Scientific Papers. Series D. Animal Science. Vol. LVII, 2014
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN-L 2285-5750

THE EFFECTS OF HELIUM-NEON LASER WITH DIFFERENT ENERGY DOSES ON CRYOPRESERVED RAM SEMEN QUALITY IN VITRO EXAMINATION

[1]Mostafa A.R. IBRAHIM, [2]Stela ZAMFIRESCU, [2]Andreea ANGHEL, [2]Nicu DOBRIN, [1]Ibrahim ABDELRAZEK, [1]Mohamed E. EL-SHARAWY, [4]El-Shenawy El- sefiy, [3]Dorina MOCUTA

[1]KafrelSheikh University, Faculty of Agriculture
[2]Ovidius University in Constanta
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest
[4]Animal Production Research Institute

Abstract

The aim of the study was to investigate the effects of different energy doses of helium–neon (He–Ne) laser irradiation on the functions and functional quality of sheep spermatozoa during in vitro liquid storage. In the study of the quality of stored turkey semen was found to be improved significantly following He-Ne laser irradiation and irradiation with He-Ne laser prevented their in vitro liquid storage-dependent damage. It was found also in another study, that irradiation increased the sperm motility index, viability, and cell energy charge. Frozen ram sperm samples in the present study, were thawed in a water bath at 37°C for 30 seconds. Samples pool was divided into three aliquots: one represented the control and the others two was irradiated with He–Ne laser at two different energy dose (3.96 and 6.12 J/cm^2). Motility, viability, osmotic-resistance, acrosomial and DNA intactness were evaluated. The lower dose of laser energy resulted to be ineffective (P<0.05) than other irradiated samples and control. No significant difference between the control and the irradiated samples for viability and osmotic resistance, acrosome integrity and DNA integrity was found. However, the semen samples irradiated with 6.12 J/cm^2 showed a slight increase in sperm progressive motility, viability, osmotic resistance, acrosome and DNA integrity, respect to the semen samples irradiated at low energy doses and control semen samples. Further studies are needed to assess the effect of higher doses of He-Ne laser irradiation on the improvement of the quality ram semen after freezing-thawing process.

Keywords: *laser irradiation, DNA, semen, acrosome*

INTRODUCTION

The widespread application of artificial insemination (AI) and realization of its full potential in sheep depends largely on the use of frozen semen, and thus, on the availability of techniques that result in acceptable fertility (Donovan et al., 2004). Cryopreservation changes the behavioral and functional capacity of spermatozoa; leading to a reduction in motility (Ibrahim et al., 1982) reduced ability of sperm to traverse the cervix and decreased viability in the female reproductive tract (Salamon et al., 1995). This usually results in unacceptably low conception rates in ewes inseminated with frozen semen (Gillan et al., 1999). Thus development of new procedures aimed at improving the quality of cryopreserved spermatozoa is a suitable goal to be pursued. In previous studies it was showed that laser irradiation improved the quality of refrigerated semen in rabbit and turkey species (Iaffaldano et al., 2005; Iaffaldano et al., 2010). Iaffaldano et al. (2013) also found that the effects of post-thaw Helium–Neon (He–Ne) laser irradiation on mobility and functional integrity of frozen/thawed chicken, pheasant and turkey spermatozoa were investigated. Cytochrome C oxidase (COX) activity was also determined as a measure of the effect of irradiation on mitochondrial bioenergetics. The effects of post-thaw Helium–Neon (He–Ne) laser irradiation on mobility and functional integrity of frozen/thawed chicken, pheasant and turkey spermatozoa were investigated. Cytochrome C oxidase (COX) activity was also determined as a measure of the effect of irradiation on mitochondrial bioenergetics.

Every sperm cell consists of a head (acrosome), which contains tightly packed condensed DNA, followed by a short neck containing mitochondria (midpiece), and a thin tail (flagellum), which is responsible for the motility of the cells. The moving speed of a

spermatozoon depends upon energy supply. Spermatozoa maintain low energy consumption during storage in cauda epididymis. These cells are motile but unable to fertilize an egg. Enhanced adenosine-5′-triphosphate (ATP) production becomes critical at the time of fertilization. Motility is activated only upon ejaculation, and so-called "hyperactivation" takes place in the oviduct.

Activation of sperm flagella motility involves both energy metabolism in mitochondria and the motile apparatus of the cells. Mammalian spermatozoa can produce ATP both by anaerobic glycolysis and aerobic breathing. It is well documented that low- power laser irradiation of spermatozoa can increase their motility as well as the ATP amount in cells. Clearly evidenced study results showed that, human sperm motility as well as velocity can be improved by He-Ne laser irradiation. Second, it was found that the irradiation stimulated nonmotile and badly moving but live spermatozoa to move. Later, an important study in this particular field was done by (Breitbart et al., 1996). Stimulation of motility of bull, ram, mouse, and human spermatozoa as well as mouse oocytes by irradiation with visible light of laser and non-laser origin at 632.8, 660, and 780 nm as well as with broad band visible light 400–800 nm were studied. It was found that irradiation of human sperm with broad band visible light (400–800 nm) caused a significant increase in hyperactivated motility, but not in total motility, of human sperm.

A rapid increase in intracellular Ca^{2+} concentration and hyperactivated motility caused by irradiation were significantly reduced when voltage-dependent Ca^{2+} channel was blocked or when Ca^{2+}-deficient medium was used. Biochemical and topological analysis evidenced that fertilizing increased in irradiated spermatozoa.

The quality of stored turkey semen was found to be improved significantly following He-Ne laser irradiation 25 and irradiation with He-Ne laser prevented there *in vitro* liquid storage-dependent damage It was found, that irradiation increased the sperm motility index, viability, and cell energy charge. It was concluded that laser irradiation might be a useful technique for enhancing the quality of semen in long-term storage (Tiina, 2012).

Therefore, the aim of this study was to investigate whether and how two energy doses of laser irradiation (3.96 and 6.12 J/cm^2) can improve the qualitative characteristics and energetic profiles of ram spermatozoa after freezing-thawing process.

MATERIALS AND METHODS

20 sexual mature sheep bucks housed in a private farm were used. Semen from bucks was randomly collected via artificial vagina and pooled to avoid individual differences (5–10 ejaculates/pool; 1 pool/week). Semen was prediluted 1:1 with Tris–citric acid–glucose (TCG) extender (Tris: 250 mmolL^{-1}; citric acid: 88 mmolL^{-1}; glucose: 47 mmolL^{-1}). Frozen ram sperm samples were thawed in a water bath at 37°C for 30 seconds. Each pool was divided into three aliquots: one represented the control and the others two was irradiated with He–Ne laser at two different energy dose (3.96 and 6.12 J/cm^2). Motility, viability, osmotic-resistance, acrosomial and DNA intactness were evaluated (Table 1).

At the same time of storage, cytochrome C oxidase activity (COX) and energetic charge (EC) were assessed on control and irradiated samples to evaluate the energetic functions of spermatic cells.

COX activity was determined spectrophotometrically as Iaffaldano et al. (2010) by dual beam dual wave length system in lysated sperm cells. Energetic charge was measured, as Iaffaldano et al. (2013), by using high performance liquid chromatrography (HPLC) and was defined as the sum of ATP, ADP and AMP fractions, using the following equation:

$$EC = [ATP] + 0.5[ADP]/([ATP] + [ADP] + [AMP])$$

RESULTS AND DISCUSSIONS

The lower dose of laser energy resulted to be ineffective ($P<0.05$) than other irradiated samples and control (Table 1)

No significant difference between the control and the irradiated samples for viability (47.96 ± 2.18 vs. 45.77 ± 1.81 and 49.06 ± 1.66), osmotic resistance (37.94 ± 3.08 vs. 36.45 ± 2.85 and 39.43 ± 1.87), acrosome integrity

(37.89 ± 2.83 vs. 36.68 ± 2.68 and 40.68 ± 1.07) and DNA integrity (98.50 ± 0.29 vs. 97.79 ± 0.35 and 98.74 ± 0.20) was found. However, the semen samples irradiated with 6.12 J/cm^2 showed a slight increase in sperm progressive motility, viability, osmotic resistance, acrosome and DNA integrity, respect to the semen samples irradiated at low energy doses and control semen samples.

In parallel, the effect of irradiation on biochemical parameters of samples was evaluated by measuring the activity of cytochrome oxidase (COX) and the energetic charge (Figure 1).

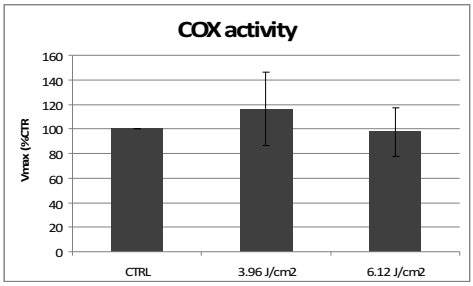
As for parameters reported in Table 1, no significant difference in mean values for both COX activity and Energetic charge between control and laser treated sperm samples was found. This could be mostly due to the extreme variability of semen samples which resulted in an unpredictable effect of laser treatment. Thus, to overcome such a problem, a comparison of all parameters obtained for each single pool is going on.

Also, Iaffaldano et al. (2005) showed an increased cell energetic charge in stored turkey semen after laser irradiation. Previous studies reported that He–Ne laser irradiation on isolated mitochondria resulted in the increase of ATP level (Passarella et al., 1984), RNA (Greco et al., 1989) and DNA synthesis (Pastore et al., 2000), generation of new mitochondria (Maxwell et al., 1996), enzyme activation (Faustini et al., 2004) and modifications in the substrate enzyme interaction. In sperm cells ATP, generated in the mitochondria, is primarily required for sperm motility. Thus the mitochondria can play roles other than just energy supply which are needed to maintain the contractibility of the tail, e.g. the regulation of the calcium flux and membrane potential.

Table 1. Sperm qualitative parameters (%) of cryopreserved and irradiated ram semen

Semen treatment	Semen parameters (%)					
	Mass motility	Progressive motility	Viability	Osmotic resistance	Acrosome integrity	DNA integrity
Control						
	53.17 ± 3,17a	44.5 ± 2.44a	47.96 ± 2.18a	37.94 ± 3.08a	37.89 ± 2.83a	98.50 ± 0.29a
Time 1 (3.96 J/cm^2)						
	43.67 ± 1,96b	36.50 ± 1.04b	45.77 ± 1.81a	36.45 ± 2.85a	36.68 ± 2.68a	97.79 ± 0.35a
Time 2 (6.12 J/cm^2)						
	52.83 ± 1.83a	45.17 ± 1.87a	49.06 ± 1.66a	39.43 ± 1.87a	40.68 ± 1.07a	98.74 ± 0.20a

a-b Different superscript letters within the same column indicate a significant difference ($P < 0.05$).



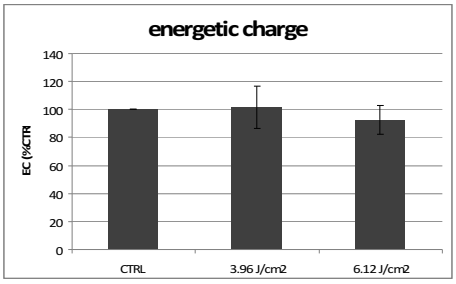


Figure 1. COX activity and Energetic charge of cryopreserved and irradiated ram semen

CONCLUSIONS

The lower dose of laser energy resulted to be ineffective than other irradiated samples and control. No significant difference between the control and the irradiated samples for viability and osmotic resistance, acrosome integrity and DNA integrity was found. However, the semen samples irradiated with 6.12 J/cm^2 showed a slight increase in sperm progressive motility, viability, osmotic resistance, acrosome and DNA integrity, respect to the semen samples irradiated at low energy doses and control semen samples.

Further studies are needed to assess the effect of higher doses of He-Ne laser irradiation on the improvement of the quality ram semen after freezing-thawing process.

REFERENCES

Breitbart H., Levinshal T., Cohen N., Friedmann H., Lubart R., 1996. Changes in calcium transport in mammalian sperm mitochondria and plasma membrane irradiated at 633 nm (He-Ne laser). J.Photochem. Photobiol.B.Biol., 34, p.117–121

Donovan A., Hanrahan J.P., Kummen E., Duffy P., Boland, M.P., 2004. Fertility in the ewe following cervical insemination with fresh or frozen-thawed semen at a natural or synchronised oestrus. Animal Reproduction Science 84 (3-4), p. 359-368.

Faustini M., Torre M.L., Stacchezzini S., Norberti R., Consiglio A.L., Porcelli F., Conte U., Munari E., Russo V., Vigo D., 2004. Boar spermatozoa encapsulated in barium alginate membranes: a microdensitometric evaluation of some enzymatic activities during storage at 18◦C. Theriogenology 61, p.173–184.

Gillan L., Maxwell W.M., 1999. The functional integrity and fate of cryopreserved ram spermatozoa in the female tract. Journal of reproduction and fertility. Supplement 54, p. 271-283

Greco M., Guida G., Perlino E., Marra E., Quagliariello E., 1989. Increase in RNA and protein synthesis by mitochondria irradiated with helium–neon laser. Biochem. Biophys. Res. Commun., 163, p.1428–1434.

Greco, M., Perlino, E., Pastore, D., Guida, G., Marra, E., Quagliariello, E., 1991. Helium–neon laser irradiation of rat liver mitochondria gives rise to a new subpopulation of mitochondria: isolation and first biochemical characterization. J. Photochem. Photobiol. B: Biol. 10, 71–78.

Iaffaldano N., G. Paventi, R. Pizzuto, S. Passarella, S. Cerolini, L. Zaniboni, M. Marzoni, A. Castillo, M.P. Rosato, 2013. The post-thaw irradiation of avian spermatozoa with He–Ne laser differently affects chicken, pheasant and turkey sperm quality. Journal of Animal Reproduction Science

Iaffaldano N., Meluzzi A., Manchisi A., Passarella S., 2005.) Improvement of stored turkey semen quality as a result of He-Ne laser irradiation. Animal Reproduction Science, 8 (3-4), p. 317-325

Iaffaldano N., Rosato M.P., Paventi G., Pizzuto R., Gambacorta M., Manchisi A., Passarella S., 2010. The irradiation of rabbit sperm cells with He-Ne laser prevents there in vitro liquid storage dependent damage. Animal Reproduction Science, 119 (1-2), p. 123-129

Ibrahim M.A., Lajos K., Belatoth M., 1982. Morphological alterations of bull spermatozoa during freezing and thawing. Acta Veterinari Hungarica, 30/4, p. 235-243.

Maxwell W.M.C., Stojanov T., 1996. Liquid storage of ram semen in the absence or presence of some antioxidants. Reprod. Fertil. Dev., 8, p.1013–1020

Passarella S., Casamassima E., Molinari S., Pastore D., Quagliariello E., Catalano I.M., Cingolani A., 1984. Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitroby helium–neon laser. FEBS Lett. 175, p.95–99.

Pastore D., Greco M., Passarella S., 2000. Specific helium-neon laser sensitivity of the purified cytochrome c oxidase. Int. J. Radiat. Biol., 76, p.863–870.

Salamon S., Maxwell W.M.C., 1995. Frozen storage of ram semen I. Processing, freezing, thawing and fertility after cervical insemination. Animal Reproduction Science, 37 (3-4) , p. 185-249

Tiina I., Karu I., 2012. Lasers in Infertility Treatment: Irradiation of Oocytes and Spermatozoa, Photomed Laser Surg. 2012 May 3

Vacca, R.A., Marra, E., Quagliariello, E., Greco, M., 1993. Activation of mitochondrial DNA replication by He–Ne laser irradiation. Biochem. Biophys. Res. Commun. 195, p.704–709.