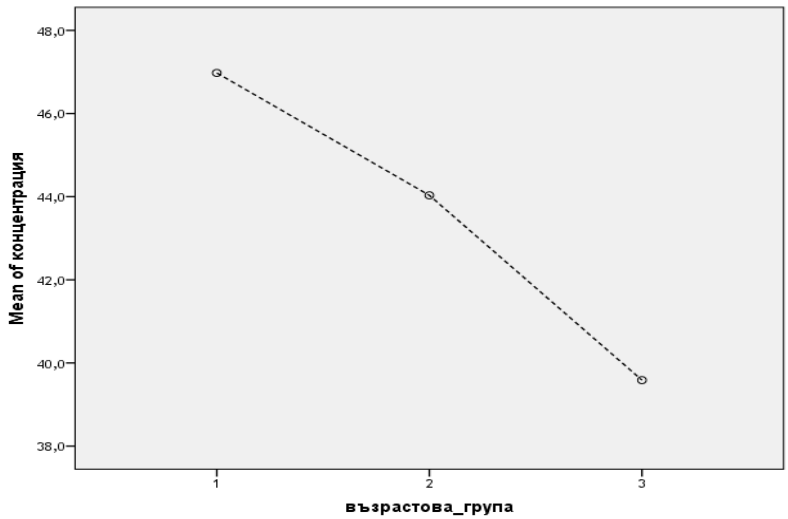

Fig. 2. Correlations between the mean values of semen concentration with the age of patients.

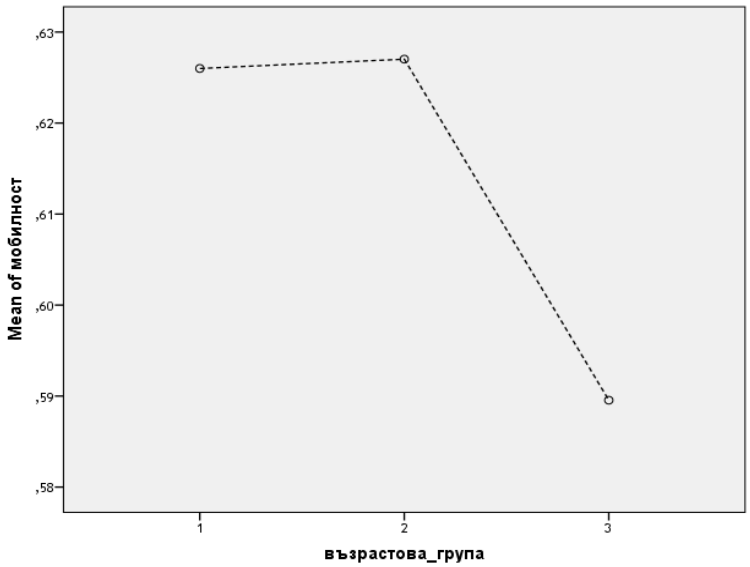

Fig. 3. Correlations between the mean values of sperm motility with the age of patients.

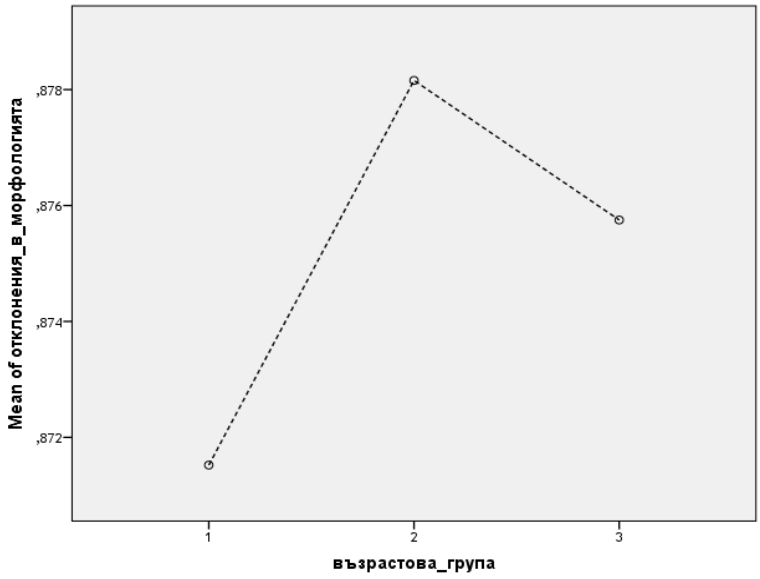

Fig. 4. Correlations between the mean deviations of sperm morphology with the age of patients.

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