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Efficacy of 1.48 µm Diode Laser in Assisted Hatching Technique in Vitro Fertilization

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Abstract: ***Assisted hatching is a laboratory technique makes an artificial opening in thick zona to make the embryonic cells can hatch out and attach to the uterine wall and form pregnancy. Laser assisted hatching used in conjugation with intra-cytoplasmic insemination fertilization. Material and methods: The study was conducted at Kamal al-Samarrai Specialist Hospital of fertility and IVF-Baghdad during the period from 3rd July 2016 to 10th November 2017. Fifty nine infertile couples have been enrolled in this study and were included in ICSI cycle. The patients subdivided into 3 groups according to the duration of laser exposure (2, 4.5 and 8 µs). Results: younger women had higher successful clinical pregnancy compared to older women (40% vs. 27.6%), 4.5 µs had the highest successful clinical pregnancy 57.9% compared to 2 µs and 8 µs, with an PR (95%CI) 8.25 (1.797 – 37.881). Conclusion: Age has modest effect to predict successful clinical pregnancy outcome, in which the older the age of women the lower probability of clinical pregnancy, in multivariate analysis 4.5 µs (and consequently 6.1 µm) was 7 folds associated with better clinical pregnancy compared to 8 µs.***

Keywords: Assisted Hatching Technique, Clinical Pregnancy, Diode Laser

1. Introduction

Since the dawn of civilization, infertility was one of the main concerns of mankind. Among all branches of medicine this field was probably the biggest issue in the daily life of typical human family. Over eighteen centuries the cure was mainly based on simple and scientifically-unsupported interventions, in many cases it was linked to spiritual and religious matters. During the first half of the twenty century some medications were introduced which mainly involved certain hormones to enhance the productivity (1). Not until 1978 when Robert Edward introduced the IVF mechanism (2).

Since the introduction of laser in 1961 many researchers used lasers in different fields of medicine (3), among those fields laser in gynecology. Many gynecological diseases now effectively and safety treated by various lasers with different parameters (4). Zona pellucida (ZP) hatching is natural process occurred after expansion of blastocyst and allows the embryo to implant in to the uterine cavity. Implantation rate has remained low and one of the causes of implantation failure could be failure in normal ZP hatching process. The cells that make up the early embryo and enclosed within a flexible membrane (shell) called the zona pellucid. It dissolves during normal development, allowing the embryonic cells to escape or hatch out of the shell, only upon hatching the embryonic cells implant within the lining of the uterus to form pregnancy (5). Implantation rate has remained low and one of the causes of the implantation failure could failure in normal ZP hatching process. The pregnancy cannot occur unless the woman embryo hatches (5).

Assisted Hatching (AH) was introduced more than two decades ago. Assisted hatching is a laboratory technique makes an artificial opening in thick zona to make the embryonic cells can hatch out and attach to the uterine wall and form pregnancy, it showed an increase in the chances of implantation (6, 7). The first pregnancy following AH was reported in 1988. Laser assisted hatching (LAH) which was proposed in early the 90s appears to be safer compared to other AH techniques. The techniques involved were in using intra-cytoplasmic insemination ICSI fertilization and using laser AH in 1992 (8).

Laser is an acronym of Light Amplification of Stimulated Emission of Radiation. A typical laser device simply consists of three parts. They are an active material, an optical cavity and a pumping source. The active materials may take a solid, liquid or gas phase. The optical cavity is simply composed of a totally reflective mirror on one side of the active material and a partially reflective mirror at the other side of the active materials. The pumping source is either optical or chemical or electrical in its origin (9).

The mechanism of laser light generation involves first pumping the atoms or molecules to higher energy levels within the energy level structure of the active material. Since the trend of nature is to head to the lowest energy levels the excited atoms or molecules of the active material relaxes back to lower energy levels. This relation mostly occurs spontaneously with the emission of the excess energy in the form of light wave packets (photons) after certain time in all directions (Incoherent) forming the ordinary day to day light. The other process of relaxation is via stimulation by some of the spontaneously emitted photons causing the excess energy to be emit light wave packets (photons) in one

direction (coherent) forming the laser light. Those stimulated emitted light photons are overlapped specially and temporally thus they are coherent (10).

Laser treatment is controlled by four variables: power, wavelength, spot size, and duration of action. The wavelength, measured in nanometers (nm), is specific to the active medium of the laser. Different wavelengths are selectively absorbed by target tissue chromophorms (11). The spot size of a laser affects depth of tissue penetration because larger spot sizes has less scatter effects and penetrate deeper (11).

Typically during ICSI, the sperm tail is positioned under the glass micro capillary injection needle .The needle is brought down and across the tail causing it to break and immobilizing the sperm (12). A rather unique application of laser is to identify and select viable sperm for ICSI. Usually motility is used as an indicator of living sperm. However in severe cases factor such as asthenozoospermia, no motile sperm may be evident. This makes it very difficult to identify and select viable sperm for ICSI.A single laser pulse applied at the tip of a sperm tail can aid in distinguishing living non motile sperm from dead sperm. The tail of viable sperm will curl, whereas the non-viable sperm will not respond to the laser pulse. Fertilization rates would be expected to be correspondingly higher if better sperm are selected for injection (13). Optical trapping uses a single beam non-contact laser to move sperm during after immobilization or during ICSI. The optical tweezers can hold actively moving sperm and determine their velocity. Laser used in optical trapping may be either infrared or ultraviolet. Advantage of this technique includes ease, no requirement for sophisticated micromanipulation skills or additional expensive disposable equipment. It may also be used for polar body extraction or chromosomal manipulation. Disadvantages include increased exposure eryo to laser, possible ultraviolet exposure depending on wavelength utilized and a potential adverse effect on the sperm (13).

The first 1.48µm laser to receive US FDA approval for clinical use in assisted hatching was the ZILOS- tk in 2004. This was followed by the Octax laser in 2006 and the Saturn Active laser system in 2008 (14). The goal of the present work is to study the efficacy of 1.48µm diode laser at various exposure times on improving hatching as an assisted laser hatching mechanism in vitro fertilization to improve implantation and increase the chance of pregnancy.

2. Materials and Methods

Study design

The study was conducted at Kamal al-Samarrai Specialist Hospital of fertility and IVF-Baghdad during the period from 3rd July 2016 to 10th November 2017. Fifty nine infertile couples have been enrolled in this study and were included in ICSI cycle. Women were allocated in 2 groups according to their age group 1 age (20-30 years), group 2 (30-40) years, and these groups subdivided in 3 groups according to the duration of laser exposure (2, 4.5 and 8 µs). The patients were included in the age between 20 – 40 years and with male cause of infertility.

Laser manipulation was performed using 1.48nm non-contact diode laser with duration 2, 4.5 and 8 µs. The size of hole made in zona was measured to be 6 – 20 µm according to duration of laser and depending on the thickness of zona of each individual embryo. The software for imaging and archiving used in the present work is Octax EyeWare from Octax Microsoft GmbH. This software may be used for controlling a variety of microscopic devices including Octax including Octax Laser Shot. The main concept of octax Eyeware is the transfer of data between different areas. The areas of the software are represented by five pages. They are namely; Database, Quick fix, Image, Video and Report. Report with the video page, it is possible to live monitoring of a camera and control the laser parameters within the attached Octax Laser Shot. In the case of the Data page facilities for recalling and managing data documented on patient data and measurements records.

In this experimental studied women who presented at or were referred to Kamal AL-Samaria hospital undergoing IVF or ICSI. All infertile couples were subjected to full history complete general and gynecological examination and full infertility investigation, including: husband's seminal fluid analysis, hormonal assay, transvaginal ultrasound and hysteroscopy or laparoscopy for tubal patency and uterine cavity and exclusion of pelvic pathology. All infertile women in this study the cause of infertility was male cause. After all in investigation then undergoing IVF or ICSI, all subject had underwent controlled ovarian hyper stimulation (with long protocol or antagonist protocol).

For long protocol started GnRh analog at day 21 of previous cycle, on day two of next cycle started ovarian stimulation with recombinant FSH or and HMG continuous with analog till the size of 2-3 follicles reach 16 – 18 mm and endometrium thickness more than 8 mm and hormonal assay (E_2 LH) give the HCG (OVETRIAL®), then after 34-36 hours and under general anesthesia, by trans vaginal ultrasound oocytes retrieved.

For antagonist protocol started ovarian stimulation at day 2 (CD2) of cycle with recombinant (FSH, LH and HMG) (follitrip®, gonall f®, menegon ®75 IU) till day 5-6 of stimulation in fixed protocol or till the size of 2-3 follicles 13-14mm and E_2 300 – 400 then started the antagonist injection (cetrolics® or orgalics® 0,25mg subcutaneous) till the day prior to HCG (ovetrial® 600iu or GnRh analog decapeptel® 0.3 mg. Then under general anesthesia by transvaginal ultra sound retrieval oocytes.

Follicles from both ovaries are aspirated by ovum aspiration needle (Cook®, Australia) started with right ovary followed by left one and FF given directly to the embryologist to identify the number and the quality of the retrieved cumulus-oocytes complexes. Once the oocyte-cumulus complexes are collected they are rinsed with flushing media to get rid of any blood residual from the follicular aspirate, then graded and transferred into drops of universal IVF media overlaid

by mineral oil in an incubator at 5-6% CO_2 with 37°C, in air at 95% humidity.

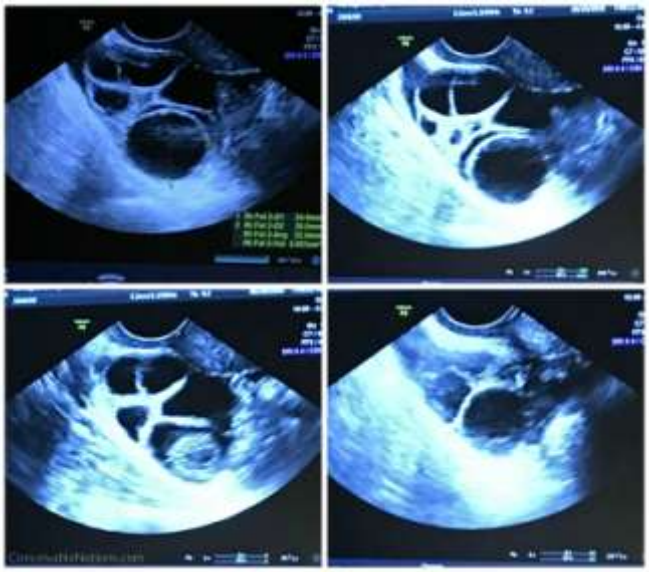

Figure 1: US findings during IVF protocol

Oocyte and sperm Preparation and Evaluation of Fertilization

Intracytoplasmic sperm injection usually performed by an embryologist 4–6 hours after oocyte aspiration. The sperm of patient's husbands usually collected at the day of oocyte retrieval by masturbation into a dry, clean and sterile plastic dish after 2-5 days of abstinence, and then the sample is transported to the laboratory at once and placed in an incubator at 37◦c for 30 minutes to allow liquefaction. If the husband is azoospermic, the sperm was obtained surgically from testis, epididymus or Vas deferens.

After oocyte retrieval, cumulus corona cells were denuded by combined oocyte maturity. Only the mature metaphase II (MII) oocytes that have extruded the first polar body and enzymatic (hyaluronidase) and mechanical treatment and carefully assessed for with normal morphology were suitable for microinjection.

Approximately 12-17 hours after microinjection procedure, examination of injected oocytes have been done to confirm fertilization by the existence of two pronuclei (2PN). The fertilization rate can be calculated from the following equation:

FR% = (number of fertilized oocyte/Total number of injected oocyte)*100

Around 48 hours after ICSI procedure, the number and size of blastomeres together with degree of fragmentation were recorded for each embryo to determine their quality. Those with good morphology are transferred into the uterine cavity at day two or three post ICSI.

Statistical analysis:

Discrete variables presented using there number and percentage used to present the data, chi square test used to analyze the discrete variable or Fisher exact test. Binary logistic regression analysis used to calculate the odd ratio (OR) and their 95% confidence intervals, when the outcome can be categorized into 2 binary levels, and if appropriate probability plot used to present the relationship. SPSS 20.0.0, GraphPad Prism 7.0 software package used to make the statistical analysis, p value considered when appropriate to be significant if less than 0.05

3. Results

Women with younger age group had higher percentage 40% to have successful clinical pregnancy compared to older age 27.6%, however it was not statistically significant as illustrated in table 1.

Table 1: The effect of age group compared to outcome of pregnancy

Outcome	Age group		All	P value
	20 – 30	30 – 40 years		
Number	30	29	59	-
Failure	18 (60.0%)	21 (72.4%)	39 (66.1%)	0.314
Success	12 (40.0%)	8 (27.6%)	20 (33.9%)	

There was inverse correlation between increased age and lower probability of success clinical pregnancy; however age has modest effect to predict success pregnancy as illustrated in figure 2.

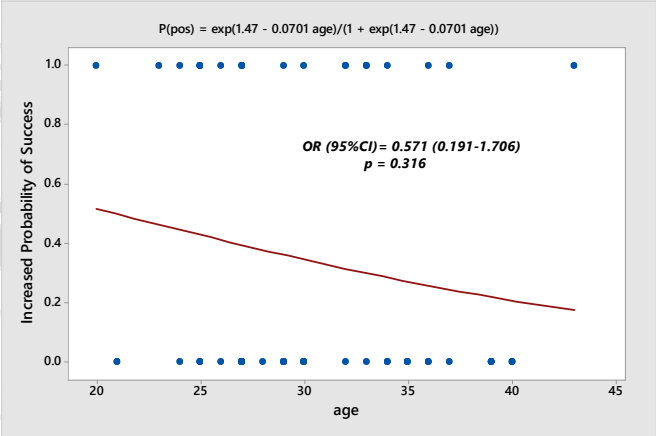

Figure 2: Probability of success clinical pregnancy and it relation to age

Oocytes exposed to 4.5 µs had higher percentage of successful clinical pregnancy (57.9%), followed by 2 µs (31.6%) and the lowest was 8 µs (14.3%), as illustrated in table 2.

Table 2: The effect of lase exposure on clinical pregnancy outcome

Outcome	Duration of diode laser 1.48µm exposure			All	P value
	2 µs	4.5 µs	8 µs		
Number	19	19	21	59	-
Failure	13 (68.4%)	8 (42.1%)	18 (85.7%)	39 (66.1%)	0.014
Success	6 (31.6%)	11 (57.9%)	3 (14.3%)	20 (33.9%)	

4.5 µs was the only strong and significant predictor of successful pregnancy compared to 8 µs by 8.25 folds (ranging from 1.8 – 37.9 folds), while 2 µs had modest effect

of better successful pregnancy compared to 8 µs by 2.769 folds, as illustrate in table 3.

Table 3: Correlation between duration of laser exposure on outcome of pregnancy

Duration	OR	95%CI	P value
2 µs	2.769	0.583 – 13.162	0.200
4.5 µs	8.250	1.797 – 37.881	0.007
8 µs	1.0	-	-
OR: odd ratio, 95%CI: 95% confidence interval			

4.5 µs had similar effect on successful pregnancy in both age groups, while 2 µs had better outcome in the younger age group compared to older age, while 8 µs was slightly higher successful pregnancy in older age as illustrated in table 4

Table 4: Distribution of the effect of lase exposure on clinical pregnancy outcome stratified based on age group

Age group	Outcome	Duration of diode laser 1.48µm exposure			P value
		2 µs (6.1 µm)	4.5 µs (11.0 µm)	8 µs (20.0 µm)	
20-30	Number	10	10	10	-
	Failure	5 (50.0%)	4 (40.0%)	9 (90.0%)	0.038
	Success	5 (50.0%)	6 (60.0%)	1 (10.0%)	
30-40	Number	9	9	11	0.079
	Failure	8 (88.9%)	4 (44.4%)	9 (81.8%)	
	Success	1 (11.1%)	5 (55.6%)	2 (18.2%)	

In multivariate analysis 4.5 µs remain independently predict higher probability of successful clinical pregnancy by 7.379 folds after excluding the effect of age, as illustrated in table 5.

Table 5: Multivariate analysis of the predictors of successful pregnancy

Duration	OR	95%CI	P value
2 µs (6.1 µm)	2.372	0.481 – 11.686	0.288
4.5 µs (11.0 µm)	7.379	1.578 – 34.499	0.011
8 µs (20.0 µm)	1.0	-	-
Age	0.948	0.848 – 1.061	0.353
OR: odd ratio, 95%CI: 95% confidence interval			

4. Discussion

In the current study laser therapy was effective in increasing the implantation rate to 33.9% for all patients, also 4.5 µ second appear to be the best exposure time to yield the highest pregnancy rate 57.9% compared to all other groups (2 and 8 µs), these findings was in agreement with Balaban *et al* (2006) in which they reported 40.9% successful pregnancy rate for women implanted using laser-assisted hatching compared to 27.3% for women without assisted hatching ($p<0.05$). (15) Our finding was in agreement with recent meta-analysis in 2017 in which they reported that LAH was significantly with increased clinical pregnancy OR (95%CI) = 1.65 (1.24 – 2.19), and with increased rate of implantation OR (95%CI) = 1.59 (1.06 – 2.38). (16) One the primary causes of lower rate of implantation of the embryo is failure of hatching in IVF/ICSI procedure. (17) The possible mechanism for this enhanced pregnancy outcome in LAH are secondary medium conditions or cryopreservation procedures might make the embryonic zona Pellucida ZP harden and/or thicken, which might lead to hatching difficulty. By thinning, drilling or other methods, AH could mechanically promote the hatching process. Studies in human and animal models found that AH resulted in earlier hatching than in non-assisted hatching embryos. Facilitation of earlier embryonic hatching might be particularly important given that the short window of endometrial receptivity appeared to be shifted 1–2 days earlier in cycles with ovarian stimulation for assisted reproduction treatment compared with natural cycles. The artificial gap produced by hatching may also serve as a channel for the exchange of metabolites, growth factors, and messages between the embryo and the endometrium. (16)

5. Conclusion

Age has modest effect to predict successful clinical pregnancy outcome, in which the older the age of women the lower probability of clinical pregnancy, In multivariate analysis 4.5 µs (and consequently 6.1 µm) was 7 folds associated with better clinical pregnancy compared to 8 µs, while 2 µs had modest effect to predict better clinical pregnancy compared to 8 µs, and this effect was independent of the effect of age.

6. Recommendations

We recommend using 4.5 µs first line in all age groups, while in younger women (20 – 30 years) it is reasonable to use 2 µs in such women, while using 8 µs is a second choice in older women (30 – 40 years).

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