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FRAGMENTATION OF SPERM DNA: CLINICAL SIGNIFICANCE, REASONS, METHODS OF EVALUATION AND CORRECTION

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A review of the main causes of male infertility in the aspect of the relationship with the degree of sperm DNA fragmentation is presented. Information is provided on the main methods for assessing sperm DNA fragmentation and its effect on male fertility. The effect of oxidative stress on the integrity of sperm DNA structure, the reparative capabilities of antioxidant therapy, and the effect of varicocele on male fertility are described.

Keywords: sperm DNA fragmentation; assessment methods; oxidative stress; varicocele.

ФРАГМЕНТАЦИЯ ДНК СПЕРМАТОЗОИДОВ: КЛИНИЧЕСКАЯ ЗНАЧИМОСТЬ, ПРИЧИНЫ, МЕТОДЫ ОЦЕНКИ И КОРРЕКЦИИ

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Представлен обзор основных причин мужского бесплодия в аспекте взаимосвязи со степенью фрагментации ДНК сперматозоидов (ФДНКС). Приведены сведения об основных методах оценки ФДНКС и ее влиянии на мужскую фертильность. Описано воздействие оксидативного стресса на целостность структуры ДНК сперматозоидов, репаративные возможности антиоксидантной терапии, а также влияние варикоцеле на мужскую фертильность.

Ключевые слова: фрагментация ДНК сперматозоидов; методы оценки; оксидативный стресс; варикоцеле.

INTRODUCTION

Infertility is a problem that affects 15% of sexually active couples engaging in unprotected intercourse, which is approximately 48.5 million couples worldwide. One in eight couples faces problems when planning their first child, and one in six faces problems when planning their second. Notably, the share of male factor infertility varies in different countries worldwide, ranging from 20%–70% [1–4].

According to the World Health Organization, infertility is the inability to achieve clinical pregnancy by a sexually active couple despite regular sexual life for one year or more, without contraceptive use [5]. The primary reasons for the decreased male fertility are congenital or acquired abnormalities of the genitourinary organs, malignant neoplasms and infections of the genitals, increased temperature in the scrotum, endocrine disorders, genetic abnormalities, and immunological factors [6]. The annual increase in the number of oncological diseases, including in young men, negatively affects their fertility that could be significantly reduced or completely lost because of chemotherapy and radiation therapy [7]. Nevertheless, in approximately 40% of infertile men,

the cause of infertility remains unknown (idiopathic male infertility). Moreover, in male idiopathic infertility during the anamnesis, diseases that violate the spermatogenesis are not diagnosed; no changes during physical examination are detected; and no violations of hormonal, genetic, and biochemical parameters are observed. Presumably, idiopathic male infertility might be indirectly caused by imbalanced environmental factors, processes that accumulate oxygen free radicals, or genetic and epigenetic abnormalities. Therefore, detecting new genetic factors of male infertility in idiopathic infertility is one of the priorities of modern andrology [8, 9].

Male infertility is a multifactorial pathological condition affecting approximately 7% of the male population. The genetic landscape of male infertility is complicated because the histological phenotypes of sperm and supremacy are extremely heterogeneous, and at least 2000 genes are involved in spermatogenesis [9].

Over the past years, the significance of determining the degree of sperm DNA fragmentation (SDF) has garnered attention. It is believed that this is a separate entity of genetic imbalance of a sperm that causes imbalances in male fertility and affects the probability of conception during the natural reproductive cycle, and reduces the effectiveness of assisted reproductive treatment procedures (ART) [10–12]. The integrity of the genome is continuously imbalanced by both endogenous by-products of metabolism and exogenous factors. Depending on indicators, such as the cell type, the cell cycle stage, and the type of DNA damage, the sperm has several ways to repair damaged DNA, and an incorrect repair can have negative consequences. Double-stranded DNA breaks are induced endogenously during spermatogenesis both at the meiotic stage (to facilitate the formation of meiotic crossovers) and during spermatogenesis when the chromatin of round haploid spermatids is compacted by replacing histones with protamine. The term "damage of the sperm DNA" refers to several defects in the chromatin structure, including breaks in one or two helices of the DNA molecule, deletions, the formation of additional bonds in the helix or between the helices of DNA, and incorrect placement of protamine because of defective crosslinking of DNA and proteins.

SDF is a unique form of genetic damage of the male gamete DNA, which can lead to problems with fertility and embryonic development. The higher the number of damages, the lower the integrity of the genetic material and the probability of pregnancy. Notably, measuring the integrity of a sperm sex chromatin has been the subject of numerous studies last decade, which revealed that excessive sperm SDF disrupts male fertility.

CLINICAL SIGNIFICANCE OF DETERMINING SPERM DNA FRAGMENTATION

The integrity of sperm DNA is a crucial factor for successful fertilization and proper development during pregnancy. Pathological SDF means the loss of structural integrity of the entire DNA molecule of the sperm. Even though the ability to fertilize is preserved, with defective DNA, its primary functions are disrupted, which is both a factor in reducing the probability of fertilization and a factor that increases the risk of miscarriage and the birth of a child with various genetic abnormalities.

Chromatin damage can occur at any stage of spermatogenesis, spermiogenesis when passing through the epididymis, and in vitro during the preparation of sperm for fertilization. It is critical to note that defective sperm containing damaged DNA might retain its fertilization ability. However, increased SDF can cause idiopathic male infertility, ART procedure failures, and recurrent miscarriages during the natural reproductive cycle. Moreover, sperm DNA damage is associated with an increased risk of oncogenetic diseases and transmission of genetic defects to offspring [10, 11]. Nonetheless, the use of balanced antioxidant complexes has been proven to reduce the negative effects of increased secretion of reactive oxygen species (ROS) and improve the quality of ejaculate, thereby reducing the risk of unsatisfactory ART procedural results [10–14].

A group of authors spermatologically analyzed 461 men with infertility. Notably, in 23% of the surveyed patients, SDF frequency was more than 15%, ranging from 15.1%–30% in 18% of patients and exceeding 30% in 5%. The number of sperms with fragmented DNA in severe forms of azoospermia is higher than the less expressed disorders of spermatogenesis. The negative dynamics between the sperm concentration changes and the SDF frequency was revealed. The results confirmed the assumption of a correlation between spermatological parameters (concentration, motility, and morphology of spermatozoa) and the frequency of SDF. Therefore, the

SDF indicator has an absolute diagnostic and prognostic value for married couples with reproductive disorders [3].

Furthermore, SDF is directly proportional to decreased fertilization rate, reduced embryonal quality, decreased frequency of pregnancy, and an increased risk of miscarriage.

CAUSES OF PATHOLOGICAL FRAGMENTATION OF SPERM DNA

The primary causes of pathological SDF that lead to sperm nuclear apparatus damage include various types of intoxication, occupational hazards, adverse environmental factors, poor lifestyle, alimentary factors, varicocele, infectious and inflammatory diseases of the scrotum, smoking, and drug use. Cannabis use negatively affects the spermatogenesis process, starting from the meiotic stage to spermiogenesis and possibly sperm maturation in the testes of men with infertility [15, 16]. In addition, the administration of certain drugs, particularly from the group of serotonin reuptake inhibitors, can cause an increase in the degree of pathological SDF [17].

Other causes of SDF may be pathological apoptosis, excessive ART production, and a decrease in the number of seed antioxidants. Moreover, the toxic effects of drugs and factors, such as xenobiotics; increased temperature in testis tissues (fever, varicocele); and old age, were associated with sperm DNA strand damage [10].

Furthermore, one of the possible and probable causes of pathological SDF could be oxidative stress. According to numerous literature data, one of the factors that can reduce male fertility is the hyperproduction of the so-called ROS, including ozone, nitric oxide, and free radicals. All these agents can damage the membranes of sperm, reducing their mobility and disrupting their fertilizing ability. Generally, the pathophysiology of infertility disorders associated with oxidative stress has the following path of development: hyperproduction of ROS causes modification of nuclear DNA, destroying lipids and proteins of the plasma mitochondrial membrane. In addition, imbalances in the plasma membrane structure alter its fluidity, thereby impairing the sperm mobility and the acrosomal reaction necessary for the penetration of the eggshell by the sperm [18].

METHODS FOR EVALUATING SPERM DNA FRAGMENTATION

Damage to sperm DNA can be caused by several factors, both external and internal. One of the problems associated with assessing the degree and type of SDF is the disunity and high variability of the results obtained using different detection methods. Generally, most tests currently developed and implemented in clinical practice for evaluating SDF have a relatively high sensitivity.

Notably, various methods of verification of DNA fragmentation are used to detect sperm DNA damage, such as structural analysis of sperm chromatin (SCSA), chromatin dispersion test (SCD), nick-end labeling of dUTP using terminal deoxynucleotidyl transferase (TUNEL) and gel electrophoresis (Comet), and coloring with aniline blue and chromomycin A3 [19–22].

TUNEL method

The TUNEL method is an evaluation method for determining the degree of SDF. Analysis of the terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) quantifies the inclusion of fluoresceinated dUTP in single- and double-stranded DNA breaks by tagging the 3'-OH ends of TdT. The TdT-mediated label method of the dUTP end of a DNA break measures the extent of DNA damage by including the insertion of a DNA probe (a modified nucleotide) at the site of the DNA damage. This method allows us to determine the proportion of sperms with DNA damage that carries a modified nucleotide embedded in the DNA break. The recommendations for standardization and harmonization provide information according to which TUNEL is a reliable test for measuring SDF and suitable for conducting multicenter studies. In addition, the TUNEL method enables determination of the degree of complete and partial SDF, which is crucial for assessing its severity and the effectiveness of a treatment [20, 22–25].

SCSA method

The SCSA method or Evenson chromatin structure determination analyzes the sperm's chromatin structure. The research principle is based on measuring the susceptibility of DNA to denaturation. The sperms with denatured DNA are quantified using flow cytometry. The study is performed using a fluorescent DNA marker. They apply 1024 channels (degrees) of both red and green fluorescence. The SCSA test is a rapid measurement using flow cytometry that provides reliable statistics with exceptional accuracy and repeatability. Analysis of several

experimental studies indicates that SCSA is an effective method for determining the integrity of sperm DNA, which has been confirmed by numerous publications on the use of the SCSA test in clinical practice [20, 22, 26].

SCD method

The SCD method analyzes the chromatin dispersion of sperm. This method is used to measure the susceptibility of DNA to denaturation, and the number of sperm cells with fragmented DNA is calculated. The method is based on the dispersion of chromatin around the nucleus because of which it is possible to distinguish sperm with different degrees of SDF. Hence, to examine the dispersion of chromatin in the nuclei of a sperm, special reagents (enzymes) are used to "highlight" the heads of those sperm cells where there are breaks in the DNA. In the SCD test, the chromatin of the damaged sperm is distributed much closer to the nucleus, and in the normal sperm, it is distributed in a larger radius. Therefore, the SSD test can be used as a routine test for SDF screening [22].

The DNA-comet method (Comet)

The method of performing gel electrophoresis of individual cells or the DNA-comet technique is highly sensitive and rapid, providing highly reliable results during DNA repair system studies. In addition, it is relatively simple and feasible, and has been standardized at the international level. The method is based on the registration of different mobilities in a constant electric field of damaged DNA or DNA fragments of individual lysed cells enclosed in a thin agarose gel on a standard slide. In this case, the cell's DNA migrates, forming an electrophoretic trace that visually resembles the "tail of a comet," the parameters of which depend on the degree of DNA denaturation. The degree of DNA fragmentation in a single sperm cell is estimated by the proportion of DNA in the "tail of the comet," the length of the "tail," and the intensity of color. Nowadays, there are no exact established thresholds for the norm. Analysis of 50 sperm cells is informative and sufficient to conclude regarding the proportion of sperm with damaged DNA in the entire ejaculate [20, 22, 26].

Staining with aniline blue and chromomycin A3

This method involves measuring the level of sperm chromatin compaction based on the ratio of histones and protamines. Nonetheless, the number of sperm cells with low chromatin compaction, calculated as a percentage, is subject to review [26, 27].

INFLUENCE OF MEN'S AGE ON THE PROBABILITY OF PATHOLOGICAL FRAGMENTATION OF SPERM DNA

One of the factors that predispose to sperm DNA breaks is the age of the man. The natural aging process of the entire body directly affects the increase in sperm DNA structure imbalances. According to S.I. Moskovtsev et al. [28], compared with men younger than 30 years, SDF occurs twice as often in men older than 45 years (15.2% vs. 32.0%). Notably, SDF levels in the age groups 30–35, 35–40, and 40–45 years are 19.4%, 20.1%, and 26.4%, respectively [28]. In a meta-analysis of 26 studies involving 10,220 patients, the authors determined a negative association between the increased age in men and the degree of SDF [29]. Notably, damaged sperm DNA can affect embryonal quality, cause impaired implantation, and reduce the frequency of pregnancy [28, 29].

INFLUENCE OF NUTRITION AND ANTIOXIDANT THERAPY ON SPERM DNA INTEGRITY

Several physiological and genetic factors are associated with sperm function and infertility. ROS and oxidative stress are strongly associated with various pathologies, including aging and male infertility. Antioxidants (vitamins C, E, folic acid, L-carnitine, etc.) are extensively used in various treatment modalities to protect cells from damage caused by oxygen free radicals [30–33].

Despite the diversity of factors predisposing to male infertility, the exact cause remains unknown in several cases. When studying the idiopathic causes of male infertility at the molecular level, a significant contribution of oxidative stress was revealed, leading to an imbalance of the body's redox state caused by either too high levels of oxidants or too low levels of antioxidants [34, 35].

ROS or "free radicals" are highly reactive oxygen-derived molecules characterized by unpaired electrons in their outer valence orbit. ROS play a crucial role in signal transmission and homeostasis. They are produced by the sperm in small quantities, providing useful functional efficiency, including initiating sperm formation, regulating their maturation, and enhancing cellular signaling pathways. Howev-

er, high ROS levels can have a paradoxical effect on sperm function, leading to infertility. Some endogenous (immature spermatozoa, leukocytosis, varicocele) and exogenous (testicular hyperthermia, environmental exposure) factors have been recognized as the potential causes of increased ROS production.

Furthermore, because of an excessive amount of ROS or when the antioxidant activity is disrupted, an imbalance between oxidation and reduction occurs, causing oxidative stress – to which the spermatozoa are particularly vulnerable. They contain low levels of enzymatic antioxidants, which are not sufficient to protect sperm from high ROS levels [34].

A critical factor contributing to the repair of fragmented DNA sections is a change in nutrition, primarily filling the body's lack of polyunsaturated fatty acids. Docosahexaenoic acid is an essential polyunsaturated fatty acid of the omega 3 class – one of the most valuable polyunsaturated fatty acids for human health. Docosahexaenoic acid is a part of most body tissues, one of the most essential structural and functional components of the central nervous system, the main component of the gray matter of the brain, retina, testicles, and cell membranes of sperm. The use of docosahexaenoic acid in patients with an increased SDF index reduces sperm DNA damage and increases the antioxidant activity of the ejaculate [35, 36].

Vitamin E (α-tocopherol) is a powerful antioxidant and is a fat-soluble organic compound located in the cell membranes. It suppresses free hydroxyl radicals and superoxide anions, reducing the lipid peroxidation initiated by ROS at the plasma membrane level. A direct link has been noted between the vitamin E level in seminal plasma and the percentage of mobile sperm forms in the ejaculate. Hence, lower vitamin E levels were observed in the ejaculate of men with infertility.

Vitamin C (ascorbic acid) is a water-soluble compound, and its concentration in the seminal plasma is 10 times higher than in blood plasma. It neutralizes hydroxyl, superoxide, and peroxide radicals, protecting from endogenous oxidative damage. The seminal fluid of men with infertility with asthenozoospermia has been noted to have lower vitamin C content and a higher ROS level compared with fertile men.

Folic acid (vitamin B_9) is involved in the nucleic acid synthesis and amino acid metabolism. Nevertheless, because of its affinity to free radicals, it is possible to use this vitamin as an antioxidant in male infertility treatment. Consumption of vitamin B_9 leads to a decrease in the degree of pathological SDF [37].

Therefore, antioxidant therapy has a positive effect on the primary parameters of the ejaculate and improves its main indicators, including sperm viability that positively affects the fertilizing ability of the ejaculate, the ART results, and the frequency of live births.

Hence, the oral antioxidant supplements improve the quality of ejaculate, reduce the oxidative damage processes, and decrease the risk of potentially harmful effects, thereby favorably affecting male fertility [34, 38–40].

INFLUENCE OF VARICOCELE ON SPERM DNA

One of the factors that affect fertility and gestation is varicocele. Varicocele is considered as one of the ways of pathological SDF correction. Microsurgical varicocelectomy increases the frequency of spontaneous pregnancy and improves the ART results (including cases of unsuccessful ART attempts in the anamnesis). Notably, varicocelectomy has been proven to reduce oxidative stress effects and the degree of SDF, thereby helping to mitigate reproductive losses (especially in the first trimester of pregnancy). Therefore, assessing the level of SDF in patients with varicocele will help evaluate and predict the probability of conception in this category of patients. Surgical treatment for varicocele can improve sperm DNA integrity, increasing the chances of conception, or improving the prognosis of ART procedures [12, 21, 22, 32, 41].

A study by G. Pourmand et al. [32] included 100 men with infertility and left grade II varicocele. After the examination, all patients underwent varicocelectomy using the Marmara method. Patients were divided into two groups, and those in group 1 did not receive sperm-producing therapy during the postoperative period. Patients in group 2 were given a complex of micronutrients for 6 months from the first postoperative day. A spermogram and additional sperm tests were performed and evaluated before the operation and 6 months after it. According to the authors, surgical treatment for varicocele improved the integrity of sperm DNA and increased the probability of impregnation or the effectiveness of ART procedures in group 1. Nevertheless, surgical treatment,

along with complex of micronutrients (in group 2 patients) had the most significant positive effect on both the primary parameters of the spermogram and the degree of SDF. Therefore, the authors recommended performing additional tests, especially to determine the level of SDF, when examining all men with fertility disorders. Nonetheless, it is imperative to assess SDF in patients with varicocele, even with normal zoospermia, to accurately predict the ability to conceive in this category of patients [32].

CONCLUSION

Therefore, determining the degree of SDF plays a crucial role in the andrology practice because it helps to accurately predict the probability of pregnancy, its course, and results, both during the natural reproductive cycle and in vitro fertilization or intracytoplasmic sperm injection protocols. Notably, TUNEL is one of the most optimal methods for evaluating SDF. Moreover, antioxidant therapy and varicocele surgery can normalize the structural integrity of the sperm DNA.

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