Journal of Applied Biology & Biotechnology Vol. 3 (04), pp. 032-037, July-Aug, 2015
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2015.3407

Visible Diode Laser Enhancement of Exotic DNA Uptake by Fowl Sperm

Essam A. El-Gendy[1], Mona M. Abdelaziz[2], Mohamed M. Abdelfattah[2], Mohamed S. Salama[3], YhiaM. Badr[2]

[1] Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt. [2] Department of Laser Applications in Metrology, Photochemistry and Agriculture, National Institute of laser Enhanced Sciences, Cairo University, Giza 12613, Egypt. [3] The Unit of Genetic Engineering, Faculty of Sciences, Ain Shams University, Cairo 11566, Egypt.

ARTICLE INFO

Article history:
Received on: 02/07/2015
Revised on: 14/07/2015
Accepted on: 28/07/2015
Available online: 24/08/2015

Key words:
fowl sperm, laser irradiation, lipofectin, exotic DNA.

ABSTRACT

An experiment was conducted to assess the use of low power laser irradiation and lipofectin to enhance fowl sperm uptake of exogenous DNA. Semen samples of 10 roosters were collected and pooled. The pooled sample was diluted with a semen extender and then divided into 6 aliquots for 6 different treatments. The treatments included different combinations of the inclusion of exogenous DNA (bacterial plasmid pUC18), the exposure to low power laser irradiation and the transfection with lipofectin (5%). Laser irradiation was by using visible diode laser (650 nm) at energy dose of 4 J/cm^2. The recognition of the plasmid DNA in the sperm was by using two specific oligonucleotides (forward and reverse) to prime a 420-bp fragment on the pMB1 *rep* of the plasmid. The results indicated that low power laser irradiation enhanced the sperm uptake of the plasmid DNA. Also, lipofectin enhanced the introduction of the plasmid DNAinto the sperm, whether the semen was laser irradiated or not.

1. INTRODUCTION

Gene transfer technology is a potent biotechnological potentially universal tool that allows for the generation of GM animals useful for biomedical and veterinary purposes, for research on the mechanisms of gene function or for the genetic improvement by formation of new phenotypes that increase the economic value of the animals [1-4]. Sperm-mediated gene transfer (SMGT) technique is based on the ability of sperm cells to bind and internalize exogenous DNA molecules under appropriate conditions and to transfer them into the oocyte during fertilization [5, 6]. Although SMGT seems applicable in avian species, the detailed knowledge of the sperm uptake of DNA is not entirely understood. Also, the method of increasing the efficiency of DNA integration into the sperm chromatin is not entirely developed. Francolini *et al.* [7] attributed the significance of SMGT is that the sperm is used as a natural vector of genetic material for transporting the target DNA. The exogenous DNA molecules bind to the sperm head in the subacrosomal region and in the proximity of the equatorial area. Once bound to the cell membrane, DNA molecules are taken up [8]. The ability of sperm cells to bind DNA seems a general feature in different animal species [5]. Lavitrano *et al.*[9] showed that the incorporation of DNA into the sperm is mediated by DNA receptors located on spermatozoa which are specific DNA-binding proteins (DBPs) of 30-35 KD. El-Gendy *et al.* [4] reported that the ability of sperm to associate DNA molecules was positively influenced by sperm motility. Also, DNA/sperm ratio was a parameter determining number of DNA molecules incorporated into the sperm. The more is the DNA/sperm ratio, the more is the number of DNA molecules associated with sperm. Camaioni *et al.* [10] showed that when mouse spermatozoa were briefly exposed to radioactively labeled DNA pSV2CAT plasmid, radioactivity was detected by high-resolution autoradiography on the surface and within the nucleus of the spermatozoa. El-Gendy *et al.* [4] demonstrated that rooster sperm was capable of binding DNA molecules, and the efficiency of DNA to pass into sperm nuclei could be greatly enhanced with the presence of a liposome reagent. The nucleus of sperm is the actual organelle entering the oocyte during the process of fertilization. The evidences suggested that the transgene was preserved inside the limited space of nucleus and integrated into the de-condensed chromosomes at the fusion of two nuclei. Nakanishi and Iritani [11] reported that while spermatozoa incubated with liposome, the binding position of foreign DNA and spermatozoa was no longer limited to the front of acrosome.

The investigators further radioactive-labeled the sperm and found that 52% of the sperm were labeled. It was also reported that the motility of chicken spermatozoa transfected with liposome declined slightly and the hatchability after artificial insemination was 90%. El-Gendy *et al.* [12] carried out an experiment to develop transgenic chickens using sperm incubated with the plasmid pUC18 as an exogenous DNA.

** Corresponding Author*
Essam A. El-Gendy, Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt
Email: essamaelgendy@yahoo.com

Two lines of chickens were used as parental flocks, and lipofectin was used in different concentrations to facilitate the sperm cell uptake of the plasmid DNA. Hens were inseminated with the treated semen. The plasmid DNA was recognized in spermatozoa of both lines in most of the treatments with different amplification rates. However, plasmid DNA was highly fused into the sperm cells when it was introduced in a complex with lipofectin. Also, the plasmid DNA band was highly amplified in the progeny which resulted from sperm cells incubated with the plasmid and lipofectin (5%). The results revealed the success of the development of F_1 chickens by SMGT. The SMGT-derived offspring formed 40 and 50% of the F_1 generation in both lines.

On the other hand, low-power laser light, like sunlight, is non-ionizing and has unique properties that can influence the biological activities under certain conditions [13]. It accelerates proliferative processes in irradiated tissues [14, 15]. Also, Karu [16] and Wong-Riley *et al.* [17] reported that the irradiation of red and near infrared light can lead to the activation of mitochondrial respiratory chain components and the initiation of a signaling cascade which promotes cellular proliferation and protection. Grossman *et al.*[18]reported that the anti-oxidant enzymes have maximum light absorption in the red spectrum range and therefore their activities can be initiated by it. Karu [19] reported that the light must be absorbed by the endogenous chromophores of cells or tissues for actions. Gao and Xing [20] revealed that low power laser irradiation (LPLI) promotes proliferation of multiple cells, mainly through the activation of mitochondrial respiratory chain and the initiation of cellular signaling. Mitochondria are the center of many diverse cellular functions integrating signals between the organelle and the nucleus. Yakimenko *et al.* [21] used low intensity (λ=633 nm) light irradiation on hatching chicken and quail eggs to determine its influence on embryonic and post-embryonic development. Hatching rates significantly increased by 3.66-4.05 % and chick mortality significantly decreased by 1.25-3.23 % in layer and broiler chickens compared to the untreated controls. The hemoglobin in chick blood was increased during embryonic stage and decreased in post embryo period. The liver peroxide levels were changed and cytochrome P-450 enzyme system was activated without adversely affecting liver energy metabolism. The objective of this study was to assess the use of a low power diode laser irradiation to enhance the sperm uptake of exogenous DNA in the presence of lipofectin as a transfection reagent.

2. MATERIALS AND METHODS

2.1 Semen Source

Ten adult males of an experimental control chicken line [22] were used as the source of semen. Semen was collected by the dorsal-abdominal massage described by Lake [23], and contamination of semen with the cloacae products was avoided.

2.2 Exogenous DNA and Transfection Reagent

The exogenous DNA used in this experiment was the bacterial plasmid pUC18 (GenBank/ EMBL accession number #L09136, Sigma-Aldrich corporate, 10115 Berlin, Germany). It is a cloning vector isolated from *E.coli* and is 2686 bp long. It contains three regions as described by Yanisch-Perron *et al.* [24]. Region 1 is the pMB1 *rep*, which is responsible for the replication of the plasmid. Region 2 is the *bla* gene which encodes for beta-lactamase that confers resistance to ampicillin. Region 3 is *E.coli* operon *lac* containing CAP protein binding site, promoter P_{lac}, *lac* as a repressor binding site and 5'-terminal part of the *lacZ* gene encoding for the N-terminal fragment of beta-galactosidase. Two specific oligonucleotides (Table 1) were used to recognize a 420-bp fragment on pMB1 *rep* region of the plasmid pUC18. Lipofectin (cat.No.18292-011, 1 ml, Invetrogen, USA) was used for transfection. It is a 1:1 (w/w) liposome formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) and dioleoylphophotidylethanolamine (DOPE) in membrane filtered water [25].

Table 1: Lengths and sequences of the forward and reverse primers, specific for pMB1 replicon of plasmid pUC18 and the primer recognition site on plasmid.

Primer	Length (b)	Sequence	Recognition site
Forward	18	5'-TGACGCCGGGCAAGAGCA-3'	1130-1147
Reverse	20	5'-GGCCGAGCGCAGAAGTGGTC-3'	1531-1550

2.3. Laser Source

A continuous wave diode laser source (Model HLM 1230) was used to produce a wavelength of 650nm. The output power averaged 6mW, and was measured using a high sensitivity sensor (PM-450B Optical Power Meter). For stability, the laser source was turned on 15 minutes before the exposure.

2.4 The Experimental Design

The semen was individually collected from the birds three times, three days apart to make three replicates. In each, the individual samples were immediately intermingled to form a pooled semen sample. The pooled semen was diluted (1:4, v:v) using Beltsville poultry semen extender (BPSE) of Sexton [26] to a concentration of 1×10^9 spermatozoa/ml and then divided into 6 aliquots and placed in 6 polystyrene cuvettes to form 6 different treatments. In treatment 1 (T_1), the semen aliquot was kept untreated (control treatment). In treatment 2 (T_2), the semen aliquot was exposed to 650nm continuous wave diode laser of irradiation at energy dose of 4J/cm^2.In treatment 3 (T_3), the semen aliquot was mixed with the plasmid pUC18 (2.5µg plasmid/100µl diluted semen). In treatment 4 (T_4), the semen aliquot was laser irradiated at energy dose of 4J/cm^2 and mixed with the plasmid pUC18. In treatment 5 (T_5), the semen aliquot was mixed with the plasmid pUC18 and lipofectin (2.5µg plasmid + 5µg lipofectin/ 100µl diluted semen). In treatment 6 (T_6), the semen aliquot was laser irradiated at energy dose of 4J/cm^2 and mixed with the plasmid pUC18 and lipofectin (2.5µg plasmid + 5µg lipofectin/ 100µl diluted semen). Samples were kept at 37°C during the preparation of all treatments. They were subsequently stored at the

same temperature in a shaking incubator at 150 rpm for 120 minutes. After 2 h of incubation for all treatments, each semen aliquot was washed by adding 500µl of BPSE solution and mixed thoroughly, and then centrifuged at 4000rpm for 5 minutes. The supernatant was removed and the pellets (sperm cells) were re-washed using the same procedure. In T_1, sperm were also subjected to the washing procedure and used as a negative control. A sample of plasmid DNA was also used as a positive control.

2.5 DNA Extraction

DNA was extracted from washed sperm of all treatments, according to the manufacturer's instructions (Qiagen DNeasy Blood & Tissue Kit, cat. no. 69504, Qiagen GmbH D.4072724 Hilden, Germany). In this concern, 200µl of semen from each treatment were added to 10ml of buffer 1 (Table 2) in a Corex centrifuge tube and vortexed for 10 s at full speed.

Table 2: The chemical composition of the buffers used in DNA extraction.

Buffer	Constituent	
Buffer 1	150 mM	NaCl
	10 mM	EDTA, pH=8.0
Buffer 2	100 mM	Tris-Cl, pH 8.0
	10 mM	EDTA
	500 mM	NaCl
	1%	SDS
	2%	β-mercaptoethanol

The contents were then centrifuged for 10 min at 4000 rpm (2500 xg) and the supernatant was removed carefully, leaving approximately 1 ml of pellet and buffer 1 mixture. The pellet was resuspended and vortexed for 10 s at full speed and transferred into a 2 ml microcentrifuge tube. Then, 0.5 ml buffer 1 was added to the Corex tube and vortexed for 10 s at full speed to collect any sample adhering to the walls of the tube and transferred into the same microcentrifuge tube. The microcentrifuge tube was centrifuged for 2 min at full speed in a microcentrifuge and the supernatant was removed carefully. The pellet was resuspended again in 300 µl of buffer 2 (Table 2). One hundred µl of proteinase K was added to suspended sperm and the content was incubated for 2 h at 56°C in a shaking waterbath to disperse the sample. Then, another 20µl of proteinase K were added to the sperm suspension and incubated for a further 2 h at 56°C in the shaking water bath to disperse the sample.

Then, 400µl of buffer AL were added to the sample, and mixed thoroughly by a vortex. Then, 400 µl of ethanol (96–100%) were added, and mixed thoroughly by a vortex. Then, 650 µl of the mixture, including any precipitate were transferred into DNeasy mini spin column, placed in a 2 ml collection tube and centrifuged at 6000 xg for 1 min and the flow was discarded. This step was repeated until the sample has been loaded. The DNeasy mini spin column was placed in another 2 ml collection tube, and 500 µl of buffer AW1 were added, and the contents were centrifuged for 1 min at 6000 xg and the flow was discarded. The DNeasy mini spin column was placed in a another 2 ml collection tube, and 500µl of buffer AW2 was added, and the contents were centrifuged for 3 min at 20,000 xg to dry the DNeasy membrane, and the flow was discarded. The DNeasy mini spin column was placed in a clean 1.5ml microcentrifuge tube and 200µl of buffer TE were pipetted directly onto the DNeasy membrane, and the contents were incubated at room temperature for 1 min, and then centrifuged for one min at 6000xg to elute and kept at -20ºC until use.

The DNA solution of each treatment was diluted by the elution buffer TE (1 µl DNA solution /100µl TE buffer). Then, DNA concentration was determined using a double beam UV/visible spectrophotometer (JENWAY Double Beam 6800, UK) at 260nm, according to Sambrook *et al.* [27]:

DNA conc. (µg/µl) = 50µg /1000 x A_{260} x dilution factor

A_{260} is the spectrophotometer reading at 260 nm, the dilution factor was 100 and the value of 50µg represents DNA concentration (µg/ml) for each unit of the spectrophotometer reading.

2.6 Recognition of Exogenous DNA in the Sperm

The polymerase chain reaction (PCR) was applied to all sperm treatments, to recognize the existence of the plasmid DNA in the sperm. The PCR was also applied to the non-treated (negative control) semen and to the plasmid DNA (positive control). The PCR analysis was performed in 20µl reaction solution (Table 3). Samples were subjected to initial denaturation at 95ºC for 5 min, and 35 cycles of denaturation at 95ºC for one min, followed by the annealing at 65ºC for one min and the extension reaction at 72ºC for 2 min and the final extension was at 72ºC for 7 minutes.

The agarose gel (2%) was prepared by melting one g agarose in 50 ml 1X TBE buffer and inoculated with 1µl ethedium bromide (10mg/ml). The PCR products were mixed with 2µl loading dye and loaded to the garose gel. Also, a Gelpilot 100-pb DNA marker (cat # 2399035, Qiagen GmbH D.4072724 Hilden, Germany) was loaded. The loaded products were then subjected to the electrophoresis at 50 volt for 1.5 h and DNA fragments were visualized on the gel under UV trans-illuminator.

Table 3: The polymerase chain reaction (PCR) components.

Reaction components	Amount
DNA sample	1 µl (100 ng)
Master Mix [1]	10 µl (2x)
Forward primer	2 µl (0.2 µmol)
Reverse primer	2 µl (0.2 µmol)
Distilled water	5 µl
Total volume	20 µl

[1], Source: Qiagen GmbH, D.4072724 Hilden, Germany.

3. RESULTS AND DISCUSSION

The electrophoresis of the total sperm DNA before and after running PCR is photographed (Figure1). The plasmid DNA was not recognized in the sperm-PCR product and this was considered a negative control. However, the plasmid DNA was recognized by the fragment detected at 420 bp in the plasmid-PCR product and was considered a positive control. This result reveals that the presence of the 420 bp in the sperm-PCR products denotes to the successful detection of the plasmid.

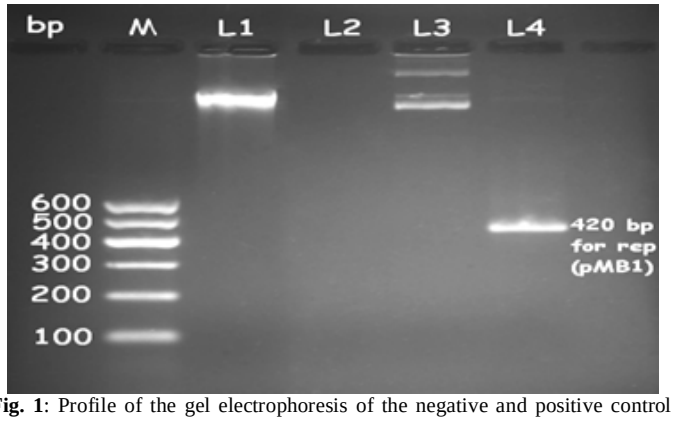

Fig. 1: Profile of the gel electrophoresis of the negative and positive control treatments. L1: the sperm DNA not subjected to PCR analysis. L2: the PCR product of sperm DNA, where semen was diluted with BPSE and no recognition of the 420-bp fragment (negative control). L3: the plasmid pUC18, not subjected to PCR analysis. L4: the PCR product of plasmid pUC18, where the specific primers recognized the 420-bp fragment of pMB1 replicon (positive control). M is the lane of the 100-bp DNA marker.

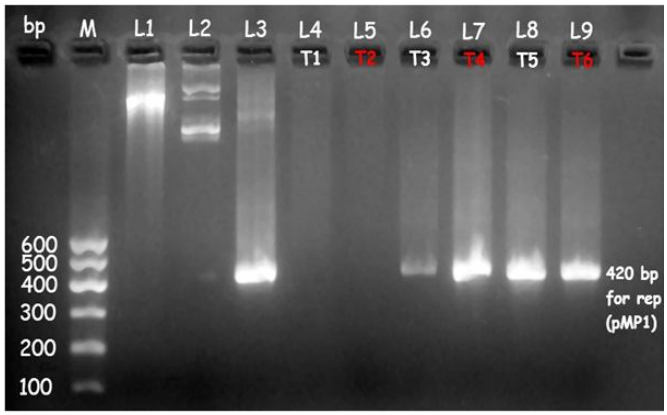

Fig. 2: Evidence of the fusion of the plasmid pUC18 into fowl sperm. L1: the sperm DNA not subjected to PCR analysis. L2: the plasmid pUC18, not subjected to PCR analysis. L3: the PCR product of the plasmid pUC18 (positive control). L4: the PCR product of sperm DNA, where semen was diluted with BPSE (T_1) and no recognition of the 420-bp DNA fragment (negative control). L5: the PCR product of sperm DNA, where semen was diluted with BPSE and laser irradiated at 4 J/cm^2 (T_2). L6: the PCR product of sperm DNA, where semen was diluted with BPSE and incubated with the plasmid pUC18 (T_3). L7: the PCR product of sperm DNA, where semen was diluted with BPSE, laser irradiated and incubated with plasmid pUC18 (T_4). L8: the PCR product of sperm DNA, where semen was diluted with BPSE and incubated with the plasmid pUC18 and lipofectin (T_5). L9: the PCR product of sperm DNA, where semen was diluted with BPSE, laser irradiated and incubated with the plasmid pUC18 and lipofectin (T_6). M is the lane of the 100-bp DNA Marker.

In figure 2, the 420-bp fragment of the plasmid DNA was detected in the sperm-PCR product in the semen diluted with BPSE and incubated with the plasmid pUC18 (T_3) and also in the semen diluted with BPSE, incubated with the plasmid pUC18 and laser irradiated (T_4). Also, the 420-bp fragment was detected in the sperm-PCR product of the semen diluted with BPSE and incubated with a mixture of the plasmid and lipofectin 5% (T_5) and in the sperm-PCR product of the semen diluted with BPSE, incubated with a mixture of the plasmid and lipofectin 5% and laser irradiated (T_6). The amplification rates of 420-bp fragment were almost similar in T_4, T_5 and T_6 and more than that recognized in T_3. These results indicate that laser irradiation of the semen enhanced the fusion of the plasmid into the sperm (T_4 vs. T_3). Also, lipofectin 5% enhanced the fusion of the plasmid into the sperm (T_5vs T_6), whether the semen was laser irradiated or not (T_6 vs. T_5).

Lipofectin transfection reagent is a small unilamellar liposome containing N-[l-(2,3,-dioleyloxy)propyl]-N,N,N-trimethylamrnonium chloride (DOTMA) and spontaneously interacts with DNA to form lipid-DNA complex with 100% entrapment of DNA [25]. DOTMA facilitates the fusion of the complex through the plasma membrane of tissue culture cells resulting in both uptake and expression of the DNA. This interprets the high fusion rate of the plasmid into the sperm cells resulting in high amplification rates of 420-bp fragment when lipofectin was added in T_5 and T_6 compared to PCR fragment recognized in T_3 where lipofectin was not added. Walker *et al.*[28] reported that all chemical DNA delivery systems contain compounds with one or more positive charges. In addition, many delivery formulations require the presence of a neutral lipid such as dioleoyloxyphosphatidyl ethanolamine (DOPE). The function of the positively charged compounds is to interact with the negatively charged phosphate backbone of DNA molecule. Neutralizing the charge promotes collapse of the DNA into a more compact structure. Because the resulting complexes have a net positive charge, they interact with negatively charged biological membranes. What happens next is unclear, but somehow the positively charged DNA complex particle enters the cell. Entry may occur directly through the plasma membrane or via an intermediate endosome [29, 30]. According to Axelord *et al.* [31], DOPE facilitates membrane fusion so DNA molecules can internalize into the cytoplasm.

Lanes *et al.* [32] reported that DNase activity in seminal plasma was present in all sources of semen tested: ejaculated semen, semen from the lumen of the testes, and semen from macerated testes. The presence of DNase in seminal plasma is likely the cause of failure of other attempts to produce transgenic animals using SMGT. Carballada and Esponda [33] revealed that DNase is the main source of inhibition of DNA uptake by mammalian spermatozoa. Sato *et al.* [34] stated that it was noteworthy that DNase activity was completely inhibited by 40 mM EDTA. Similarly, 50 mM EDTA diminished DNase activity in chicken and mouse semen, this organic chelator eliminates free divalent cations (Ca^{2+} and Mg^{2+}) in the medium, which exert the activity of DNase I. However, Nechaevsky and Ivanov [35] reported that DNase II is only active at acidic pH and in the absence of bivalent cations. Therefore, this enzyme does not appear to play an important role in the interaction between sperm and exogenous DNA. El-Gendy *et al.* [4] eventually showed the rooster sperm uptake of the plasmid pUC18, as an exo-gene, with a high rate in the presence of lipofectin (5%). It was reported that lipofectin coated the plasmid and kept it intact. El-Gendy *et al.* [12] successfully developed the F_1 transgenic chickens by SMGT and using lipofectin as a transfection reagent. Also, Collares *et al.* [36] reported successful transmission of an exogenous DNA to chickens by SMGT after removing seminal plasma and treating the

exogenous DNA with dimethylsulfoxide or N,N-dimethylacetamide. Lubart *et al.* [37] indicated that there is an accelerated exchange and uptake of Ca^{2+} between the medium and the bovine sperm cells during irradiation with 6-8 J/cm^2 He-Ne (633 nm). Lubart *et al.* [38] revealed that the plasma membrane is not affected by He-Ne irradiation, and therefore suggested that Ca^{2+} transportation rate into the cells due to irradiation at 633 nm is determined by the mitochondrial Ca^{2+} uptake. These findings could provide an explanation to the role of LPLI in enhancement the sperm uptake of exogenous DNA during the process of SMGT which could be due to the alteration in the uptake of Ca^{2+} between the medium and the sperm cells during laser irradiation. In addition, Iaffaldano *et al.* [39] reported that the He-Ne laser irradiation of rabbit sperm cells prevented the damage in spermatozoa due to liquid storage.

4. CONCLUSION

This research aims at the development of a technique for the delivery of an exotic gene into sperm cells, so that the generation of genetically modified (GM) animals would be possible. One major concern about the development of GM animals is the production of biochemical compounds, such as enzymes and vaccines for potential medical and veterinary purposes. Another concern is the genetic buildup of the animal to carry a gene that sponsors the superior productivity or the survivability in adverse environmental climates. The results indicated that low power laser irradiation enhanced the sperm uptake of the plasmid DNA. Also, lipofectin enhanced the introduction of the plasmid DNA into the sperm, whether the semen was laser irradiated or not.

5. REFERENCES

1. Lillico SG, McGrew MJ, Sherman A, Sang HM. Transgenic chickens as bioreactors for protein-based drugs. Drug Discovery Today. 2005; 10: 191-196.
2. Smith K, Spadafora C. Sperm-mediated gene transfer: applications and implications. BioEssays: news and reviews in molecular, cellular and developmental biology. 2005; 27: 551-562.
3. Lavitrano M, Busnelli M, Cerrito MG, Giovannoni R, Manzini S, Vargiolu A. Sperm-mediated gene transfer. Reproduction, Fertility and Development. 2006; 18: 19–23.
4. El-Gendy EA, Gad AY, Mostageer A. Sperm-mediated gene transfer in poultry 1. The relationship with cock sperm viability. Arab Journal of Biotechnology. 2007; 10: 1-12.
5. Zani M, Lavitrano M, French D, Lulli V, Maione B, Sperandio S, Spadafora C. The Mechanism of Binding of Exogenous DNA to Sperm Cells: Factors Controlling the DNA Uptake. Experimental Cell Research. 1995; 217: 57-64.
6. Gandolfi F. Sperm-mediated transgenesis. Theriogenology. 2000; 53: 127-137.
7. Francolini M, Lavitrano M, Lamia CL, French D, Frati L, Cotelli F, Spadafora C. Evidence for nuclear internalization of exogenous DNA into mammalian sperm cells. Molecular Reproduction and Development. 1993; 34: 133-139.
8. AnzarM,Buhr MM. Spontaneous uptake of exogenous DNA by bull spermatozoa. Theriogenology.2006;65:683-690.
9. Lavitrano M, French D, Zani M, Frati L, Spadafora C. The interaction between exogenous DNA and sperm cells. Molecular Reproduction and Development. 1992; 31: 161-169.
10. Camaioni A, Russo MA, Odorisio T, Gandolfi F, Fazio VM, Siracusa G. Uptake of exogenous DNA by mammalian spermatozoa: specific localization of DNA on sperm heads. Journal of Reproduction and Fertility. 1992; 96: 203-212.
11. Nakanishi A, Iritani A. Gene transfer in the chicken by sperm-mediated methods. Molecular Reproduction and Development. 1993; 36: 258-261.
12. El-Gendy EA, Ahmed MM, El-Tantawy SM. Development of transgenic chickens by sperm-mediated gene transfer. African Journal of Biotechnology. 2013; 12: 2755-2763.
13. Karu TI. Molecular mechanism of the therapeutic effect of low-intensity laser radiation. Laser in Life Sci. 1988; 2: 53–74.
14. Mester E, Mester AF, Mester A. The biomedical effects of laser application. Lasers in Surgery and Medicine. 1985; 5: 31-39.
15. Tafur J, Mills PJ. Low-intensity light therapy: exploring the role of redox mechanisms. Photomedicine and Laser Surgery. 2008; 26: 323-328.
16. Karu TI. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. Journal of Photochemistry and Photobiology B: Biology. 1999; 49: 1-17.
17. Wong-Riley MT, Bai X, Buchmann E, Whelan HT. Light-emitting diode treatment reverses the effect of TTX on cytochrome oxidase in neurons. NeuroReport. 2001; 12: 3033-3037.
18. Grossman N, Schneid N, Reuveni H, Halevy S, Lubart R. 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species. Lasers in Surgery and Medicine. 1998; 22: 212-218.
19. Karu TI. Mitochondrial signaling in mammalian cells activated by red and near-IR radiation. Journal of Photochemistry and Photobiology. 2008; 84: 1091-1099.
20. Gao X, Xing D. Molecular mechanisms of cell proliferation induced by low power laser irradiation. Journal of Biomedical Science. 2009; 16: 4.
21. Yakimenko I, Besulin V, Testik A. The effects of low intensity red laser irradiation on hatching eggs in chicken and quail. International Journal of Poultry Science. 2002; 1: 6-8.
22. El-Gendy EA. A model for the genetic employment of chickens local to warm climate 1. Crossing with a fast growing strain and growth patterns of the crossbreds. International Journal of Poultry Science.2009;8: 299-306.
23. Lake PE. Fowl semen as collected by the massage method. The Journal of Agricultural Science. 1957; 49: 120-126.
24. Yanisch-Perron C, Vieira J, Messing J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mpl8 and pUC19 vectors. Gene. 1985;33:103-119.
25. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM, Danielsen M. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proceedings of the National Academy of Sciences. 1987; 84: 7413-7417.
26. Sexton TJ. A new poultry semen extender 1. effect of extension on the fertility of chicken semen. Poultry Science. 1977; 56: 1443-1446.
27. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual. NY, USA: Cold Spring Harbor Laboratory Press, Cold Spring Harbor; 1989.
28. Walker S, Sofia MJ, Axelrod HR. Chemistry and cellular aspects of cationic facial amphiphiles. Advanced Drug Delivery Reviews. 1998; 30: 61-71.
29. Leventis R, Silvius JR. Interactions of mammalian cells with lipid dispersions containing novel metabolizable cationic amphiphiles. Biochimica et Biophysica Acta (BBA) – Biomembranes. 1990; 1023: 124-132.
30. Behr JP. Gene transfer with synthetic cationic amphiphiles: prospects for gene therapy. Bioconjugate Chemistry.1994;5: 382-389.
31. Axelrod HR, Sofia MJ, Walker S. Chemistry and cellular aspects of cationic facial amphiphiles. Advanced Drug Delivery Reviews. 1998; 30: 61-71.
32. Lanes CF, Sampaio LA, Marins LF. Evaluation of DNase activity in seminal plasma and uptake of exogenous DNA by spermatozoa of the

Brazilian flounder Paralichthys orbignyanus. Theriogenology. 2009; 71: 525-533.
33. Carballada R,Esponda P. Regulation of foreign DNA uptake by mouse spermatozoa. Experimental Cell Research.2001;262: 104-113.
34. Sato F, Soh T, Hattori MA, Fujihara N. Evaluation of deoxyribonuclease activity in seminal plasma of ejaculated chicken semen. Asian Journal of Andrology. 2003; 5:213-216.
35. Nechaevsky YV, Ivanov VA. DNase II in spermatozoa of the loach misgurnus fossilis L. Biochemistry. 1999; 64: 488-493.
36. Collares T, Campos VF, DeLeon PM, Cavalcanti PV, Amaral MG, Dellagostin OA,Deschamps JC,Seixas FK. Transgene transmission in chickens by sperm-mediated gene transfer after seminal plasma removal and exogenous DNA treated with dimethylsulfoxide or N,N-dimethylacetamide. Journalof Bioscience. 2011;36:613-620
37. Lubart R, Friedmann H, Levinshal T, Lavie R, Breitbart H. Effect of light on calcium transport in bull sperm cells. Journal of Photochemistry and Photobiology B: Biology. 1992; 15: 337-341.
38. Lubart R, Friedmann H, Sinykov M, Cohen N, Breitbart H. Changes in calcium transport in mammalian sperm mitochondria and plasma membranes caused by 780 nm irradiation. Lasers in Surgery and Medicine. 1997; 21: 493-499.
39. Iaffaldano N, Rosato MP, Paventi G, Pizzuto R, Gambacorta M, Manchisi A and Passarella S. The irradiation of rabbit sperm cells with He–Ne laser prevents their in vitro liquid storage dependent damage. Animal Reproduction Science.2010;119: 123-129.

How to cite this article:

El-Gendy E.A., Abdelaziz M.M, Abdelfattah M.M, Salama M.S, Badr Y.M. Visible Diode Laser Enhancement of Exotic DNA Uptake by Fowl Sperm. J App Biol Biotech. 2015: 3 (04): 032-037.