Results. A relationship between high-risk disease and male gender, multifocal lesions, localization in the lower third of the thyroid lobe, superficial location of the tumor, and stromal fibrosis was showing in Sak S. D.`s study. According to the results of studies, size of tumor larger than 1.0 cm and multifocal lesions can be considered an indicator of the aggressive course of PTC. AIT complicates the cytological diagnosis of nodal masses. Cellular atypia, anisonucleosis, and pleomorphism are observed in both AIT and PTC, so differential diagnosis in the case of a combination of these pathologies is more difficult. The basis for this relationship is the molecular, hormonal and histopathological similarity of these diseases. Galectin-3 is a regulator of normal cell proliferation, and its overexpression leads to malignant cell transformation and metastasis. The neoplastic transformation of benign tissue is a complex process influenced by numerous factors. Humoral mechanisms of cancer development are associated with the IgG4 subclass. Regulatory Treg cells are a subgroup of CD4+ cells that suppress the immune response, playing an important role in avoiding the host's antitumor immune response. It is reported that a decrease in CD4+ leads to oncological transformation. The BRAF mutation has also been shown to cause overexpression of many other molecular tumour markers, such as VEGF and MET.

Conclusions. The level of molecular genetic markers associated with participation in apoptosis, increased proliferation rate and angiogenesis, as well as the duration of inflammation can be used to establish the diagnosis, predict the course and assess the risk of PTC metastasis.

Key words: thyroid glands, papillary carcinoma, combine pathology, metastasis, molecular markers.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

Received 20.03.2023
Accepted 28.08.2023

DOI 10.29254/2077-4214-2023-3-170-88-98
UDC 612.613.1:57.086.13

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DOES CRYOPRESERVATION UNIFORMLY AFFECT THE DNA FRAGMENTATION RATE OF SPERMATOZOA OF MEN WITH DIFFERENT STATES OF SPERMATOGENESIS? A SYSTEMATIC REVIEW AND META-ANALYSIS

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Cryotechnologies have become an integral part of infertility treatment methods. However, the question of the impact of cryopreservation on human sperm DNA fragmentation levels remains open. The aim was to conduct a systematic review and meta-analysis of studies on the effect of the cryopreservation method (a method of rapid cooling and vitrification) on the occurrence of DNA damage in spermatozoa of men with different states of spermatogenesis. The search was carried out in Medline, Embase, PubMed and Google Scholar databases. The results of 52 studies from 32 scientific papers were analysis in the present study. DNA damage in spermatozoa of men with different states of spermatogenesis was measured before and after cryopreservation using one of these methods: SCSA, SCD, TUNEL and Comet Assay. It was found that cryopreservation, regardless of the method used, leads to an increase of 7.73±2.04% in the DNA fragmentation rate of spermatozoa, regardless of the state of spermatogenesis of men. Cryopreservation increases normospermia sperm DNA fragmentation compared to pathospermia (SMD=8.76%, 95% CI 6.22 – 11.31%, p<0.00001 and SMD=4.01%, 95% CI 2.23 – 5.79%, p<0.00001, respectively). The greatest effect of cryopreservation on DNA fragmentation compared to fresh sperm was determined by the TUNEL method. Using this method, 8.08±2.38% of cells with the mentioned pathology were identified (SMD=8.08%, 95% CI 5.69 – 10.46%, p<0.00001). Determining the degree of DNA damage of spermatozoa before their banking can be a useful marker of the effectiveness of their cryopreservation, as it has prognostic value regarding the effectiveness of infertility treatment by assisted reproductive technology methods.

Key words: *DNA fragmentation rare, DNA integrity, DNA damage, cryopreservation, rapid freezing, vitrification, human spermatozoa.*

Connection of the publication with planned research works.

This article is a part research work of the Institute of Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine №: III-4, 2.2.6. 128 «Determination of cryoresistance of reproductive cells depending on their species specificity and morphological and functional characteristics», state registration number 0120U100546.

Introduction.

Cryopreservation is an integral component of assisted reproductive technologie (ART) [1]. Highly effective methods of low-temperature storage of spermatozoa opened the way to banking and wide clinical application of spermatozoa in ART methods. For all that, the study of the morphological, ultrastructural and functional characteristics of spermatozoa remains relevant [2, 3, 4].

Cryopreservation of germ cells is recommended for men with dangerous professions: soldiers, rescuers, firefighters, policemen, and patients with oncological diseases before radiation and chemotherapy, which are associated with a high risk of reduced fertility, in order to preserve the reproductive potential of the family. Surgical interventions on the organs of the reproductive system can lead to ejaculatory dysfunction and insufficient function of the testicles, therefore complex operations on the genitals are also a medical indication for sperm cryopreservation [5].

Cryopreservation of spermatozoa is a necessary procedure before diagnostic testicular biopsy [6].

Indication for cryopreservation is the artificial insemination, in which the use of frozen-warmed spermatozoa is more effective than fresh spermatozoa. Thus, sperm accumulation is carried out in cases of oligo-, astheno- and teratozoospermia. For this, several ejaculates are cryopreserved. After they are heated, the fraction of actively motile spermatozoa is separated from all accumulated samples, which allows to increase the number of spermatozoa in the dose used for artificial insemination [7].

Modern approaches in the field of reproductive medicine use cryopreservation not only for medical, but also for social indicators, for example, under the conditions of a married couple's conscious choice of career and postponing conception and bearing a child to a later reproductive period. However, the patients should be informed about possible influence of cryopreservation on the genetic characteristics of reproductive cells [8].

To date, there is no single universal approach that would guarantee 100% survival of male reproductive cells. Cryopreservation protocols are constantly being improved in order to secure high rates of motility, morphological integrity and fertilizing ability of spermatozoa [9]. Specialists face the question of ensuring the stability of the genetic apparatus of male cells after cryopreservation, since the correct DNA replication and expression of sperm genes after oocyte fertilization are crucial for the further development of the embryo and the birth of a healthy child [10].

The frequency of DNA fragmentation of spermatozoa can be a predictor of the success of fertilization of oocytes by the method of intracytoplasmic sperm injection [11], qualitative characteristics of embryos [12], which ultimately affects the cumulative level of live birth [13].

Today, the question of the DNA condition of cryopreserved spermatozoa remains open. A number of reports showed significant changes in DNA integrity after freezing-thawing, for example, in male smokers [14], while other studies did not reveal such changes [15]. The difference in the obtained results can be explained by the use of spermatozoa from the ejaculates of men with different states of spermatogenesis, which are characterized by heterogeneous initial morphological and functional characteristics, and in the implementation of cryopreservation methods that differ in the choice of cryoprotectant, exposure, speed of cooling and rewarming, approaches to cryoprotectant removal, and the use of rehabilitative media.

The aim of the work.

To analyze whether cryopreservation by different methods affects the increase in the frequency of DNA fragmentation of spermatozoa of men with different states of spermatogenesis.

Object and research methods.

Literature search strategy.

A systematic, comprehensive search of the literature published in the MEDLINE-PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) and a manual search of the reference lists of the retrieved articles was conducted in accordance with the preferred reporting items for systematic reviews and objective analyses. We searched the following electronic databases: using the following keywords: "DNA integrity", "DNA fragmentation", "DNA damage", "cryopreservation", "quick freezing", "vitrification", "human spermatozoa".

Inclusion and exclusion criteria.

Studies that presented the relationship between DNA damage of spermatozoa of men with different states of spermatogenesis (normozoospermia and pathospermia) before and after cryopreservation were included in the meta-analysis if they met the following criteria: cryopreservation was performed by one of two methods (rapid and/or vitrification). Sperm DNA condition was detected by SCSA, SCD, TUNEL, or Comet Assay.

Studies were included if data on the DNA fragmentation rate, expressed as mean ± standard deviation (SD), could be directly extracted from the original article. The exclusion criteria were: (1) lack of primary data; (2) conference abstracts; (3) small sample size (n<10). Studies were excluded if: 1) the articles did not indicate the state of spermatogenesis of men in whom the DNA fragmentation rate was studied; 2) the level of DNA fragmentation before and after cryopreservation was not compared; 3) no SD value; 4) the results were published in the form of a literature review.

The analyzed data included sample sizes and outcome parameters with mean and standard deviation. Measurement results were converted to SMD with 95% CI. Heterogeneity was assessed using the I2 statistic to

Table – The main characteristics of the studies used in meta-analysis

Author, year	Number of samples	Method of cryopreservation	Method of determination of DNA fragmentation	Normo- or pathospermia	Reference
Agha-Rahimi A 2014a	30	Rapid	Comet Assay	Normozoospermia	[16]
Agha-Rahimi A 2014b	30	Vitrification	Comet Assay	Normozoospermia	[16]
Aizpurua 2017a	18	Rapid	TUNEL	Normozoospermia	[17]
Aizpurua 2017b	18	Vitrification	TUNEL	Normozoospermia	[17]
Alqawasmeh 2021	101	Rapid	SCD	Normozoospermia	[18]
Amor 2018a	29	Rapid	TUNEL	Normozoospermia	[19]
Amor 2018b	14	Rapid	TUNEL	Pathospermia	[19]
Bateni 2014	19	Rapid	TUNEL	Normozoospermia	[20]
de Paula 2006a	30	Rapid	TUNEL	Normozoospermia	[21]
de Paula 2006b	47	Rapid	TUNEL	Pathospermia	[21]
Di Santo 2010a	15	Rapid	TUNEL	Normozoospermia	[22]
Di Santo 2010b	15	Rapid	TUNEL	Pathospermia	[22]
Duru 2001a	16	Rapid	TUNEL	Normozoospermia	[23]
Duru 2001b	16	Rapid	TUNEL	Pathospermia	[23]
Ekrami 2020	25	Rapid	SCD	Normozoospermia	[24]
Frainais 2010a	20	Rapid	TUNEL	Normozoospermia	[25]
Frainais 2010b	20	Rapid	TUNEL	Pathospermia	[25]
Karabulut 2018	72	Rapid	TUNEL	Normozoospermia	[26]
Khalili 2014a	34	Vitrification	TUNEL	Normozoospermia	[27]
Khalili 2014b	34	Vitrification	TUNEL	Pathospermia	[27]
Khodayari Naeini 2014	10	Rapid	SCD	Normozoospermia	[28]
Le 2019a	20	Rapid	SCD	Normozoospermia	[29]
Le 2019b	20	Rapid	SCD	Pathospermia	[29]
Le 2019c	20	Rapid	SCD	Normozoospermia	[29]
Le 2019d	20	Rapid	SCD	Pathospermia	[29]
Le 2019e	20	Rapid	SCD	Normozoospermia	[29]
Le 2019f	20	Rapid	SCD	Pathospermia	[29]
Liu 2018	150	Rapid	TUNEL	Normozoospermia	[30]
Lusignan 2018	27	Rapid	TUNEL	Normozoospermia	[31]
Marchiani 2014	36	Rapid	TUNEL	Normozoospermia	[32]
McEvoy 2014	164	Vitrification	SCD	Normozoospermia	[33]
Meseguer 2011	210	Rapid	SCD	Normozoospermia	[34]
O'Neill 2019a	53	Rapid	SCD	Normozoospermia	[35]
O'Neill 2019b	53	Vitrification	SCD	Normozoospermia	[35]
Raad 2018a	100	Rapid	SCD	Normozoospermia	[36]
Raad 2018b	100	Rapid	SCD	Normozoospermia	[36]
Raad 2018c	100	Rapid	SCD	Normozoospermia	[36]
Raad 2018d	100	Rapid	SCD	Normozoospermia	[36]
Raad 2018e	100	Rapid	SCD	Normozoospermia	[36]
Rahiminia 2017	20	Vitrification	TUNEL	Normozoospermia	[37]
Ribas-Maynou 2014	44	Rapid	TUNEL	Normozoospermia	[38]
Riva 2018a	18	Rapid	TUNEL	Normozoospermia	[15]
Riva 2018b	18	Rapid	TUNEL	Normozoospermia	[15]
Saeednia 2015	25	Rapid	SCD	Normozoospermia	[39]
Saeednia 2016	25	Rapid	TUNEL	Normozoospermia	[40]
Shabani Nashtaei 2018	22	Not indicated	SCD	Normozoospermia	[41]
Slabbert 2015	35	Rapid	TUNEL	Normozoospermia	[42]
Thompson-Cree 2003	46	Rapid	Comet Assay	Pathospermia	[43]
Thomson 2010	240	Rapid	TUNEL	Normozoospermia	[44]
Toro 2009	7	Rapid	TUNEL	Normozoospermia	[45]
Vutyavanich 2012	20	Rapid	Comet Assay	Normozoospermia	[46]
Yan 2020	22	Rapid	SCSA	Normozoospermia	[47]

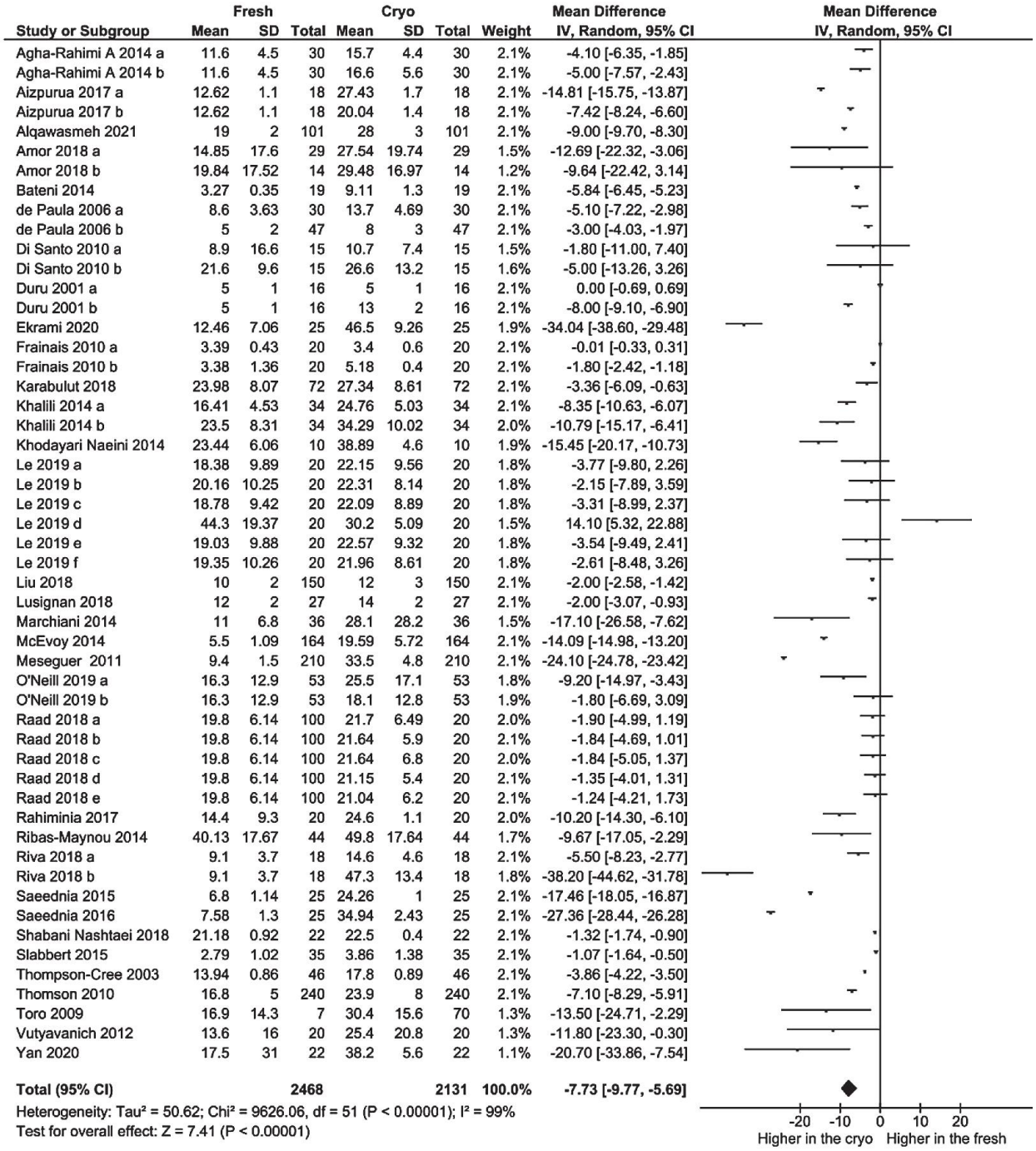


Figure 1 – Basic characteristics of the eligible studies included in the present meta-analysis.

quantify the percentage of total variation across studies. The random effects were used as analytical model, because statistically significant heterogeneity of the data was found (if the I2 value was greater than 50%, the final score was analyzed in the random effect model). A sensitivity analysis was performed to assess whether any individual study affected the stability of the meta-analytic results by sequentially removing an individual study.

Statistical analysis was performed using RevMan 5.4 (Inverse Variance statistical method).

Research results.

Acceptable studies.

The literature search yielded information on 156 potentially relevant studies. Based on the information in the title or abstract, 43 of those were removed from the meta-analysis. Other 67 were removed due to the lack of full access to the research results, 14 open access articles were excluded due to the review nature of the work and the lack of original data. For the meta-analysis, 33 papers were used, which indicated the state of spermatogenesis in men whose spermatozoa were cryopreserved using the rapid and/or vitrification method, and the level of DNA fragmentation was determined by the methods of SCSA (n=1), SCD (n=20), TUNEL (n=27), and Comet Assay (n=4).

The studies of DNA fragmentation rate of fresh and cryopreserved human spermatozoa were divided into comparing groups: depending on the spermatogenesis state (normo- and pathospermia); depending on the

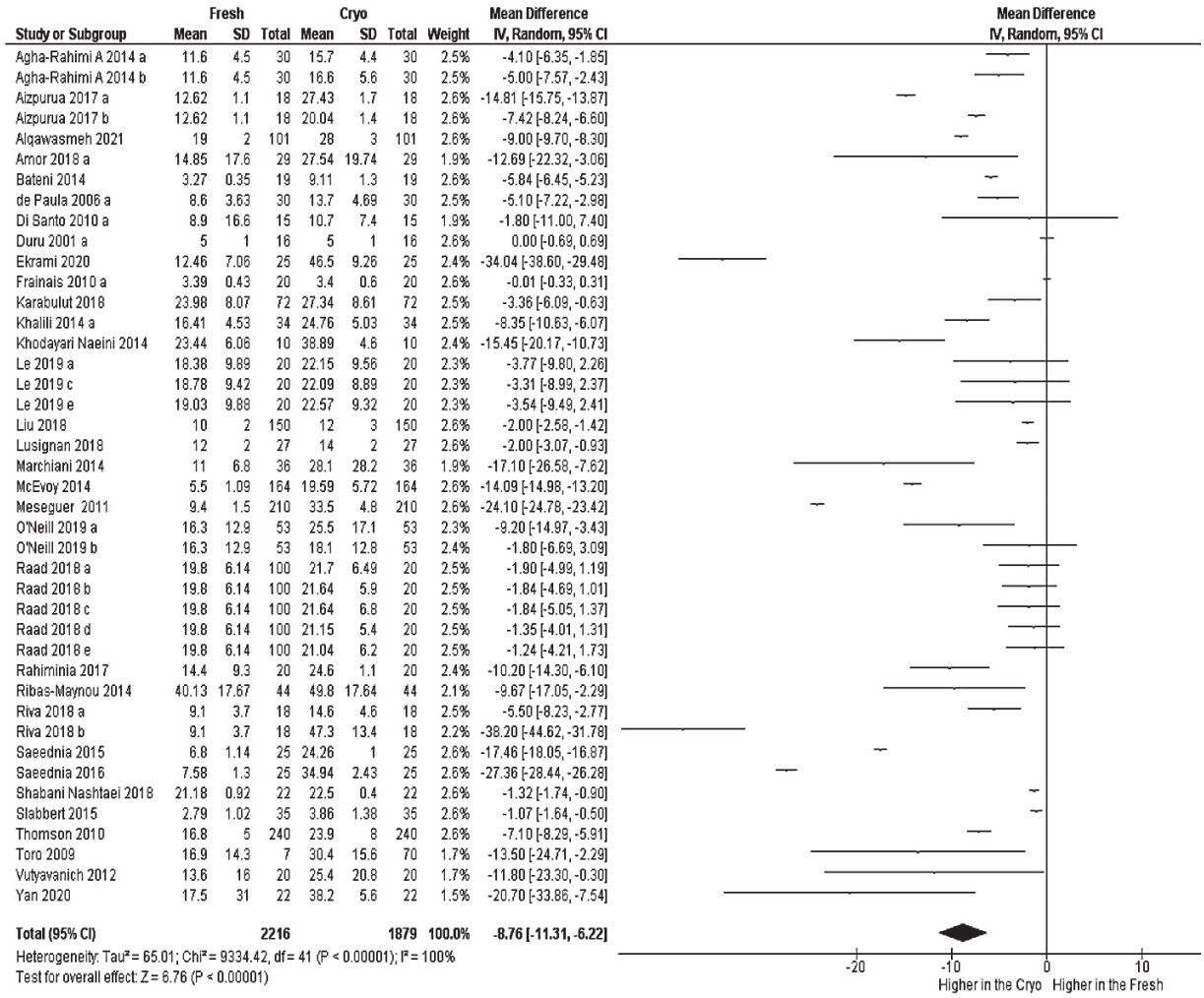


Figure 2 – The effect of cryopreservation of human spermatozoa with normozoospermia on the DNA fragmentation rate. The method of cryopreservation and the method of determination of DNA fragmentation rate were not taken into account.

cryopreservation method and depending on the method of its determination (SCSA, SCD, TUNEL or Comet Assay).

The total sample of the analyzed scientific works indicating the method of cryopreservation, the method of determining DNA fragmentation and the state of spermatogenesis in men is presented in **table.**

After cryopreservation, the frequency of DNA fragmentation increased by 7.73±2.04% compared to the rate for native male germ cells (number of comparisons n=52). The data are presented as a "forest plot", that is, a representation of the results of individual studies in the form of a diagram, which consists of a series of horizontal segments that reflect the confidence intervals of the studied value estimated in the analyzed works **(fig. 1).**

Thus, taking into account all the works included in the meta-analysis, the DNA fragmentation was rate

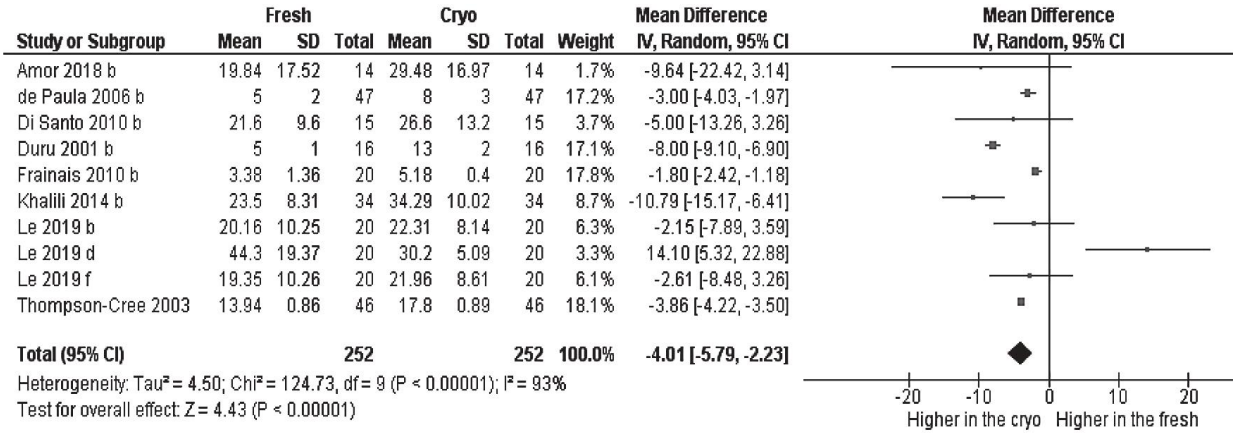


Figure 3 – The effect of cryopreservation of human spermatozoa with pathospermia on the DNA fragmentation rate. The method of cryopreservation and the method of determination of DNA fragmentation rates were not taken into account.

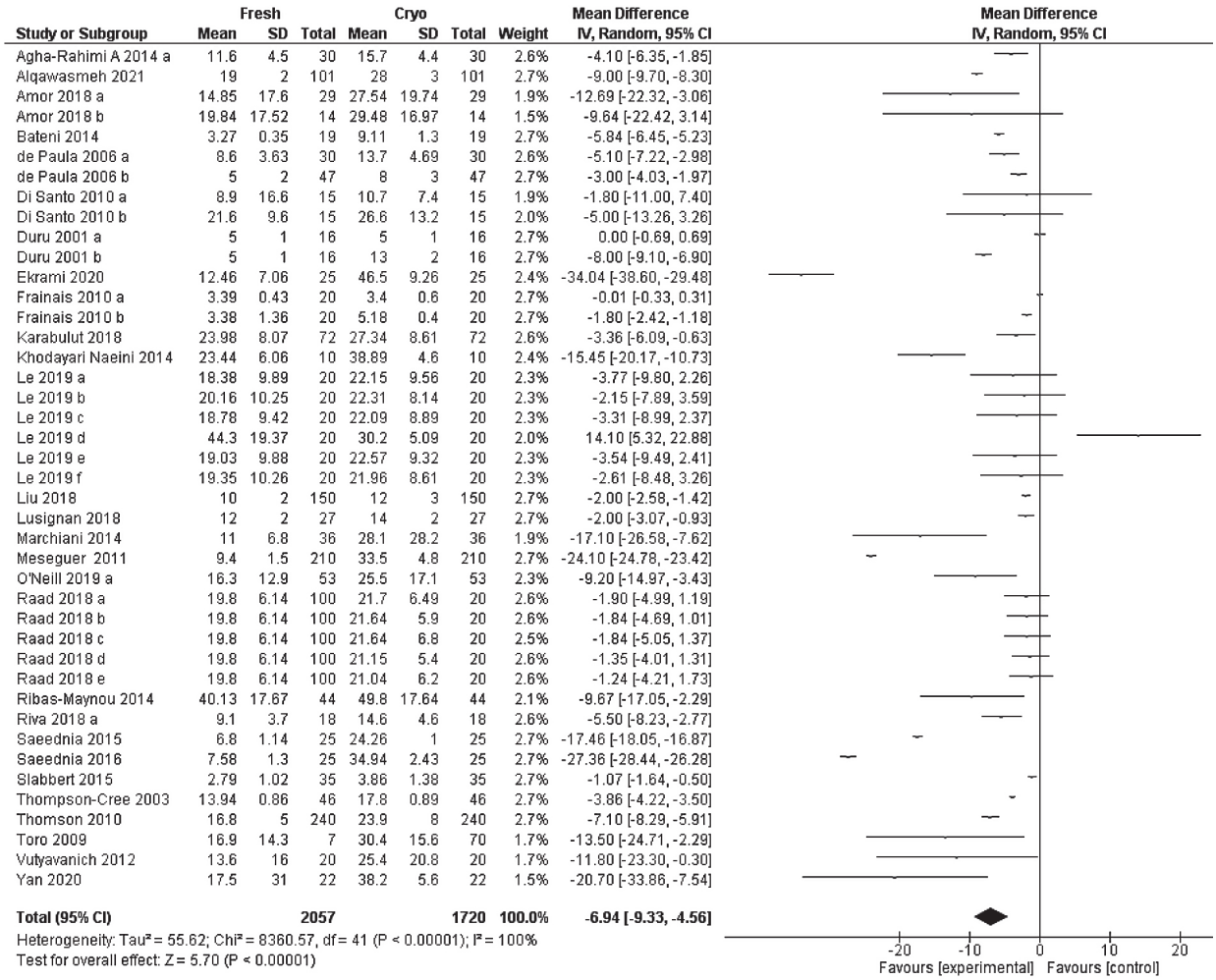


Figure 4 – The effect of cryopreservation by rapid freezing on the DNA fragmentation rate of spermatozoa. The method of determination of DNA fragmentation rate and the state of spermatogenesis were not taken into account.

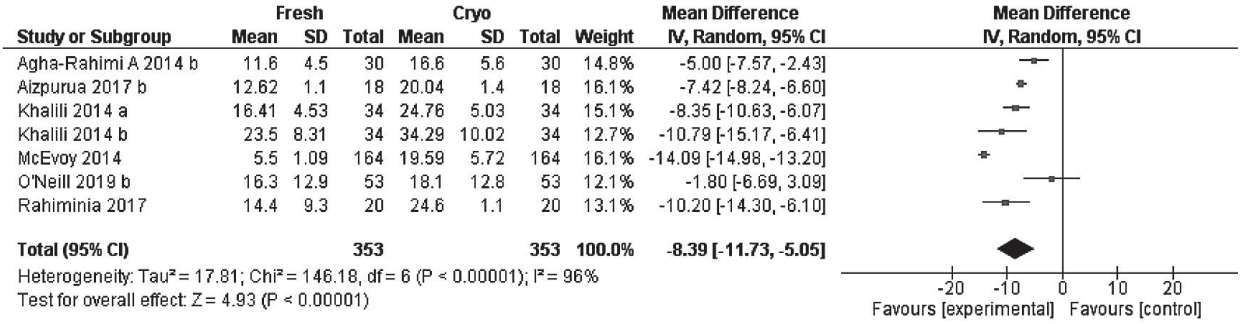


Figure 5 – Effect of cryopreservation by vitrification on the DNA fragmentation rate of spermatozoa. The method of determination of DNA fragmentation rate and the state of spermatogenesis were not taken into account.

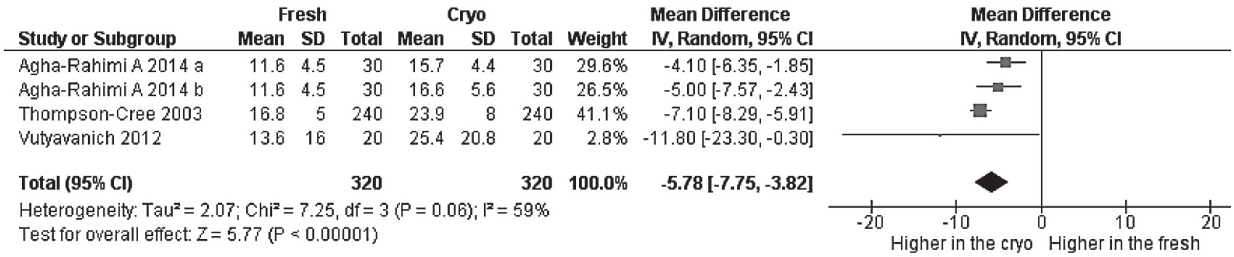
significantly higher, compared to fresh samples after freezing-warming, regardless of the used cryopreservation method, the state of spermatogenesis of men and methodical approaches to determine the indicated parameter.

DNA fragmentation of human sperm under conditions of normo- and pathospermia.

Comparison of the effect of cryopreservation on DNA fragmentation of spermatozoa depending on their morphological and functional characteristics included works in which the rate of DNA fragmentation was determined by one of the specified methods (SCSA, SCD, TUNEL, Comet Assay) and cryopreservation was performed both by a rapid method or by vitrification **(fig. 2, fig. 3)**. Cryopreservation increased the DNA fragmentation rate of spermatozoa by 8.76±2.54% and 4.01±1.78% compared to fresh cells in the case of normo- and pathospermia, respectively (SMD=8.76%, 95% CI, 6.22 – 11.31%, P<0.00001 and SMD=4.01%, 95% CI, 2.23 – 5.79%, P<0.00001 respectively). The number of comparisons in the samples was 42 and 10 under conditions of normo- and pathospermia, respectively. Thus, cryopreservation has a significant effect on the DNA fragmentation rate of human spermatozoa with normozoospermia.

DNA fragmentation of human spermatozoa depending on the cryopreservation method.

Comparison of the effect of cryopreservation on DNA fragmentation of human spermatozoa depending



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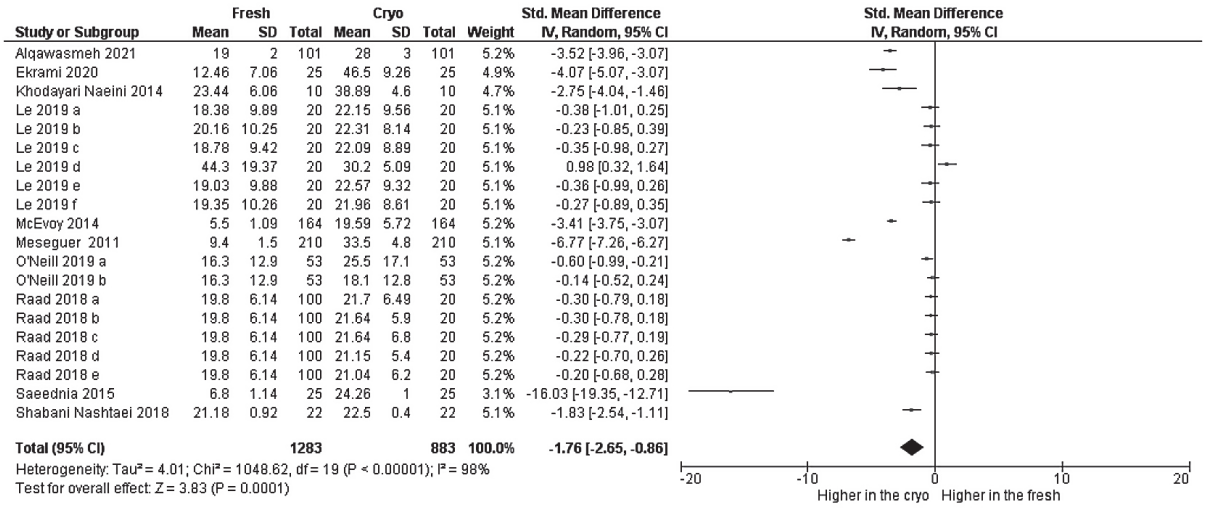

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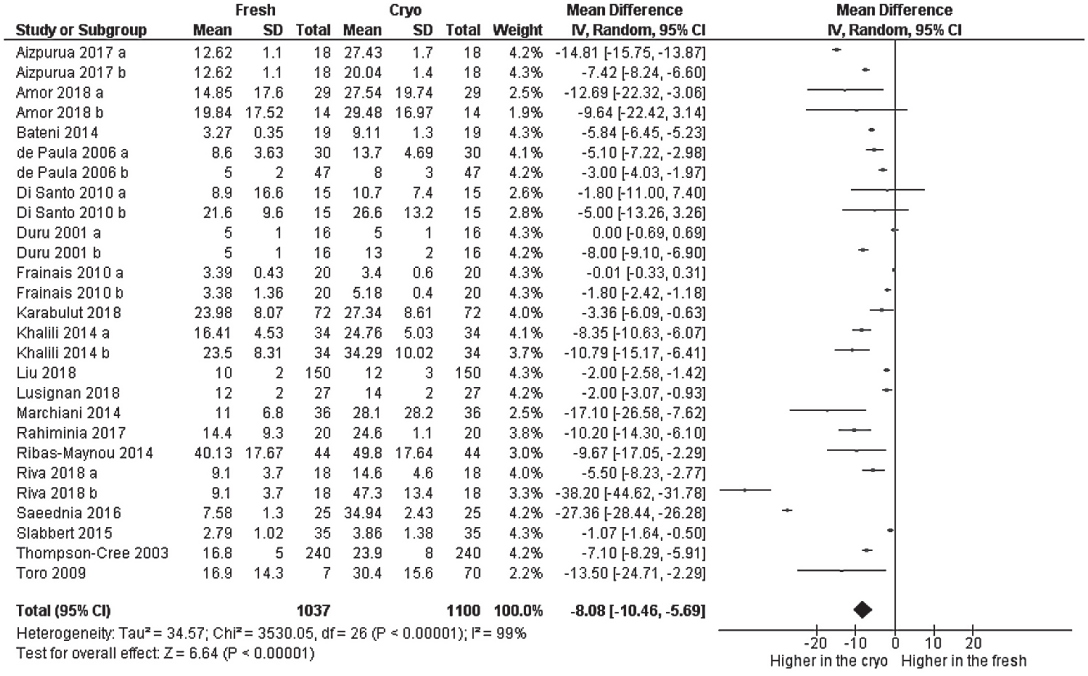

C

Figure 6 – Determination of the effect of cryopreservation on the DNA fragmentation rate of spermatozoa by the Comet Assay (a), SCD (b), TUNEL (c) methods. The method of cryopreservation and the state of spermatogenesis were not taken into account.

on the cryopreservation method (rapid freezing or vitrification) is important when choosing a strategy for the preparation of male germ cells for long-term biobanking.

In the case of rapid freezing, the DNA fragmentation rate in spermatozoa compared to fresh cells increased by 6.94±2.38% (SMD=6.94%, 95% CI 4.56 – 9.33%, p <0.00001), number of comparisons n=42 **(fig. 4).**

In case of vitrification, the corresponding indicator was 8.39±3.34% (SMD=8.39%, 95% CI 5.05 – 11.73%, p <0.00001), number of comparisons n=7 **(fig. 5).**

Thus, vitrification of human spermatozoa results in a slight increase in sperm DNA fragmentation compared

to rapid freezing, although this difference is not significant. However, the vitrification protocol may be a potential risk factor for DNA fragmentation.

DNA fragmentation of human spermatozoa depending on the method of its determination.

The Comet Assay method revealed 5.78±1.96% of cells with DNA damage (SMD=5.78%, 95% CI 3.82 – 7.75%, p=0.06) **(fig. 6a).**

According to the SCD method, 7.00±4.44% of male germ cells with DNA fragmentation were detected (SMD=7.00%, 95% CI 2.55 – 11.44%, p <0.00001), the number of comparisons n=20 **(fig. 6в).**

The greatest effect of cryopreservation on DNA fragmentation compared to fresh sperm was determined by the TUNEL method. Using this method, 8.08±2.38% of cells with the specified pathology were identified (SMD=8.08%, 95% CI 5.69 – 10.46%, p <0.00001), the number of comparisons n=27 **(fig. 6c).**

It should be noted that when determining the DNA fragmentation rate of spermatozoa by the SCD method, the spread of values was almost twice as large compared to the TUNEL method, although the number of comparisons in the samples is approximately the same. Of course, the number of comparisons of works in which the Comet Assay method was used to determine DNA fragmentation, is quite small (p=0.06; I^2 =59%).

Discussion of research results.

The results of the conducted meta-analysis prove that cryopreservation reduces the integrity of DNA. This statement was confirmed by other authors who reported about increasing sperm DNA fragmentation rate after cryopreservation [21, 48].

Based on the results of the meta-analysis, it can be stated that regardless of the cryopreservation method, initial morphological and functional characteristics and methods of determination, the DNA fragmentation rate in cryopreserved cells was significantly higher compared to fresh cells.

Currently, studies analyzing the relationship between the choice of cryopreservation method and sperm DNA damage at different rates of spermatogenesis have shown conflicting results.

The effects of slow freezing and vitrification on sperm DNA integrity in the absence of cryoprotectant were compared and it was found that cryopreservation does not damage DNA integrity [49]. Another study showed that slow cryopreservation causes DNA fragmentation indirectly by disrupting the mitochondrial membrane potential and activating caspases 3, 8, and 9. Cryopreservation increases the activation of caspase 3, which leads to DNA fragmentation. The authors also found a connection between caspase activation, externalization of phosphatidylserine and DNA fragmentation [26].

The conclusion about an increase in the frequency of DNA fragmentation under conditions of normozoospermia, compared to pathospermia, somewhat contradicts the established opinion [50], since it was shown that spermatozoa of fertile men were more resistant to damage than samples of infertile men [51].

We can explain this by the fact that in the process of cryopreservation, spermatozoa with DNA damage have a high cryosensitivity, and therefore a low survival rate. After cryopreservation, spermatozoa are isolated from the sample by the “swim up” method. The damaged spermatozoa do not float, thus they cannot be analyzed further.

The question of choosing a method for assessing the degree of DNA fragmentation is relevant: first of all, it should be highly sensitive. According to the results of our meta-analysis, the TUNEL method was the best among compared. A 2018 meta-analysis on the detection of the degree of DNA fragmentation in infertile men showed the same sensitivity of the methods used [52]. In a meta-analysis, we compared methods for analyzing the chromatin structure (Sperm Chromatin Structure Assay, SCSA), a flow cytometric test in which sperm DNA breaks are assessed indirectly using DNA denaturation followed by staining with the fluorescent dye acridine orange [53]); the SCD method, which analyzes the so-called halo, which is observed in spermatozoa with a normal DNA structure [54]; the TUNEL method, which quantifies the incorporation of fluorescent dUTP into single- and double-stranded DNA breaks, marking the 3’-OH-end of TdT [55] and DNA gel electrophoresis of individual cells (Comet Assay), which allows determining the content of high- and low-molecular-weight DNA by halo of low-molecular-weight DNA [56].

Unfortunately, the degree of fragmentation by the SCSA method was analyzed in only one paper. Most of the studies were performed using the TUNEL and SCD methods. This is due to the fact that the majority of scientific studies of DNA fragmentation of human spermatozoa have been conducted using the TUNEL method, which has the ability to detect both single- and double-strand breaks. In clinical research, the SCD method is mainly used, as it is the most simple, convenient and informative.

Conclusions.

Cryopreservation of human spermatozoa, regardless of their initial morphological and functional characteristics, leads to an increase in the level of DNA fragmentation by 7.73±2.04%. Cryodamage of DNA integrity depends on the initial state of spermatogenesis. Thus, the DNA fragmentation rate in normozoospermia is higher by 4.75% than in pathospermia. The greatest effect of cryopreservation on DNA fragmentation compared to fresh sperm was determined by the TUNEL method.

Determining the degree of DNA damage of spermatozoa before their banking can be a useful marker of the effectiveness of their cryopreservation, as it has prognostic value regarding the effectiveness of infertility treatment by ART methods.

Prospects for further research.

The results of the presented work can be used in counselling patients on infertility in reproductive medicine clinics.

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ЧИ ОДНАКОВО ВПЛИВАЄ КРІОКОНСЕРВУВАННЯ НА РІВЕНЬ ФРАГМЕНТАЦІЇ ДНК СПЕРМАТОЗОЇДІВ ЧОЛОВІКІВ З РІЗНИМ СТАНОМ СПЕРМАТОГЕНЕЗУ? СИСТЕМАТИЧНИЙ ОГЛЯД ТА МЕТА-АНАЛІЗ

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Резюме. Кріотехнології стали невід'ємною складовою методів лікування безпліддя. Проте питання впливу кріоконсервування на рівень фрагментації ДНК сперматозоїдів людини залишається відкритим.

Мета дослідження. Провести систематичний огляд та мета-аналіз досліджень щодо впливу методу кріоконсервування (спосіб швидкого охолодження та вітрифікації) на виникнення пошкоджень ДНК сперматозоїдів чоловіків з різним станом сперматогенезу.

Об'єкт і методи дослідження. Пошук здійснювали в базах даних Medline, Embase, PubMed та Google Scholar. У статті проаналізовано 52 дослідження, які були викладено у 32 наукових статтях.

Результати та їх обговорення. Пошкодження ДНК сперматозоїдів чоловіків із різним станом сперматогенезу вимірювали перед та після кріоконсервування за допомогою одного з чотирьох методів: SCSA, SCD, TUNEL та Comet Assay. Було з'ясовано, що кріоконсервування, незалежно від використаного способу, призводить до збільшення на 7,73±2,04% рівня фрагментації ДНК сперматозоїдів, незалежно від стану сперматогенезу чоловіків. Кріоконсервування збільшує фрагментацію ДНК за нормоспермії у порівнянні з патоспермією, (ССР=8,76%, 95% ДІ 6,22 – 11,31%, p<0,00001 та ССР=4,01%, 95% ДІ, 2,23 – 5,79%, p<0,00001, відповідно). Найбільший ефект кріоконсервування на фрагментацію ДНК у порівнянні з нативною спермою визначався методом TUNEL. За цим методом було визначено 8,08±2,38% клітин з вказаною патологією (ССР=8,08%, 95% ДІ 5,69 – 10,46%, p<0,00001).

Висновки. Визначення ступеня пошкодження ДНК сперматозоїдів перед їх банкуванням може стати корисним маркером ефективності їх кріоконсервування, оскільки має прогностичне значення щодо ефективності лікування безпліддя методами допоміжних репродуктивних технологій.

Ключові слова: фрагментація ДНК, цілісність ДНК, пошкодження ДНК, кріоконсервування, швидке заморожування, вітрифікація, сперматозоїди людини.

DOES CRYOPRESERVATION UNIFORMLY AFFECT THE DNA FRAGMENTATION RATE OF SPERMATOZOA OF MEN WITH DIFFERENT STATES OF SPERMATOGENESIS? A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract. Cryotechnologies have become an integral part of infertility treatment methods. However, the question of the impact of cryopreservation on human sperm DNA fragmentation levels remains open.

Aim. To conduct a systematic review and meta-analysis of studies on the effect of the cryopreservation method (a method of rapid cooling and vitrification) on the occurrence of DNA damage in spermatozoa of men with different states of spermatogenesis.

Object and methods. The search was carried out in Medline, Embase, PubMed and Google Scholar databases.

Results. The results of 52 studies from 32 scientific papers were analysis in the present study. DNA damage in spermatozoa of men with different states of spermatogenesis was measured before and after cryopreservation using one of these methods: SCSA, SCD, TUNEL and Comet Assay. It was found that cryopreservation, regardless of the method used, leads to an increase of 7.73±2.04% in the DNA fragmentation rate of spermatozoa, regardless of the state of spermatogenesis of men. Cryopreservation increases normospermia sperm DNA fragmentation compared to pathospermia (SMD=8.76%, 95% CI 6.22 – 11.31%, p<0.00001 and SMD=4.01%, 95% CI 2.23 – 5.79%, p<0.00001, respectively). The greatest effect of cryopreservation on DNA fragmentation compared to fresh sperm was determined by the TUNEL method. Using this method, 8.08±2.38% of cells with the mentioned pathology were identified (SMD=8.08%, 95% CI 5.69 – 10.46%, p<0.00001).

Conclusions. Determining the degree of DNA damage of spermatozoa before their banking can be a useful marker of the effectiveness of their cryopreservation, as it has prognostic value regarding the effectiveness of infertility treatment by assisted reproductive technology methods.

Key words: DNA fragmentation rare, DNA integrity, DNA damage, cryopreservation, rapid freezing, vitrification, human spermatozoa.

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Conflict of interest:
The Authors declare no conflict of interest.

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Received 27.03.2023
Accepted 28.08.2023

DOI 10.29254/2077-4214-2023-3-170-98-109
UDC 616.314-085-74

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MODERN ADHESIVE SYSTEMS AND TECHNIQUES OF THEIR APPLICATION FOR DIRECT RESTORATION OF HARD DENTAL TISSUES DEFECTS

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The widespread use of adhesive systems in many branches of modern dentistry is often accompanied by complications associated with their physical, mechanical and biological characteristics, mistakes at the stages of adhesive preparation, and the irrational choice of bonding agents for individual clinical situations. The work aimed to study the known physical, mechanical and clinical properties of modern adhesive systems, the latest trends and approaches to their improvement, and prospects for the further development of restorative dentistry. The most popular adhesive systems for direct photocomposite restorations remain bonds of the V and VII generations, which are used according to the classic protocols of total etching or self-etching, respectively. The obtained results of laboratory and clinical studies of these adhesive systems are often contradictory and emphasize the problem of creating a reliable connection between the photocomposite and the dentin of the teeth. In order to increase the clinical effectiveness of direct photocomposite restorations, universal adhesive systems and self-adhesive composites were developed, which have extended indications for use in certain clinical situations and significantly simplify adhesive preparation. However, the existing experience of using these new materials also demonstrates the development of complications, even in the short period of operation of restorations. To date, no adhesive systems have an optimal set of physicomechanical and clinical characteristics for the direct restoration of defects in the hard tissues of teeth with photocomposite materials. The known results of laboratory and clinical studies of similar adhesive systems demonstrate significant discrepancies regarding the indicators of adhesive strength, the tightness of the dentin-photocomposite interface, the success of restorations, which is explained by the lack of standardized methods of their testing and clear criteria for evaluating clinical effectiveness.

Key words: *adhesive systems, photocomposite restorations, adhesive preparation technique, clinical effectiveness, adhesive strength.*

Connection of the publication with planned research works.

This work was carried out as part of the SRW of the Department of Stomatology, «Optimization of approaches to the diagnosis, treatment and prevention of dental diseases», state registration number 0120U104631.

Introduction.

In order to restore the anatomical shape of the teeth, photocomposite materials are widely used. A reliable connection of restorations and hard tooth tissues is provided by adhesive systems (AS), which are monomers of organic resins with small inorganic filler content. Several requirements are put forward to AS. In particular, they must be bioinert, have high physical and