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# LABORATORY STUDY OF CHANGES IN SPERMOGRAM INDICATORS CONSIDERING THE LEVEL OF SPERM DNA FRAGMENTATION

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Abstract. The relationship between changes in semen parameters in the presence of sperm DNA fragmentation in men of reproductive age with impaired reproductive function was investigated. It has been established that changes in semen analysis indicate the presence of astheno-, oligo- and teratozoospermia, dyskinesis, and are dependent on the level of sperm DNA fragmentation. Thus, the more severe the changes in the key parameters of the spermogram, the greater the probability that the frequency of sperm DNA fragmentation will be higher than normal.

Keywords: male infertility, sperm DNA fragmentation, semen analysis.

The increase in the share of the male factor in the structure of childless marriage necessitates a deeper and more detailed study of the existing problem. Today it is known that at least 30-50 % of all cases of severe male infertility are caused by genetic factors. [1, p. 2444-2448; 2, p. 163-167]. Sperm DNA fragmentation is a relatively recently discovered cause of male infertility, and as the name implies, that is breaks or damages sperm DNA strands, and the more such damage, the lower the degree of integrity of the genetic material and the less chance of pregnancy. [3, p. 24-27; 4, p. 83-91].

Given the numerous data that anomalies of chromatin in sperms are often

associated with reduced sperm indicators, and sperms evaluated as "morphologically normal" (by light microscopy) may have a damaged DNA, and vice versa [4, p. 83-91; 5, p. 2-10], it was of interest to determine the relationship between sperm DNA fragmentation level and the degree of impaired fertility of the ejaculate.

Aim: to assess changes in the main sperm indexes under the influence of sperm DNA fragmentation in men of reproductive age

#### Material and methods

Totally 70 men aged from 22 to 43 years have been included in investigation who underwent examination on planning childbirth. All men have presented an agreement in written form for participation in the studies. The agreement has been approved by Institutional Committee on Bioethics and corresponded to the bases of Helsinki Declaration and corresponded to ethic, moral and legal requirements of the Order No 281 of Ministry of Health of Ukraine from 01.11.2000.

Examined men have been divided into three groups. The first group (control) included 20 men with a normal level of sperm DNA fragmentation, who had 1-2 children. The second group (comparison) consisted of 27 infertile patients with a normal level of sperm DNA fragmentation. The third group was composed of 23 infertile men with a high level of sperm DNA fragmentation.

All men underwent a comprehensive study that included the semen analysis according to WHO recommendations [6] and determination of the level of sperm DNA fragmentation.

Semen analysis was performed over standard technique recommended by WHO. During ejaculate analysis volume, color, consistency, pH, concentration of spermatozoa in 1 ml ejaculate and their total amount, degree of motility and microscopic investigation of stained specimen have been assessed.

To assess sperm DNA fragmentation level technique Sperm Chromatin Dispertion test has been used. Sperm DNA fragmentation level up to 30 % corresponded to standard values, higher values – than 30 % of calculated 500 spermatozoa.

Statistical analysis of obtained data was performed using computer programs

set STATISTICA (StatSoft Statistic v.7.0.). Statistical significance of compared indices with distribution different from standard, assessed by Kolmogorov-Smirnov test, has been established using Wald-Wolfowitz runs test at the significance level of 0.05.

#### Results

The first (control) group was characterized by ejaculate fertility preserved accordingly to the standard values, recommended by WHO.

While examining native and stained specimen under microscope in II group of patients active spermatozoa decreased by 52 %, fixed forms and immotile sperm increased by 130 % and 21 %, respectively, compared to control group values. Concentration of spermatozoa in 1 ml and their total amount in ejaculate of II group patients has been decreased by 37 % on average and 24 % compared to the values of control group. Pathologic forms increased by 60 % secondary to decreasing standard forms of spermatozoa by 26 % concerning values of control group; teratozoospermia index was in conformity with admissible values.

Thus, the detected changes in spermograms in men of II group indicate the presence of a small astheno- and teratozoospermia on the background of moderate oligozoospermia, which, in turn, indicates a slight decrease in ejaculate fertility.

In the study of ejaculate indices in men of III group there was a decrease in the number of active spermatozoa by 65 % and 48 % relative to the values of the control and II groups and an increase in the not motile spermatozoa by 26% relative to the control indicators.

Significantly increased the number of fixed sperm forms by 188 % and 25 % relative to control and II groups, respectively, the appearance of dyskinetic forms. The concentration of sperm in 1 ml decreased by 58 % and 33 %, and the total number of sperm in the ejaculate - by 55 % and 40 % relative to the values of the control and II groups, respectively. There was also a decrease in the number of normal sperm forms by 41 % and 21 % and an increase in pathological forms by 95% and 22 % relative to the values of control and II groups, respectively.

In the analysis of spermograms of men in III group, significant astheno- and

oligozoospermia, slight dyskinesis, marked teratozoospermia were observed, which obviously contributes to a significant decrease in ejaculate fertility in men of this group.

As a result of the study of the level of sperm DNA fragmentation, it was found that in men II groups, although changes in semen analysis were observed, but the number of fragmented sperms was on average 16%. In men of III group, the level of sperm DNA fragmentation exceeded the norm and averaged 38%.

Thus, the data obtained by us confirm the assumption of a certain relationship between the basic parameters of sperm (their concentration, motility and morphology) and the frequency of their nuclear DNA fragmentation. The less motile and more morphologically anomalous sperms in the ejaculate were found, the greater the likelihood of increased frequency of DNA fragmentation in them.

#### Conclusions

1. The revealed changes in the semen analysis in men with reproductive function disorders indicate the presence of astheno-, oligo- and teratozoospermia, dyskinesis depending on the level of sperm DNA fragmentation.

2. The degree of impaired spermatogenesis directly correlates with the level of DNA fragmentation in male gametes. The heavier the pathoospermia, the greater the probability that the frequency of DNA fragmentation in sperm will be above normal.

3. The index of sperm DNA fragmentation has significant diagnostic and prognostic value in married couples with impaired reproduction, especially for men with sperm counts that are close to normal, in who no obvious causes of infertility are detected and in cases of failed attempts of IVF, ICSI or habitual miscarriages.

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