

Evaluation of Effectiveness of Laser Assisted Hatching Pregnancy Rates on Fresh IVF / ICSI Cycles

Taze IVF/ICSI Sikluslarında Lazer Asiste Hatching'in Etkinliğinin Değerlendirilmesi

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INTRODUCTION

Infertility is one of the crucial and critical conditions in reproductive life, described as a year of an unprotected sexual relationship without pregnancy and concerns about 12% to 15% of reproductive couples (1-3). The escape of the blastocyst from the zona pellucida (hatching) is one of the principal physiological events affecting implantation process and hardening of the zona pellucida makes implantation difficult. A thick zona pellucida can be correlated to old age, poor embryo quality or freezing and thawing processes (4-6). At present, although significant advances have been made in artificial reproductive techniques such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) embryo implantation rates are still low between about 20-25% with blastocyst transfer in IVF-ICSI programs and it is estimated that up to 85% of embryos are not implanted. Gamete quality and characteristics of the embryo (ie, chromosomal structure and cytoplasm quality) are principally associated with embryo implantation and development (7,8). Moreover, the hatching problems in many euploid embryos with full developmental potential are not dependent on the implants used (9).

In order to increase implantation rates, up to now, many methods have been suggested and practiced including increased endometrial receptivity and embryo implantation capacity to improve embryo transfer (ET) (10). Assisted hatching (AHA) is thought of as another way to improve embryo implantation capacity and may include artificial distortion of the zona pellucida, zona thinning, zona drilling (i.e., forming a hole in the zona), total avoidance of the zona, and practice of chemicals or laser (11-13). In the last ten years, AHAs have been used for advanced age, frozen-thawed embryos, and IVF-ET failures. Nevertheless, the effects of AHA are still debatable (14).

Zeng and his colleagues conducted a meta-analysis of relevant work published in PubMed, EMBASE and Cochrane Central databases until March 2017. This meta-analysis was primarily used to evaluate the effect of laser-assisted incubation on assisted reproductive outcomes. Analyze 12 randomized controlled trials (more than 2574 participants) included. In summary, this meta-analysis shows that LAH is associated with a higher clinical pregnancy rate, embryo implantation rate, and multiple pregnancy rate in women with cryopreserved thawed embryos. However, it is unlikely that LAH will increase live birth rates and low rates.

In the present study, we aimed to examine pregnancy and live births (take home babies) in patients enrolled in an IVF/ICSI-ET program, and to determine possible differences between the patients treated via LAHA and the group who were not.

MATERIALS and METHODS

After receiving the approval of the Local Ethics Committee, a retrospective analysis was conducted by analyzing the data of 315 cycles of a total of 315 patients treated at IVF Center. The study was conducted complying with the tenets of the Declaration of Helsinki. We included patients in our study group who underwent 100 LAHA treatments between January 2009 and December 2010 and 215 control groups during the same period. The causes of declining fertility among the groups (female, male, or unknown) were similar in both groups. There was no difference between the control group and the LAHA group in terms of demographics. Patients were informed about LAH and written informed consent was obtained.

In our study group we included 100 LAHA patients in our center. Including criteria to working groups was excessive cytoplasmic fragmentation or delayed development with increased initial follicle stimulating hormone (FSH) levels (≥ 10 mIU / mL) or abnormal / poor morphology embryos with recurrent IVF failure (at least two failed ET therapies) rate B-HCG measurements were made on the twelfth day. Good quality embryo is a condition used by embryologists. However, this definition may not always give the same result as the defined shape. Sometimes the pregnancy outcomes of high quality embryos may be negative. Sometimes a bad embryo can be a healthy pregnancy. Because this evaluation is a subjective evaluation. One of the most important factors that affect embryo quality is genetic. This assessment does not give us this information. We used this definition that our embryologists used, without any scoring in our study.

LAHA manipulations were performed using a suture-Tm3 system using 2-3 pulses of 0.8 millisecond with 400 voltage duration. The size of the hole made in the zona was measured to be 5-10 μ m, depending on the zona thickness of each individual embryo (The study group consisted of 100 patients with repeated IVF failures (minimum two unsuccessful ET treatments), elevated baseline follicle-stimulating hormone (FSH) levels (≥ 10 mIU/mL), or with embryos with an abnormal/weak morphology, excessive cytoplasmic fragmentation, or a delayed development rate.)

Four types of protocols were used (long, short, antagonist, and microdose) with urinary and recombinant FSH. The dose and duration relied on the requirements of the individual patient. The laser-assisted hatching (LAHA) method was applied in at least one of these patients with non-touch laser system (Saturn™, Saturn Laser System; Research Instruments, Penryn, Cornwall, UK)

The control group consisted of 215 patients who were treated at our IVF center during the same time period, who underwent at least two unsuccessful ETs, or had a baseline FSH level of ≥ 10 mIU/mL. None of the patients in the control group underwent LAHA.

Following the follicle aspiration, two embryos having the best quality were selected, and transfers were performed on Day 5.

Statistical Analysis

Statistical analysis was conducted using the SPSS for Windows version 11.5 software (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to analyze the normality of the distribution of continuous variables. Descriptive data were expressed as mean \pm standard deviation or median (minimum-maximum) for continuous categorical variables, while the number of cases was presented in percentage (%). The Student's t-test was used to examine significant differences in the mean values between the groups, while the Mann-Whitney U test was conducted to investigate significant differences in the median values between the groups. Categorical variables were assessed applying the Pearson's chi-square or Fisher's exact chi-square tests. A p value of < 0.05 was regarded to be statistically significant.

RESULTS

The study group including 100 LAHA-treated patients was compared to the control group including 215 non LAHA-treated patients. Patient demographic characteristics and the controlled ovarian hyperstimulation protocols used are presented in Table 1. LAHA, laser - assisted hatching; E₂, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Table 1. Clinical parameters and applied protocols.

Variables	LAHA (+) (n = 100)	LAHA (-) (n = 215)	p-value
Age	32.8 \pm 5.2	34.7 \pm 5.2	0.003
FSH (mIU/mL)	6.7 (0.2-14.4)	7.0 (0.7-14.8)	0.298
E ₂ (pg/mL)	37.2 (8.0-363.0)	40.8 (5.1-455.6)	0.123
LH (mIU/mL)	4.4 (0.1-17.2)	4.8 (0.6-14.8)	0.033
Protocol			
long	80 (80.0%)	171 (79.5%)	0.924
microdose	7 (7.0%)	36 (16.7%)	0.019
antagonist	10 (10.0%)	6 (2.8%)	0.007
short	3 (3.0%)	2 (0.9%)	0.331

No statistically significant differences were found in the beta human chorionic gonadotropin (β hCG) positivity ($p=0.194$ and $p<0.05$, respectively), clinical

pregnancies ($p=0.812$ and $p<0.05$, respectively), live births ($p=0.642$ and $p<0.05$) between the patient and control groups (Table 2).

Table 2. β hCG positivity, clinical pregnancies, live births, and implantation rates among cases in the LAHA (+) and LAHA (-) groups.

Variables	LAHA (+) (n = 100)	LAHA (-) (n = 215)	p-value
β hCG Positivity	16 (16.0%)	48 (22.3%)	0.194
Clinical Pregnancies	14 (14.0%)	28 (13.0%)	0.812
Live Births	9 (9.0%)	23 (10.7%)	0.642

LAHA, laser - assisted hatching; β hCG, beta human chorionic gonadotropin. In the patients aged 37 or older, there was no statistically significant difference in the β hCG positivity ($p=0.291$), clinical pregnancies ($p=0.705$), live births

($p=1.000$), and implantation rates ($p=0.625$) between the patient and control groups (Table 3). AHA, assisted hatching; β hCG, beta human chorionic gonadotropin.

Table3. β hCG positivity, clinical pregnancies, live births, and implantation rates in patients aged 37 or older

Variables	Age ≥ 37 years AHA (+) (n = 30)	Age ≥ 37 years AHA (-) (n = 137)	p-value
β hCG Positivity	4 (13.3%)	30 (21,9.%)	0.291
Clinical Pregnancies	3 (10.0%)	10 (7.3%)	0.705
Live Births	1 (3.3%)	8 (5.8%)	1.000

In addition, in the patients with three or more IVF failures, we found no statistically significant differences in the β hCG positivity ($p=1.000$), clinical pregnancies ($p=1.000$), live births ($p=1.000$) and implantation rates ($p=0.622$) between the patient and control groups.

However, among the patients with a FSH level of ≥ 10 mIU/mL, the β hCG positivity ($p=0.036$) and clinical pregnancy rates ($p=0.037$) were statistically significantly lower in the patient group. On the other hand, we found no statistically significant differences in the live birth ($p=0.082$) and implantation rates ($p=0.07$) between the LAHA-treated patients with a FSH level of ≥ 10 mIU/mL and non-LAHA-treated patients.

DISCUSSION

The main finding of this study was that LAHA did not improve the biochemical, clinical, and take home baby rates in fresh IVF/ ICSI - ET patients. The main limitation of this study involve the small sample size due to the current numbers accrued thus far, which is underpowered for the detection of significant differences in rare, but important clinical outcomes, such as β hCG positivity, clinical pregnancies live births and implantation rates. Its retrospective design, only LAHA method applied all participants, small sample size, and the lack of body mass index (BMI) and pregnancy outcomes can be regarded as the other limitations

There is no agreement about the effectiveness of AHA to develop the result of the assisted reproduction (15,16). AHA is a routine practice in some IVF clinics, while in some clinics it is only considered in certain situations (17). Antinori et al. (18) stated a marked improvement in pregnancy percentage after assisted hatching in patients receiving first IVF. However, failure to show significant impact on non-selective assisted hatching was reported by Cohen et al. and Hurst et al. (19,20). Our data revealed that that biochemical, clinical, and take home baby rates were not significantly different between the LAHA and control groups.

In their study, Horng et al. analyzed 120 IVF/ICSI cycles of women aged above 37 years. Participants were divided into two groups: in group 1, the embryo was transferred after culture, without LAHA; and Group 2 had LAHA before embryo transfer. The authors found no statistically significant difference between the two groups at the implantation rate (7.3% and 6.7%, $p = 0.89$, respectively) and pregnancy rate (16.3% and 14.2%, $p = 0.86$, respectively). (21). Consistent with our study results, the authors concluded that LAHA had no use in increasing the implantation and pregnancy rates in advanced aged women.

In a prospective, randomized-controlled trial, 203 IVF-ET patients with aged ≤ 39 years with normal FSH and E_2 levels, and no more than one IVF-ET failure with a good embryo quality were divided into two groups, those who were treated via LAHA and those who were not (22). Positive β hCG (%61 and %64 per cycle, respectively, $p=0.30$), clinical pregnancy (53% and 54% per cycle, respectively, $p=0.90$), (13% and 16% per pregnancy, respectively), and live birth rates (46% and 45% per cycle, respectively, $p=0.90$) were found to be similar between the two groups. In another recent study, the patients were divided into two groups of less than 35 years of age and over 35 years of age (23). Consistent with our findings, the authors stated that laser-assisted zona thinning of Day 3 embryos did not have advantageous result in clinical pregnancy and implantation rates.

Furthermore, in their prospective, randomized trial, Petersen et al. assessed the outcomes of quarter-laser zona thinning-assisted hatching (qLZT-AHA) to enhance the implantation of embryos in 150 patients with implantation failure story (24). Patients were administered ICSI and then divided into two groups as qLZT-AHA recipients and non-recipients. The implantation rate in patients with persistent implantation failure was significantly higher ($p=0.02$) in the qLZT-AHA group than the non-qLZT-AHA group. However, there was no significant difference in patients with a single implantation failure. Contrary to the results of the present study, this study showed that qLZT-AHA was a useful approach to rise embryo implantation rates in cases with repeated implantation failures.

In another prospective, randomized trial, Valojerdi et al. studied 410 patients with an advanced age (≥ 37 years), 795 patients with repeated implantation failures (≥ 2 cycles), and 180 patients with frozen-thawed embryos. Participants in the study were divided equally and working and control groups were established (12). On the ET day, the zona pellucida of chosen embryos was opened with a laser, which was not implemented in the control group. As in the present study, clinical pregnancy and implantation rates were alike in both groups to those with advanced age or recurrent implantation failure. However, in the frozen-thawed embryo patients, the clinical pregnancy and implantation rates in the study group were statistically higher than in the control group. Ge H.S. et al also stated that assisted hatching by thinning zona did not have a significant effect on clinical pregnancy or live birth rates in the 387 fresh embryo transfer cycle (25).

Hatching failure or inability to escape from the surrounding zona pellucida is one of the reasons responsible for the low implantation rate in the assisted reproductive process. Although 20 years have passed since the administration of AH in assisted reproduction, the indications for its usage is still unclear.

In conclusion, The hatching process of blastocysts still remains unclear. The majority of morphologically normal blastocysts undergoes hatching problems. Our study results showed that LAHA, an assisted reproductive technology, did not significantly increase the β hCG positivity, clinical pregnancy, and take home babies rates. Routine performance of assisted hatching on all embryos in fresh IVF/ICSI patients is neither scientific nor reasonable. Assisted hatching may be clinically useful and that individual ART programs should evaluate their own patient populations to identify which subgroups may benefit from the procedure. We think that large scale, prospective, randomized, controlled studies are necessary to determine whether clinical practice of our LAHA is appropriate.

Conflict of interest

No conflict of interest was declared by the authors.

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