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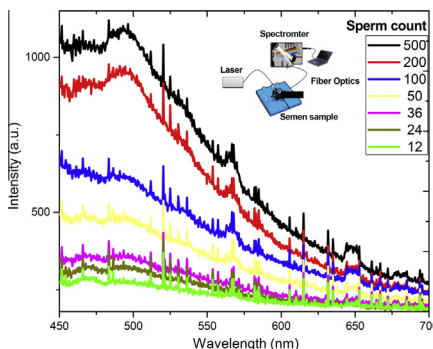
Laser researches on livestock semen and oocytes: A brief review



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GRAPHICAL ABSTRACT



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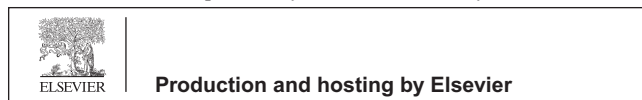
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ABSTRACT

This article presents a brief review of the past and present literature pertinent to laser effects on sperm motility parameters, improvement of oocyte maturation and characterization of semen in livestock. The aim was, on one hand, to make the readers aware of such knowledge and on the other hand to trigger the interest of the animal reproduction scientific community in attempting some laser techniques that have not yet been fully exploited in the field of artificial insemination. With respect to the conventional methods, laser is a more sensitive and less costly technology that can be used for improving artificial insemination and embryo production system. Since 1980s, laser treatment came on the biological samples scene; its applications have continuously been developed thereafter. Exploitation of laser light by various researchers for improving the reproductive efficiency of sperm cells and the maturation rate in different livestock is

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demonstrated herein. Laser irradiation, in principal, can increase the production of adenosine triphosphate (ATP) and consequently increases the energy provided to the cell. Since sperm motility and oocyte maturation depend on the energy consumption, an increase in the energy supply to the cells will be of great importance. In addition, the authors also discuss the use of laser spectrochemical analytical techniques, such as laser induced breakdown spectroscopy (LIBS) and laser induced fluorescence (LIF), in characterization of semen samples.

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Introduction

Over the past two decades, the assisted reproductive technologies (ARTs), namely artificial insemination (AI), *in vitro* maturation (IVM), *in vitro* fertilizations (IVF), intracytoplasmic sperm injection (ICSI), and somatic cell nuclear transfer (SCNT), for different species have been evolved effectively [1–6].

Artificial insemination offers many advantages to commercial livestock production and is routinely used in several domestic animals such as cattle, sheep and horses [7]. The technique represents a very important and a promising issue to increase animal production. Improvement of sperm motility is expected to have direct qualitative and quantitative impacts on AI of livestock. As it is well known, the role of a spermatozoon is to deliver the male's genetic material to the oocyte during fertilization; consequently motility is considered as one of the most important parameters. For motility, spermatozoa require development of a tail (flagellum). Mammalian sperm is characterized by the fusion of mitochondria in a mitochondrial sheath located around the apical portion of the tail. In different species [8] a direct relationship between motility and mitochondrial activity was shown.

Evaluation of oocytes quality is of high importance, since improvement of maturation rate represents the corner stone of the above mentioned technologies. Many researchers conducted experiments and suggested procedures for improving oocytes maturity using different types of media such as a basal medium (M199) [9], and DMEM/F12-based media [10] and/or different incubation times [11,12]. The success of *in vitro* produced embryo depends on the quality and competence of gametes involved in oocyte maturation, fertilization, and early embryonic development.

During the last two decades of the 20th century, the effects of laser on biological tissues have been studied widely [13–17]. It has been clearly demonstrated that low level laser irradiation, also known as photobiomodulation, has pronounced biological effects. Laser irradiation of fibroblasts [18], and other biological structures, such as the neuromuscular junction [19] are some examples of such biological application of lasers. In addition of studying the cells' response to laser light irradiation, recent studies dealt with laser effects on spermatozoa [20–23], and oocytes [24,25]. The positive effects of low level laser irradiation include the increase of cellular metabolism and improvement of structural characteristics, as has been confirmed in the literature [13,26,27].

Many studies have focused on utilization of laser effect on sperm motility parameters. Notwithstanding, there have been very few studies concerning the effects of laser on oocyte maturation. Over the last couple of years there has been an exponential growth in the areas of utilization of lasers (pulsed or continuous wave (CW)) which is reflected by an increasing number of publications, and thus its utility as an assisting technology has been proved. Below, a summary is presented of literature pertinent to the effect of laser irradiation on improving AI, oocyte maturation, and embryo production system in domestic animals.

Laser and improvement of sperm parameters

Sperm consists of a head containing condensed DNA, followed by a short neck (midpiece) containing mitochondria and a thin tail (flagellum) responsible for motility [11,25]. The spermatozoon motility depends on energy supply. Both of energy metabolism in mitochondria and the motility system of the cells are involved in the activation of the sperm flagellum. ATP on the other hand can be produced by mammalian spermatozoa via both of aerobic and anaerobic glycolysis [28–30]. Previously published works showed the potential of low-power laser irradiation of spermatozoa in increasing their motility and raising the ATP amount in cells. The first paper published on this topic was that of Goldstein [31]. Thereafter, it has been clearly evidenced that He–Ne

Table 1 Types of lasers used in animal reproduction researches and relevant parameters.

Laser	Operation mode	Wavelength (nm) (color)	Photon energy (eV)	Application	Species and reference
DPSS	CW	405 (violet)	3.07	Molecular analysis (LIF)	Buffalo [68]
Nd:YAG	CW and pulsed	532 (green)	2.34	Irradiation	Buffalo [20]
second harmonic He-Ne	CW	632.8 (red)	1.96	Irradiation (activation and/or sterilization)	Bovine [21,23,62], chicken [39], Turkey [53,54], rabbit [55]
Diode laser	CW	655 (red)	1.90	Irradiation	Dog [49,50]
Nd:YAG	Pulsed	1064 (IR)	1.17	Elemental analysis (LIBS)	Buffalo [68]

DPSS: Diode pumped solid state laser.

CW: Continuous wave.

IR: Infrared.

laser irradiation (at wavelength 632.8 nm) of active living human sperm improved their motility and speed [32–35]. During the years 1996 and 1997 other works have been published dealing with coherent light (laser) and incoherent light (visible) motility stimulation of bull, ram, mouse and human spermatozoa [36–41]. The hyperactivated motility of human sperm irradiated with visible light (400–800 nm) has been increased significantly while the concentration of intercellular Ca^{2+} accompanied with a reduction in the hyperactivated motility increased very fast when voltage-dependent Ca^{2+} channel was blocked [41]. Biochemical and topological analysis of bovine sperm cells showed change in sperm metabolism due to low-level laser treatment; however it has been mentioned that more studies are necessary to establish an optimal dose to increase the fertility potential of these cells [42]. Wenbin et al. [43] found that laser irradiation increased sperm fructose fermentation, respiration, the amount of phosphorus (^{32}P) uptake and the Ca^{2+} absorption, thus there was an increase of motility and survival time of spermatozoa. According to Zan-Bar et al. [40], the effects of light are mediated through reactive oxygen species (ROS). Indeed, although high ROS level can lead to cell death (by ATP depletion and lipid peroxidation), at low level, ROS can play a major role in activation of many cellular processes. In the case of spermatozoa, ROS, including superoxide anion, H_2O_2 , and reactive nitrogen species as nitric oxide (NO) can cause sperm hypercapacitation and the acrosome reaction [44–46]. The energy delivery time is, in fact, an essential parameter in laser irradiation, since there should be enough time for the cell to obtain more metabolites from the medium needed for reactions where laser irradiation is involved in. This is similar to the mechanisms of photodynamic therapy (PDT), where the presence of oxygen is needed for the production of an oxygen singlet ($^1\text{O}_2$) [47,48]. However, the laser light photon energy E ($E = hc/\lambda$ where h is Planck's constant, c is the speed of light and λ is the wavelength) is a decisive parameter for the time needed to obtain the required effect. Table 1 lists parameters of lasers mostly used in animal reproduction applications.

In the year 2005, Corral-Baqués et al. [49] demonstrated that irradiating dog sperm with a 655 nm diode laser light at 4.00, 6.00, and 10.00 J/cm^2 improves its motility features and seems to maintain its functional characteristics up to 45 min after irradiation. In 2009 the same research group extended their investigations on the effects of low-level laser irradiation

on dog spermatozoa and its dependence on the laser output power [50]. The results showed that irradiation with different output powers had different effects on semen parameters including motility, average velocity, linear coefficient and beat cross frequency. Yazdi et al. [51] reported on the effect of 830 nm diode laser irradiation on human sperm motility. Significant increase in the irradiated sperm motility has been obtained after exposure to 4.00 and 6.00 J/cm^2 for 60 and 45 min respectively.

Zan-Bar et al. [40] reported the effect of red laser light (660 nm) on ram and tilapia sperm. They found that the use of such red light irradiation led to a slight increase in motility and fertility in ram spermatozoa compared with higher values for the two parameters in tilapia sperm. In an interesting study, Abdel-Salam et al. [20] reported that irradiation of buffalo semen with green laser light (532 nm) at 0.31 J/cm^2 and 0.38 J/cm^2 doses improved semen quality such as motility, progressive, VCL (curvilinear velocity), VSL (progressive velocity), VAP (average path velocity), and ALH (mean amplitude of lateral head displacement) for short exposure times from 4 to 5 min. They interpreted the obtained improvement in semen quality as being due to the shorter wavelength used where the photon energy is higher than in case of using red laser with longer wavelength and low photon energy.

Laser and semen storage

It is well known that semen of domestic animals cannot be stored for longer time without a loss of fertilizing capability even if oxygenated and stored with appropriate diluents at reduced temperature [52]. Recent article [53] reported that He Ne laser irradiation has differential action on biostimulation of turkey, chicken and pheasant spermatozoa. This work is considered the first elucidate of the possibility for restoration of motility of cryopreserved avian spermatozoa by biostimulation provided via He Ne laser irradiation. Previously, the quality of stored turkey semen was found to be improved significantly following He-Ne laser irradiation with energy doses ranging from 3.24 J/cm^2 to 5.40 J/cm^2 and in particular at fluences close to 4.00 J/cm^2 laser prevented *in vitro* liquid storage-dependent damage [54]. It was found in another study [55] that laser irradiation increased the sperm motility parameter, viability, and cell energy charge in rabbit semen. The authors concluded that laser irradiation might be a viable technique for enhancing the quality of semen in long-term storage.

Previous studies by Passarella et al. [13] demonstrated that irradiation with He–Ne laser of energy density 5 J/cm^2 led to an increase in proton electrochemical potential and ATP synthesis in isolated mitochondria. The increase of ATP synthesis has been interpreted by the authors as being due to laser induced protomotive force. Sperm-fertilizing capacity can be affected by changing sperm motility due to all factors enhancing or hindering ATP production or availability. Iaffaldano et al. [55] found that He–Ne laser irradiation improved rabbit sperm preservation during liquid storage modulating sperm qualitative functions that may be related to biostimulation of rabbit spermatid cells and increased cytochrome c oxidase activity.

Laser and bacterial contamination

The extent of microbial contamination is an important parameter to consider in quality control of semen that is used for artificial insemination or direct mating. Semen is an ideal medium for establishment and growth of many microorganisms including bacteria and fungi. Other sources of contamination include inflammatory foci in the genital tract, the skin of animals, semen collection, the processing equipment, animal handlers and laboratory personnel during manipulation. Bartlett [56] considered that low number of bacteria in frozen semen, capable of being transferred during coitus, should be accepted as “normal” because the endometrium of healthy animals could be highly resistant to microbial infection. Perez and Thibier [57] reported that there was no evidence of a relationship between the presence of potentially pathogenic bacteria and the fertilizing ability of semen. To minimize these adverse effects, antibiotics are included in the composition of the ram semen extenders to prevent bacterial growth [58,59]. Addition of variable alternatives antibiotics in extenders to control microbial contamination in semen has been weakened by the increasing appearance of antibiotic resistant strains and a growing list of opportunistic and potential pathogens in semen [60]. Novel methods for controlling the contaminants such as the use of antibodies (monoclonal and genetically-engineered antibodies), anti-idiotypic vaccines, visible wavelengths radiation, and exploitation of natural inhibitors in the semen should be looked at as variable alternatives or supplements to antibiotic treatment [61]. Hussein et al. [62] reported that in Friesian bulls semen medium, reduced bacterial growth and improved semen quality were observed 2, 4, and 8 min after irradiation by light emitting diode (LED) (680 nm, 10 mW) and diode laser (DL) (660 nm, 100 mW). It has been found that such red wavelengths improved semen quality in some of the exposed samples. This coincided with the previous work [63], which showed the importance of red wavelength for the increase of semen viability.

Laser and oocyte maturation

In all assisted reproductive technologies, namely IVM, ICSI, and SCNT, the evaluation of the oocytes quality is of high importance; however the improvement of the maturation rate represents the corner stone of these technologies. In fact there are very few studies concerning the effects of laser on oocyte maturation. Soares et al. [24] used He–Ne Laser (632 nm) to irradiate bovine oocytes. These authors found that laser is capable of modulating events in granulosa cells that may lead

to changes in oocyte. Also, Moreno-Millan and Ocaña-Quero [64] used low laser power to treat immature oocyte, and found that He–Ne laser with doses of 0.40 J/cm^2 and 2.00 J/cm^2 affected the maturation process negatively and caused nuclear damage. Researches indicate that the mechanisms involved in laser interaction with biological cells are due to photons absorption by cellular photoreceptors that trigger chemical reactions such as glycolysis and oxidative phosphorylation. This could accelerate RNA transcription and DNA replication. In addition, it is widely known that laser light accelerates mitosis and affects different metabolic processes through changes induced in the mitochondrial membrane [65]. As mentioned by Karu [66] the absorption of different light wavelengths by cytochromes from the internal mitochondrial membrane increases ATP synthesis. Oocyte suffers from numerous transformations during its maturation to be ready for fertilization and following embryo development. These transformations are mainly induced by granulosa cells, which facilitate communication to oocyte through the gap junctions [34]. Previously published results, using He–Ne laser for irradiation, revealed that the mitochondrial membrane potential has been increased [26,17]. It is most probable that the same mechanism takes place also in case of using other laser wavelengths. Shorter wavelength laser light is especially a good candidate to induce effects similar to those obtained by He–Ne laser irradiation at shorter irradiation time and lower energy doses. This is mainly due to the fact that the shorter is the laser light wavelength the higher is its photon energy. For example, the He–Ne laser photon energy is 1.87 eV while the second harmonic of Nd:YAG laser 532 nm (green) and the diode pumped solid state laser, DPSS, 405 nm (violet) have photon energies of 2.25 eV, 2.92 eV respectively. Consequently the effect of shorter wavelength laser irradiation will be higher on the mitochondria [24]. This is in agreement with what has been reported by Fujiwara et al. [34] who interpreted the induced chemical energy as being due to the absorption of laser energy by proteins of mitochondrial respiratory chain.

Laser characterization of semen

Smuk et al. [67] studied random laser action in bovine semen when excited by a Q-switched frequency doubled Nd:YAG laser, without any analytical investigations. Abdel-Salam and Harith [68] used laser spectrochemical analytical techniques such as laser induced breakdown spectroscopy (LIBS) and laser induced fluorescence (LIF) for characterization of semen samples. Via LIBS those authors obtained information about the elemental seasonal variations in the seminal plasma. Ca, Mg, Zn and Fe were found to be higher in winter than in summer in buffaloes' seminal plasma. They also found that elements' concentrations in seminal plasma have direct relation to the sperm parameters and consequently LIBS can be used for indirect assessment of semen parameters. LIF that is normally used for detection of selective species and studying structure of molecules has been exploited by the same authors to estimate sperm count in buffalo semen samples [68]. The sperm count could be correlated to the intensity of the fluorescence emission and provided the basis for instrumentation for *in situ* rapid determination of sperm counts with no need for conventional microscopic or time consuming imaging techniques in the laboratory.

Conclusions

As mentioned above, the aim of this brief review was to demonstrate what have been published in the topic of improvement of sperm parameters and maturation rate of domestic animals oocytes via laser irradiation. Such improvement can contribute effectively in enhancement of *in vitro* embryo production. The publications in this field are still very limited, and investigated mainly the irradiation effect of red laser ($\lambda = 632.8$ nm) to improve the system of sperm parameters and *in vitro* embryo production [20,24,33,49,68]. However, negative effects on sperm parameters and on the maturation process of the oocyte have been obtained using such red laser for irradiation [50,64].

We do expect that the use of the shorter wavelengths, e.g. $\lambda = 532$ nm and $\lambda = 405$ nm is promising and more reasonable than longer laser wavelength for biostimulative purposes because they will be better absorbed by the cellular chromophores. However, studies indicated that the cellular photoreceptors are capable of absorbing photons that may trigger chemical reactions at certain wavelengths [24–26,56]. The laser fluence (J/cm^2) is the key point of the impacts on the biological cells. However, and as mentioned by Passarella and Karu [69] the overall mechanism of light interaction with the biological samples needs more research work to be fully understood. In view of the photosensitivity of numerous molecules, it is possible to exploit photobiomodulation in photomedicine and biotechnology [69]. In the above review it has been shown that lasers show up in the field of livestock reproduction as an easy, time saving, less costly and effective technique in addition of having the possibility of its use *in situ*, namely in cattle farms and veterinary clinics.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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