

THE EFFECT OF LOW-DOSE IONIZING RADIATION (COMPUTER TOMOGRAPHY) ON THE FUNCTIONAL PARAMETERS OF MALE GAMETES

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Abstract

Human semen (native and processed) was treated with different doses of ionizing radiation. The influence of the irradiation on motility, DNA fragmentation and level of apoptosis of spermatozoa has been investigated. The obtained results point out that the treatment of the samples with dosages usually used in medicinal diagnostics (computer tomography) does not have any effect on the total motility of the spermatozoa, but does influence certain specific kinetic parameters – amplitude of lateral head displacement (ALH) and beat/cros frequency (BCF). We did not observe any differences in the percentage DNA fragmentation between the experimental and the control groups. Irradiation with low-dose ionizing emissions increases the proportion of gametes in early apoptosis stage and the effect is dose-dependent.

Key words: spermatozoa, low-dose ionizing radiation, motility, apoptosis

Introduction. Infertility is an important medico-social issue, which affects around 10–15% of the couples in reproductive age. In some cases, it is due to the so-called “male factor” [1,2]. Different diseases and exogenic factors (including ionizing radiation) influence the spermatogenesis and may lead to reduced quality of the semen, most often displayed in low number and motility of the

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spermatozoa. In the available literature, we could not find enough data about the direct effect of the ionizing radiation on the spermatozoa. Apart from the medical aspect, such investigations are also important in purely fundamental scientific perspective. The male gametes are terminally differentiated cells, with a haploid chromosome number, and their DNA is specifically associated with protamines and not histones. The spermatozoa have overall negative charge and the effect of different electromagnetic waves on them poses an interesting point for investigation.

The aim of the present study is to investigate the effect of low-dose ionizing radiation (computer tomography) on the overall motility, kinetic parameters, as well as the levels of DNA fragmentation and apoptosis of male gametes.

Material and methods. Collection and initial evaluation of the semen samples. In the current investigation we have used donor semen (after obtaining an informed consent from the donors). It was collected after 48–96 h of sexual abstinence. After liquification, the semen was evaluated according to the uniformly accepted criteria (volume of the sample, pH, motility, morphology and concentration of spermatozoa) [1]. Only normozoospermic ejaculates have been used further in the investigation (native and processed). Each of them has been separated into four samples – three experimental (treated with different doses of radiation) and one control (untreated).

Semen processing. In order to separate the fraction of normokinetic gametes we have used density gradient centrifugation [2].

Ionizing radiation treatment. The experimental samples have been subjected to ionizing radiation with computer tomograph LightSpeed VCT XT (General Electric). We have applied three irradiation dosages (Total exam DLP) – 1710.18, 2173.12 and 2826.08 mGy/cm. The absorbed dose per slice (CTDI_w) varied between 38.12 and 62.99 mGy (usually used in medical practice [3]). The duration of the exposure was between 240 and 300 s. After the irradiation, the functional parameters of the spermatozoa in the experimental and the control samples have been studied.

Evaluation of the kinetic parameters of the gametes. Spermatozoa motility was investigated with computer assisted sperm analysis (SCA – sperm class analyzer) in 4-well Leja chamber on every 2 h post-treatment [2]. Apart from total motility, the following additional kinetic parameters have also been studied: curvilinear velocity (VCL), straight velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat/cross frequency (BCF).

DNA fragmentation. For this analysis, we have used GoldCyto Sperm Kit (GoldCyto Biotech Co), according to the manufacturer's instructions. The method is based on *Sperm chromatin dispersion (SCD)* test [4,5]. The spermatozoa are added to pre-treated inert agarose microgel, without fixation. The initial treatment with acid denatures the DNA of the spermatozoa with fragmented

DNA. The lysis buffer removes most of the nuclear proteins and in the absence of multiple breakages, large nucleoids with extensive halos are formed from DNA that has left the nuclear membrane. Nucleoids from spermatozoa with fragmented DNA, either do not have halos of dispersion, or they are minimal.

Investigation of the level of apoptosis. Flow cytometric analysis on SONI MA 900 (Multi-Application Cell Sorter) with FITC Annexin V Apoptosis Detection Kit (BD) was performed 3 h after the irradiation according to manufacturer's instructions. This investigation allows to detect vital, necrotic, early and late apoptotic cells [2].

Statistical analysis. The Prism 6 software was used to perform the statistical analysis. The results are reported as the mean $\pm$ the standard deviation (SD). One-way ANOVA analysis with 0.05 significance level and Dunn's multiple comparison test were used to determine the differences between the groups. The hypothesis testing is two-tailed and values of $p \leq 0.05$ were considered significant [6].

Results and discussion. Motility. For the current investigation, 32 donor ejaculates have been used from males aged 26–41 years. The semen volume varied between 1.8 and 4.3 ml, the concentration of spermatozoa from 32 to 95 mln/ml and the overall motility was above 60%. After density gradient centrifugation we have isolated the fraction of morphologically normal spermatozoa with overall motility of 88–94%. It has been established that the treatment with low doses of ionizing radiation does not influence the motility of the gametes. The only case when such effect was detected, was after long-term incubation of the isolated normokinetic gametes. After 24 h a tendency of the untreated spermatozoa to retain better overall motility was observed (Fig. 1).

During our studies, it has been found that the ionizing radiation affects certain kinetic parameters of the spermatozoa. Part of the experimental samples showed increased ALH and BCF. ALH gives information of the degree of lateral displacement of the heads of spermatozoa along their average line of movement [7] which can be displayed in its maximum or mean value. BCF is a measurement of the average rate at which the sperm trajectory crosses the average path. Both parameters are derived from VAP [8] and reflect the sperm beating frequencies. In the available literature, there is conflicting data regarding the influence of ionizing radiation on these parameters. Some investigations show similar to our results of increase in both ALH and BCF in normozoospermic [9] and astenozoospermic patients [10]. Different factors may be responsible for these observations, such as change in the activity of the ion channels involved in flagellar beating and increased metabolic rate [9]. On the contrary, other authors do not report significant differences in the above kinetic parameters of spermatozoa after treatment [11].

DNA fragmentation. DNA fragmentation (sDF) is one of the most common aberrations of the genetic material in the form of single or double strand breakages. It is a result of various processes (defects in DNA condensation during

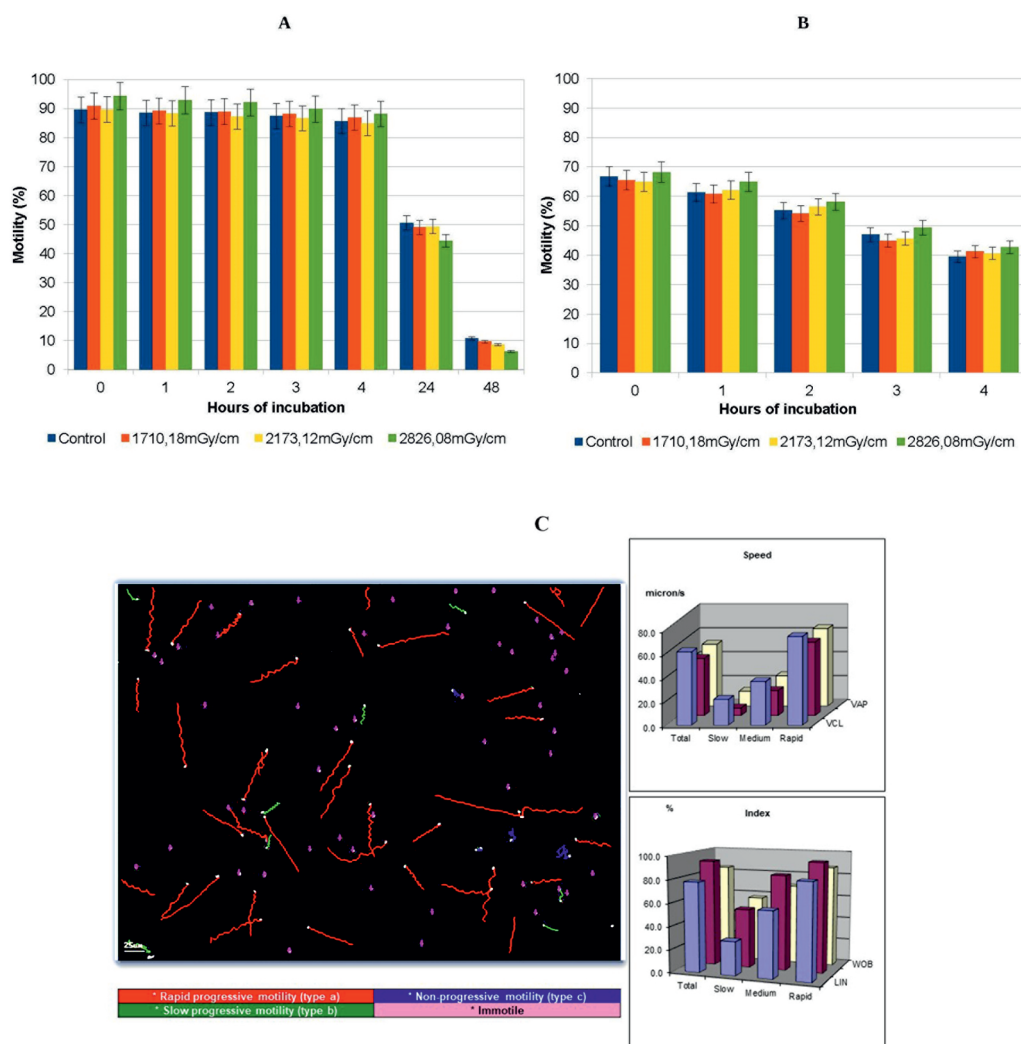


Fig. 1. Motility and survival rate of male gametes (native and processed semen) after treatment with different doses of ionizing radiation: A: processed isolated normokinetic spermatozoa ($n = 14$); B: native semen ($n = 18$); C: kinetic characteristics of human spermatozoa (fraction of motile gametes) 2 h post-treatment (representative CASA analysis of one sample); $p \geq 0.05$

spermatogenesis, apoptosis, oxidative stress) and is associated with a number of pathological conditions of the organism and also with external factors [12]. In the current research, with the help of SCD-test we have evaluated the influence of low-dose ionizing radiation on the DNA fragmentation of male gametes. The obtained results show that there is no difference in the level of DNA fragmentation in the experimental and the control groups of spermatozoa (Fig. 2). It should be noted, that after 6 h of incubation in all groups (irradiated and untreated) an increase in percentage of the male gametes with DNA fragmentation was observed

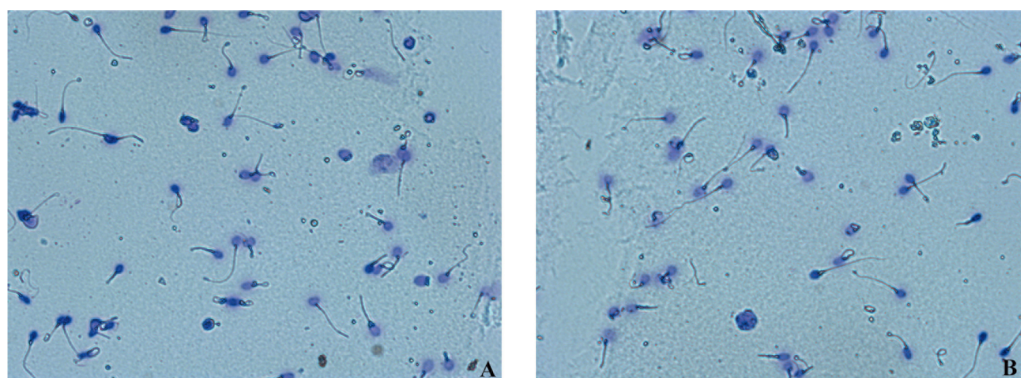


Fig. 2. DNA fragmentation of spermatozoa (SCD-test, 200 $\times$). A: control sample; B: experimental sample of treated with ionized radiation (computer tomography) semen. (The results are representative of one ejaculate)

which suggests that it is due not to the radiation exposure, but to the incubation in *in vitro* conditions. Moreover, most scientists share the point that the use of spermatozoa for ART procedures should be prolonged no more than 2–3 h after collection and processing [5].

Apoptosis. The apoptosis, or programmed cell death has a key role in the process of spermatogenesis. Its biological significance in ejaculated spermatozoa has not yet been elucidated [13]. Data exists, that the process of cryopreservation can induce apoptosis in the male gametes of man and certain domestic animals. The obtained results from our investigation, showed that the treatment with ionizing radiation may initiate programmed cell death in spermatozoa. An increase in the percentage of gametes in early stage of apoptosis, compared to vital ones, in all three experimental groups, treated with different doses of radiation was observed (Table 1, Fig. 3). Some authors suggest a positive link between the elevated levels of spermatozoa apoptosis with increased DNA fragmentation. In our investigation we could not find such a relationship, probably other mechanisms for the initiation of the process are involved. Interestingly, we did not detect a significant increase in the late apoptotic/necrotic cells compared to the control group. It is important to be mentioned that according to the manufacturer's instructions, the apoptosis detection kit does not differentiate between necrotic and late apoptotic cells. Both express annexin V and do not have an intact plasma membrane letting PI inside their cytoplasm, thus reacting positive to the two markers. In addition, we have performed the flow cytometry three hours post-exposure to the radiation, therefore a more accurate analysis including different time periods is needed to elucidate this matter.

However, in the study of DNA fragmentation bias can be introduced as each cell has to be examined visually, and they could be miscounted. While flow cytometry permits examination of thousands of cells in a few seconds, thereby

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Influence of treatment with low-dose radiation on the level of apoptosis in male gametes (native semen, $n = 9$), $p \leq 0.05$ compared to the control

Treatment groups	Percentage cells		
	Vital	Early apoptotic	Late apoptotic or necrotic
Control (untreated)	53.4 ± 9.5	13.9 ± 6.8	29.1 ± 6.4
1710.18 mGy/cm	$27.6 \pm 6.3^*$	$35.4 \pm 7.5^*$	33.0 ± 7.1
2173.12 mGy/cm	$25.7 \pm 7.1^*$	$37.7 \pm 8.1^*$	34.4 ± 6.8
2826.08 mGy/cm	$21.3 \pm 7.9^*$	$39.2 \pm 8.7^*$	36.5 ± 7.6

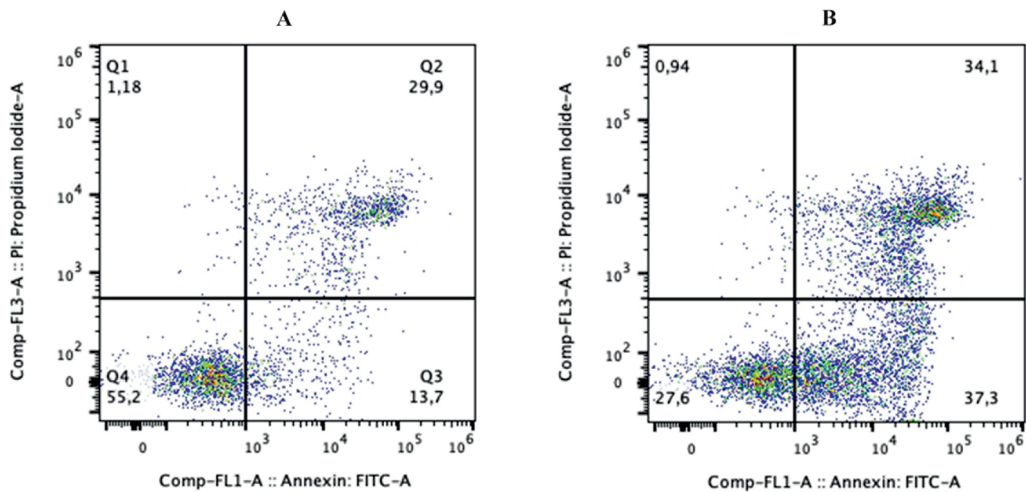


Fig. 3. Flow cytometric analysis of native human semen: A: control sample; B: experimental group treated with 1710.18 mGy/cm ionizing radiation; Q1 (annexin V negative, PT positive) – necrotic cells; Q2 (annexin V positive, PI positive) – late apoptotic or necrotic cells; Q3 (annexin V positive, PT negative) – early apoptotic cells; Q4 (annexin V negative, PT negative) – vital cells. (Representative results of one ejaculate)

enormously increasing precision [14]. It is important to be noted that our investigations are preliminary and pilot and additional research is needed to elucidate the mechanisms of action of the low-dose ionizing radiation on spermatozoa. Further studies in the field of molecular biology and transcriptome profile analysis of the male gametes and possible epigenetic alterations would also be very intriguing [15].

Conclusion. In conclusion, the obtained results from our investigation point out that the low-dose exposure to ionizing radiation applied routinely in medical diagnostics do not significantly influence the motility and the DNA fragmentation level of male gametes. In addition, there is an increase in certain kinetic parameters – the amplitude of lateral head displacement and the beat cross frequency and also elevated percentage of spermatozoa in early apoptotic stages, compared to the untreated samples. Further investigations are needed to explain the effect

of the irradiation to late apoptotic/necrotic cells and the molecular mechanisms behind the observed processes.

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