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Photostimulation Applications to Increase Spermatological Parameters

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Abstract

Today, it is an important issue to preserve genetic resources and transfer them to future years. The process of freezing semen, which has a wide range of uses such as reproductive biotechnology, protection of the lineage of species and clinical applications, is of great importance in this sense. Freezing the semen in a way that will provide sufficient fertility will make artificial insemination practices more practical and economical, and more accurate and reliable pedigree records will be kept. In addition, it will be possible to use animals with high genetic capacity in large populations. During the freezing-thawing of sperm, membrane lipid phase change, osmotic-mechanical stress and free oxygen radicals occur. As a result, structural deformations in membrane structures and cell organelles and breaks in DNA occur. These negative effects can be reduced by adding antioxidants, various cryoprotectants and chemical substances to semen extenders, and sperm functions are improved after solution. The developments in the light of recent research have been in the direction of increasing the fertility abilities of sperm cells in the cryopreservation process by irradiating them in different spectrum areas. On the other hand, lasers are involved in many areas of our lives due to their linearity, single wavelength, stable radiation and high power. Laser sources are gaining more and more acceptance in various industries thanks to their narrow focusing advantages. Recently, it has been shown that under intense radiation of red LED (Light Emitting Diode) lights, the resistance and capacitation of porcine sperm cells increase in vitro and fertility ability increases in vivo. Consistent results have also been obtained in species such as humans, dogs, bulls, rams and rabbits using devices and systems such as low-energy laser and visible light lamps with wavelengths ranging from 400 nm to 800 nm.

Keywords: Laser; LED; Photostimulation; Semen

Abbreviations: Laser: Light Amplification by Stimulated Emission of Radiation, LED: Light Emitting Diode, CASA: Computer-Assisted Sperm Analysis, VAP: Average Path Velocity (μm/s), VCL: Curvilinear Velocity (μm/s), VSL: Straight-Line (rectilinear) Velocity (μm/s), ROS: Reactive Oxygen Species, CO2: Carbon dioxide, DNA: Deoxyribo Nucleic Acid, RNA: Ribo Nucleic Acid, ATP: Adenosine Triphosphate

Introduction

Laser, Working Principle of Laser, Types of Lasers

A laser is a coherent and focused beam of photons. This beam of light is more intense than the light emanating from any source. The laser has a single wavelength. All of the laser waves are of the same frequency and are in phase with each other. Light consists of energy packets called photons. The energy of light is carried by photons. Photons have the property of behaving like both waves and particles. In addition, the wavelength of the photons determines the color of the light.

Light consists of energy packets called photons. The energy of light is carried by photons. Photons have the property of behaving like both waves and particles. In addition, the wavelength of the photons determines the color of the light. The principle of operation of the laser is based on the excitation of photons. Some gases are used to obtain laser light. The most commonly used gas is carbon dioxide. When the carbon dioxide atom is excited by electricity, light or another way, in other words, when energy is given, the electrons of the atom are excited and go from a low energy level to a high energy level. But this excited electron cannot stay in this high energy level forever. Therefore, the excited electron goes from a high energy level to a low energy level. During the transition, it releases energy as photons as much as the difference between the high energy level and the low energy level. The released photon excites the electron of another atom, causing the same photon to be released. The excitation process continues with the energizing of the system. By means of mirrors placed at both ends of the laser, photons are reflected on both sides, thereby stimulating more atoms. The beamed photons create the laser beam **[1].**

Lasers are classified as high and low power lasers according to their energy densities. High power lasers are also called hot lasers due to the thermal effect they produce and are mostly used in surgical applications. Low-energy lasers act photochemically. They are called low-power laser, low level laser, soft laser.

Argon laser: They are mostly used in eye diseases. It is frequently used in retinal hemorrhages, detachment and glaucoma.

CO_2 laser: It is the most suitable type of laser for microsurgical uses.

Neodymium YAG (Ytrium Aluminium Oxide Garnet) laser: It is especially used in tumor treatment and endoscopy.

Helium-Neon (He-Ne) laser: It acts on a wide tissue mass with high dispersion and low absorption. It is the most suitable type of laser for transcutaneous irradiation treatments. It ensures the proliferation of collagen fibers and cells. It reduces pain **[2].**

Photostimulation: Photostimulation artificially activates biological compounds, cells, tissues and even whole organisms, for this purpose light is used. This method is effective for investigating various relationships between different biological processes **[3].**

Effect of Photostimulation on Spermatozoa Motility and Oxidative DNA Damage

The ability to successfully fertilize the oocyte relies on the motility ability of the spermatozoa. Sperm motility in both humans and animals has been used as a metric for the viability of sperm samples.

Current methods of assisted reproduction in humans and animals rely on the use of drugs to increase fertility or the direct injection of sperm into the oocyte. These methods are not always successful and additional ways of improving fertilization can be exploited. Many studies have shown that low doses of red light cause an increase in sperm motility and overall fertilization potential in several species **[4-7].** One study showed that red light from LEDs can improve sperm quality by increasing the speed of sperm cells and the mitochondrial membrane potential **[7]**. On the other hand, it has been shown that excitation of 630 nm laser light can lead to the generation of reactive oxygen species (ROS) such as hydrogen peroxide **[8]**. Low levels of ROS allow the sperm acrosome to react with the oocyte **[9],** acting as an important messenger in fertilization. However, high levels of ROS can damage cell DNA. Therefore, it is possible that light stimulation causes oxidative DNA damage, which may be more visible in nuclear DNA located near the sperm midpiece, where the cellular mitochondria mass is concentrated. Previous studies have shown that DNA damage can lead to male infertility and that ROS-induced oxidative stress is the main cause of DNA fragmentation **[10,11].** There

-fore, careful evaluation of DNA damage is important.

For viable fertility to occur, laser irradiation must not cause DNA damage in sperm cells. Spermatozoan DNA damage has been associated with developmental problems and difficulties in embryonic implantation, as well as fertilization dysfunction. The inability of a cell to cope with ROS has been associated with both DNA damage and decreased sperm motility **[12].**

In a study by Daryl Preece et al (2017), it was shown that exposure to red light can increase sperm motility while producing little or no DNA damage to sperm cells **[13].** Since sperm morphology and energy differ significantly between species, it is anticipated that the effect of photostimulation may also vary significantly between species.

Effects of Laser Irradiation on Spermatozoa Functions in Animals

Cryopreservation of spermatozoa has led to wider application of artificial insemination. However, sperm quality decreases after cryopreservation, which reduces their fertility **[14].** Exposure to various stresses during cryopreservation physically alters sperm cells and also leads to changes in chemical components necessary for energy metabolism of sperm to support motility **[15].** Sperm mitochondrial damage occurs due to destabilization of sperm cell membranes during freeze-thaw procedures, resulting in impaired sperm motility **[16].** To date, most research has been done to preserve spermatozoa **[17]** using suitable extenders **[18]** to improve freeze-thaw processes and/or various additives **[19]** during the freeze-thaw process. Sperm mitochondrial function may be adversely affected by the use of some cryoprotectants, namely soy lecithin, as previous findings have revealed **[20]**. Therefore, various other strategies aimed at improving sperm mitochondrial function need to be evaluated. Application of photobiostimulation with a low-intensity helium-neon (He-Ne) laser has shown to increase the motility of spermatozoa **[21].** Photobiostimulation of sperm was first reported in 1969 **[22].** This has been proven in several species {human **[23]**, mouse **[24]**, sheep **[25]** and dog **[26]** semen}.

On the other hand, there are other animal species where laser Irradiation has positive effects on spermatozoa functions {bull **[27]**, bison **[28]**, stallion **[29]**, turkey **[30]**}.

Possible Mechanisms of Laser Action in Spermatozoa

Laser irradiation of sperm leads to increased respiration, fructose fermentation, ^{32}P uptake and Ca^{2+} absorption, which increases motility and prolongs sperm survival **[31].** Photobiostimulation occurs when light is absorbed by porphyrins or cytochromes and the absorbed energy is transferred to oxygen molecules leading to the generation of reactive oxygen species (ROS) **[32].** ROS is known as a double edged sword in animal reproduction because higher levels of ROS can cause sperm death due to depletion of ATP and lipid peroxidation causing oxidative stress. Besides, moderate levels of ROS regulate various physiological functions of sperm such as hyperactivation, capacitation, acrosomal reaction, and zona binding **[33].**

Oxidative phosphorylation in sperm mitochondria leads to the formation of ATP, which is mainly required for sperm motility, and it has been shown that the energetic load of the cell increases after laser irradiation **[34].** As a result of long-term storage, sperm mitochondrial senescence occurs, resulting in reduced sperm motility due to reduced ability to produce ATP through mitochondrial respiration **[35]**. In isolated mitochondria, He-Ne laser irradiation causes an increase in ATP synthesis **[36]**, RNA **[37]** and DNA synthesis **[38]**. Sperm mitochondrial ATP is primarily required for sperm motility **[39]**, in addition, mitochondria are also involved in maintaining sperm tail contractility, such as regulation of membrane potential and calcium flux **[40]**.

In conclusion, photostimulation applications are a low-cost method that can be used to improve spermatological parameters. The beneficial effects of photostimulation on spermatozoa include sperm viability, acrosomal integrity, hypo-osmotic swelling response, mitochondrial function, and CASAbased sperm parameters (progressive motility, sperm velocity - VAP, VCL, VSL, distance). The increase in these sperm characteristics may be related to the fact that laser irradiation results in higher cytochrome c oxidase activity, ATP levels and mitochondrial membrane potential, which increases sperm survival. In addition, laser irradiation needs to be standardized, fertility experiments should be performed with laser irradiated spermatozoa and gene expression patterns in laser-treated sperm of various species.

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