Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2022.36690

Turk J Obstet Gynecol 2022;19:130-7

Comparison of the impact of laser-assisted hatching on fresh cleavage and blastocyst embryo transfer and association with pregnancy outcomes

Lazer destekli yuvalamanın taze klivaj ve blastokist embriyo transferleri üzerindeki etkisinin karşılaştırılması ve gebelik sonuçları ile ilişkisi

Selin Hazır[1], İskender Kaplanoğlu[2], Asena Ayça Özdemir[3], Oya Aldemir[2], Runa Özelci[2], İnci Kahyaoğlu[2], Serdar Dilbaz[2]

[1]*Mersin University Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Mersin, Turkey*

[2]*University of Health Sciences Turkey, Etlik Zübeyde Hanım Women's Health Training and Research Hospital, Clinic of Reproductive Endocrinology and Infertility, Ankara, Turkey*

[3]*Mersin University School of Medicine, Department of Medical Education, Mersin, Turkey*

Abstract

Objective: Assisted hatching (AH) techniques can improve live birth (LB) and clinical pregnancy (CP) rates. Since there are limited data regarding this subject, we investigated the impact of laser-assisted hatching (LAH) on fresh embryo transfer (ET) and association with pregnancy outcomes in unselected patient population.

Materials and Methods: This retrospective study included the fresh ETs performed at our center between April 2010 and April 2019. Among 3.782 fresh ETs, 3.286 underwent LAH (n=1.583 at cleavage stage and n=1.703 at blastocyst stage) while 496 underwent non-assisted hatching (NAH) (n=213 at cleavage stage and n=283 at blastocyst stage). The ETs were performed at the blastocyst or cleavage stages, and single or double embryos were transferred. LB rate was the primary outcome, while secondary outcomes were the pregnancy test, monozygotic twinning (MZT), and CP rates.

Results: The LAH and NAH groups showed similar LB, pregnancy test, CP, and MZT rates at cleavage and blastocyst stages. On the other hand, LAH significantly affected LB rates at the blastocyst stage (20.6% at blastocyst stage vs. 16% at the cleavage stage, p=0.001).

Conclusion: In conclusion, LAH does not improve reproductive outcomes of fresh blastocyst-stage and cleavage-stage ETs. However, LAH significant impacts LB rates in the blastocyst stage than the cleavage stage.

Keywords: In vitro fertilization, laser-assisted hatching, fresh embryo transfer, cleavage stage, blastocyst stage

Öz

Amaç: Destekli yuvalama teknikleri canlı doğum ve klinik gebelik oranlarını iyileştirebilir. Bu konuyla ilgili sınırlı veri olduğundan, seçilmiş olmayan hasta popülasyonunda lazer destekli yuvalamanın taze embriyo transferi üzerindeki etkisini ve gebelik sonuçları ile ilişkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Bu retrospektif çalışmaya merkezimizde Nisan 2010 ile Nisan 2019 tarihleri arasında gerçekleştirilen 3,782 taze embriyo transferleri dahil edildi. Klivaj aşamasındaki 3,286 embriyoya lazer destekli yuvalama işlemi uygulanırken, 496 embriyo (klivaj aşaması n=213, blastokist aşaması n=283) kontrol grubu olarak değerlendirildi. Embriyo transferleri klivaj (n=1,583) ve blastokist (n=1,703) aşamalarında gerçekleştirildi ve tek veya iki embriyo transfer edildi. Birincil sonuç olarak canlı doğum oranı, ikincil olarak ise gebelik testi, klinik gebelik ve monozigotik ikizlik oranları gruplar arasında karşılaştırıldı.

PRECIS: Impact of laser-assisted hatching on outcomes of fresh cleavage vs. blastocyst embryo transfer.

Address for Correspondence/Yazışma Adresi: PhD Selin Hazır,
Mersin University Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Mersin, Turkey
Phone: +90 537 626 74 84 **E-mail:** dr.emb.selin@gmail.com **ORCID ID:** orcid.org/0000-0002-0714-5929
Received/Geliş Tarihi: 22.02.2022 **Accepted/Kabul Tarihi:** 30.03.2022

Bulgular: Lazer destekli yuvalama ve kontrol grubu klivaj ve blastosist aşamalarında benzer canlı doğum, gebelik testi, klinik gebelik ve monozigotik ikizlik oranları gösterdi. Öte yandan, lazer destekli yuvalama, blastokist aşamasında canlı doğum oranlarını önemli ölçüde etkiledi (blastokist aşamasında %20,6 ve klivaj aşamasında %16, p=0,001).

Sonuç: Sonuç olarak, lazer destekli yuvalamanın taze klivaj ve blastokist evresi embriyo transferlerinin üreme sonuçlarını iyileştirmediği gösterildi. Bununla birlikte, lazer destekli yuvalamanın, blastokist aşamasındaki canlı doğum oranlarını klivaj aşamasına kıyasla anlamlı düzeyde artırmış olduğu saptandı.

Anahtar Kelimeler: Tüp bebek, lazer destekli yuvalama, taze embriyo transferi, klivaj aşaması, blastokist aşaması

Introduction

Assisted hatching (AH) methods involve the handling of zona pellucida (ZP) and are implemented as part of assisted reproductive technologies (ART)[1]. The ZP is a coat enveloping the oocyte; it prevents polyspermy and protects the embryo before implantation[2]. After fertilization, hatching of ZP is crucial for implantation in the receptive endometrium. Failure to hatch is one of the primary reasons for failure to implantation[3].

To date, different AH techniques have been developed to increase implantation ratios in women going through intracytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF)[4]. The AH techniques can be performed chemically, mechanically, or by laser[5,6]. All AH methods are implemented to create a gap in the ZP or to thin the ZP for supporting the embryo during hatching when the blastocyst is ready for expansion and implantation[7].

Among the AH methods, laser-assisted hatching (LAH) has become popular since it is relatively simple and less time-consuming[8]. Also, in this method, the target can be precisely controlled, allowing the creation of gaps in the ZP less risk of injury to the embryo[9]. As per the Society for Assisted Reproductive Technology report[10], AH was applied in 56.3% of cleavage stage and 22.8% of blastocyst stage fresh embryo transfer (ET) patients in 2010. However, the retrospective and prospective studies assessing the impact of AH on reproductive outcomes[11,12] gave conflicting results. While some studies reported that AH might improve the clinical pregnancy (CP) and the multiple pregnancy rates, others reported either no improvement or a decrease in implantation and live birth (LB) rates[13,14]. On the other hand, it was recommended by the American Society for Reproductive Medicine[15] that AH might be clinically beneficial for women aged 38 or older, who went through at least two IVF/ICSI cycles, or those who have poor-quality or cryopreserved embryos. Although several studies[16-18] investigated the impact of AH on patients who underwent frozen-thawed cycles, patients who experienced repeated implantation failure (RIF), and those with advanced maternal age, little data obtained in the literature respecting the impact of AH in an unselected patient population undergoing IVF/ICSI.

We examined the impact of LAH on the clinical outcomes, including pregnancy test, CP, LB, and monozygotic twinning (MZT) ratios in a general patient group undergoing IVF/ICSI.

Materials and Methods

Patients

This study was performed at the infertility clinic of the University of Health Sciences Turkey, Etlik Zubeyde Hanim Women's Health Training and Research Hospital and approved by the Ethical Review Committee (17.01.2020-01/20). Patients who went through fresh IVF/ICSI cycles at this center between April 2010 and April 2019 constituted the target population of this study. In total, 3.782 fresh ETs were performed. Among these, 3.286 underwent LAH (n=1.583 at cleavage stage and n=1.703 at blastocyst stage), 496 underwent NAH (n=213 at cleavage stage, and n=283 at blastocyst stage). While one of the main inclusion criteria was single or double fresh ETs on day 3 or day 5, patients who underwent frozen-thawed embryo cycles were excluded.

Controlled Ovarian Stimulation, ICSI, and Embryo Culture

Women partners were stimulated using gonadotropin-releasing hormone (GnRH) antagonist or agonists after evaluating the ovarian reserve. The medication doses were determined based on the patients' body mass index (BMI), age, antral follicle counts, and basal serum follicle-stimulating hormone (FSH) levels. Oocyte maturation was induced by injecting 10.000 IU of human chorionic gonadotropin (hCG) (Pregnyl, Schering-Plough, Turkey) subcutaneously. When at least three follicles ≥16-18 millimeter in size, transvaginal ultrasound-guided oocyte pick-up was performed 36 hours after the hCG injection. Retrieved oocytes were amassed in G-IVF plus medium (Vitrolife, Gothenburg, Sweden) covered by 3 mL Ovoil (Vitrolife, Gothenburg, Sweden) in an atmosphere of 5% O_2, 6% CO_2, and 95% humidity at 37 °C for 2-4 hours.

The mature oocytes were inseminated by ICSI. Fertilization was confirmed as the presences of the two distinct pronuclei and second polar body 16-18 h after insemination. Zygotes were cultured in 30 µL drops of G-TL medium (Vitrolife, Gothenburg, Sweden) covered by 3 mL Ovoil (Vitrolife, Gothenburg, Sweden) in an atmosphere of 5% O_2, 6% CO_2, and 95% humidity at 37 °C.

Luteal support was initiated on the oocyte retrieval day by administering 100 mg progesterone in oil (Progestan, Kocak, Istanbul) daily or vaginal progesterone (Crinone® 8% progesterone vaginal gel, Merck, Germany). Luteal support lasted until 10-12 weeks of gestation. Pregnancy was accepted

positively when serum level of hCG was ≥10 IU/L 2 weeks after oocyte retrieval.

The LAH Procedure, Embryo Morphology, and Embryo Transfer

The LAH was applied to each embryo by creating a hole using an infrared diode laser (1.480 nm/400 mW, 40x objectives, Saturn 3 Laser System, Research Instruments Ltd., Cornwall, UK) and a non-contact 630-650 nm pilot laser on day 3 of embryo development. Cleavage embryos were graded based on their quality[19]. They were graded as grade 1 if they had 6-8 blastomeres on day 3 with <10% fragmentation without morphological abnormalities. The cleavage embryos were accepted as grade 2 if they had uneven blastomeres with mild variation in refractility and <10% fragmentation. Embryos with 3-6 blastomeres and 20-50% fragmentation were graded as grade 3, while those with less than three blastomeres and >50% fragmentation were graded as grade 4. Thus, the grade 1 and 2 embryos were categorized as good quality ones, while grade 3 and 4 were accepted as poor quality for the cleavage stage. Therefore, the grade 4 embryos were not transferred.

Blastocysts were graded as per the Gardner classification[20] and scored based on expansion status, inner cell mass (ICM), and trophectoderm (TE) development. The expansion status was graded as follows: Early blastocysts were graded as grade 1, blastocysts as grade 2, full blastocysts as grade 3; expanded blastocysts as grade 4; hatching blastocysts as grade 5 and hatched blastocysts as grade 6. The ICM was graded based on the presence of tightly packed cells (A), loosely packed cells (B), and very few cells (C). The TE was graded as A if several cells formed a cohesive epithelium, B if few cells formed a loose epithelium, and C in the presence of only very few large cells. Blastocysts having a grade of at least 3BB were classified as good-quality embryos. The ET was performed by transabdominal ultrasonography guidance on day 3 or day 5 of embryo development. When we have at least three good-quality embryos on day 3, the embryos were cultured to the blastocyst stage in the G-TL medium (Vitrolife, Gothenburg, Sweden) and transfers were performed on day 5. The other ETs were performed on day 3.

Clinical Outcomes

Serum hCG levels were measured two weeks after ETs. CP was described as the presence of gestational sac via vaginal ultrasonography by six weeks of pregnancy.

Statistical Analysis

The normal distribution of the continuous parameters was tested by the Shapiro-Wilk test. If the variables were not normally distributed, the Mann-Whitney U test was used to compare the LAH and NAH groups. The Fisher's Exact and chi-square tests

Table 1. Demographic data and controlled ovarian stimulation parameters of cleavage stage embryo transfer cycles

Parameters	LAH (n=1.583)	NAH (n=213)	p*
	Mean ± SD (min-max)	Mean ± SD (min-max)	
Maternal age	32.04±5.09 (18-48)	33.25±3.74 (25-44)	**<0.001**
BMI	29.43±9.17 (16.4-316)	28.32±4.14 (19.1-43.7)	**0.014**
Antral follicle count	11.59±7.6 (0-45)	11.24±8.29 (1-30)	0.121
Endometrial thickness on hCG day (mm)	7.86±1.78 (5-15.6)	7.76±1.35 (5-11.6)	0.739
Endometrial thickness on OPU day (mm)	7.94±1.81 (5-15.4)	7.66±1.29 (5-11.5)	0.251
Total oocyte count	10.63±7.41 (1-52)	10.47±7.82 (1-44)	0.466
Mature oocyte count	7.21±5.63 (1-36)	7.67±5.87 (1-30)	0.232
2PN	3.78±3.31 (0-23)	4.03±3.92 (1-28)	0.620

*p-value calculated by Mann-Whitney U test, LAH: Laser-assisted hatching, NAH: Non-assisted hatching, SD: Standard deviation, BMI: Body mass index, hCG: Human chorionic gonadotropin, mm: Millimeter, OPU: Oocyte pick-up, PN: Pronuclei

were implemented to analyze the categorical data. Descriptive statistics of the continuous variables were expressed as medians, means, standard deviations, interquartile ranges, and minimum and maximum values. The categorical parameters were given as frequencies (n) and percentages (%). The IBM SPSS statistics software was used for all statistical analyses.

Results

During the study period, 3,782 IVF/ICSI cycles were performed. In total, 1,796 patients underwent ET at the cleavage stage. Among these patients, 1,583 underwent LAH while 213 underwent NAH. The demographic data and controlled ovarian stimulation parameters of the patients are displayed in Table 1. The maternal age was significantly higher, and BMI was significantly lower in the NAH group than in the LAH group (p<0.001, p=0.014). Two groups were similar regarding other parameters. The embryo grades, numbers of transferred embryos, and reproductive outcomes in two groups are shown in Table 2.

While the number of grade 2 embryos was significantly higher in the LAH group, the number of grade 3 embryos was significantly higher in the NAH group (p<0.001 and p<0.001). However, the two groups were similar regarding reproductive outcomes, including the rates of pregnancy tests, clinical pregnancy, and live birth (p=0.311, p=0.368, p=0.23). The MZT rates were also similar between the LAH and NAH groups.

Our review revealed that 1986 patients underwent ET at the blastocyst stage. Among these patients, 1703 underwent LAH while 283 underwent NAH. Data of these patients are presented in Table 3.

There was a significant difference between the two groups regarding BMI, endometrial thickness on hCG and oocyte retrieval days, and the number of embryos with 2 pronuclei (2PN) embryos (p=0.005, p<0.001, p<0.001, p=0.007).

The blastocyst scores and reproductive outcomes are displayed in Table 4. The two groups were significantly different concerning blastocoel expansion, ICM, and TE scores, and numbers of transferred embryos (p<0.001, p<0.001, p<0.001, p=0.012). However, the two groups were similar regarding the pregnancy test, clinical pregnancy, live birth, and MZT rates (p=0.498, p=0.231, p=0.208, p=1).

Comparison of the pregnancy test, CP, LB, and MZT rates for investigating the effects of LAH on reproductive outcomes following fresh cleavage-stage or blastocyst-stage ETs revealed no significant difference regarding pregnancy test, clinical pregnancy, and MZT rates between cleavage and blastocyst stage ETs (p=0.249, p=0.698, p=0.735). In contrast, there was a statistically significant difference concerning live birth rates. The live birth rates were significantly higher in the blastocyst-stage ETs than cleavage-stage ETs (p=0.001) (Table 5).

Discussion

In our retrospective review, we analyzed the impact of LAH on fresh ETs in a general IVF population. Our findings indicate that LAH does not advance pregnancy outcomes of fresh cleavage and blastocyst-stage ETs. However, it increased a remarkable enhancement in the LB rate at the blastocyst stage ETs. The LAH procedure did not significantly reproductive outcomes, including CP, pregnancy test, LB, and MZT rates, irrespective of embryo morphology.

Several studies, which analyzed the impact of LAH on reproductive outcomes, but the results are conflicting[16,17,21,22]. Most of these studies[16-18] focused on the effects of LAH on specific patient populations, including those who underwent

Table 2. Embryo grades and reproductive outcomes of cleavage-stage embryo transfer cycles

Parameters		LAH n (%)	NAH n (%)	Total n (%)	p
Embryo grade	1	632 (40.5)	78 (36.6)	710 (40.0)	**<0.001**
	2	773 (49.5*)	83 (39.0)	856 (48.2)	
	3	157 (10.1)	52 (24.4*)	209 (11.8)	
Embryo transfer	1	1024 (65.6)	167 (78.4*)	1191 (67.1)	**<0.001**
	2	538 (34.4*)	46 (21.6)	584 (32.9)	
Pregnancy test	Positive	582 (37.3)	87 (40.8)	669 (37.7)	0.311
	Negative	980 (62.7)	126 (59.2)	1106 (62.3)	
Clinical pregnancy	Positive	459 (29.4)	69 (32.4)	528 (29.7)	0.368
	Negative	1103 (70.6)	144 (67.6)	1247 (70.3)	
Live birth	Positive	250 (16.0)	41 (19.2)	291 (16.4)	0.230
	Negative	1312 (84.0)	172 (80.8)	1484 (83.6)	
Monozygotic twinning	Positive	30 (1.9)	4 (1.9)	34 (1.9)	1.00**
	Negative	1532 (98.1)	209 (98.1)	1741 (98.1)	

p-values were calculated by chi-square test, *p-values lower than 0.05, **p-values calculated by Fisher's Exact test, LAH: Laser-assisted hatching, NAH: Non-assisted hatching

frozen-thawed IVF/ICSI-ET cycles, those with advanced maternal age, and patients previously diagnosed with RIF. Zeng et al.[21] conducted a systematic review analyzing twelve randomized controlled trials and concluded that LAH was affiliated with higher CP and implantation rates and a higher risk of multiple pregnancies in women receiving thawed embryos. In a retrospective trial, Hiraoka et al.[16] studied the impact of the ZP openings with different sizes in LAH performed on high-quality blastocysts originated from slow frozen-thawed cleavage stage embryos in women with RIF. They detected a remarkable improvement in pregnancy, implantation, and delivery rates with the opening of 50% of the ZP (74%, 52%, 65%) while the improvements in the control (17%, 10%, 13%; p<0.01) and 40 µm ZP opening (43%, 27%, 38%, p<0.04) groups were less significant[18]. These authors also reported significantly lower delivery rates in the control group than the 50% ZP opening and the 40 µm ZP opening groups[16]. Another randomized trial, Wan et al.[17] performed a quarter ZP opening by LAH and investigated its impact on the clinical parameters after transferring vitrified-warmed blastocysts originating from low-grade cleavage stage embryos. These researchers reported a remarkable increase in the CP and implantation rates while the LB rates did not change significantly (p=0.034, p=0.021, p>0.05). Ng et al.[4] showed in a retrospective trial conducted on vitrified-warmed blastocyst transfers that LAH did not impact the rates of implantation (26.2% vs. 27.3%), conception (38.7% vs. 42.1%), clinical pregnancy loss, LB, CP and MZT. They also reported that five pairs of dichorionic/diamniotic twins developed from single ETs.

Only a few studies have analyzed the effects of LAH on fresh ETs[18,23,24]. In a study by Sagoskin et al.[23] conducted ZP drilling by LAH on the day of fresh ET in women going through the transfer of cleavage embryos (day 3) in a selected patient population with good prognostic factors, including normal serum FSH and E2 levels, maternal age ≤39, have good-quality embryo on day 3 and history of no more than one failed IVF/ICSI cycle. Patients with unfavorable prognostic factors were excluded from this study. The presence of spontaneous pregnancy loss (13% vs. 16%), fetal cardiac activity (53% vs. 54%), and LB (47% vs. 46%) rates were similar between the groups who underwent LAH and those who did not undergo AH. These authors concluded that LAH did not benefit this selected patient population. In a prospective randomized study, Razi et al.[24] performed LAH to open a hole in ZP on day 2 of embryo development in patients undergoing ICSI due to male factor infertility during their initial IVF/ICSI cycle. Comparison of LB and CP rates between LAH and NAH groups revealed insignificant differences (11.11%

Table 3. Demographic and controlled ovarian stimulation parameters of the blastocyst-stage embryo transfer cycles

	LAH (n=1.703)	NAH (n=283)	
Parameters	**Mean ± SD (min-max)**	**Mean ± SD (min-max)**	**p**
Maternal age	30.65±4.77 (19-50)	31.3±5.68 (19-47)	0.135
BMI	28.74±13.42 (16.4-316)	29.33±5.33 (19-43.5)	**0.005**
Antral follicle count	13.01±8.49 (0-31)	13.14±9.46 (0-30)	0.439
Endometrial thickness on hCG day (mm)	8.1±1.92 (5-16.5)	7.32±1.45 (5-11.5)	**<0.001**
Endometrial thickness on OPU day (mm)	8.12±2.5 (5-17)	7.3±1.36 (5-12)	**<0.001**
Total oocyte count	10.21±6.63 (1-42)	10.73±7.87 (1-43)	0.856
Mature oocyte count	7.32±5.08 (0-31)	7.71±5.67 (1-32)	0.629
2PN	4.94±3.42 (1-25)	4.75±3.92 (1-20)	**0.007**

p-values were calculated by Mann-Whitney U test, LAH: Laser-assisted hatching, NAH: Non-assisted hatching, SD: Standard deviation, BMI: Body mass index, hCG: Human chorionic gonadotropin, mm: Millimeter, OPU: Oocyte pick-up, PN: Pronuclei, min: Minimum, max: Maximum

vs. 8.6%, p=0.6, and 20% vs. 23.9%, p=0.3). Additionally, these authors reported that there were multiple pregnancies (twin) in both LAH and control groups. One congenital anomaly was present in the LAH group. Tannus et al.[18] worked on patients with advanced maternal age (i.e., mean age 41.1±1.1). This retrospective study showed that LAH was affiliated with reduced LB and CP rates in fresh ETs performed during cleavage but not the blastocyst stage. In a retrospective study by Xu et al.[25] have evaluated the effect of LAH on the low-grade cleavage stage embryos. They reported the total blastocyst rate (50.7% vs. 40.2, p<0.001), usable blastocyst rate (31% vs. 18.6%, p<0.001) were significantly higher in the LAH group. Additionally, CP rates were not different between groups (49.4% vs. 40%, p>0.05).

This study found that clinical outcomes were similar between NAH and LAH when the latter was performed in cleavage-stage ETs. Our study also showed that LAH did not improve the pregnancy test (p=0.311), LB (p=0.230), and CP (p=0368) rates in cleavage-stage ETs. Similarly, we did not detect a remarkable difference in the positive pregnancy test (p=0.498), CP (p=0.231), and LB (p=0.208) rates in blastocyst-stage ETs. On the other hand, LAH significantly improved LB rates in blastocyst-stage ETs than the cleavage-stage ETs (p=0.001). A study by Schwärzler et al.[26] analyzed the pregnancy outcomes of blastocyst-stage and cleavage-stage ETs. Additionally, it was reported that[27] the blastocyst could improve the synchronization between embryo and endometrium and permit the selection of more advanced embryos considered the most appropriate for transfer. Also, it is known that blastocyst transfer leads to relatively higher LB and implantation rates. In line with these findings, we found that LB rates were remarkably higher in blastocyst-stage ETs than in cleavage-stage ETs. The differences in the previously published reports and our study results can be ascribed to the differences in the study population, AH timing, and technique.

The use of micromanipulation techniques in ART is related to a higher risk of MZT. Several researchers have reported that multiple factors, including maternal age, prolonged embryo culture until the blastocyst stage, embryo biopsy for preimplantation genetic testing, fresh or frozen-thawed ET, ovarian stimulation, and ZP manipulation as ICSI and AH might account for this increased risk[28-31]. However, several authors reported that blastocyst transfer was related to an increased risk of MZT[27], the others did not report such an association[30,31]. Our results also revealed an insignificant difference in MZT rates between cleavage and blastocyst stage ETs.

Table 4. Blastocyst scores and reproductive outcomes of blastocyst stage embryo transfer cycles

Parameters		LAH n (%)	NAH n (%)	Total n (%)	p
Blastocoel expansion	3	439 (25.8)	176 (62.2*)	615 (35.7)	**<0.001**
	4	717 (42.1*)	69 (24.4)	786 (45.6)	
	5	260 (15.3*)	29 (10.2)	289 (16.8)	
	6	26 (1.5)	9 (3.2)	35 (2.0)	
Inner cell mass score	A	148 (8.7)	64 (22.6*)	212 (10.7)	**<0.001**
	B	611 (35.9)	107 (37.8)	718 (36.2)	
	C	944 (55.4*)	112 (39.6)	1.056 (53.2)	
Trophoectoderm score	A	22 (1.3)	66 (23.3*)	88 (4.4)	**<0.001**
	B	609 (35.8)	131 (46.3*)	740 (37.3)	
	C	1.072 (62.9*)	86 (30.4)	1.158 (58.3)	
Embryo transfer	1	1239 (72.8)	226 (79.9*)	1.465 73.8)	**0.012**
	2	464 (27.2*)	57 (20.1)	521 (26.2)	
Pregnancy test	Positive	668 (39.2)	105 (37.1)	773 (38.9)	0.498
	Negative	1.035 (60.8)	178 (62.9)	1.213 (61.1)	
Clinical pregnancy	Positive	511 (30.0)	75 (26.5)	586 (29.5)	0.231
	Negative	1.192 (70.0)	208 (73.5)	1.400 (70.5)	
Live birth	Positive	350 (20.6)	49 (17.3)	399 (20.1)	0.208
	Negative	1.353 (79.4)	234 (82.7)	1.587 (79.9)	
Monozygotic twinning	Positive	30 (1.8)	5 (1.8)	35 (1.8)	1.00**
	Negative	1.673 (98.2)	278 (98.2)	1951 (98.2)	

p-values were calculated by chi-square test, *p-values lower than 0.05, **p-values calculated by Fisher's Exact test, LAH: Laser-assisted hatching, NAH: Non-assisted hatching

Table 5. Effect of LAH on cleavage and blastocyst stage ET

		Day 3 AH n (%)	Day 5 AH n (%)	Total n (%)	p
Pregnancy test	Positive	582 (37.3)	668 (39.2)	1250 (38.3)	0.249
	Negative	980 (62.7)	1035 (60.8)	2015 (61.7)	
Clinical pregnancy	Positive	459 (29.4)	511 (30.0)	970 (29.7)	0.698
	Negative	1103 (70.6)	1192 (70.0)	2295 (70.3)	
Live birth	Positive	250 (16.0)	350 (20.6*)	600 (18.4)	**0.001**
	Negative	1312 (84.0*)	1353 (79.4)	2665 (81.6)	
Monozygotic twinning	Positive	30 (1.9)	30 (1.8)	60 (1.8)	0.735
	Negative	1532 (98.1)	1673 (98.2)	3205 (98.2)	

p-values were calculated by chi-square test, *p-values lower than 0.05. LAH: Laser-assisted hatching, ET: Embryo transfer, AH: Assisted hatching

Study Limitations

Our study has several limitations such as its retrospective design, completed at a center and consisted of small patient population. Our some results were not reached statistical significance because of the small patient population. Larger prospective and multi-center studies must enhance our knowledge on the effect of LAH on fresh ETs.

Conclusion

In conclusion, our findings were shown that LAH has insignificant impact on the rates of CP, MZT and pregnancy test between cleavage and blastocyst stage ETs, but a significant effect on LB rate in blastocyst stage ETs.

Ethics

Ethics Committee Approval: This study was performed at the Infertility Clinic of the University of Health Sciences, Etlik Zubeyde Hanim Women's Health Training and Research Hospital and approved by the Ethical Review Committee (17.01.2020-01/20).
Informed Consent: Retrospective study.
Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: O.A., R.Ö., S.D., Concept: S.H., İs.K., A.A.Ö., O.A., R.Ö., İ.K., S.D., Design: S.H., İs.K., A.A.Ö., O.A., R.Ö., İ.K., S.D., Data Collection or Processing: S.H., İ.K., A.A.Ö., O.A., R.Ö., S.D., Analysis or Interpretation: S.H., A.A.Ö., İ.K., Literature Search: S.H., İs.K., O.A., R.Ö., İ.K., S.D., Writing: S.H., İs.K., A.A.Ö., O.A., R.Ö., İ.K., S.D.
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study received no financial support.

References

1. Cohen J, Malter H, Fehilly C, Wright G, Elsner C, Kort H, et al. Implantation of embryos after partial opening of oocyte zona pellucida to facilitate sperm penetration. Lancet 1988;2:162.
2. Lefièvre L, Conner SJ, Salpekar A, Olufowobi O, Ashton P, Pavlovic B, et al. Four zona pellucida glycoproteins are expressed in the human. Hum Reprod 2004;19:1580-6.
3. Mercader A, Simón C, Galán A, Herrer R, Albert C, Remohí J, et al. An analysis of spontaneous hatching in a human endometrial epithelial coculture system: is assisted hatching justified? J Assist Reprod Genet 2001;18:315-9.
4. Ng EH, Naveed F, Lau EY, Yeung WS, Chan CC, Tang OS, et al. A randomized double-blind controlled study of the efficacy of laser-assisted hatching on implantation and pregnancy rates of frozen-thawed embryo transfer at the cleavage stage. Hum Reprod 2005;20:979-85.
5. Cohen J, Alikani M, Trowbridge J, Rosenwaks Z. Implantation enhancement by selective assisted hatching using zona drilling of human embryos with poor prognosis. Hum Reprod 1992;7:685-91.
6. Hsieh YY, Huang CC, Cheng TC, Chang CC, Tsai HD, Lee MS. Laser-assisted hatching of embryos is better than the chemical method for enhancing the pregnancy rate in women with advanced age. Fertil Steril 2002;78:179-82.
7. Kutlu P, Atvar O, Vanlioglu OF. Laser assisted zona thinning technique has no beneficial effect on the ART outcomes of two different maternal age groups. J Assist Reprod Genet 2010;27:457-61.
8. Hammadeh ME, Fischer-Hammadeh C, Ali KR. Assisted hatching in assisted reproduction: a state of the art. J Assist Reprod Genet 2011;28:119-28.
9. Mantoudis E, Podsiadly BT, Gorgy A, Venkat G, Craft IL. A comparison between quarter, partial and total laser assisted hatching in selected infertility patients. Hum Reprod 2001;16:2182-6.
10. Kissin DM, Kawwass JF, Monsour M, Boulet SL, Session DR, Jamieson DJ, et al. Assisted hatching: trends and pregnancy outcomes, United States, 2000-2010. Fertil Steril 2014;102:795-801.
11. Hirotoshi M, Hidehiko M, Noriko F, Junko M, Azusa K, Tatsuhiro T. Relevance of the site of assisted hatching in thawed human blastocysts: a preliminary report. Fertil Steril 2010;94:2444-7.
12. Martins WP, Rocha IA, Ferriani RA, Nastri CO. Assisted hatching of human embryos: a systematic review and meta-analysis of randomized controlled trials. Hum Reprod Update 2011;17:438-53.
13. Primi MP, Senn A, Montag M, Van der Ven H, Mandelbaum J, Veiga A, et al. A European multicentre prospective randomized study to assess the use of assisted hatching with a diode laser and the benefit of an immunosuppressive/antibiotic treatment in different patient populations. Hum Reprod 2004;19:2325-33.

14. Frydman N, Madoux S, Hesters L, Duvernoy C, Feyereisen E, Le Du A, et al. A randomized double-blind controlled study on the efficacy of laser zona pellucida thinning on live birth rates in cases of advanced female age. Hum Reprod 2006;21:2131-5.
15. Practice Committee of the American Society for Reproductive Medicine; Practice Committee of the Society for Assisted Reproductive Technology. Role of assisted hatching in in vitro fertilization: a guideline. Fertil Steril 2014;102:348-51.
16. Hiraoka K, Fuchiwaki M, Horiuchi T, Murakami T, Kinutani M, Kinutani K, et al. Effect of the size of zona pellucida opening by laser assisted hatching on clinical outcome of frozen cleaved embryos that were cultured to blastocyst after thawing in women with multiple implantation failures of embryo transfer: a retrospective study. J Assist Reprod Genet 2008;25:129-35.
17. Wan CY, Song C, Diao LH, Li GG, Bao ZJ, Hu XD, et al. Laser-assisted hatching improves clinical outcomes of vitrified-warmed blastocysts developed from low-grade cleavage-stage embryos: a prospective randomized study. Reprod Biomed Online 2014;28:582-9.
18. Tannus S, Cohen Y, Henderson S, Son WY, Tulandi T. The Effect of Assisted Hatching on Live Birth Rate Following Fresh Embryo Transfer in Advanced Maternal Age. Reprod Sci 2019;26:806-11.
19. Baczkowski T, Kurzawa R, Głabowski W. Methods of embryo scoring in in vitro fertilization. Reprod Biol 2004;4:5-22.
20. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 2000;73:1155-8.
21. Zeng M, Su S, Li L. The effect of laser-assisted hatching on pregnancy outcomes of cryopreserved-thawed embryo transfer: a meta-analysis of randomized controlled trials. Lasers Med Sci 2018;33:655-66.
22. Charis N, Marta W, Taryn N, Sarah G, Julius H, Zhong-Cheng L, et al. Assisted hatching of vitrified-warmed blastocysts prior to embryo transfer does not improve pregnancy outcomes. J Ovarian Res 2020;13:88.
23. Sagoskin AW, Levy MJ, Tucker MJ, Richter KS, Widra EA. Laser assisted hatching in good prognosis patients undergoing in vitro fertilization-embryo transfer: a randomized controlled trial. Fertil Steril 2007;87:283-7.
24. Razi MH, Halvaei I, Razi Y. Laser assisted zona hatching does not improve live birth rate in patients undergoing their first ICSI cycles. Iran J Reprod Med 2013;11:1021-6.
25. Xu W, Zhang L, Zhang L, Jin Z, Wu L, Li S, et al. aser-assisted hatching in lower grade cleavage stage embryos improves blastocyst formation: results from a retrospective study. J Ovarian Res 2021;14:94.
26. Schwärzler P, Zech H, Auer M, Pfau K, Göbel G, Vanderzwalmen P, et al. Pregnancy outcome after blastocyst transfer as compared to early cleavage stage embryo transfer. Hum Reprod 2004;19:2097-102.
27. Kaur P, Swarankar ML, Maheshwari M, Acharya V. A comparative study between cleavage stage embryo transfer at day 3 and blastocyst stage transfer at day 5 in in-vitro fertilization/intra-cytoplasmic sperm injection on clinical pregnancy rates. J Hum Reprod Sci 2014;7:194-7.
28. Papanikolaou EG, Fatemi H, Venetis C, Donoso P, Kolibianakis E, Tournaye H, et al. Monozygotic twinning is not increased after single blastocyst transfer compared with single cleavage-stage embryo transfer. Fertil Steril 2010;93:592-7.
29. Sharara FI, Abdo G. Incidence of monozygotic twins in blastocyst and cleavage stage assisted reproductive technology cycles. Fertil Steril 2010;93:642-5.
30. Mateizel I, Santos-Ribeiro S, Done E, Van Landuyt L, Van de Velde H, Tournaye H, et al. Do ARTs affect the incidence of monozygotic twinning? Hum Reprod 2016;31:2435-41.
31. MacKenna A, Schwarze JE, Crosby J, Zegers-Hochschild F. Factors associated with embryo splitting and clinical outcome of monozygotic twins in pregnancies after IVF and ICSI. Hum Reprod Open 2020:1-6.