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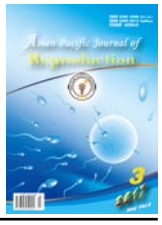

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Laser irradiation effects and its possible mechanisms of action on spermatozoa functions in domestic animals

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ABSTRACT

This article presents a review pertains the laser irradiation effects and its possible mechanisms of action on spermatozoa functions in domestic animals. To improve artificial insemination, laser is sensitive and cost effective technique, when compared to other conventional methods. Laser may have both positive and negative effects on spermatozoa functions. Since the effects of light are mediated by reactive oxygen species, and the levels of these reactive oxygen species following irradiating spermatozoa with laser may be responsible for determining the effects of laser on sperm. Dose of laser may be regarded as of great significance and this dosage of laser may be responsible for determining its effects on spermatozoa. Optimum dosage of laser for improving seminal attributes may vary among various species and this need to be standardized in each of them. The beneficial effects include improving sperm livability, acrosomal integrity, hypo-osmotic swelling response, mitochondrial function and computer-aided sperm analysis parameters. The increase in cytochrome c oxidase activity, ATP levels and mitochondrial membrane potential, in laser irradiated cells may be responsible for enhanced sperm quality parameters. Improving fertility with laser irradiated spermatozoa has been reported in few species like boar and need to be elaborated in other species. In conclusion laser may be regarded as an easy, cheap and time saving technology for improving artificial insemination; in addition, laser may have various potential applications in the field of reproductive biotechnology as well as in livestock farms and veterinary polyclinics.

1. Introduction

Cryopreservation of spermatozoa has led to wider application of artificial insemination. But following cryopreservation, sperm quality reduces which in turn decreases its fertility[1]. Exposure to various stresses during cryopreservation alters sperm physically and also leads to changes in chemical components which are needed for energy metabolism of sperm to support motility[2]. Sperm mitochondrial damage occurs due to destabilization of sperm membranes during freezing-thawing procedures, leading to impaired sperm motility and their capability to survive in the female genital tract[3]. To date, most of the researches have been focused to find out suitable extenders to improve procedures for freezing[4] and/or to protect spermatozoa by use of various additives[5,6], during freezing-thawing process[7]. Sperm mitochondrial function could negatively be affected by use of certain cryoprotectants namely soy lecithin, as revealed by previous findings[8]. So various other strategies aimed to improve sperm mitochondrial function need to be evaluated. Regarding this photobiostimulation of spermatozoa with a low-intensity helium-neon (He-Ne) laser has been shown to improve its motility[9]. Photobiostimulation of sperm was first reported in 1969[10], which has been proven in various species such

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as human[11], mouse[12], sheep[13], dog[14,15], avian[16], and rabbit[17] sperm.

2. Effects of laser irradiation on spermatozoa functions in domestic animals

The effects of laser irradiation on spermatozoa functions in various domestic animals are presented as follows.

2.1. Cattle

Livestock production depends highly on milk-producing animals particularly on cattle and buffalo and contributes to agricultural income-generation, food security, agro-industries and animal products' and animal production trade. As reported by Austin[18] and Chang[19], spermatozoa must reside inside the genital tract of female in order to become capable of fertilizing an oocyte, however many efforts have been made to eliminate the need of female reproductive tract in capacitation process. A variety of *in vitro* capacitating methods for mammalian spermatozoa including exposure to bovine serum albumin, serum, follicular fluid, cumulus cells, adrenal extracts and Sendai virus[20,21]. Ocafia-Quero *et al.*[22] studied effects of He-Ne laser irradiation with fluences from 2 to 16 J/cm^2 at 632 nm on acrosome reaction in bull spermatozoa. They revealed that laser irradiation significantly increases percentage of acrosome reacted spermatozoa and decreases percentage of *in vitro* dead spermatozoa at 90 min of incubation in comparison to other capacitation agents and the control group. The number of acrosome reacted spermatozoa increased and percentage of dead spermatozoa was proportional to intensity of fluencies. In another study, Fernandes *et al.*[23] used aluminum gallium indium phosphide laser with a wavelength of 660 nm and power of 30 mW, to study the effect of low-level laser irradiation on sperm motility, plasma membrane integrity and acrosomal integrity in cryopreserved bull semen (*Bos taurus indicus*). Bull semen was divided into three groups *viz.*, control group without lased irradiation, a 4J group exposed to a laser irradiation dose of 4 joules (133 s of irradiation), and a 6J group exposed to irradiation dose of 6 joules (200 s of irradiation), prior to freezing. The results revealed that there was an increase in percentage of membrane intact (live) and acrosome intact spermatozoa in 4J and 6J group. However, in preserving sperm motility, the dose of 4 joules was more effective than 6 joules.

2.2. Buffalo

Buffalo is one of the favorite animals in many countries, contributing around 52% of total milk products. It is presumed that enhancing its spermatozoa motility will have a promising effect on artificial insemination both qualitatively and quantitatively. Since motility of spermatozoa depends on amount of energy consumption, a higher energy supply to sperms will have a significant effect on their motility. Abdel-Salam *et al.*[24] studied the effect of laser irradiation (fluences ranging from 0.076 to 0.380 J/cm^2) at 532 nm on buffalo semen. They found that low laser irradiation doses (0.31 and 0.38) J/cm^2 for 4-5 min improved progressive motility, sperm velocity (VAP, VCL, VSL), distance (DAP, DCL, DVL), and ALH.

2.3. Ram

Cryopreservation modifies sperm functional and behavioral capacity, reduces sperm motility, their capacities to pass through the cervix and decreases the number of viable spermatozoa in female reproductive tract[25]. This is one of the leading reasons for lower pregnancy rates in ewes inseminated with frozen-thawed semen[26]. It becomes necessary to find out some new alternative techniques to cryopreservation for improving quality of frozen-thawed semen. On exposing tilapia and ram spermatozoa with red light 630-670 nm and white light 400-800 nm, revealed an increase in motility and viability of tilapia sperm with both wavelengths while as only red light was effective in increasing sperm motility and viability in case of ram[13]. Irradiating cryopreserved ram semen after thawing to He-Ne laser with two different energy doses (3.96 and 6.12 J/cm^2) reported that lower dose was ineffective in enhancing semen quality as compared to other irradiated samples and control. The comparison among various parameters *viz.*, sperm viability, osmotic resistance, acrosomal integrity and DNA integrity between control and irradiated samples revealed no significant difference[27]. In another study, using similar laser and doses of energy (3.96 and 6.12 J/cm^2) revealed that He-Ne laser irradiation leads to enhanced ram sperm motility, viability, mitochondrial function and hypo-osmotic swelling response at energy dose rate of 6.12 J/cm^2, in contrast to the dose of 3.96 J/cm^2 which reduces the quality of seminal attributes relative to the control sample[28]. Iaffaldano *et al*[7] conducted a research, in which frozen-thawed ram semen was irradiated with helium-neon laser irradiation with fluences ranging from 3.96-9 J/cm^2. Irradiation at 6.12 J/cm^2 significantly increased sperm motility and viability, however in other parameters such as osmotic resistance, DNA and acrosome integrity, no significant changes were reported. In sperms irradiated with laser, higher cytochrome c oxidase activity and ATP levels were reported than non-irradiated samples. Moreover, cytochrome c oxidase activity and ATP levels were positively correlated, and they also showed positive correlation with motility, suggesting that mitochondria-laser light interactions were responsible for enhanced semen quality in laser irradiated semen samples.

2.4. Horse

Brandˆao *et al*[29] subjected fresh and frozen equine spermatozoa with laser irradiation (650-nm) at energy dose of 6 J/cm^2 for 120 s. The results revealed that in fresh semen samples, beat cross frequency was significantly higher for spermatozoa treated with

laser than without laser treated. Immediately after thawing (0 min), mitochondrial membrane potential was significantly lower in spermatozoa treated with laser before freezing as compared to control. However, 2 h after thawing, plasma and acrosomal membrane integrity was significantly higher in spermatozoa treated with laser before freezing than control. In spermatozoa treated with laser after thawing, no significant difference in sperm parameters was observed 2 h after thawing, however, immediately after thawing, percentage of progressive motility was significantly lower than spermatozoa treated with laser before freezing and cryopreserved control samples.

2.5. Dog

Subjecting fresh dog semen to irradiation doses of 4, 6 and 10 J/cm^2 with a 655-nm continuous-wave diode laser and assessing it for various motility attributes with computer-aided sperm analysis (CASA) and sperm function tests, revealed that average path velocity (VAP), linear coefficient (Lin), beat cross frequency and functional tests were significantly different when compared to the control[14]. Corral-Baqués *et al.*[15] irradiated fresh dog semen with continuous wave diode laser (655 nm) with an average output power of 6.8 mW, 15.4 mW, 33.1 mW and 49.7 mW, respectively, and studied various motility parameters using CASA, at 0 and 45 min after irradiation. They reported that semen motility parameters were affected differently with various output powers, the most intense effects were observed with highest output power. Significant changes in motile sperm subpopulation were attributed to different output powers used.

2.6. Boar

Both intrinsic and extrinsic factors determine boar sperm quality, which in turn affects their reproductive performance. The intrinsic factors are breed[30], age[31] and size of testis[32] and extrinsic factors include semen handling[33], nutrition[34,35], environment[36], semen collection rhythm[37], temperature and photoperiod[38]. Daily variations in either natural or artificial light could only effect semen production in boar in extreme cases, particularly during dark hours[39]. Subjecting boar semen samples prior to AI to photo-stimulation could be a way to override reproductive performance variations in boars. Yeste *et al.*[40] observed the effect of different red light LED regimens on sperm quality and reproductive performance of boars. The best pattern was 10 min light, 10 min rest and 10 min of further light (10-10-10 pattern), which resulted in increase in majority of CASA based motility parameters with no change in sperm viability and acrosomal integrity in liquid stored boar semen. There was higher reduction in sperm quality parameters in non-exposed samples than laser irradiated samples following incubation for 90 min at 37 ℃. Increase in percentage of sperm with high mitochondrial membrane potential due to laser exposure may be responsible for enhanced seminal attributes. Subjecting sperm to *in vitro* capacitation, percentage of spermatozoa with capacitation-like changes in membrane structure were higher in samples exposed to laser irradiation as compared to control ones. Laser treated (10-10-10 pattern), semen samples prior to artificial insemination resulted in significantly higher farrowings rates and both total and live-born piglets.

2.7. Rabbit

Production of commercial rabbits for meat production depends exclusively on artificial insemination programs, but this technique is limited due to the fact that rabbit spermatozoa can be stored for a short length of time. Because of reduced survival of rabbit spermatozoa following long-term liquid storage and freeze-thawing procedures, all inseminations in rabbit are carried with fresh spermatozoa[41,42]. Thus development of novel procedures for enhancing quality of sperm is a goal to be pursued. Irradiating rabbit sperm cells with He-Ne laser prevents *in vitro* storage damage. He-Ne laser irradiation improved rabbit sperm preservation during liquid storage modulating sperm qualitative functions. This effect may be related to the evidence of energetic biostimulation of rabbit spermatic cells and to an improved cytochrome c oxidase activity[17].

2.8. Poultry

Fertilizing capacity of cryopreserved poultry semen shows greater variation, which is responsible for its limited application for production or preservation of genetic stocks[43]. After cryopreservation, poultry spermatozoa are unable to maintain motility due to sensitivity to freeze-thaw procedures. One of the suitable goals to be achieved is developing new techniques for improving motility of avian spermatozoa after freezing thawing procedures. Differential action following He-Ne laser irradiation of frozen-thawed turkey, chicken and pheasant spermatozoa has been reported. This work is first which elucidate the possibility for restoration of sperm motility in cryopreserved poultry spermatozoa following biostimulation with He-Ne laser irradiation[44]. Previous works reported enhancement in quality of stored turkey semen following He-Ne laser irradiation with energy doses of 3.24 J/cm^2 to 5.40 J/cm^2 and particularly fluencies near to 4.00 J/cm^2 significantly reduced vitro liquid storage-dependent damage[16].

Microbial load is one of the essential quality attributes to be considered in semen used for artificial insemination or direct mating. Semen is an ideal medium for growth of microbes including bacteria and fungi and various other sources of its contamination are animals skin, mishandling of artificial vagina during semen collection, the processing equipment, animal handler, semen collector and laboratory personnel during manipulation. As reported by Bartlett[45], in frozen-thawed semen, a lower number of bacteria are accepted as normal due to high resistance of the endometrium of healthy animals to microbial infection. Normally antibiotics are added in

the dilutor to check the growth of microorganisms[46,47], as these microbes impair semen quality due to production of reactive oxygen species. Due to application of a variety of antibiotics in extenders for reducing microbial growth, there is an emergence of antibiotic resistant strains and opportunistic and potential pathogens[48]. One of the novel methods for checking bacterial contamination in semen dilutors is use of laser irradiation. Hussein *et al.*[49] reported reduced microbial growth and enhanced semen quality in Friesian bulls following irradiating semen media with light emitting diode (680 nm, 10 mW) and diode laser (660 nm, 100 mW).

In bovine semen, random laser action by a Q-switched frequency doubled Nd: YAG laser was studied by Smuk *et al.*[50]. Laser induced breakdown spectroscopy (LIBS) and laser induced fluorescence (LIF) are some of the laser spectrochemical analytical techniques used for characterization of semen samples[51]. Using LIBS, Abdul-Salam and Harith[51] used laser spectrochemical analytical techniques such as laser LIBS and laser induced LIF for characterization of semen samples. With the help of LIBS, it was possible to obtain information regarding the seasonal variations of elements in seminal plasma, and it was found that in buffalo bull seminal plasma, Ca, Mg, Zn and Fe were higher during winter season than summer season and the levels of these elements in seminal plasma were directly related to sperm parameters, suggesting that LIBS can be used as an indirect assay for semen parameters. LIF is generally used for identification of selective species and molecular structure studies, has been used for estimation of sperm concentration in semen samples. Sperm concentration could be correlated to the intensity of the emitted fluorescence and thus provided the basis for in situ rapid determination of sperm numbers, thus avoiding the use of microscopic or other time consuming imaging procedures in the laboratory[51].

3. Possible mechanisms of laser action on spermatozoa

Irradiation of sperm with laser leads to its increased respiration, fructose fermentation, ^{32}P uptake and the Ca^{2+} absorption, which increases motility and prolongs sperm survivability[52]. Photobiostimulation occurs when the light is absorbed by porphyrins, flavins or cytochromes and absorbed energy is transferred to oxygen molecules leading to generation of reactive oxygen species (ROS)[53]. ROS are responsible for mediating the effects of light[13]. ROS are known as double edged swords in animal reproduction as higher levels of ROS can lead to sperm death due to depletion of ATP and lipid peroxidation leading to oxidative stress[55] and moderate ROS levels regulate various physiological functions of sperm such as hyperactivation, capacitation, acrosomal reaction and zona binding[54,55]. Studies have shown elevated intracellular Ca^{2+} levels and its transport occurs in irradiated mouse[56] and bull spermatozoa[12,57]. This enhanced intracellular Ca^{2+} transport was responsible for regulating sperm motility, capacitation and the acrosome reaction[58] and in presence of calcium ions, fertilizing capacity of mouse sperm was improved by He-Ne laser irradiation[12]. Calcium mediates sperm activation through its action on axoneme and accessory cytoskeletal components[59,60]. In sperm mitochondria, oxidative phosphorylation leads to generation of ATP, which is primarily needed for sperm motility and it has been shown that energetic charge of cell was increased following laser irradiation[16].

As a result of prolonged storage, sperm mitochondrial ageing occurs, which leads to reduced sperm motility due to reduced capability to generate ATP through mitochondrial respiration[61,62]. In isolated mitochondria, He-Ne laser irradiation leads to an increase in ATP synthesis[63], RNA[64] and DNA synthesis[65], new mitochondria generation[66], activation of enzymes[67], modifications in substrate-enzyme interaction[68], and proton electrochemical potential[16] and it is evident that laser-induced protomotive force may be directly responsible for extra ATP synthesis. The sperm mitochondrial ATP is primarily needed for sperm motility[69], in addition, mitochondria also play a role in maintaining sperm tail contractability, *eg*. regulation of the membrane potential and flux of calcium[70,71]. Electron transfer chain is the common and final pathway in mitochondrial energy metabolism. Embedded in inner mitochondrial membranes is the electron transfer chain with two mobile carriers (Cytochrome C and Coenzyme Q) and four multimeric enzymatic complexes (complexes I, II, III, and IV)[72]. A model proposed by Karu[73] revealed components of the respiratory chain (*i.e.* flavines, cytochromes) after absorbing light, cause the respiratory chain activation and the oxidation of the NAD pool, leading to changes in both mitochondrial and the cytoplasmic redox status. This alteration in redox status may impair transport of ions due to effect on permeability of membrane, which leads to changes in the ratio of Na^+ /H^+, increase in Na^+, K^+, activity of ATPase, which in turn impairs Ca^{2+} flux. Ca^{2+} flux influences the levels of cyclic nucleotides, which in turn modulates synthesis of DNA and RNA, leading to cell proliferation. In particular, sperm motility is related with enzymatic activities of the sperm mitochondrial respiratory chain complexes, in which the apparent rate-limiting step is catalyzed by cyclo-oxygenase (COX) enzyme (or complex IV). A huge amount of oxygen in cells is consumed by COX, which is involved in reduction of dioxygen, acts as redox-linked proton pump and is also responsible for generation of a trans-membrane electrochemical gradient via coupling the free energy of water formation and eventually synthesis of ATP[74,75]. Sperm ATP contents showed positive correlation with progressive motility and COX activity was correlated with both mass and progressive motility[7].

Laser seems to alter somatic cell mitochondrial respiratory chain by changing the electric potential of cell membranes, their permeability to calcium, sodium and potassium ions or by elevating the activity of COX and ATP [9,63,76-78]. Positive correlations have been observed between COX activity with viability and acrosome intact

spermatozoa. COX enzyme (or complex IV) appears to be involved in catalyzing rate-limiting step in sperm mitochondrial respiratory chain complexes, which are related to spermatozoa motility. Various attributes responsible for enhancing or reducing ATP availability or production particularly those that mediate mitochondrial function, could affect fertilizing capability of spermatozoa by changing motility[16]. COX activity is enhanced with He-Ne laser irradiation, which leads to proton pumping, needed for generation of the electrochemical proton gradient for driving ATP synthesis[7]. In avian semen, a high rate of species specific response to He-Ne laser biostimulation in terms of COX activity has been observed. Irradiation leads to enhanced COX activity in pheasant and turkey sperm, but not in chicken sperm. Variations originating from different energy metabolism of spermatozoa following photo-stimulation may be responsible for differential response of avian spermatozoa to irradiation[44]. It is reported that while turkey spermatozoa chiefly depend on mitochondrial oxidative metabolism for ATP and higher glycolysis activity has been observed in chicken spermatozoa for provision of ATP in anaerobic conditions[59]. Reduced Km values after He-Ne laser irradiation, indicates increased substrate enzyme affinity, and confirms the findings, where Km values for purified COX were declined in response to He-Ne laser subjection[67]. Time-related reduction in sperm quality during refrigeration was declined by laser irradiation treatment and this may be due to elevated activity of COX and augmentation of EC in laser irradiated spermatozoa[17]. Thus by acting as a potent antioxidant, COX is involved in scavenging excess free radicals, generated in mitochondria.

4. Conclusion

In conclusion, laser is a cost effective and more sensitive technology which can be used for enhancing the artificial insemination system. The beneficial effects of laser on spermatozoa include increase sperm livability, acrosomal integrity, hypo-osmotic swelling response, mitochondrial function and CASA based sperm parameters (progressive motility, sperm velocity (VAP, VCL, VSL), distance (DAP, DCL, DVL), and ALH. The increase in these sperm attributes may be related to the fact that laser irradiation leads to higher cytochrome c oxidase activity, ATP levels and mitochondrial membrane potential, which in turn lead to enhanced sperm survival. Enhancing sperm survival using various doses of laser irradiation need to be standardized, in addition to this, fertility trials with laser irradiated spermatozoa and gene expression patterns in lased treated sperms in various species need to be conducted. Laser may be considered as double edged sword like ROS, as laser may have beneficial as well as harmful effects on spermatozoa of various species depending upon the dose of irradiation used. Dose of laser irradiation may be of paramount importance. Red light has been found to have more negative effects on sperm quality parameters[15]. Application of shorter laser wavelengths may be more reasonable for biostimulative purposes than longer laser wavelengths, because former are better absorbed by cellular chromophores than latter. To sum up, laser in the field of semen biology may be regarded as an easy, time saving, less costly and effective technique, in addition to other fields of reproduction like reproductive biotechnology and laser may have the possibility of its use in livestock farms and veterinary polyclinics.

Conflict of interest statement

We delcare that we have no conflict of interest.

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