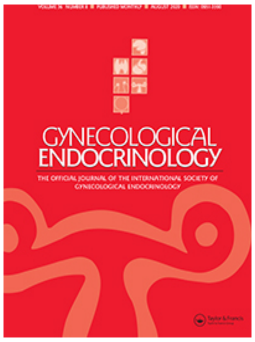

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ASSISTED REPRODUCTIVE TECHNOLOGY

Laser irradiation pretreatment improves endometrial preparation of frozen-thawed embryo transfer in recurrent implantation failure patients

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ABSTRACT

Recurrent implantation failure (RIF) remains a clinical dilemma. Helium-Neon (He-Ne) laser irradiation has recently become more popular under certain clinical conditions. Given the unique therapeutic effects, we were interested in determining whether pretreatment with He-Ne laser irradiation prior to frozen-thawed embryo transfer (FET) would improve the microcirculation and cause the release of growth factors and cytokines, thus improving endometrial receptivity and the clinical pregnancy rates. Patients chose for themselves whether to proceed with ($n = 29$) or without ($n = 31$) pretreatment with He-Ne laser irradiation prior to FET. The clinical pregnancy rate (37.9%) and implantation rate (20.3%) were higher in the laser-treatment group than in the control group (35.5% and 15.9%, respectively, $p = .844$ and .518, respectively). The live birth rate was higher in the laser-treatment group (27.6% vs. 25.8%, respectively, $p = .876$) and the miscarriage rate was lower in the laser-treatment group (18.2% and 27.3%, respectively, $p = .611$). No side effects or complications from laser irradiation were encountered in patients who received the laser treatment. We concluded that pretreatment with He-Ne laser prior to FET may be an alternative choice for RIF-affected women; however, additional well-designed prospective studies are necessary to determine the precise clinical value of this treatment.

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He-Ne laser irradiation; recurrent implantation failure; frozen-thawed embryo transfer; pregnancy

Introduction

Embryo implantation is a crucial and complex step in the reproductive process which affects the likelihood of success for couples undergoing *in vitro* fertilization (IVF) and embryo transfer (ET) cycles [1]. Despite advances in assisted reproductive technologies, the average embryo implantation rate in IVF is approximately 25%, and approximately 20% of patients experience recurrent implantation failure (RIF) [2]. It is estimated that up to two-thirds of implantation failures are due to defects in endometrial receptivity [1]. Several adjuvant interventions have been introduced to improve endometrial receptivity, such as mechanical endometrial scratching. The scratching and subsequent healing of the endometrium may increase uterine receptivity and promote successful embryo implantation by inducing an inflammatory response, involving the secretion of cytokines, growth factors and immune cells, which are crucial for the implantation process [3,4]. Atosiban treatment before embryo transfer has also been suggested to decrease the uterine contractility. The advantage of these interventions, however, is still controversial and has poorly defined advantages in improving IVF-ET outcome. Therefore, the management of RIF-affected patients remains challenging.

Successful embryo implantation requires a delicate interaction between the embryo and the receptive endometrium. Decidual vascular adaptation of the receptive endometrium, by increasing the microcirculation and angiogenesis, necessary for implantation, can alter endometrial receptivity and improve the embryo implantation rate [5]. Several identified molecular mediators, including regulatory protein HOXA 10, cytokine leukemia inhibitory factor (LIF) and growth factor are also recognized to play a pivotal role in embryo implantation [1,6,7]. Achieving a successful embryo implantation therefore includes improving the endometrial microcirculation, as well as the modulation of growth factors, pro-inflammatory cytokines, and regulatory proteins. A comprehensive strategy to improve the endometrial receptivity in the peri-implantation endometrium warrants intensive research activity.

Helium-neon (He-Ne) laser (632.8 nm) was originally developed by Soviet scientists in 1981 for the treatment of cardiovascular diseases by improving microcirculation and reducing of the area of infarction [8,9]. It is a low-energy laser that has therapeutic efficacy on various other clinical conditions, such as vitiligo repigmentation [10,11], chronic pain and fibromyalgia [12], wound healing [13] and orthodontic pain [14]. With the photomodulatory effects of He-Ne laser irradiation, hypoxic tissue would be improved by the increased release of NO from monocytes, and the resulting vasodilatation and improved microcirculation [15,16]. *In vitro* studies have revealed that He–Ne laser-

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treated keratinocytes stimulate melanocyte proliferation, induce various growth factor synthesis, and result in the growth of fibroblasts and keratinocytes [10,17,18]. Although the mechanisms have not been fully elucidated, He-Ne laser irradiation has attracted increasing attention in recent years and is especially famous for its bio-stimulation effects on microcirculation and on the release of certain cytokines and growth factors [9].

Given the unique biological and therapeutic effects of He-Ne laser irradiation, we were interested in determining whether pretreatment with He-Ne laser irradiation before FET in RIF-affected women would improve the microcirculation and cause the release of growth factors and cytokines in the peri-implantation endometrium, thus improving endometrial receptivity and the clinical pregnancy rates. To address this question, we investigated the effect of He-Ne laser irradiation prior to FET in RIF-affected women and specifically analyzed implantation rates and clinical pregnancy outcomes. This knowledge may enable clinicians to improve this critical aspect of modern reproductive therapies.

Material and methods

Patients and design

This prospective study included patients with a history of at least two previous IVF failures after the transfer of good-quality embryos at the Reproductive Center of the Kaohsiung Veterans General Hospital in Taiwan from December 2016 to January 2018. Ethical approval was obtained from the Institutional Review Board at the Kaohsiung Veterans General Hospital (VGHKS16-CT10-16). All participants provided written informed consent. There are no conflicts of interest between the authors and any products used in this study.

Patients with recurrent implantation failure who underwent an IVF/embryo transfer cycle using cryopreserved embryos and failed to conceive after at least two embryo transfers comprising a minimum of four top-quality embryos and meeting the following inclusion criteria were prospectively recruited: (i) age 18–45 years; (ii) body mass index (BMI) $\leqq$ 29 kg/m^2. Exclusion criteria were: (i) severe endometriosis; (ii) uterine anomaly; (iii) adenomyosis; and (iv) with systemic diseases such as autoimmune disorders.

Treatment protocol

Before the endometrium preparation, all RIF-affected patients were informed that pretreatment with He-Ne laser irradiation may improve their pregnancy outcomes but may also require additional time and expense. Patients themselves chose whether to proceed with (laser group) or without (controls) pretreatment with He-Ne laser irradiation prior to frozen-thawed embryo transfer. The patients who chose the laser group had He-Ne laser irradiation performed by intravenous blood irradiation by He-Ne laser (Medipark medical He-Ne laser irradiator TR-CB6, Gyeonggi-do, Korea) with a power of 1–3 mW and an exposure period of 60 min. This procedure was carried out by feeding a plastic laser-catheter into a suitable vein of the elbow or forearm. A series of 10 treatments were carried out five days per week, with a weekend break. Frozen-thawed embryo transfer for patients who received laser treatment proceeded the following menstrual cycle. Hormone therapy for embryo transfer started with 8 mg estradiol (E2) valerate (Progynova; Schering, Germany) taken orally each day for 14 days. On the 13th to 15th day of oral E2 treatment, the endometrial thickness was evaluated. If the thickness was > 8 mm, luteal and progesterone support with crinone vaginal gel (Merck Serono, Switzerland), 90 mg/d, was administered to patients. Embryo transfer was performed 3 days later. The transfer of blastocysts was performed on the fifth day. Once pregnancy was achieved, exogenous estrogen and progesterone supplementation were continued until 8 weeks gestation. The patients were followed up with ultrasonography to determine fetal viability until approximately 8 weeks gestation. Clinical pregnancy was defined by the presence of an intrauterine gestational sac and live fetus upon transvaginal ultrasound (TVS) at 7 weeks gestation.

Outcome parameters

The primary outcome measures were implantation rate (IR) and clinical pregnancy rate (PR). The IR was defined as the number of gestational sacs per number of embryos transferred. The PR was defined as the presence of one or more gestational sacs at 3 weeks, after a positive human chorionic gonadotropin (hCG) test, along with a fetal heartbeat. Miscarriage was defined as a loss of a clinical pregnancy before 13 weeks gestation. Anti-Mullerian hormone (AMH) was evaluated prior to and post to He-Ne laser irradiation. The implantation rate, clinical pregnancy rate, miscarriage rate and live birth rate were compared between the two groups.

Endometrial biopsy

All of the endometrial biopsies were performed on the 7th day after the LH surge of the patient's natural menstrual cycle, detected by daily urine LH monitoring, beginning from day 9 of the cycle. All biopsies were obtained with the use of a Pipet Curet (Cooper Surgical, Trumbull, CT, USA). Samples were evaluated for HOXA 10 and Leukemia inhibitory factor (LIF) expression by quantitative real-time PCR.

Statistical analysis

The outcome measures and associated clinical variables were analyzed using the chi-square test, Mann–Whitney statistical tests and two-tailed Student's *t*-test as appropriate. The Statistical Package for Social Sciences (version 18.0; SPSS, Chicago, IL, USA) was used for statistical analysis. A p value of .05 was considered to be significant.

Results

A total of 60 subjects with RIF were recruited into this study. There were 29 patients in the laser group and 31 patients in the control group. The basic characteristics of the patients, including patient age, body mass index (BMI), number of patients with primary infertility, number of previous failed cycles, AMH level, indication for IVF and basal sex hormone levels, were comparable between the two groups, and no significant differences were detected (Table 1). Treatment side effects and complications were not found in both groups.

In the laser-treatment group, the clinical pregnancy rate and implantation rate per cycle were 37.9% and 20.3%, respectively, which were higher than the rates in the control group (35.5% and 15.9%, respectively), but this was not a statistically significant difference ($p = .844$ and .518, respectively). The live birth rate was also higher in the laser treatment group than in the control group (27.6% vs. 25.8%, respectively, $p = .876$); however, it did not differ significantly. The miscarriage rate was lower in the laser-treatment group than in the control group (18.2% and 27.3%, respectively, $p = .611$) (Figure 1). No side effects of the laser were encountered in patients receiving the laser treatment. In the laser-treatment group, however, one patient did develop an ectopic pregnancy and another patient terminated her pregnancy due to trisomy 18, confirmed by amniocentesis.

The AMH level showed no significant difference between pre-laser and post laser treatment (2.5 ± 1.92 vs. 2.5 ± 2.36, $p = .904$)

Table 1. Patient characteristics.

	Control group ($n = 31$)	Laser group ($n = 29$)	p value
Age (year)	38.5 ± 3.89	39.3 ± 4.23	.452
Body mass index (kg/m^2)	23.2 ± 4.10	21.6 ± 3.16	.098
Weight (kg)	60.3 ± 10.98	55.4 ± 9.86	.077
Number of patients with primary infertility, n (%)	16 (51.6%)	17 (58.6%)	.586
Etiology of infertility			.607
Tubal factor	9 (29.0%)	6 (20.7%)	
Male factor	7 (22.6%)	6 (20.7%)	
Anovulation	3 (9.7%)	7 (24.1%)	
Unexplained	5 (16.1%)	3 (10.3%)	
Combined	7 (22.6%)	7 (24.1%)	
Number of previous failed cycles	3.7 ± 1.18	3.9 ± 2.24	.698
AMH (ng/ml)	3.4 ± 4.02	2.5 ± 1.92	.360
Number of embryo transferred	2.2 ± 0.67	2.0 ± 0.56	.295
Endometrium thickness on the first day of progesterone supplementation (mm)	11.7 ± 2.41	10.8 ± 1.88	.132

Values are expressed as the mean ± standard deviation, and the number of positive findings/the total number in the group, as well as the resulting percentages.

(Figure 2(A)). The mRNA expressions of HOXA-10 and LIF in the endometrium at the peri-implantation period were determined by real-time RT-PCR before and after laser treatment. There was a trend for the increased expression of HOXA-10 and LIF after laser-treatment, although this did not reach statistical significance (Figure 2(B)).

Discussion

Recurrent implantation failure remains a clinical dilemma in reproductive medicine and is emotionally and financially burdensome for patients. Intensive research on ways to improve endometrial receptivity and pregnancy outcomes is therefore warranted. He-Ne laser irradiation has recently become more popular under certain clinical conditions. Our study suggested that pretreatment with He-Ne laser irradiation may improve endometrial receptivity for frozen-thawed embryo transfer and possibly future embryo implantation in patients with recurrent implantation failure. Regulatory protein HOXA 10 and cytokine LIF expression in the endometrium were slightly elevated after laser treatment, suggesting that pretreatment with He-Ne laser irradiation prior to endometrial preparation for frozen-thawed embryo transfer may be an alternative choice for patients experiencing recurrent implantation failure.

Embryo implantation is a complex and intriguing process. Several studies have suggested that there is an alternation of endometrial receptivity in patients with unexplained RIF, and therefore, there are likely several approaches to improving endometrial receptivity [7]. Local endometrial scratching is the best known and most promising method to increase secretions of cytokines, interleukins and growth factors that favor the implantation process [19]. Previous studies indicate that endometrial

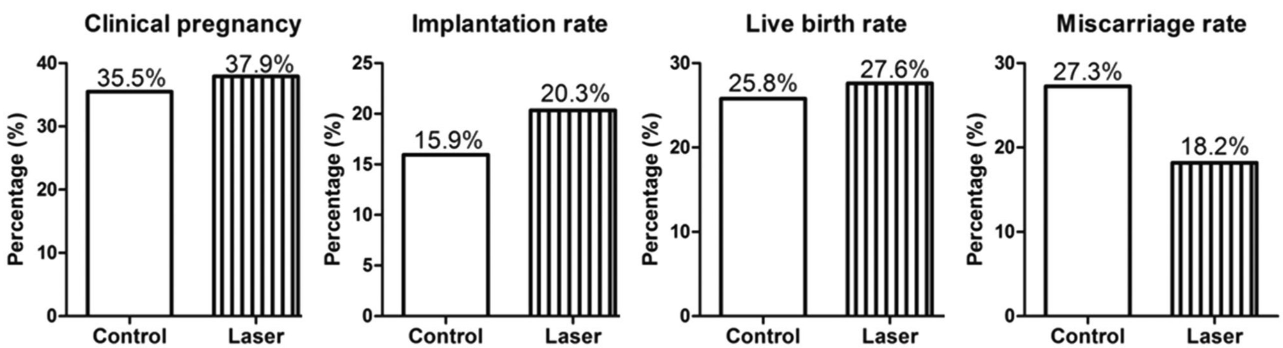

Figure 1. Frozen–thawed embryo transfer pregnancy outcomes in the control group and in the He-Ne laser irradiation group in patients with RIF.

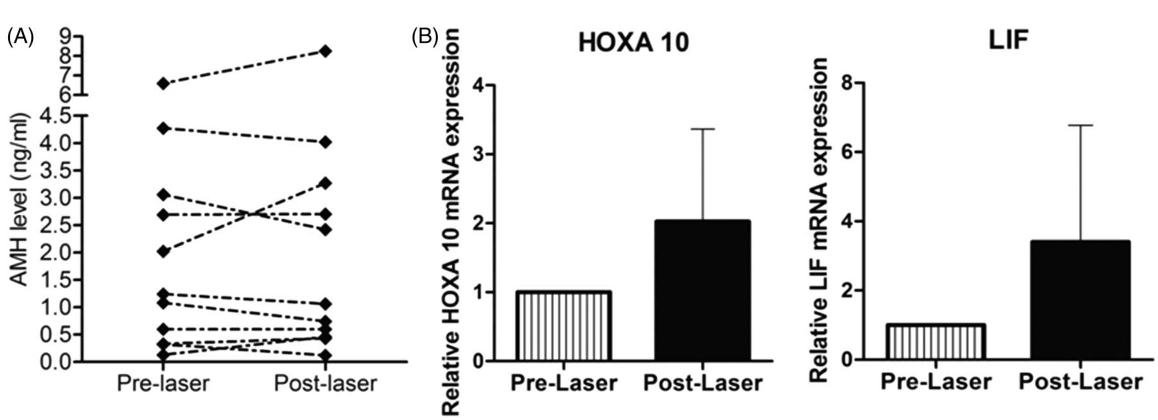

Figure 2. (A) The mRNA expressions of HOXA-10 and LIF in the endometrium at the peri-implantation period before and after He-Ne laser irradiation treatment. (B) Pre-laser and post-laser treatment of AMH level in patients treated with He-Ne laser irradiation treatment.

injury is associated with an improvement in live birth and clinical pregnancy rates in women with more than two embryo transfers failure [4]. However, the effect of endometrial injury on adverse events such as miscarriage, multiple pregnancies and vaginal bleeding remains unclear, so determining which women will benefit from the procedure is difficult [19]. He-Ne laser irradiation has been used clinically for more than 20 years, and its use yields improved immunologic activity and anti-inflammatory effects [8,20]. It trigger cellular proliferation, differentiation and apoptosis [21]. In our study, we attempted pretreatment with He-Ne laser irradiation prior to endometrial preparation for frozen-thawed embryo transfers for RIF-affected patients. The use of He-Ne laser irradiation in RIF-affected women resulted in the improvement of microcirculation and the release of growth factors and cytokines in the peri-implantation endometrium, thus improving endometrial receptivity. This suggests that pretreatment with He-Ne laser irradiation before endometrial preparation of frozen-thawed embryo transfer is an alternative choice for RIF-affected patients.

He-Ne laser (632.8 nm) is a low energy laser emitting radiation in the visible light spectrum (power with a range of 10^{-3} to 10^{-1}W), and has been demonstrated to have therapeutic roles in rheumatoid arthritis [22], wound healing [23], postherapeutic neuralgia [24], recovery of nerve injury [25], and vitiligo [26]. Cafaro et al. showed that laser acupuncture could possibly be used, without serious side effects, for improving salivary flow rates in patients with Sjögren's syndrome [27]. Low-level laser therapy and energy transfer within the cell has numerous effects, including: increasing its total energy and increasing metabolism, causing structural and cellular change, improved electrolyte exchange, increased circulation of blood and lymph, increased nerve excitability, activation of the respiratory chain and increased ATP-synthesis within mitochondria [28,29]. Currently, there are no reports concerning serious side effects of intravenous laser blood irradiation, perhaps due to the low power of energy administered. The results of the present study suggest that laser therapy may be a viable approach for patients with recurrent implantation failure by improving the microcirculation and the release of growth factors and cytokines in the peri-implantation endometrium. This may be of clinical relevance in improving endometrial receptivity for embryo transfer and possibly future embryo implantation.

Our study had limitation because it was not a randomized study. Patients who had experienced more failed cycles would have more motivation to choose the laser treatment, and therefore, it might interfere with the clinical results. Other limitations include the small sample size. Additional large prospective and randomized trials are required.

We concluded that He-Ne laser irradiation may improve endometrial receptivity, the implantation rate and the clinical pregnancy rate in RIF-affected women. Additional large, well-designed prospective studies are worthwhile and necessary.

Disclosure statement

The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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