



UDC: 616.697.008.3-071

BIOMETRIC ANALYSIS OF SPERMOGRAMS OF MEN OF DIFFERENT AGE GROUPS IN NORMAL AND PATHOLOGY IN LVIV REGION, UKRAINE

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Tarnovska, A. V., & Heneha, A. B. (2022). Biometric analysis spermograms of men of different age groups in normal and pathology in Lviv region, Ukraine. *Studia Biologica*, 16(3): 49–60. doi:[10.30970/sbi.1603.686](https://doi.org/10.30970/sbi.1603.686)

Background. Analysis of the causes of infertility revealed an increase in the proportion of male factors over the past 20 years by an average of 10–12 %. World Health Organization data, which summarize the observations of many authors in recent years, show a decrease in male reproductive potential. The ejaculate parameters of the modern average man are steadily declining. For example, the concentration of germ cells in the seminal fluid has decreased three times, and this trend continues: the concentration of sperm decreases annually by about 2 %, and their motility – by 1.5 %. Not only is infertility a pressing problem in modern medicine, but also an important social issue, as it is associated with the mental state of men and the preservation of the family.

Materials and Methods. Spermograms were obtained during the study of patients in the clinic of reproductive medicine “Alternative Clinic” (Lviv, Ukraine). A total of 50 men were surveyed, including 16 men aged 20 to 29 (junior age group), 17 men aged 30 to 39 (middle age group) and 17 men 40 to 49 years old (senior age group), respectively. We found that out of the 16 men in the junior group, 4 spermograms corresponded to asthenozoospermia, and 12 – normozoospermia, of the 17 middle-aged group, 7 spermograms corresponded to asthenozoospermia, and 10 – normozoospermia and 10 men of the senior group to 15 spermograms asthenozoospermia, 2 – normospermia. Spermograms were evaluated by the following indicators: ejaculate count, viscosity, sperm count per 1 mL of ejaculate, sperm motility in categories “A” and “B”, the number of morphologically normal and morphologically degenerative sperm, Farris index. For control we took spermograms of patients with normozoospermia.

Results. Analyzing spermograms according to the studied indicators, we found a decrease in the amount of ejaculate in older men relative to the norm, which may indicate a lack of function of the seminal vesicles, prostate. According to the criteria for



the number of sperm in 1 ml of ejaculate and the total number of sperm in the whole ejaculate, it was shown that in the spermograms of patients with asthenozoospermia of different age, these figures are much lower than normal. This indicates a decrease in male fertility in these diseases. Examining sperm motility according to the following criteria: "A" – fast translational movements and "B" – slow, sluggish translational movements, we found a decrease in these indicators compared with the norm in men of all ages with asthenozoospermia. This indicates a decrease in the quality of ejaculate in men, and, consequently, reduce the likelihood of fertilization. Examining sperm morphology, we assessed the number of normal and degenerative sperm. We have shown that in men of all ages with asthenozoospermia, the number of morphologically normal sperm is lower than in the control, and the number of morphologically degenerative sperm is increasing. Probably, such pathology can lead to a decrease in the possibility of fertilization and increases the likelihood of malformations in the fetus, if fertilization has occurred. Evaluating the spermograms of men in the study groups according to the Farris index, we found a significant reduction in this number in men with asthenozoospermia. This indicates a low probability of fertilization.

Conclusions. Male infertility (in particular, asthenozoospermia) can be caused by many different diseases in which there are deviations from the norms of qualitative and quantitative indicators of ejaculate. According to the data obtained from the observation of men, there is a tendency for the reduction of ejaculate volume, concentration and motility of sperm, as well as their morphological status.

Keywords: asthenozoospermia, normozoospermia, spermogram, male infertility

INTRODUCTION

Infertility is one of the common problems facing many couples today. In particular, the number of such couples in Ukraine is about one million. The male factor is the cause of childlessness in 50 % of marriages (Hrynychuk, 2007; Ivaniuta & Ivaniuta, 2015). Genital diseases, endocrine, immune, and genetic changes have a negative effect on the generative capacity of men. Dysfunction of the blood-testicular barrier due to infectious and inflammatory processes occurring in the testicles and prostate of men is one of the most common causes of male infertility. There are two main causes of male infertility: a decreased sperm quality and testicular pathology, respectively, two forms of disorders – secretory and obstructive. In inflammatory diseases, there is often a decrease in the number of sperm, a decrease in their motility due to changes in the biochemical parameters of sperm and blood, in particular, an increase in the concentration of prostatic acid phosphatase and antisperm antibodies (Eskenazi *et al.*, 2001).

In both men and women, fertility decreases with age for a number of reasons. However, most discussions centre on women and their age, when the chances of getting pregnant are the best. And for men, the simplest minimum requirements are set - to have at least a few active sperm. In theory, men do not lose the reproductive ability until death. However, mutations are more common in sperm than in eggs. Due to oxidative stress (smoke, for example), DNA can break down into fragments, making such sperm inoperable (ESHRE Capri Workshop Group, 2008; Kehoe *et al.*, 2009).

Both men's general health and sperm quality deteriorate with age. After 35 years, the ability of sperm to fertilize is twice lower than at a younger age. After 40, the level of the sex hormone testosterone drops significantly.

In addition, over the past 40 years, the quality of male sperm in developed countries has deteriorated significantly – the number of motile sperm has decreased by 20 %. Probably, this is due to the increasing tendency for the occurrence of the male genitalia diseases (Eskenazi *et al.*, 2001). In addition, there is a deterioration in quantitative and qualitative indicators of spermograms in otherwise healthy men (Sukhikh & Bozhedomov, 2009; Tsvetkova *et al.*, 2012). The average number of sperm in a healthy man's ejaculate has halved during the past 50 years, and the average ejaculate volume has decreased by one third (Tarnovska *et al.*, 2018).

It is known that a decrease in the fertility of ejaculate can be observed without any deviations from the normal parameters of routine spermatological examination. About 30 % of cases (Chaika & Chernysheva, 2001; Gorpinchenko, *et al.*, 2010, Gorpinchenko *et al.*, 2019) of spermogram studies do not give an explicit answer about the root cause of reduced fertility, because changes in this function occur at the functional molecular biological or biological level. 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 comprehensive laboratory examination of ejaculate is necessary.

The problems of protecting the reproductive systems of the male body from the influence of negative factors that cause a decrease in male fertility remain topical nowadays. Thus, it is important to build models of probable causes of reduced reproductive capacity of men, as well as to study the age factor of male fertility in relation to other factors.

The aim of this work was to compare spermograms of men of junior (20–29 years), middle (30–39 years) and senior (40–50 years) age groups in normal and asthenozoospermia. To achieve this goal, the following tasks were solved: to analyze spermograms of men of different age groups (20–29 years, 30–39 years and 40–50 years), to determine whether the age factor contributes to a reduced male fertility and to make a comparative characterization of asthenozoospermia according to normal rates for men of different ages.

Not only is infertility an urgent problem of modern medicine, but it also has an important social significance, as it is associated with the psychological state of men and the preservation of the family.

It is now generally accepted that environmental reproductive health is thought to be impaired in men. In this regard, the concept of human reproductive health as an integral indicator of the state of the environment and an indicator of environmental ills has been developed (Moskowitz *et al.*, 2001). The influence of negative environmental factors on both fetal development and the adult body is associated with a decrease in spermatogenesis and the development of infertility (Pastukhova, 2008). It is known that spermatogenesis is one of the most dynamic processes in the body, which makes it extremely sensitive to damaging agents of both endogenous and exogenous genesis (Bragina *et al.*, 2002). Infertility can also be the result of acute and chronic inflammatory processes of various etiologies (Arya & Dibb, 2016; Condic, 2002).

MATERIALS AND METHODS

Macroscopic, microscopic, biochemical and immunological studies of ejaculate are crucial for the diagnosis of functional disorders of the gonads and judgments about male fertility.

Spermograms were obtained during the study of patients in the clinic of reproductive medicine "Alternative Clinic". Sperm samples were collected in accordance with

WHO requirements by masturbation 2–6 days of abstinence from sexual intercourse (World Health Organization, 2010). A total of 50 men were surveyed, including 16 men aged 20 to 29 (junior age group), 17 men aged 30 to 39 (middle age group) and 17 men 40 to 50 years old (senior age group), respectively. We found that out of 16 men in the junior group, 4 spermograms corresponded to asthenozoospermia, and 12 – normozoospermia, of the 17 middle-aged group, 7 spermograms corresponded to asthenozoospermia, and 10 – normozoospermia and 17 men of the senior group to 15 spermograms asthenozoospermia, 2 – normospermia.

Asthenozoospermia is characterized by reduced motility of sperms, less than 32 % progressively motile (according to WHO 2010 criteria or category A + B according to previous WHO standards) or with total motility below 40 % (World Health Organization, 2010).

For control we took spermograms of patients with normozoospermia.

In order to assess the possibility of in vitro fertilization, the Farris index is determined. It allows you to show the number of fast and motile, sedentary and immobile sperm.

$$\text{Farris index} = \frac{\text{Ejaculate volume} \cdot \text{number of sperm in 1 mL} \cdot \% \text{ motile sperm}}{100}$$

At the clinic “Alternative”, patients filled out questionnaires and were introduced to the rules of the procedure. Measures were taken to ensure patient safety, respect for human rights, human dignity and moral and ethical standards in accordance with the principles of the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine, ICH GSP and current regulations of Ukraine.

Statistical analysis of research results was performed using batch analysis programs (MS Excel Data analysis). The main statistical indicators were calculated according to the direct quantitative data obtained in the research results (mean – M; standard error of mean – SEM). To assess the reliability of the difference between the statistical characteristics of the two alternative data sets, the Student’s *t*-test was calculated.

A significant feature is a difference in the reliability $p > 0.95$ (or significance level $p < 0.05$). The results of the analysis were displayed in the diagrams.

RESULTS AND DISCUSSION

The research results are presented in the form of a diagram (**Fig. 1 A–H**). Spermograms were evaluated by the indicators as described below.

The first indicator is the amount of ejaculate (**Fig. 1A**). Normally it is in the range of 2-5 ml, but there are significant fluctuations. Ejaculate volume less than 1 ml is characteristic of androgen deficiency. This can also be associated with the deformation of the seminal vesicles and vas deferens. According to researchers, the average amount of ejaculate in healthy men should be 3.7 mL. Excessive amounts of ejaculate (more than 7-8 mL) are generally accompanied by a decrease in sperm concentration.

Thus, we have shown that in patients with asthenozoospermia aged 20–29 years, ejaculate volume was 3.58 mL (median 3.3, quartile 2.8, 3; 3.4), aged 30–39 years, 3.52 mL (median 3; quartile 2, 3, 4.6), and aged 40–50 years, 2.87 mL (median 2.8; quartile 2.1, 2.8, 3.6), which is a deviation from the norm.

This indicates a lack of function of the seminal vesicles, the prostate gland. In general, the volume of semen is lower than normal, indicating a weak ability to fertilize.

The second criterion used to test sperm was the viscosity of the semen (**Fig. 1B**). According to this criterion of spermograms, the viscosity of ejaculate in asthenozoospermia of the studied diseases is within normal limits.

The third criterion is the number of sperm in 1 mL of ejaculate (**Fig. 1C**). According to this criterion, it is shown that in the spermograms of patients with asthenozoospermia of different age groups, the number of sperm in 1 mL of semen is much lower than normal. This indicates a decrease in male fertility in these diseases.

The fourth criterion is the total number of sperm in the whole ejaculate (**Fig. 1D**). According to this criterion in asthenozoospermia, the total number of sperm is significantly lower than normal.

The fifth criterion is sperm motility (**Fig. 1E**). The motility of each sperm is classified into categories "A", "B", "C" and "D". We evaluated sperm motility according to the following criteria: "A" – fast translational movements and "B" – slow, sluggish translational movements.

Thus, we have shown that in the case of asthenozoospermia in men of the junior age group, sperm motility according to criteria "A" and "B" is 18 % and 19 %, (4 men out of the 16 investigated) respectively (norm 34 % and 18 %), the middle age group – 16 % and 21 % (7 men out of the 17 investigated) (norm 36 % and 18 %), and the senior age group – 18 % and 17 % (15 men out of the 17 investigated) (norm 28 % and 24 %) respectively. Sperm motility is very important in assessing the quality of ejaculate. The probability of fertilization decreases with a decrease in the number of normal motility sperm in the ejaculate.

The sixth indicator is the morphology of sperm (**Fig. 1F**). We evaluated the morphology of sperm by the number of normal and degenerative sperm. Thus, we have shown that in the case of asthenozoospermia the number of morphologically normal sperm is less than in the control and is 35 % in men of 20–29 years of age, 19 % in men of 30–39 years, and 17 % in men of 40–50 years, while in controls – 36–37 %. However, against the background of a decrease in the number of morphologically normal sperm, the number of morphologically degenerative sperm in younger men increases (80 % in asthenozoospermia and 63 % in normozoospermia); in the middle-age group (68 % with asthenozoospermia and 65% with normozoospermia); in the senior age group (65% with asthenozoospermia and 64 % with normozoospermia). Therefore, in asthenozoospermia there is an increase in the number of pathological forms of sperm above normal. Severe asthenozoospermia dramatically reduces the chances of fertilization and increases the likelihood of fetal malformations if fertilization has occurred.

The seventh criterion is an indicator of Farris's fertility (**Fig. 1G**).

We have shown that in the case of asthenozoospermia in younger men the Farris index is significantly lower than normal (75 at 160) ($p = 0.99$), in middle-aged men the Farris index is 130 at 159 ($p = 0.078$), and in older men the Farris index is 78 at normal 112 ($p = 0.78$). This indicates a low probability of fertilization.

The causes of a decrease in quantitative and qualitative parameters of sperm remain unknown. There are studies (Gorpinchenko & Romanyuk, 2016) that suggest that life-style factors (stress, smoking, alcohol, environmental chemicals that have estrogenic activity, urbanization, etc.) and consumption of meat with female hormones negatively affect the male reproductive system, which is the most vulnerable and least protected.

Analysis of the causes of infertility revealed an increase in the proportion of male factors over the past 20 years by an average of 10–12 %. WHO data, which summarize

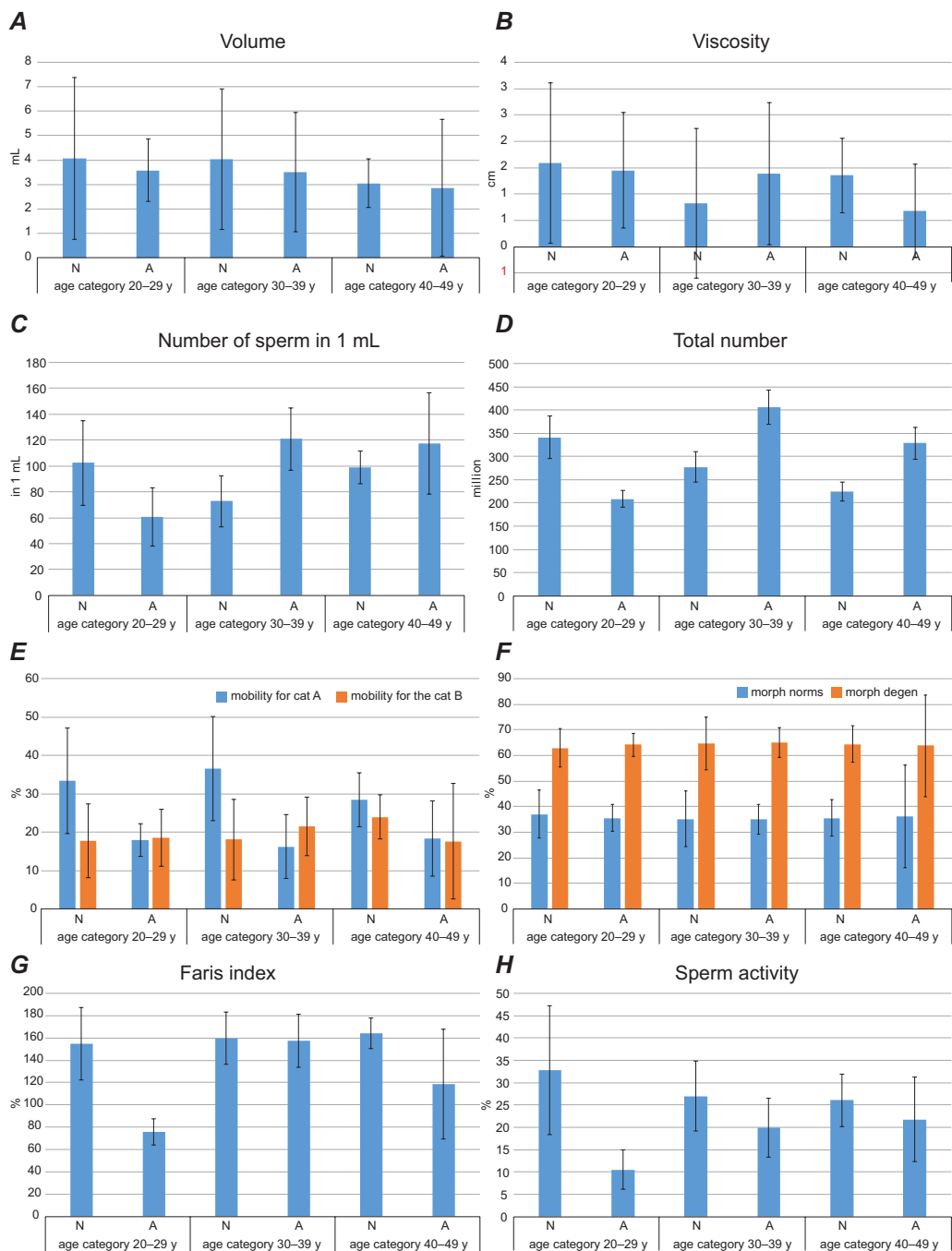


Fig. 1. Analysis of spermatograms of men of junior (20–29 years), middle (30–39 years) and senior (40–49 years) age groups with normozoospermia (N) and asthenozoospermia (A): A – volume of seminal fluid; B – viscosity; C – the number of sperm in 1 mL of ejaculate; D – total count; E – sperm motility in categories “A” and “B”; F – the number of morphologically normal sperm and degenerative sperm; G – the Farris index; H – the number of active sperm

the observations of many authors in recent years, show a decrease in male reproductive potential. The ejaculate parameters of an average contemporary man are steadily declining. For example, the concentration of germ cells in the seminal fluid has decreased three times, and this trend continues: the concentration of sperm decreases annually by about 2 %, and their motility – by 1.5 % (Weber *et al.*, 2005; World Health Organization, 2010).

It is believed that male fertility (ability to conceive) is provided by a certain amount of sperm (from 20 to 100 million/mL). Normally, at least 50 % of sperm remain motile 2 hours after ejaculation, and more than half of the original number of motile sperm is still alive 24 hours after ejaculation.

Male infertility can be caused by numerous disorders. Thus, the absence or small number of sperm in the ejaculate may be due to an obstruction or congenital absence of seminal ducts, testicular damage or varicocele (abnormal varicose veins of the testes, which impairs blood flow from the testis, causing fever in the scrotum and impaired spermatogenesis) (Acacio *et al.*, 2002; Yatskiv & Tarnovska, 2012). The temperature can also rise when visiting the sauna, or due to acute respiratory viral infections (Punab *et al.*, 2017; Ozelci *et al.*, 2016; Amini, Kahrobaie *et al.*, 2020). External factors also have a significant impact: the habit of wearing tight underwear and outerwear, as well as prolonged sitting can cause venous plethora in testicular tissue and, as a consequence, the development of chronic hypoxia. Excessive sexual activity, including daily or more frequent ejaculations, can lead to a decrease in sperm count, and abstinence from sexual intercourse for 5–7 days is undesirable, as an increase in sperm count is accompanied by a decrease in sperm motility due to an increase in “old” cells. Sperm adhesion can occur periodically in most men, but the recurrence of these changes is the evidence of an autoimmune reaction or infection (Bragina *et al.*, 2000; Matzuk & Lamb, 2008; Lunyova *et al.*, 2010). Secretory disorders of the seminal vesicles, prostate, and other accessory glands in the male gonads can lead to liquefaction of semen. Retrograde ejaculation is sometimes found in men, accompanied by the ejaculation of semen into the bladder. Such phenomena occur after prostatectomy, as well as diabetes and neurological disorders. There are reports in the literature of impaired sperm structure; the reasons may be testicular injuries, surgery or mumps in the past, severe allergic reactions, radiation exposure, the use of certain drugs (Bozhemov *et al.*, 2002; Gerasimov & Polumiskov, 2003; Gołąb *et al.*, 2002). Bad habits (smoking and alcohol consumption) can also lead to lower testosterone levels, decreased libido and potency, resulting in impaired sperm motility. It is shown that the assessment of sperm quality is one of the main methods of determining a man’s reproductive abilities and the functional state of his genitourinary system. Therefore, the basic analysis of andrology – spermogram has a wide range of diagnostic possibilities.

CONCLUSIONS

It has been found that the average number of sperm in a healthy man’s ejaculate has halved in the past 50 years, and the average ejaculate volume has decreased by one third. It is known that the multicomponent composition of the internal male genitalia is in constant adjustment due to age changes, functional activity and the influence of various factors. Studies in men of different ages have shown a tendency for the reduction of ejaculate volume, sperm concentration and motility, as well as their morphological status.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: Measures were taken to ensure patient safety, respect for human rights, human dignity and moral and ethical standards in accordance with the principles of the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine, ICH GSP and current regulations of Ukraine.

Animal studies: This article does not contain any studies with laboratory animals performed by the any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [T.A.V.; H.A.B.]; methodology, [T.A.V.; H.A.B.]; validation, [T.A.V.; H.A.B.]; formal analysis, [T.A.V.; H.A.B.]; investigation, [T.A.V.; H.A.B.]; resources, [T.A.V.; H.A.B.]; data curation, [T.A.V.; H.A.B.]; writing – original draft preparation, [T.A.V.; H.A.B.]; writing – review and editing, [T.A.V.; H.A.B.]; visualization, [T.A.V.; H.A.B.]; supervision, [T.A.V.; H.A.B.]; project administration, [T.A.V.; H.A.B.]; funding acquisition, [-].

All authors have read and agreed to the published version of the manuscript.

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БИОМЕТРИЧНИЙ АНАЛІЗ СПЕРМОГРАМ ЧОЛОВІКІВ РІЗНИХ ВІКОВИХ ГРУП У НОРМІ ТА ЗА ПАТОЛОГІЙ У ЛЬВІВСЬКІЙ ОБЛАСТІ, ЛЬВІВ

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Вступ. Аналіз причин непліддя виявив збільшення питомої ваги чоловічого фактора за останні 20 років у середньому на 10–12 %. Дані ВООЗ, що узагальнили спостереження багатьох авторів за останні роки, свідчать про зменшення репродуктивного потенціалу чоловіків. Параметри еякуляту сучасного середньостатистичного чоловіка неухильно знижуються. Наприклад, концентрація статевих клітин у сім'яній рідині зменшилась утричі, і ця тенденція зберігається: концентрація сперматозоїдів знижується щорічно приблизно на 2 %, а їхня рухливість – на 1,5 %. Непліддя не тільки є актуальною проблемою сучасної медицини, але й має важливе соціальне значення, оскільки з ним пов'язаний психічний стан людини та збереження сім'ї.

Матеріали та методи. Спермограми отримано під час дослідження пацієнтів у клініці репродуктивної медицини “Альтернатива клініка” (Львів, Україна). Загалом було обстежено 50 чоловіків: з них 16 чоловіків віком від 20 до 29 років (молодша вікова група) та по 17 чоловіків віком від 30 до 39 років (середня вікова група) і 40 до 50 років (старша група) відповідно. Зразки сперми збирали згідно з вимогами ВООЗ методом мастурбації після 2–6 днів утримання від статевого акту (Всесвітня організація охорони здоров'я). Унаслідок досліджень нами виявлено, що з 16 чоловіків молодшої групи у 4 спермограми відповідали астенозооспермії, а 12 – нормозооспермії, зі 17 середньої вікової групи у 7 спермограми відповідали астенозооспермії, а у 10 – нормозооспермії та зі 17 чоловіків старшої групи у 15 спермограми відповідали астенозооспермії, 2 – нормоспермії. Спермограми оцінювали за такими показниками: кількість еякуляту, в'язкість, кількість сперматозоїдів в 1 мл еякуляту, рухливість сперматозоїдів за категоріями “А” та “В”, кількість морфологічно нормальних і морфологічно дегенеративних сперматозоїдів, індекс Фарріса. За контроль ми брали спермограми пацієнтів із нормозооспермією.

У “Альтернатива клініка” пацієнти заповнювали анкети та були ознайомлені з правилами проведення процедури. Були передбачені заходи зі забезпечення безпеки здоров'я пацієнта, дотримання його прав, людської гідності та морально-етичних норм відповідно до принципів Гельсінської декларації, Конвенції Ради Європи про права людини і біомедицину, Належної клінічної практики (ICH GCP) і чинних нормативно-правових актів України.

Статистичну обробку результатів досліджень проводили з використанням персонального комп'ютера за допомогою програми пакету аналізу. Обчислювали основні статистичні показники по безпосередніх кількісних даних, отриманих у результаті досліджень (середнє арифметичне значення – M ; стандартна похибка середнього арифметичного – m). Для оцінювання достовірності різниці між статистичними характеристиками двох альтернативних сукупностей даних обчислювали коефіцієнт Стьюдента.

Достовірною вважається різниця за показника достовірності $p > 0,95$ (або рівні значимості $p < 0,05$). Результати обробки виводили у вигляді діаграм.

Результати. Аналізуючи спермограми за досліджуваними показниками ми виявили зниження кількості еякуляту у чоловіків старшої вікової групи щодо норми, що може свідчити про недостатню функцію сім'яних пухирців, передміхурової залози. За критеріями кількості сперматозоїдів в 1 мл еякуляту та загальної кількості сперматозоїдів у всьому еякуляті з'ясовано, що у спермограмах пацієнтів, хворих на астенозооспермію різних вікових груп, ці показники значно нижчі за норму. Це свідчить про зниження фертильності чоловіків із цими захворюваннями.

Досліджуючи рухливість сперматозоїдів за такими критеріями: “А” – швидкі поступальні рухи та “В” – повільні, в'ялі поступальні рухи, ми виявили зниження цих показників порівняно з нормою у чоловіків усіх вікових груп, хворих на астенозооспермію. Це свідчить про зниження якості еякуляту чоловіків і, як наслідок, про зниження ймовірності запліднення. Досліджуючи морфологію сперматозоїдів, ми оцінювали за кількістю нормальних і дегенеративних сперматозоїдів. Нами встановлено, що у чоловіків усіх вікових груп, хворих на астенозооспермію, кількість морфологічно нормальних сперматозоїдів є менша порівняно з контролем, а кількість морфологічно дегенеративних сперматозоїдів зростає. Ймовірно, така патологія може призвести до зниження можливості запліднення і збільшує вірогідність вад

розвитку у плода, якщо запліднення відбулося. Оцінюючи спермограми чоловіків досліджуваних груп за індексом Фарріса, ми виявили суттєве зниження цього показника у чоловіків, хворих на астенозооспермію. Це свідчить про низьку ймовірність запліднення.

Висновки. Чоловіче непліддя (зокрема, астенозооспермія) може бути спричинене безліччю різноманітних захворювань, за яких спостерігають відхилення від норм якісних і кількісних показників еякуляту. Згідно з отриманими даними, зі збільшенням віку чоловіків виявлено тенденцію до зниження показників об'єму еякуляту, концентрації та рухливості сперматозоїдів, а також їхнього морфологічного стану.

Ключові слова: астенозооспермія, нормозооспермія, спермограма, чоловіче непліддя