ANALYSIS OF VARIANCE SPERMOGRAM INDICATORS OF MEN DIFFERENT AGE GROUPS WITH ASTENOZOOSPERMIA, TERATOZOOSPERMIA AND AZOOSPERMIA

Antonina Tarnovska
Ph.D., Assistant Professor, Ivan Franko National University of Lviv, Ukraine
e-mail: antonina.tarnovska@lnu.edu.ua, orcid.org/0000-0002-1626-3919

Anastasiia Heneha
Ph.D., Assistant Professor, Ivan Franko National University of Lviv, Ukraine
e-mail: anastasiya.heneha@lnu.edu.ua, orcid.org/0000-0003-3516-426X

Summary

In recent decades, a decline in male fertility due to deterioration in sperm quality has been noted around the world. This is probably explained by the tendency to increase the diseases of the male genital organs and, as a result, the increase in the percentage of male infertility. In addition, there is a deterioration of the quantitative and qualitative indicators of spermograms in practically healthy men. The average number of sperm in the ejaculate of a healthy man has halved over the past 50 years, and the average volume of ejaculate has decreased by one third. It is known that the multicomponent composition of the internal male genital organs is in constant restructuring due to age changes, functional activity and the influence of various factors. That is why it is important to take into account both physiological and age-related changes in a man's ability to conceive.

Diagnosis of male infertility includes clinical research methods and methods of laboratory-instrumental examination. Among the latter, the most important for finding out the functional state of the gonads and the fertilizing ability of sperm is the study of ejaculate. The object of our research was spermograms obtained during the examination of patients at the "Alternative Clinic" reproductive medicine clinic.

The purpose of the work was using biometric analysis to the different indicators of men spermograms of younger, middle and older age groups with normozoospermia (N), asthenozoospermia (AZS), teratozoospermia (T) and azoospermia (A). The task of the research was to analyze the main indicators of spermograms of men of different age groups in normal and pathological conditions, to conduct a one-factor and two-factor variance analysis of the influence of the studied diseases and age factor.

After analyzing the main indicators of spermograms of men of younger, middle and older age groups with asthenozoospermia, azoospermia, and teratozoospermia, it was established that the indicators that undergo the greatest deviations in the studied diseases are the mobility of spermatozoa according to categories A and B, the morphology of spermatozoa, the Farris fertility index, and the activity of spermatozoa.

After conducting a one-factor variance analysis, we established that the share of the influence of the studied diseases on the overall variability of such indicators of spermograms as the mobility of spermatozoa according to category A and B and the morphology of spermatozoa in the spermograms of men of the studied age groups is within 63–98% of the total contribution (younger age group), 60–96% (middle age group) and 75–96% (older age group). The share of influence of unaccounted factors is within 2–40% of the total contribution. The share of the influence of the studied diseases in the overall variability of such indicators as the Farris index

and sperm activity in the spermograms of men of all studied groups is significantly reduced and is within 22–44% of the total contribution. Instead, the influence of unaccounted factors (56–78% of the total contribution) is growing significantly. This may indicate the presence of concomitant diseases and other pathologies in the men who took part in the research.

After conducting a two-factor variance analysis, we established that the shares of the influence of the studied diseases on the overall variability of such indicators of spermograms as motility of spermatozoa according to category A and B, morphology of spermatozoa, Farris fertility index and activity of spermatozoa are decisive and are within the range of 90.7–99.9% of the total contribution, the share of the influence of the age factor on the variability of spermogram indicators of men of different age groups is insignificant.

Key words: spermogram, normozoospermia, asthenozoospermia, teratozoospermia, azoospermia, infertility, age factor.

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1. Introduction

In recent decades, a decline in male fertility due to deterioration in sperm quality has been noted throughout the world. This is probably explained by the tendency to increase diseases of the male genital organs. In addition, there is a deterioration of the quantitative and qualitative indicators of the spermogram in practically healthy men *(Gorpynchenko, Gurzhenko, et all, 2019; Amini, Kahrobaie, et all, 2020).* The average number of sperm in the ejaculate of a healthy man has halved over the past 50 years, and the average volume of ejaculate has decreased by one third *(Tarnovska, Henega, et all, 2022)*.

The reasons that lead to a decrease in the quantitative and qualitative parameters of sperm remain unknown. There are studies that give reason to believe that lifestyle factors (stress, smoking, alcohol, exposure to environmental chemical factors that have estrogenic activity, urbanization, etc.) negatively affect the male reproductive system, which is the most vulnerable and least protected *(Yatskiv, Tarnovska, 2012; Tarnovska, Henega, et all, 2018).*

It is known that a decrease in the fertilizing capacity of the ejaculate can be observed without any deviations from the normal parameters of a routine spermatological examination. In about 30% of cases, the study of the spermogram does not give an unequivocal answer about the root cause of reduced fertility, because changes in this function occur at the functional molecular-biological or biological level *(World Health Organization, 2010)*. Thus, in order to establish the fact of male infertility and its probable cause, along with objective and other types of examination of the patient, a complex laboratory study of ejaculate is first of all necessary *(Arya, Dibb, 2016; Gorpinchenko, Romanyuk, 2016).*

Despite the large base of research, the problem of male fertility decline has not yet been resolved, the cause and relationship of the decrease in quantitative and qualitative ejaculate parameters, the decrease in the fertile capacity of the ejaculate in the absence of deviations from the normal parameters of the ejaculate have not been definitively established, the relationship and mutual influence have not been investigated these parameters on each other *(Danis, Samplaski, 2019)*. The problems of protecting the reproductive systems of the male body from the influence of negative factors that lead to a decrease in male fertility, building models of the probable causes of the decrease in male reproductive capacity remain relevant. It is also important to study the age-related factor of male fertility decline in relation to other factors *(Tarnovska, Heneha, 2022)*.

The aim of the work was the biometric analysis of spermograms of men of different age groups with normozoospermia, asthenozoospermia, teratozoospermia and azoospermia. The task of the study: to analyze the main indicators of spermograms of men of the younger (20–29 years), middle (30–39 years) and older (40–50 years) age groups with normozoospermia, teratozoospermia, azoospermia and asthenozoospermia, to conduct one-factor and two-factor variance analysis of the influence of the studied diseases and the age factor on spermogram indicators of men of younger, middle and older age groups.

2. Research materials and methods

Spermograms were obtained during examination of patients at the "Alternative Clinic" reproductive medicine clinic.

Spermograms were evaluated according to indicators: volume of ejaculate, viscosity, number of spermatozoa in 1 ml of ejaculate; the total number of sperm in the ejaculate; motility of spermatozoa according to movement categories A and B; morphology of spermatozoa (percentage of morphologically normal and morphologically altered spermatozoa), Farris fertility index and active sperm count.

Statistical data processing was carried out using the Excel program (in particular, the "Data Analysis" package, calculating the main statistical indicators from direct quantitative data obtained as a result of research (arithmetic mean value – M; standard error of the arithmetic mean m). To assess the reliability of the difference between statistical the characteristics of two alternative sets of data were calculated by the Student's coefficient. The difference is considered reliable at a reliability index of $p \geq 0.95$ (or a significance level of $P < 0.05$). The processing results were displayed in the form of diagrams.

3. Results and discussion

Spermograms were obtained during examination of patients at the "Alternative Clinic" reproductive medicine clinic. In total, we examined 132 men: 56 men aged 20 to 29 years (younger age group), 51 men aged 30 to 39 years (middle age group) and 25 men aged 40 to 50 years (older age group).

We established that in the younger age group (56 patients), 15 men had spermograms consistent with asthenozoospermia, 11 with azoospermia, 15 with teratozoospermia, and 15 with normozoospermia. In the middle age group (51 patients), 13 men had spermograms consistent with asthenozoospermia, 7 with azoospermia, 15 with teratozoospermia, and 16 with normozoospermia. In the older age group (28 patients), 6 men had spermograms consistent with asthenozoospermia, 8 with azoospermia, 5 with teratozoospermia, and 10 with normospermia. As a control, we took the spermograms of patients with normozoospermia.

Having analyzed the results of spermograms of men of different age groups, we found out that the main indicators of deviations in these spermograms are the mobility of spermatozoa according to movement categories A and B; sperm morphology (percentage of morphologically normal and morphologically altered spermatozoa), Farris fertility index and number of active spermatozoa.

The first criterion is sperm motility, which we evaluated according to indicators: A – fast translational movements and B – slow, sluggish translational movements.

In men of the younger age group, patients with teratozoospermia and asthenozoospermia, the mobility of spermatozoa according to categories A and B is 33% and 22%, 18% and 19%, respectively, at the norm of 33% and 18%. When suffering from azoospermia, the mobility of spermatozoa according to categories A and B in men of this age group is 0%.

In middle-aged men with teratozoospermia, sperm mobility in categories A and B is 31% and 22%, respectively, at the norm of 36% and 18%, and in asthenozoospermia, this criterion is 16% and 22%, respectively, at the norm of 36 % and 18%. With azoospermia, these indicators are close to zero.

In men of the older age group, patients with teratozoospermia, the mobility of spermatozoa according to category A and B is 32% and 18%, respectively, with a norm of 29% and 24%, in case of asthenozoospermia, this criterion is 18% and 17%, respectively, with a norm of 29% and 24%, and with azoospermia, these indicators are close to zero (Fig. 1).

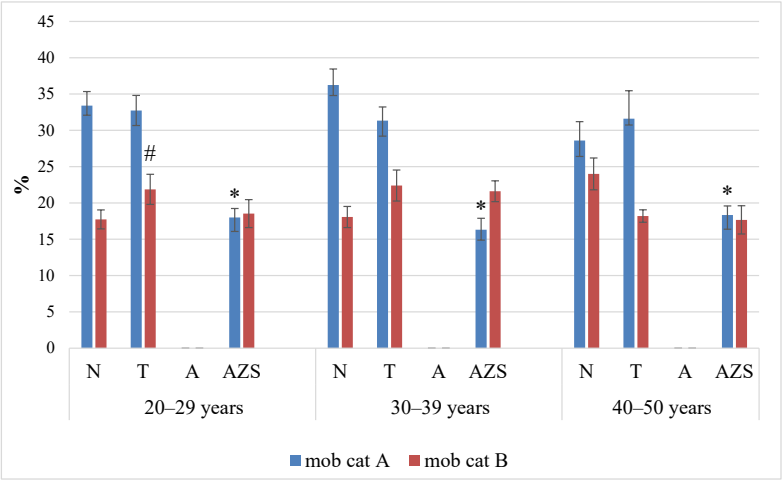


Fig. 1. Comparison of the mobility of spermatozoa by category A and B in the spermograms of men of the younger age group (20–29 years), the middle age group (30–39 years), and the older age group (40–49 years), (N – normozoospermia, T – teratozoospermia, A – azoospermia, AZS – asthenozoospermia). Spermograms of men with normozoospermia were taken as controls
*** Significantly compared to the control (mobility according to category A), p≥0.95**
 # Significantly compared to the control (mobility according to category B), p≥0.95

The next stage of the study of spermograms is the morphology of spermatozoa, which characterizes the reproductive capacity of sperm. We evaluated the morphology of spermatozoa by the number of normal and degenerate spermatozoa.

We found that in the spermograms of men of the younger age group, patients with teratozoospermia, the number of morphologically normal spermatozoa is less compared to controls and is 20% morphologically normal spermatozoa and 80% morphologically degenerate spermatozoa (control: 37% morphologically normal spermatozoa and 63% morphologically degenerate spermatozoa). In the spermograms of men with asthenozoospermia of the same age group, 35% of morphologically normal spermatozoa and 65% of morphologically degenerate spermatozoa were found in 20–29-year-olds, which is also lower than the control. With azoospermia in the spermograms of men of this age group, the studied indicators are 0%.

In spermograms of middle-aged men with teratozoospermia and asthenozoospermia, the percentage of morphologically normal spermatozoa is 19% and 35%, respectively (control 36%), and the percentage of morphologically degenerate spermatozoa is 81% and 65%, respectively (control 64%). With azoospermia in the spermograms of men of this age group, the studied indicators are 0%.

In spermograms of men of the older age group, patients with teratozoospermia and asthenozoospermia, the percentage of morphologically normal spermatozoa is lower compared to controls and is 24% and 36%, respectively (control 36%), and the percentage of morphologically degenerate spermatozoa in these diseases is 76% and 64%, respectively (control 64%). With azoospermia in the spermograms of men of this age group, the investigated indicators are 0% (Fig. 2).

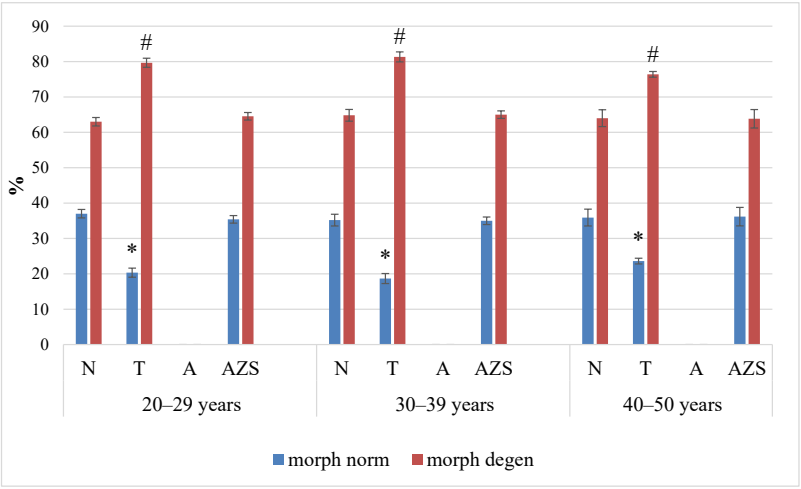


Fig. 2. Comparison of morphologically normal and morphologically degenerate spermatozoa in the spermograms of men of the younger age group (20–29 years), the middle age group (30–39 years), and the older age group (40–49 years), (N – normozoospermia, T – teratozoospermia, A – azoospermia, AZS – asthenozoospermia).
Spermograms of men with normozoospermia were taken as controls
*** Significantly compared to the control (percentage of morphologically normal sperm), p≥0.95**
 # Significantly compared to the control (percentage of morphologically degenerate spermatozoa), p≥0.95

The following criteria are the Farris fertility index (Farris index), which allows you to assess the chances of natural fertilization (determines the number of fast and mobile, slow and immobile spermatozoa) and the activity and viability of spermatozoa.

In the spermograms of men of the younger age group with teratozoospermia, the Farris index is 165%, the activity and viability of spermatozoa is 33%, in the case of asthenozoospermia, these indicators are 76% and 11%, respectively. Normally, the Farris index is 154%, the activity and viability of spermatozoa is 33%. A decrease in the studied parameters in the spermograms of men of the younger age group with these diseases indicates a low probability

of fertilization. With azoospermia in the spermograms of men of this age group, the studied indicators are 0%.

In spermograms of middle-aged men with teratozoospermia, the Farris index is 114% (normal 160%), sperm activity and viability 21% (normal 27%). This also indicates a low probability of fertilization. In spermograms of men with asthenozoospermia, the Faris index is 158% (normal 160%), sperm activity and viability 20% (normal 27%). With azoospermia in the spermograms of men of this age group, the studied indicators are 0%.

In the spermograms of men of the older age group, patients with teratozoospermia, the Farris index is 138%, the activity and viability of spermatozoa is 21%, while the norm is 164% and 26%, respectively. In the sperm samples of men of this age group, patients with asthenozoospermia, the Farris index is 119%, the activity and viability of spermatozoa is 22%, while the norm is 164% and 26%, respectively. With azoospermia in the spermograms of men of this age group, the investigated indicators are 0% (Fig. 3).

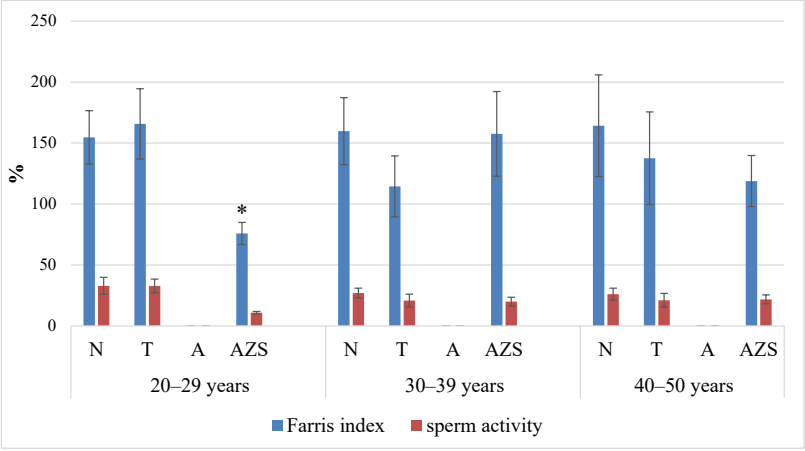


Fig. 3. Comparison of the Farris fertility index and sperm activity in the spermograms of men of the younger age group (20–29 years), the middle age group (30–39 years), and the older age group (40–49 years), (N – normozoospermia, T – teratozoospermia, A – azoospermia, AZS – asthenozoospermia).
Spermograms of men with normozoospermia were taken as controls
*** Significantly compared to the control (Farris index), p≥0.95**

Analysis of variance spermograms of men of different age groups with asthenozoospermia, teratozoospermia and azoospermia. In order to quantitatively assess the influence of teratozoospermia, asthenozoospermia, and azoospermia on the overall variability of spermogram indicators of men of younger, middle, and older age groups, 41 series of univariate variance analysis were conducted. The share of the influence of the researched diseases in the overall variability of the sperm motility index according to category A in men of the younger age group is 82% of the total contribution. In men of the middle and older age groups, the share of the influence of the studied diseases on the overall variability of the sperm motility index according to category A probably decreases and amounts to 78% and 75%, respectively, of the total contribution.

The share of the influence of the studied diseases in the overall variability of the sperm motility indicator by category B in men of the younger age group is 63% of the total contribution (the share of the influence of unaccounted factors is 37%), while the share of the influence of the studied diseases in the overall variability of the motility indicator is for men of the middle and older age groups spermatozoa according to category B make up 75% of the total contribution in the older age group (the share of influence of unaccounted factors 25%) and 60% of the total contribution in the middle age group (the share of influence of unaccounted factors 40%).

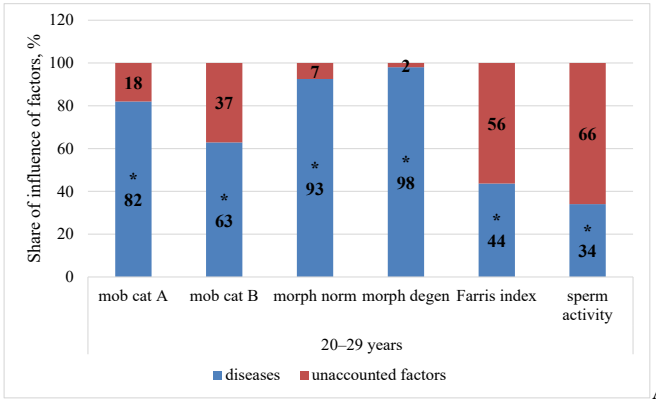
The share of the influence of the studied diseases in the total variability of the indicator of morphologically normal spermatozoa in men of the younger age group is 93% of the total contribution (the share of the influence of unaccounted factors is 7%), while in men of the middle and older age groups the share of the influence of the studied diseases in the total variability of the indicator of morphologically normal spermatozoa are likely to decrease and constitute 86% of the total contribution for the middle age group (the share of influence of unaccounted factors 14%) and 85% of the total contribution for the older age group (the share of influence of unaccounted factors 15%), respectively.

The share of the influence of the investigated diseases in the overall variability of the indicator of morphologically degenerate spermatozoa in men of the younger age group is 98% of the total contribution (the share of the influence of unaccounted factors is 2%), while the share of the influence of the investigated diseases in the overall variability of the indicator of morphologically degenerate spermatozoa in men of the middle and older age groups are 96% of the total contribution (the share of influence of unaccounted factors 4%) and 96% of the total contribution (the share of influence of unaccounted factors 4%), respectively.

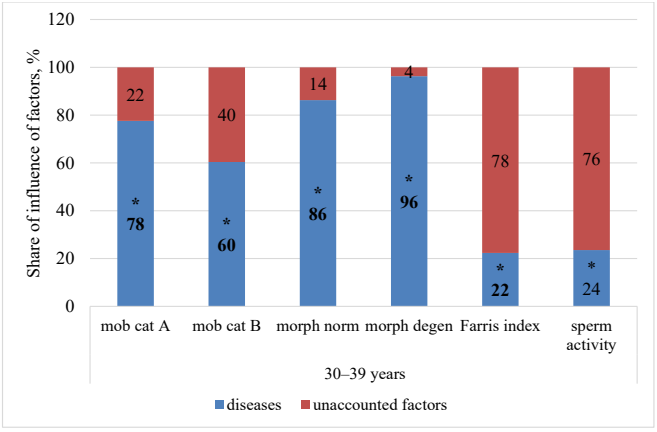
The share of the influence of the studied diseases in the total variability of the Farris fertility index in men of the younger age group is 44% of the total contribution (the share of the influence of factors not taken into account is 56%), while the share of the influence of the studied diseases in the total variability of the Farris fertility index in men of the middle and older age groups is likely decrease and make up 22% of the total contribution for men of the middle age group (the share of influence of unaccounted factors 78%) and 28% of the total contribution for men of the older age group (the share of influence of unaccounted factors 72%), respectively.

The share of the influence of the researched diseases in the overall variability of the sperm activity and viability indicator in men of the younger age group is 34% of the total contribution (the share of the influence of unaccounted factors is 66%), while in men of the middle and older age groups the share of the influence of the researched diseases in the overall variability of the activity indicator and sperm viability is 24% of the total contribution for men of the middle age group (the share of influence of factors not taken into account 76%) and 38% of the total contribution for men of the older age group (the share of influence of factors not taken into account 62%), respectively (Fig. 4).

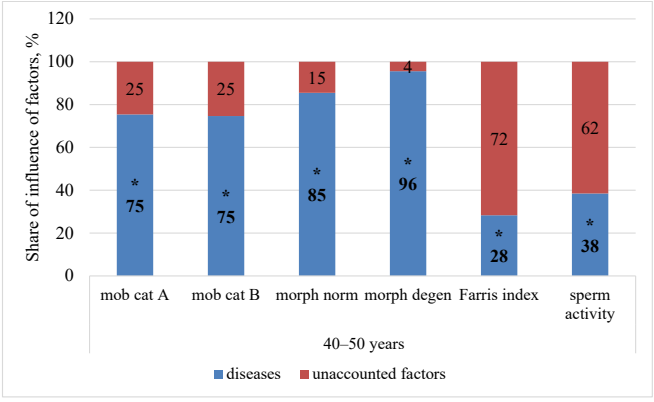
In order to quantitatively assess the influence of teratozoospermia, asthenozoospermia and azoospermia and the age factor on the general variability of spermogram indicators of men of younger, middle and older age groups, 18 series of two-factor variance analysis were conducted. Having conducted a two-factor variance analysis of the influence of the studied diseases and the age factor on the general indicators of the spermograms of men of different age groups, we found that the share of the influence of the studied diseases in the overall variability of the sperm motility index by category A and B is 98.5% of the total contribution (the share of the influence of the age factor 1%) and 95.4% of the total contribution (share of influence of unaccounted factors 4.4%, share of influence of age factor 1%), respectively.



A



B



C

Fig. 4. Results of one-factor variance analysis of the influence of the studied diseases on the general variability of spermogram indicators of men of the younger (A), middle (B) and older (C) age groups
Spermograms of men with normozoospermia were taken as controls
*** Significantly compared to the control p≥0.95**

The share of the influence of the investigated diseases in the total variability of the indicators of morphologically normal and morphologically degenerate spermatozoa in the spermograms of men of different age groups is 99.4% of the total contribution (the share of the influence of the age factor is 0.6%) and 99.9% of the total contribution (the share of the influence of the factor age 0.1%), respectively.

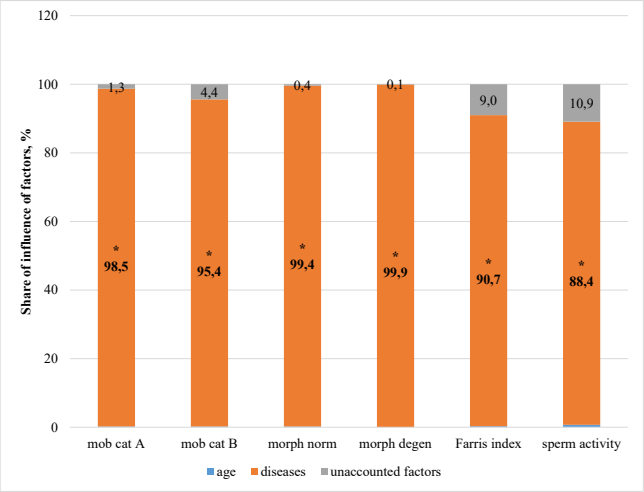


Fig. 5. Results of a two-factor variance analysis of the influence of the studied diseases and age on the general variability of spermogram indicators of men of different age groups.
Spermograms of men with normozoospermia were taken as controls
*** Significantly compared to the control, p≥0.95**

The share of the influence of the studied diseases in the total variability of the Farris fertility index in the spermograms of men of different age groups is 90.7% of the total contribution (the share of the influence of unaccounted factors 9%, the share of the influence of the age factor 0.3%).

The share of the influence of the studied diseases in the overall variability of the indicator of sperm activity and viability in the spermograms of men of different age groups is 88.4% of the total contribution (the share of the influence of unaccounted factors 10.9%, the share of the influence of age 0.7%) (Fig. 5).

4. Conclusions

It has been found that the greatest deviations in the studied groups are the mobility of spermatozoa according to categories A and B, the morphology of spermatozoa, the Farris fertility index, and the activity of spermatozoa. We found that in group with asthenozoospermia, the percentage of spermatozoa with motility criteria A and B decreases in the spermograms of men of all age groups. We also found that in the spermograms of men of middle and older age groups with teratozoospermia, the percentage of morphologically normal spermatozoa decreases, and instead, the percentage of morphologically degenerate spermatozoa increases compared to the

control. It is shown that in the spermograms of middle-aged men with teratozoospermia, the Farris fertility index is significantly reduced compared to controls. This indicates a low probability of fertilization. Men of all studied age groups with azoospermia have the lowest ability to fertilize an egg, because this pathology is characterized by the absence of spermatozoa in the ejaculate.

After conducting a univariate variance analysis, we established that the influence of the studied diseases on the overall variability of such indicators of spermograms as the motility of spermatozoa criteria A and B, as well as the morphology of spermatozoa in men of the younger age group ranges from 63% to 98% of the total contribution. The share of influence of the investigated diseases in the total variability of Farris fertility indicators and sperm activity decreases (in the range of 34–40% of the total contribution), and the share of unaccounted factors increases (in the range of 56–66% of the total contribution). It should be noted that the share of the influence of the researched diseases on the total variability of such indicators of spermograms of men of the middle age group, such as the mobility of spermatozoa according to category A and B and the percentage of morphologically normal and degenerative spermatozoa are within 60–96% of the total contribution. The share of the influence of the studied diseases on the overall variability of the Farris fertility index and the activity and viability of spermatozoa significantly decreases (it is within 22–24% of the total contribution), and the share of the influence of unaccounted factors on the overall variability of these indicators increases.

The share of the influence of the studied diseases on the overall variability of such indicators of spermograms as the mobility of spermatozoa according to category A and B and morphologically normal and degenerative spermatozoa in men of the older age group ranges from 75 to 96% of the total contribution. The share of the influence of the studied diseases on the overall variability of such indicators as the Farris fertility index and the activity and viability of spermatozoa significantly decreases (is within the range of 28–38% of the total contribution), and the share of influence of unaccounted factors increases (is within the range of 62–72% of the total contribution).

After conducting a two-factor variance analysis, we established that the shares of the influence of the studied diseases on the overall variability of such indicators of spermograms as motility of spermatozoa according to category A and B, morphology of spermatozoa, Farris fertility index and activity of spermatozoa are decisive and are within the range of 90.7–99.9% of the total contribution, the share of the influence of the age factor on the variability of spermogram indicators of men of different age groups is insignificant.

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