ABSTRACT

of the dissertation for the degree of Doctor of Philosophy (Ph.D.)
in the educational program "8D05101 – Biology"
by **Denis Vladimirovich Zadubenko**
on the topic:

"Influence of electromagnetic radiation of the visible and infrared ranges on the motility and ultrastructural organization of human spermatozoa"

Relevance of the study

Due to the high and steadily increasing demand for assisted reproductive technologies (ART), the search for methods to modulate sperm motility in vitro in order to enhance the effectiveness of ART protocols is highly relevant. Experimental studies of the effects of electromagnetic radiation (EMR) on animal spermatozoa began in the late 1980s and continue to this day. Over this period, it has been established that EMR significantly affects key cellular processes, including the production of adenosine triphosphate (ATP), the universal energy source for almost all living organisms. The influence of EMR on the activity of cytochrome c oxidase—a component of the oxidative phosphorylation pathway—has been documented. Currently, understanding the impact of EMR on sperm motility in asthenozoospermia is of particular importance due to the high prevalence of this condition among infertile men. It is necessary to determine whether EMR affects ultrastructural parameters of spermatozoa such as acrosome reaction activation, normal or pathological acrosome position, changes in the post-acrosomal sheath, chromatin condensation, the degree of nucleosome vacuolization, and mitochondrial conformations. It remains unclear whether EMR influences, or influences at all, the compaction of acrosomal and nucleosomal contents, the organization or disorganization of the sperm axoneme, and morphological changes of peripheral microtubules. A scientific consensus must be formed regarding the impact of EMR on energy-related processes in human spermatozoa and on the degree of DNA fragmentation. The search for methods to modify sperm motility using EMR to enhance ART efficiency has significant practical importance, since disorders of spermatogenesis account for approximately half of the causes of infertility. Modulating male gamete motility after cryopreservation is also a highly relevant specialized task, as the number of motile and progressively motile spermatozoa decreases after freezing.

Objects of the study:

Ejaculates of infertile men with asthenozoospermia.

Subject of the study:

Ultrastructural organization, motility, DNA fragmentation index, and mitochondrial activity of human spermatozoa after exposure to electromagnetic radiation in the visible and infrared ranges.

Purpose of the study

To investigate the potential of experimental electromagnetic radiation in the visible and infrared ranges to influence the motility and ultrastructural organization of male gametes in vitro.

Research objectives:

1. To determine the structure of spermatogenesis disorders in men from infertile couples.
2. To design and assemble an electromagnetic radiation device and conduct laboratory testing.
3. To determine the effect of electromagnetic radiation on sperm motility in asthenozoospermia and assess its influence on the ultrastructure of the sperm head, neck, and tail.
4. To evaluate experimentally the effect of electromagnetic radiation on the DNA fragmentation index and mitochondrial activity of irradiated spermatozoa.
5. To determine the optimal EMR regimen that improves sperm motility without increasing the DNA fragmentation index or causing ultrastructural abnormalities.

Research methods

Spermatogenesis disorders in infertile patients were assessed using semen analysis with Kruger morphology evaluation. Immunological causes of infertility were investigated using the mixed antiglobulin reaction (MAR test) for IgG. Sperm motility in experimental and control aliquots was evaluated using computer-assisted sperm analysis (CASA). EMR effects on sperm ultrastructure were studied using transmission electron microscopy. Mitochondrial activity was assessed by staining with vital fluorescent potential-sensitive dyes followed by confocal laser scanning microscopy. The effect of EMR on the genetic apparatus of spermatozoa was evaluated using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Wavelengths of the applied radiation were measured using high-sensitivity fiber-optic spectrophotometry; radiation power was measured with a wave-power detector.

Statistical analysis was performed using specialized software. The paired Student's t-test was applied to evaluate the effect of EMR on sperm motility. For experiments with small and dependent samples, the Wilcoxon test was used. To assess EMR effects on sperm ultrastructural organization, Fisher's exact test with the Benjamini–Hochberg correction was applied.

Scientific novelty

The structure of spermatogenesis disorders in infertile men living in Central Asia has been defined. It was found that the main abnormality in ejaculate quality is reduced sperm motility (asthenozoospermia): 38.6% of men in infertile marriages

exhibit this characteristic. Additionally, 47.1% of men in infertile marriages demonstrated normozoospermia (N=957).

A laboratory prototype of a device was developed, consisting of monochromatic LEDs, a white LED, a red laser, and an infrared diode, capable of increasing motility of human spermatozoa in vitro in asthenozoospermia. The peaks of emitted wavelengths, power, and energy density of the EMR sources used were described. EMR regimens that positively influence sperm motility without increasing the DNA fragmentation index—evaluated by TUNEL—were identified.

Using confocal laser scanning microscopy, a trend toward increased mitochondrial activity in human spermatozoa with asthenozoospermia was documented after exposure to EMR delivering a total energy dose from 0.02 to 5.15 J.

Practical significance of the study

The practical significance of the research lies in the development of a laboratory prototype device that enabled the identification of electromagnetic radiation (EMR) treatment regimens capable of increasing sperm motility in men with asthenozoospermia. An alternative method for enhancing sperm motility in vitro has been developed, which in the future may be applied in the field of assisted reproductive technologies (ART) as a tool to improve the treatment outcomes of male-factor infertility in IVF programs.

Theoretical significance

We demonstrated that the key factor in increasing sperm motility is not the duration of EMR exposure, but its power (4.46 mW). The effects of EMR on ultrastructural morphological markers were evaluated following exposure to a combination of LED, laser, and infrared radiation (LED 400–750 nm; laser 651 nm; infrared diode 933 nm; total radiation power 4.46 mW; total delivered energy 4.01 J; exposure time 15 minutes). It was shown that this EMR regimen leads to changes in the following ultrastructural markers of spermatozoa: “normal mitochondrial morphology,” “presence of acrosome,” “abnormal fibrous sheath,” and “normal acrosome morphology.” These findings may reflect artifacts of transmission electron microscopy (TEM), yet they indicate the need for further research on the safety of this regimen.

The study expands existing knowledge on the possible mechanisms underlying changes in sperm motility after EMR exposure, based on previously described phenomena by T.I. Karu—photoacceptivity of cytochrome c oxidase, ATP hyperproduction, increased levels of reactive oxygen species, and sperm capacitation—by identifying the EMR energy threshold that activates these processes in human spermatozoa.

Main statements of the dissertation submitted for defense:

1. Asthenozoospermia is the most common condition among all ejaculate abnormalities in Central Asia (Kazakhstan, Uzbekistan), with only 38.6% of infertile men (N=957) having this condition.

2. It has been shown that 47.1% of men in infertile marriages have normal ejaculate parameters (N=957).

3. A laboratory prototype of a device has been created, the main components of which are monochromatic LEDs, a white LED, a red laser, and an infrared diode, capable of increasing sperm motility in vitro in humans with asthenozoospermia. 4. Monochromatic LED light in combination with 651-nm laser and 933-nm infrared light with a radiant power density of 0.354 mW/cm2 and a total delivered energy of 1.34 J statistically significantly increased human sperm motility in vitro compared to the negative control ($P<0.05$).

5. Irradiation of spermatozoa at a radiation source power of 4.46 mW and a radiation power density of 0.354 mW/cm2 with a total transmitted wave energy of 4.01 J (400-750 LEDs, 651 nm laser, 933 nm infrared diode) showed that the used EMR mode leads to statistically significant changes in such ultrastructural markers of spermatozoa as normal mitochondria shape, presence of acrosome, abnormal fibrous layer, normal acrosome shape compared to the negative control ($P<0.05$).

6. Treatment of spermatozoa with a combination of monochromatic LED, laser, and infrared radiation does not lead to an increase in the number of single-strand and double-strand breaks in human sperm DNA, which are registered by labeling with the participation of terminal deoxynucleotidyl transferase dUTP compared to the negative control.

Relation to the plan of major scientific works

The dissertation was carried out within the framework of the priority area of scientific development approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan (“Life and Health Sciences”) and constitutes a major scientific research project.

Personal contribution of the doctoral candidate

The doctoral candidate’s contribution to each publication includes: literature review, planning and conducting experiments, data collection, interpretation and statistical analysis of results, as well as writing and discussing the findings.

Approbation of the research

The materials of the dissertation have been presented and discussed at international conferences, congresses, forums, and round tables, including:

– International Scientific Conference of Students and Young Scientists “Farabi Alemi” (2020, 2022, 2023, Almaty, Kazakhstan);

– Round Table “Prospects and Achievements of Genetic Research in Kazakhstan and the World,” dedicated to the 30th anniversary of Independence of the Republic of Kazakhstan (December 9, 2021, Almaty, Kazakhstan);

– International Youth Scientific Forum “Lomonosov” (2022, 2024, Moscow, Russian Federation);

– International Congress of the Kazakhstan Association of Reproductive Medicine (November 10–12, 2022, Nur-Sultan, Kazakhstan);

– International Forum “Modern Trends in the Sustainable Development of Biological Sciences” (March 27–28, 2024, Almaty, Kazakhstan);

– International Conference of the International Society for Reproductive Genetics “ISRG-2025” (June 6–8, 2025, Changsha, P.R. China).

The data on the structure of spermatogenesis disorders in Central Asia were submitted as reports to the official publication bodies of the World Health Organization—Population Health Division of the United Nations—and the official journal of the American Society for Reproductive Medicine.

Publications

The main content of the dissertation is reflected in 12 published works, including 2 papers in Scopus-indexed journals; 3 articles in journals included in the list of the Committee for Quality Assurance in Education and Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan; and 7 abstracts in the materials of international conferences.