https://doi.org/10.15415/jnp.2017.51018

Effects of Low-Energy Laser Irradiation on Sperm Cells Dynamics of Rabbit (Oryctolagus Cuniculus)

J.M. DE JESÚS-MIRANDA[1], L. A. MANDUJANO[2], F. MÉNDEZ[3], Y.J. CASTILLO[1], J. MULIA[1], C. GARCÍA[4], Y.E. FELIPE[4] AND D. OSORIO-GONZÁLEZ[1*]

[1]Laboratorio de Biofísica Molecular de la Facultad de Ciencias. Universidad Autónoma del Estado de México.

[2] Laboratorio de Diseño y Modelado Biofísico Molecular de la Universidad Mexiquense S. C.

[3]Laboratorio de Ecofisiología Animal de la Facultad de Ciencias. Universidad Autónoma del Estado de México.

[4]Laboratorio de Biología de la Reproducción de la Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma del Estado de México

***Email: dog@uaemex.mx**

Published online: August 07, 2017

Abstract Infertility is a world disease in which a couple is unable to achieve pregnancy. There are numerous parameters to determinate fertility; nevertheless, sperm motility is by consensus one of the most important attributes to evaluate male fertility. Contributions to a better understanding of this crucial parameter are imperative; hence, the aim of this investigation was to assess the effect of low-energy laser irradiation on sperm cell dynamics in thawed samples that were cryopreserved. We used a 405 nm blue laser beam to irradiate spermatic cells from rabbit inside a temperature-controlled dispersion chamber at 37 °C; then, we applied an image recognizing system to calculate individual sperm trajectories and velocities. We found that sperms raise its motility after irradiation suggesting that λ=405 nm is an optimal wavelength for spermatic photo-stimulation.

Keywords: sperm motility, photo-biostimulation.

1. INTRODUCTION

Infertility is considered a global public health problem in which an active sexual couple is incapable of achieving pregnancy [28]; between 10% and 15% of couples have difficulties in getting pregnant [27]. Tahmasbpour and coworkers

Journal of Nuclear Physics, Material Sciences, Radiation and Applications
Vol-5, No-1,
August 2017
pp. 187–196

CHITKARA UNIVERSITY

(2014) affirm that about 75% of infertility problems are due to disorders in sperm quality like oligospermia (low sperm count) and asthenospermia (poor sperm motility); this last term could compromise fertility [5]. Sperm count, motility, normal forms, pH, and viscosity are a few out of many factors to evaluate sperm fertility [17]; nevertheless, motility is an inherent characteristic of a good sperm since abnormal sperms cells never show good motility. As a general rule, a spermatozoon is naturally motile, although not all motile spermatozoa are fertile [14]. Sperm motility then is a vital factor to evaluate male fertility [8,19].

Sperm motility is based on metabolic and regulatory mechanisms [23]: concentration of reactive oxygen species [2] and intracellular calcium [11], energy ATP, supply products of glycolysis [21] and mitochondria as well as in flagella motile system [16].

Laser beams were used in assisted human reproduction for a first time in the 70s [25]; since then, its use has been expanded for the treatment of sperm and fertility problems [7,20]. Despite the numerous publications about sperm stimulation using lasers to improve fertility, it has been suggested that more research is needed to understand the mechanisms better, as well as the interactions and effects in biological and cryopreserved samples photo-biostimulated [14,21]. Abdel-Salam and Harith (2015) suggested that short wavelengths as λ= 532 nm and λ=405 nm could be better tools in photo-biostimulating procedures since it is easier to absorb short wavelengths than longer ones.

A good sperm quality is a good biomarker of health in general, and it is related to survival expectancy; mortality decreases as the level of normal sperms and good sperm motility rises [13]. Asthenospermia could be related to defects in mitochondrial function, as mitochondria are the main source of energy supply for spermatic movement [9]. A bigger mitochondrial load, as well as a greater sperm motility, would imply that the sperm capability of fertilizing is increased [3].

Motility, vitality, and morphology of spermatic cells are important attributes for a good spermatic function [28]. However, most of the procedures and techniques to evaluate motility are subjective estimations (Schirren, 1982a, cited by Push, 1987). Measurements of semen quality must be precise [6,12]; as mentioned above, spermatic motility is one the most important criteria to determine male fertility [8,19,24,28]. Thus, the accurate description of the distinctive features of sperm motility becomes necessary. The aim of this work was to evaluate the effect of low energy radiation on rabbit sperm cell dynamics of thawed cryopreserved samples using a 405 nm blue beam laser.

2. METHOD

2.1 Spermatic samples

Several straws containing 0.25 mL of cryopreserved sperm cells from rabbit (*Oryctogalus cuniculus*) were thawed in a warm water bath at 37 °C. Samples were obtained from the semen bank of the Laboratorio de Biología de la Reproducción, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México. The spermatic pool was obtained using the artificial vagina method in male rabbits about 18 months age; all males were sexually active with more than 80% of motility and viability, and less than 10% of morpho-abnormalities. Each sample consisted of 15 µL of the sperm pool on a slide inside a temperature-controlled dispersion chamber at 37 °C. Each sample was disposed to be irradiated using an experimental set as shown in figure 1.

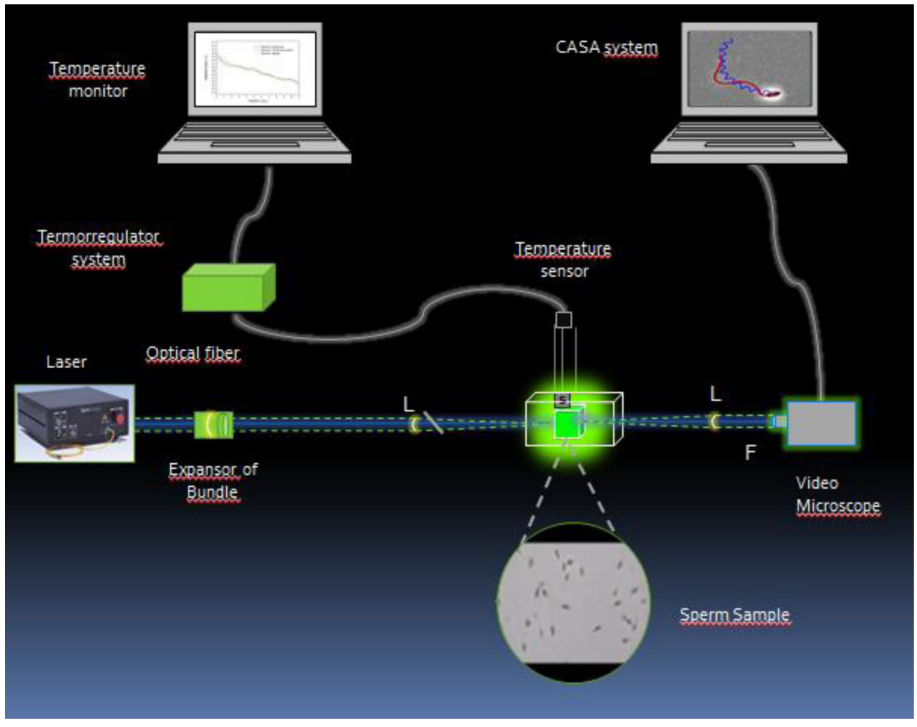

Figure 1: Experimental set. Sperm samples inside a temperature-controlled dispersion chamber (center) were irradiated with a 405 nm blue laser (left); videos were captured using a camera adapted to a microscope (right) and stored in a computer. Adapted from Beltrán *et al.,* 2016.

2.2. Spermatic stimulation

A total of 27 samples from the spermatic pool were divided into three groups of nine samples each; a) control group (non-irradiated), b) group irradiated with 4 J energy (samples irradiated for 60 s) and c) group irradiated with 6 J energy (samples irradiated for 90 s.). Irradiation was performed using a solid-state 405 nm blue beam (CW, Fermion 1 series) with an optical fiber coupled and operating at 70 mW. A lens arrangement was used to optimize the beam power density at the focal point; for this purpose, a laser beam expander was used to increase the circular diameter which was later incised in a plane-convex lens. This beam expander was adjusted with micrometric displacement to correct the divergence laser beam. The focus of the beam was made so that the beam waist hit a 40.5 mm^2 area. Videos of 30 s from each sample were recorded.

2.3. Determination of dynamic parameters

We used a Computer Assisted Sperm Analyses (CASA) to analyze sperm motility. Such system is based on the reconstruction of the individual trajectory of each sperm cell from all videotaped samples. The images of the videos were captured using a camera adapted to a microscope and stored in a computer. The calculated parameters (Fig. 2) were straight-line velocity (VSL, micrometers/s), defined as the distance a sperm travels from the first to the

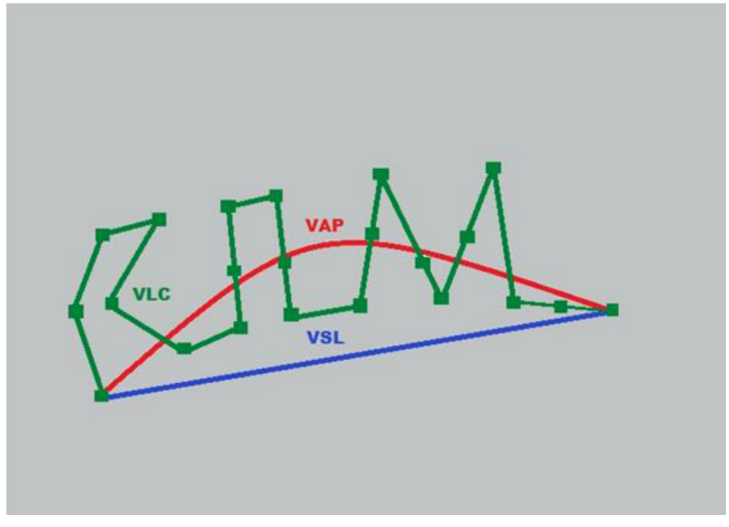

Figure 2. Schematic representation of dynamic parameters. Curvilinear (green), average (red) and straight velocities. Green frames represent images captured in videos.

end point of its travel by time; average path velocity (VAP, micrometers/s), defined as the distance traveled long its average trajectory per unit time; curvilinear velocity (VCL, micrometers/s), which description is in terms of its real trajectory; linearity (LIN = VSL/VCL), percentage ratio between straight-line and curvilinear velocities, and wobbling (WOB = VAP/VCL).

2.3. Statistical analysis

Kruskall-Wallis one-way ANOVA tests were performed using generalized linear methods at a 0.05 significance level for assessing the effect of radiation on VLS, VAP, VCL, LIN and WOB variables among groups (control, 4 J and 6 J groups). Only variables with significant differences among groups were further analyzed performing pairwise comparisons with p-value adjustment according to the number of tests carried out.

3. RESULTS AND DISCUSSION

One of the most frequent problems in assisted reproduction is to improve sperm viability; currently, there are several pharmacological treatments available, but energy stimulation using a low energy laser represents an alternative solution to the use of drugs. Although some studies have shown contradictory results because an inappropriate dose can affect the integrity of the plasma membrane or the acrosome, and even cause irreversible DNA damage, the first property to be tested in a sample that was stimulated should be the motility. The rheometry tests showed that the shear stress of the fresh samples is in the range 0.5-0.16 Pa and for samples that were thawed they were in the range 0.18-0.48 Pa as shown in Figure 3.

The shear stress values were used to calculate the viscosities which were 7.5 cP and 9.7 cP for fresh and thawed semen samples, respectively. These values were incorporated into CASA to estimate the velocities and motility parameters, which are shown in Table 1.

Motility variables VLS, VAP and VCL, were significantly different among groups; for VLS, H(2)=15.884, for VAP, H(2)=16.427, and for VCL, H(2)**Table 1.** Mean ± standard deviation of motility variables from each of the three sample groups. Significant differences in a motility variable among the groups are indicated with an asterisk; Kruskall Wallis H and its associated p-values are shown. Pairwise comparisons: equal alphanumeric characters represent non-significant differences (p>0.05), different alphanumeric characters correspond to significant differences with adjusted p-value shown (according to the number of tests performed).17.471; p<0.001 for these three test statistic values. Pairwise comparisons with adjusted p-values showed that

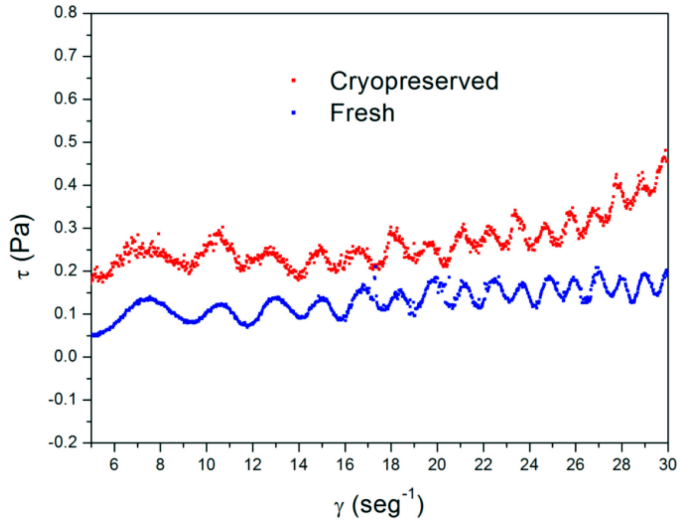

Figure 3: The graph compares the shear stress between a sample of fresh sperm (black squares) and one that was previously cryopreserved (red squares).

there are significant differences in these three variables between the control group and 4J group, and between control group and 6J group (see p-values in Table 1). These differences corroborate that laser irradiation indeed has an effect on different variables evaluating sperm motility. To evaluate the size of such an effect in each variable, we used the effect size estimate, r, as explained in Fritz *et al.*, 2012. The significant differences in VLS between control group and 4J group ($p<0.001$) have a large effect size ($r = -0.92$); however, in the significant differences between control group and 6J group ($p=0.029$), the effect size is smaller ($r = -0.61$). VAP were also significantly different between control and 4J groups ($p<0.001$, $r = -0.91$), and between control and 6J groups ($p=0.008$, $r = -0.71$). Finally, there were statistically significant differences in VCL between control and 4J groups ($p<0.001$, $r = -0.94$), and between control and 6J groups ($p=0.007$, $r = -0.71$). It is remarkable that all the calculated effect sizes are considered large in the context of this analysis.

As it was assessed, all the laser stimulated samples increased their spermatic velocities with respect to the control group; the spermatic trajectories are not predominantly linear, and they have moderate oscillations; these observations corroborate that irradiated groups have a high probability of possessing spermatic viability.

Increasing spermatic motility is imperative for assisted reproduction specialists as to achieve fecundation of the ovum, spermatozoa must undergo a process named sperm capacitation, which takes place in the female tract [29].

Table 1: Mean ± standard deviation of motility variables from each of the three sample groups. Significant differences in a motility variable among the groups are indicated with an asterisk; Kruskall Wallis H and its associated p-values are shown. Pairwise comparisons: equal alphanumeric characters represent non-significant differences ($p>0.05$), different alphanumeric characters correspond to significant differences with adjusted p-value shown (according to the number of tests performed).

Motility variable	Control group	4J Group	6J Group	Kruskall Wallis H	Kruskall Wallis p	Pairwise comparisons adjusted p
VLS* (um/s)	0.530 ±0.295 ^{A}	1.710 ±0.527 ^{B}	1.333 ±0.669 ^{B}	15.884	<0.001	Control-4J Group p<0.001 Control-6J Group p=0.029
VAP* (um/s)	0.716 ±0.252 ^{A}	2.022 ±0.598 ^{B}	1.688 ±0.694 ^{B}	16.427	<0.001	Control-4J Group p<0.001 Control-6J Group p=0.008
VCL* (um/s)	0.884 ±0.216 ^{A}	2.290 ±0.592 ^{B}	2.000 ±0.834 ^{B}	17.471	<0.001	Control-4J Group P<0.001 Control-6J Group P=0.007
LIN (dimensionless)	0.569 ±0.210	0.747 ±0.116	0.656 ±0.104	4.265	0.119	–
WOB (dimensionless)	0.790 ±0.123	0.876 ±0.064	0.846 ±0.081	2.480	0.289	–

When capacitation is achieved, spermatozoa acquire an increase in motility, which is called hyperactivation [30]. When spermatozoa come into contact with follicular fluid acrosome reaction takes place, which is the rupture of the acrosome membrane followed by the exposition of substances in the sperm head.

Increased motility in sperm samples is reported to be due to stimulation of mitochondrial ATP production by the blue laser beam; mitochondrial ATP is responsible for responding to light stimulation and propitiating the generation of reactive oxygen species (ROS). Nonetheless, this interpretation must be carefully assessed as the blue light stimulation (405nm) effects has also been reported in cell types different from sperm, where intracellular ROS production has been observed [31]. Although ROS have an important function in sperm capacitation, hyperactivation, and acrosome reaction, a correlation between high ROS concentrations and decrease in motility has been found [32].

CONCLUSIONS

The addition of cryopreservation substances to the frozen samples increased sperm viscosity and shear strength; however, blue laser bean stimulation was enough to foment sperm motility.

Low energy laser irradiation is a suitable technique to use in spermatic photo biostimulation procedures; λ= 405 nm is a properly wavelength to improve sperm motility, since control and irradiated groups were significantly different.

There were non significant differences between groups irradiated with 4 J and 6 J of energy. Trajectories were not preponderantly linear, and oscillations were considered moderate.

ACKNOWLEDGMENTS

The authors wish to thank CONACyT, COPARMEX and UNIVERSIDAD MEXIQUENSE for scholarship support and Dra. Lorena Romero Salazar from Nanothermodynamics and Complex Systems Laboratory by granting facilities for the timely development of the project.

REFERENCES

[1] Abdel-Salam, Z. y M.A. Harith. (2015). Laser researches on livestock semen and oocytes: A Brief Review. *Journal of Advanced Research.* **6,** 311–317

[2] Agarwal, A., Saleh, R.A. and M.A. Bedaiwy. (2003). Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril*. **79(4),** 829–843

[3] Anderson, M.J., and Dixson, A.F. (2002). Sperm competition Motility and the midpiece in primates. *Nature*, 416, 496

[4] Beltrán, K., de Jesús-Miranda, J.M., Castro, J.A., Mandujano-Rosas, L.A., Paulín-Fuentes, J.M. y D. Osorio-González. (2016). Using Green Fluorescent Protein to Correlate Temperature and Fluorescence Intensity into Bacterial Systems. JNPMSRA. **4(1),** 49–57

[5] Carrel, D., and Patterson, M. (2010). Reproductive Endocrinology and Infertility: Integrating Modern Clinical and Laboratory Practice. Springer. 375 p

[6] Castilla, J.A., Morancho-Zaragoza, J., Aguilar, J., Prats-Gimenez, R., Gonzalvo, M.C., Fernández-Pardo, E., Álvares, C., Calafell, L., and L. Martinez. (2005). Quality specifications for seminal parameters based on the state of the art. *Hum Reprod*. **20(9),** 2573–2578

[7] Ebner, T., Moser, M., and Tews, G. (2005). Possible applications of a non-contact 1.48 lm wavelength diode laser in assisted reproduction technologies. Hum. Reprod. Update **11(4),** 425–435.

[8] Elia, J., Imbrogno, N., Delfino, M., Mazzilli, R., Rossi, T. and F. Mazzili. (2010). The importance of the sperm motility classes-Future directions. *Open Androl J.* **2(1),** 42–4 3.

[9] Ferramosca, A., Provenzano, S.P., Coppola, L., and V. Sara. (2011). Mitochondrial Respiratory Efficiency is Positively Correlated With Human Sperm Motility. *J Urology*. **79(1),** 809–814

[10] Fritz, C.O., Morris, P.E. and J.J. Richler. (2012). Effect size estimates: current use, calculations, and interpretation. Journal of Experimental Psychology: General; **141(1),** 2–18.

[11] Gadella, B.M., and C. Luna. (2014). Cell biology and functional dynamics of the mammalian sperm surface. *Theriogenology*. **81(1),** 74–84

[12] Hartmann, R., Steiner, R., Hoffman, N., and R. Kaufmann. 1983. Human sperm motility: enhancement and inhibition measured by laser Doppler spectroscopy. *Andrologia*. **15(2),** 120–134

[13] Jensen, T.K., Jacobsen, R., Christensen, K., Nielsen, N.C. and E. Bostofte. 2009. Good Semen Quality and Life Expectancy: A Cohort Study of 43, 277 Men. *Am J Epidemiol*. **170(5),** 559–65

[14] Jeyendran, R.S. 2000. *Interpretation of semen analysis results: A practical guide*. Cambridge University Press. U.k.

[15] Karu, T.I. 2012. Laser in Infertility Treatment: Irradiation on Oocytes and Spermatozoa. *Photomed Laser Surg.* **30(5),** 239–241

[16] Karu. T.I. 2010. Mithocondríal Mechanisms of Photobiomodulation in Context of New Data About Multiple Roles of ATP. *Photomed Laser Surg.* **28(2),** 159–160

[17] Kovac, J.R., Pastuszak, A.W. y D.J. Lamb. (2013). The use of genomics, proteomics, and metabolomics in identifying biomarkers of male infertility. *Fertil. Steril*. **99(4),** 998–1007.

[18] Lubart, R., Levinshal, T., Cohen, N., Friedmann, H., y H, Breitbart. (1996). Changes in Calcium Transport in Mammalian Sperm Mitochondria and Plasma Membrane due to 633 nm and 780 nm Irradiation. *Laser. in der Medizin / Laser in Medicine*, (Conference paper) 449–453

[19] Menkveld, R. 2010. Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. *Asian J Androl*. 2010. **12(1),** 47–58.

[20] Montag, M., Rink, K., Delacretaz, G., and van der Ven, H. (2000). Laser-induced immobilization and plasma membrane permeabilization in human spermatozoa. Hum. Reprod. **15(4),** 846–852

[21] Mukai, C. and M. Okuno. Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. *Biol Reprod*. **71(2),** 540–547

[22] Passarella, S. y and T.I. Karu. (2014). Absorption of monochromatic and narrow band radiation in the visible and near-IR by both mitochondrial and non-mitochondrial photoacceptors results in photobiomodulation. *J Photochem Photobiol B.* **140,** 344–358

[23] Pereira, R., Sá, R., Barros, A. and M. Sousa. (2017). Major regulatory mechanisms involved in sperm motility. *Asian J Androl.* **19(1),** 5–14

[24] Push., H.H. The Importance of Sperm Motility for the Fertilization of Human Oocytes in vivo and in-vitro. *Andrologia.* **19(5),** 514–527

[25] Tadir, Y., Neev, J., and Berns, M.W. 1992. Laser in assisted reproduction and genetics. J. Assist. Reprod. Genet. **9(4),** 303–305

[26] Tahmasbpour, E., Balasubramanian, D. and A. Agarwal. (2014). A multi-faceted approach to understanding male infertility: gene mutations, molecular defects and assisted reproductive techniques (ART). *J Assist Reprod Genet.* **31(9),** 1115–1137.

[27] Vogt, P.H. (2004). Molecular genetic of human male infertility: from genes to new therapeutic perspectives. *Curr Pharm Des*. **10(5),** 471–500.

[28] World Health Organization. (2010). *WHO laboratory manual for the Examination and processing of human semen.* 5th ed. World Health Organization

[29] Chang, M. C. (1951). Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature*, **168(4277),** 697–698.

[30] Yanagimachi, R. (1969). In vitro capacitation of hamster spermatozoa by follicular fluid. *Journal of reproduction and fertility*, **18(2),** 275–286.

[31] Kushibiki, T., Hirasawa, T., Okawa, S., & Ishihara, M. (2013). Blue Laser Irradiation Generates Intracellular Reactive Oxygen Species in Various Types of Cells. *Photomedicine and Laser Surgery,* **31(3),** 95–104.

[32] Kuroda, S., Yumura, Y., Mori, K., Yasuda, K., Takeshima, T., Kawahara, T. & Ikeda, M. (2016). Negative correlation between presence of reactive oxygen species and Sperm Motility Index in whole semen samples of infertile males. *Revista Internacional de Andrología*. http://dx.doi.org/10.1016/j.androl.2016.08.002