STUDY ON THE RELATIONSHIP BETWEEN MALE
INFERTILITY AND DNA INTEGRITY IN SPERMATOZOA
NUCLEI

Vesela Mitkovska, Spas Dzhoglov, Doychin Boyadzhiev^{*},
Penka Vasileva, Ivan Stoyanov, Teodora Popova,
Teodora Staykova, Evgeniya N. Ivanova[#]

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Abstract

The purpose of the present study is to assess the potential relationships between the diagnoses for male infertility based on conventional sperm analysis and the levels of sperm nuclei with damaged DNA in men with reproductive problems using the acridine orange fluorescent test. Totally 87 men with reproductive problems have been investigated. Excellent and good fertility potential was established for 58.6% of the studied individuals, and fair and poor potential of male fertility – for 41.4%. Different diagnoses have been found for the men included in the study – normozoospermia (58.6%), asthenozoospermia (18.4%), oligoasthenozoospermia (11.5%), oligoasthenoteratozoospermia (9.2%) and asthenoteratozoospermia (2.3%). Statistically significant dependencies ($P < 0.001$) have been observed between the studied sperm quality parameters, as well as for infertility diagnoses done and the level of spermatozoa nuclei with DNA damage. The present study suggests that the acridine orange fluorescence test is sufficiently informative to determine the quality of the fertility potential. The obtained results could be used as a basis for future

[#]Corresponding author.

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detailed research on male reproductive health and the potential risks for its deterioration.

Key words: male infertility, semen quality, sperm DNA fragmentations

Introduction. Human reproductive health is influenced by a number of factors that negatively affect it. Environmental pollution, harmful habits, occupational hazards, the worsened overall health of individuals associated with stress, different medication and food supplements use are reasons for lower male fertility potential. There are evidences for sperm quality decline in men subjected to the adverse effects of various environmental factors [1-4]. While indicative, these studies do not reveal in detail the mechanisms that lead to the negative tendencies affecting male fertility all over the world. Genetic changes due to gene, chromosomal and genomic mutations are increasingly identified as key indicators associated with male reproductive potential impairment [5,6]. The relationship between male reproductive health and damage to DNA integrity in spermatozoa has been poorly studied in Bulgaria [7]. This fact motivates the purpose of the present study: assessment of the possible associations between the diagnoses for male infertility based on conventional sperm analysis and the levels of sperm nuclei with DNA damage in men with reproductive problems, through acridine orange fluorescent test.

Materials and methods. The study was done during the period from 2018 to 2019. It was conducted in accordance with ethical standards and after receiving informed consent from each participant.

In order to establish DNA fragmentation in the sperm nuclei, acridine orange fluorescent test was performed in a group of 87 men with reproductive problems. All participants in the study who visited a reproductive health office in Plovdiv provided a standardized semen sample. The samples were subjected to conventional sperm analysis and used to determine the conclusions (diagnosis) concerning their male fertility or infertility, according to the reference data for the qualitative semen parameters presented for Bulgarian population by STANISLAVOV and NIKOLOVA [8] and described in the Fourth edition of the WHO [9].

The diagnosis of the male fertility or infertility among the men included in the study was based on conventional sperm analysis and data reporting for the quality of spermatozoa concentration, motility and morphology. In this aspect, the proposed terms since 1999 WHO editions have been as follows: normozoospermia – normal ejaculate defined by the reference values; oligozoospermia – sperm concentration less than the reference value; asthenozoospermia – less than the reference value for mobility (less than 50% of progressive motility spermatozoa (categories “a” and “b”) or less than 25% of progressive motility spermatozoa (category “a”); teratozoospermia – less than the reference value for morphology (less than 30% of spermatozoa with normal morphology); oligoasthenoteratozoospermia – violation of the three variables (combinations of only two prefixes can be

used also); azoospermia – no spermatozoa are detected in the ejaculate; aspermia – no ejaculate is released.

The specific quantitative values on which basis the conclusions (diagnosis) of this study have been done are presented in Table 1.

T a b l e 1

Terms used in Bulgaria in an andrological laboratory according to Stanislavov and Nikolova [8]

Term	Meaning
Aspermia	Lack of semen
Hypoospermia	volume < 2 ml
Hyperspermia	volume > 6 ml
Azoospermia	lack of spermatozoa
Oligozoospermia	$< 20 \times 10^6/\text{ml}$
Polyspermia	≥ 120 spermatozoa/ml
Asthenozoospermia	$\leq 50\%$ motility
Teratozoospermia	$\leq 50\%$ spermatozoa with abnormal morphology
Necropermia	the available spermatozoa are dead by an eosin/nigrosin staining test
Cryptozoospermia	$\leq 1 \times 10^6/\text{ml}$
Globozoospermia	only round-headed sperm
OAT syndrome	Oligoasthenoteratozoospermia
Hemospermia	the presence of blood in the semen
Piospermia	the presence of pus in the sperm

Acridine orange fluorescent test was performed for establishing DNA integrity in the sperm nuclei as it was described by DZHOGLOV et al. [7]. The slides were stained with acridine orange according to VIRANT-KLUN et al. [10]. The evaluation was done by an epifluorescence microscope (Leica DM 1000) equipped with an appropriate filter (I3) and photo camera. One hundred cells were analyzed in each treatment slide where, after acridine orange staining spermatozoa with double-stranded DNA content present a green fluorescence and these with single-stranded DNA content emit fluorescence in yellow-orange to red. Fertility potential was determined by a four-step scale according to EVENSON et al. [11] as follows: excellent – when damaged spermatozoa cells were less than 15%; good – when damaged cells were between 15% and 20%; fair – when damaged cells were between 20% and 30% and poor – when damaged spermatozoa cells were more than 30%. The percentage of cells with fragmented DNA has been calculated as the ratio between the number of cells with damages and the total number of cells according to EVENSON et al. [12].

Descriptive statistics has been used to characterize the frequency of the groups compared. Nonparametric methods were applied for the group comparison. Differences between groups have been analyzed by the Pearson Chi-Square, *t*- and Gamma exact tests. Statistical significance was defined as $P < 0.001$.

Results. The data concerning the quality of fertility potential of the men included in the study are presented in Fig. 1. Microscopic visualization of sperm

Fig. 1. Number (N) and a valid percentage of individuals with DNA fragments in the spermatozoa nuclei according to the four-stages scale of Evenson et al. [11] for fertility potential: excellent – damaged cells less than 15%; good – damaged cells between 15% and 20%; Fair – damaged cells between 20% and 30%; poor – damaged cells more than 30%

Fig. 2. Microphotographs demonstrating different levels of DNA damage in spermatozoa: A – damaged cells more than 30% from an individual with 473 damaged sells per 1000 observed spermatozoa (magnification 400×); B – damaged cells between 15 and 20% from an individual with 186 damaged sells per 1000 observed spermatozoa (magnification 1000×, *in immersion*)

cells with DNA damage and different quality of fertility potential are presented in Fig. 2.

The summarized data on the conventional sperm analysis conclusions (diagnosis) in the current study are presented in Table 2.

Discussion. As could be seen in Fig. 1, for 58.6% of the individuals studied, excellent and good fertility potential has been reported. At the same time, for 41.4% of them, fair and poor potential of male fertility has been reported. It is important to note the high percentage of men with poor fertility potential, as well as the fact that over 50% of the men surveyed have DNA integrity disorders in over 15% of the spermatozoa.

T a b l e 2

Dependencies between the conventional sperm analysis conclusions and the quality of the fertility potential determined on the basis of the level of DNA damage found by acridine orange fluorescence test: $P < 0.001$; Gamma – 0.932

Conclusion	N	Fertility potential				Total – conclusion
	%	Excellent	Good	Fair	Poor	
Normozoospermia	N	40	7	1	3	51
	%	78.4	13.7	2.0	5.9	100.0
Asthenozoospermia	N	2	1	7	6	16
	%	12.5	6.3	43.8	37.5	100.0
Oligoasthenozoospermia	N	1	0	0	9	10
	%	10.0	0.0	0.0	90.0	100.0
Oligoasthenoteratozoospermia	N	0	0	0	8	8
	%	0.0	0.0	0.0	100.0	100.0
Asthenoteratozoospermia	N	0	0	0	2	2
	%	0.0	0.0	0.0	100.0	100.0
Total – fertility potential	N	43	8	8	28	87
	%	49.4	9.2	9.2	32.2	100.0

N – number of individuals; % – valid percentage of individuals with different type of fertility potential according to Evenson et al. [11]: excellent – damaged cells less than 15%; good – damaged cells between 15% and 20%; fair – damaged cells between 20% and 30%; poor – damaged cells more than 30%

Information presented in Table 2 shows that different diagnoses have been found for the men included in the study – normozoospermia (58.6%), asthenozoospermia (18.4%), oligoasthenozoospermia (11.5%), oligoasthenoteratozoospermia (9.2%) and asthenoteratozoospermia (2.3%). In terms of sperm quality, these data are evidence for the high percentage (41.4%) of men with decreased values of spermatozoa concentration and motility, as well as for the presence of cells with abnormal morphology above the normal level. Regarding the fertility potential quality, data from the study indicate that 5.9% of men with normozoospermia and 100% of those with oligoasthenoteratozoospermia and asthenoteratozoospermia have poor fertility potential (reported cells with DNA damage above 30%). In addition, 37.5% of men with asthenozoospermia and 90% of those with oligoasthenozoospermia also have poor fertility potential.

Conventional sperm analysis, with characterized indicators such as sperm concentration, motility and morphology, could not provide information about available chromosomal abnormalities and DNA destructions that are considered possible causes for decreased male reproductive potential [13]. In this aspect, the use of complex approaches will provide better accuracy and higher objectivity in evaluating the quality of male fertility [14,15].

The quality of the spermatogenesis and the fertility potential could be success-

fully assessed by the level of spermatozoa nuclei with damaged DNA by applying the acridine orange fluorescent test. According to BENCHAIIB et al. [16] DNA integrity is a suitable genetic marker for investigating male fertility. DNA damage was found in 10% of spermatozoa of the fertile men and approximately in 20–25% of the spermatozoa of those with reproductive problems [6]. A significant part of these damages is associated with the harmful influence of different environmental factors and lifestyles [5].

In the course of the present study, the biostatistical analysis of the relations between the sperm quality parameters and the DNA fragmentation levels in the spermatozoa clearly shows statistically significant relationships ($P < 0.001$). The positive value of the Gamma indicator (0.932) leads to the conclusion that the decreased quality of the investigated sperm parameters is associated with decreased quality of fertility potential. These dependencies are the basis of the recognized correlations between the quality of fertility potential and the diagnoses done by the conventional sperm analysis (Table 2).

Studies by a number of authors on male infertility based on an acridine orange fluorescence test have contradictory results. For example, CLAASSENS et al. [17] have found a correlation between abnormal sperm morphology and high levels of cells with DNA fragmentation but at the same time, TEJADA et al. [18] and EGGERT-KRUSE et al. [19] have not detected enough clear dependencies between the investigated sperm quality parameters and the levels of DNA damage by usage of the acridine orange test. However, according to a number of studies [10, 13, 20], this test is successfully applied to characterize DNA fragmentation levels in fertile and infertile men, which is distinctly confirmed by the results of our study.

Conclusions. Statistically significant dependencies have been observed between the studied sperm quality parameters and the levels of sperm nuclei with damaged DNA.

There are reasons to accept that the acridine orange fluorescence test is sufficiently informative to determine the quality of fertility potential.

The results obtained could be used as a basis for future detailed research on male reproductive health and the potential risks for its deterioration.

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*Department of Developmental Biology
Biological Faculty
University of Plovdiv “Paisii Hilendarski”
24, Tsar Asen St
4000 Plovdiv, Bulgaria*

*e-mail: e.ivanova@gmail.com
mitkovska.v@gmail.com
spas78dj@abv.bg
pepa_vasileva@abv.bg
ivan100yanov@abv.bg
teodora.irikova@gmail.com
tstaykova@yahoo.com*

**Faculty of Mathematics and Informatics
University of Plovdiv “Paisii Hilendarski”
24, Tsar Asen St
4000 Plovdiv, Bulgaria*