

BENEFICIAL ROLE OF LASER COMBINED WITH INDIRECT SWIM UP SPERM PREPARATION IN INFERTILE MEN

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Article history:		Abstract:				
Received:	April 6 th 2022	Background: A laser is a perfect therapeutic option utilized in a variety of				
Accepted:	May 6 th 2022	medical fields, including reproduction for better spermatozoa preparation and				
Published:	June 16 th 2022	activation for improving spermatozoa activity and motility, and with the				
		indirect swim-up procedure to reduce any round cells and leukocytes from				
		the sample.				
		Objectives : This research aims to study some sperm characteristics in two				
		groups, the normozoospermia infertile men and the asthenozoospermia				
		infertile group before and after activation with the indirect swim-up and laser				
		compare between both.				
		Patients and Methods: The current study was conducted on 30 semen				
		samples, divided into two groups (Asthenozoospermia and Normozoospermia				
		individuals), during the period of attendance at the infertility clinic at the High				
		Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-				
		Nahrain University. from November 2021 until March 2022. Each sperm				
		sample was separated into three portions. The first sperm in vitro was				
		prepared before activation, the second using the ISU technique, and the third				
		stage the Laser technique and ISU.				
		Result : Both techniques increased and improved sperm motility, however,				
		the laser bested the ISU alone in terms of increasing Sperm Grade A Motility				
		%, Sperm Grade B Motility %, and reducing Sperm Grade C Motility %. Sperm				
		Grade C Motility as a percentage and Sperm Grade D Motility (Immotile) as a				
		% Sperm.				
		Conclusion : The laser was significantly effective in improving sperm activity				
		and upgrading sperm motility more than the indirect swim-up technique.				

Keywords: Light Amplification by Stimulated Emission of Radiation (LASER), Indirect Swim-Up(ISU)

1. INTRODUCTION

Infertility is a common medical problem in which a couple is considered infertile, if they have not been able to conceive after 12 months of regular unprotected sexual contact, which is an important determinant for the occurrence of pregnancy (Jenkins *et al.*, 2004) Regardless of the cause this condition affects approximately 10-15 % of reproductive-aged groups (Elizabeth *et al.*, 2020). A male-infertility-related factor is identified in half of the infertile couples, along with defective sperm. Male fertility impairment can be caused by a variety of reasons, including congenital, urogenital, infection, malignancies, infections, scrotal illness, endocrine disease, genetics, and/or immunity-related infertility abnormalities. (Jungwith *et al.*, 2018).

Male factor infertility accounts for around half of all occurrences of infertility and affects approximately one in every twenty males of reproductive age (defined here as between puberty and 40 years of age) (Zhaku et al., 2019). Male infertility is caused mostly by defective sperm and/or sperm parameters such as low sperm concentration (oligozoospermia), low sperm motility (asthenozoospermia), low sperm vitalitv sperm morphology (necrozoospermia), aberrant (teratozoospermia), and sperm absence (Aspermia) (Jungwirth et al., 2012). and the lack of spermatozoa in the ejaculate (azoospermia). The principle of indirect swim-up is the approach is based on the self-active migration of sperm from a single, pre-washed cell pellet into the cytoplasm after centrifugation. The common



method for preparing sperms is the centrifugation and swim-up technique, which results in active and highly sperms but decreases concentration (Hamza et al., 2018). The swim-up principle is the most effective technique used in IVF laboratories and is selected if the sperm sample has a normal quantity of quality sperm (normozoospermic). The sperms are selected based on their motility and ability to swim out of the seminal plasma using this technique (Natali, 2011). Laser therapy looks to be a safe therapeutic option with a wide range of possible positive outcomes.

Sperm Preparation Methods: Varied sperm preparation procedures have been used to create spermatozoa with high motility, morphology, and the potential to fertilize the egg while causing little DNA damage. This approach results in low spermatozoa production and the selection of sperm based on motility, which is advantageous when the proportion of motility in the sperm is low (Hindal et al., 2018).

Indirect Swim-up Method: The indirect swim-up approach is a common procedure employed in IVF clinics since it is simple and economical. The self-active migration of spermatozoa from a single centrifuged, pre-washed cell pellet into an overlying medium is the basis of this approach (Ortega and Bosch, 2012).

LASER: Low-level laser therapy (LLLT) is a highly successful physical therapy process that is used in many sectors of medicine, including obstetrics and gynaecology, andrology, and urology, and it is indicated as an important component of the difficult treatment of infertility. According to a review of the research, LLLT is beneficial in treating male infertility. Lasers have a significant influence since they improve spermatozoa survival, motility, and movement speed. Laser therapy can help with prostatitis, and vasculitis can help with infiltrative-exudative changes as well as improving reproductive and ovulatory function. Similarly, (Ebner et al.,2001) showed that the spermatozoa samples which were immobilized by laser required a considerably shorter time for identification, aspiration, and injection in comparison to the mechanically immobilized group.

clinics at High Institute for Infertility Diagnosis and Assisted reproduction Al-Nahrain University .semen samples were taken from patients and classified into two groups according to the criteria of WHO (1999) for semen analysis; the first group is (15) Asthenozoospermia semen sample and the second group (15) normozoospermic semen sample with normal motility subjects who served as normal infertile volunteers these subjects will be admitted to the institute . after the approval of the ethical committee that must be obtained before the initiation of the study. Post-activation of each sample will be divided into two aliquots the first one using a laser at the wavelength (632.8mw) combined with indirect swim up, the second using ISU technique alone, then sperm parameters were assessed for these two techniques and the results statistically analyzed.

3. RESULTS: The seminal fluid characteristics of infertile men after activation, both ISU and laser, resulted in a highly significant rise in mean Sperm Grade A Motility % and Grade B Motility % (P 0.05), in comparison with before activation, however, the laser resulted in a more significant rise, added to that both ISU and LASER resulted in a highly significant decrease in mean Grade C Motile Sperm % and Grade D Immotile Sperm % (P 0.05) in comparison with before activation, however, the LASER resulted in a more significant decrease.

2. MATERIALS AND METHODS

The current study was conducted on 30 semen samples enrolled in this study. They were attending the infertility

 Table 3.1. Seminal fluid characteristics of all enrolled infertile men before activation, after ISU and after LASER

 activation

Total (n=30)	Before activation	After activation	ISU	After activation	LASER	Before x ISU	Before x LASER	ISU x LASER
Concentration (m/ml)	21.23±4.04	19.07±3.69		18.27±3.7	'1	0.0001^	0.0001^	0.004^
Grade A	14.50±9.32	29.50±16.37	7	29.80±18.	.09	0.0001^	0.0001^	0.909



Grade B	31.50±6.58	52.17±18.92	62.87±18.26	0.0001^	0.0001^	0.0001^		
Grade C	13.83±5.68	2.67±4.87	0.50±1.53	0.0001^	0.0001^	0.017^		
Grade D	40.83±9.01	14.83±16.21	6.83±11.78	0.0001^	0.0001^	0.0001^		
Morphology	22.33±3.26	36.07±10.25	40.63±9.96	0.0001^	0.0001^	0.0001^		

^Significant difference between two dependent means using Paired-t-test at 0.05 level.

3.DISCUSSION

Techniques for preparing sperm are an essential part of assisted reproductive technology (Kadhim et al., 2017). Meanwhile, enhanced sperm parameters increased sperm fertilization ability and ART results (Kadhim et al., 2017). The objective of sperm separation techniques is to treat spermatozoa in vitro in order to increase their functionality, i.e. motility, and to create a protective environment with the aim of maintaining or improving their functional ability for successful fertilization (Grasa et al., 2004). After the exclusion of seminal plasma, an increase in the percentages of sperm motility and progressive sperm motility is considered a normal response for sperm activity because it contains dead sperm, leukocytes, epithelial cells, debris, and microbial contamination that produce many oxygen radicals that can negatively influence sperm functions (Kadhim et al., 2017). in the current study, both techniques of activation Resulted nearly equally in reducing sperm concentration, and both techniques were effective in upgrading sperm motility. And minimizing round cell count. The use of a laser as an activation technique with an indirect swim-up greatly improved sperm motility and morphologically normal sperm percentage. According to the findings of this study, both ISU and LASER are effective in reducing semen concentration and improving sperm motility; however, LASER is superior to ISU in improving sperm quality and Morphology and significantly improving Grade A sperm motility per cent and improving Grade B motile sperm per cent; both methods reduce Grade C motile sperm per cent and Grade D immotile, and both methods reduce round cell count and leukocytes. on the other hand, it was proved that laser irradiation (at wavelength 632.8 nm) of active live human sperm enhanced motility and speed (Vesich, 1994; Lenzi et al., 1989). The laser was superior to the indirect swim-up method in upgrading the percentage of progressively motile spermatozoa and upgrading the percentage of normal morphology sperm.

CONCLUSION: The laser was significantly effective in improving sperm activity and upgrading sperm motility more than the indirect swim-up technique.

DECLARATIONS Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

- Ebner, T., Yaman, C., Moser, M., Sommergruber, M., Hartl, J., & Tews, G. (2001). Laser-assisted immobilization of spermatozoa prior to intracytoplasmic sperm injection in humans. Human Reproduction, 16(12), 2628-2631.
- Elizabeth, E. and Puscheck, Richard Scott Lucidi, (2020). Infertility Practice, Essential Overview, Etiology of Infertility,25 Medscape
- Grasa, P., Pérez-Pé, R., Baguena, O., Forcada, F., Abecia, A., Cebrián-Pérez, J. A., & Muiño-Blanco, T. (2004). Ram sperm selection by a dextran/swimup procedure increases fertilization rates following intrauterine insemination in superovulated ewes. Journal of andrology, 25(6), 982-990.
- Hamza, N. A., Selman, M. O., & Mossa, H. A. (2018). Comparison of Best Yield of in vitro Sperm Activation Techniques with New technique of Caffeine Combined with Density Gradient Centrifugation in Iraqi Patients. Journal of Pharmaceutical Sciences and Research, 10(1), 36-39.
- Hindal, A. S., Mossa, H. A., & Abood, M. S. (2018). Reactive Oxygen Species Levels in Seminal Plasma in a Sample of Iraqi Infertile Men using Advanced Stimulatory Method for Activation of Spermatozoa. *International Journal of Medical Research & Health Sciences*, 7(12), 51-55.
- Jenkins, J., Daya, S., Kremer, J., Balasch, J., Barratt, C., Cooke, I., ... & Nygren, K. (2004). European Classification of Infertility Taskforce (ECIT) response to Habbema et al., 'Towards less confusing terminology in reproductive medicine: a proposal. *Human reproduction*, *19*(12), 26872688.
- Jungwirth, A., Giwercman, A., Tournaye, H., Diemer, T., Kopa, Z., Dohle, G., ... & EAU Working Group on Male Infertility. (2012). European Association of Urology guidelines on Male Infertility: the 2012 update. *European urology*, *62*(2), 324-332.



- Junngwirth A., Diemer T, Kopa Z Krauszc Tournaye. (2018). Guidelines on Male Infertility European Association of Urology. EAU.
- Lenzi, A., Claroni, F., Gandini, L., Lombardo, F., Barbieri, C., Lino, A., & Dondero, F. (1989). Laser radiation and motility patterns of human sperm. Archives of andrology, 23(3), 229-234.
- Kadhim, A. A., Mossa, H. A., & Selman, M. O. (2017). A new sperm preparation technique by glass wool filtration combined with pentoxifylline techniques versus glass wool filtration alone for infertile and fertile men. Iraqi Journal of Embryos and Infertility Researches, 7(1), 28-36.
- 11. Natali, I. (2011) 'Sperm preparation techniques for artificial insemination-comparison of sperm washing, swim up, and density gradient centrifugation methods', Dalam: Manafi M, penyunting. Artificial insemination in farm animals. InTechOpen, pp. 115–122
- 12. Ortega, N. M. and Bosch, P. (2012) 'Methods for sperm selection for in vitro fertilization, In Vitro Fertilization-Innovative Clinical and Laboratory Aspects. IntechOpen.
- 13. Vesich, T. L. (1994). Some features of rehabilitating action of laser emission on native and cryopreserved human spermatozoa. Probl Cryobiol, 4(1), 33-5.
- 14. World Health Organisation, (1999). WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge university press.
- Zhaku, V., Sh, B., Beadini, N., & Murtezani, B. (2019). Combination of Maca, Korean ginseng extract and antioxidant therapy for male with oligoasthenozoospermia: case study. Journal of Hygienic Engineering and Design, 26(1), 28-35.