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ORIGINAL ARTICLE

Laser-assisted hatching and clinical outcomes in frozen-thawed cleavage-embryo transfers of patients with previous repeated failure

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Abstract

Assisted hatching (AH) is initially developed to provide an artificial manipulation of the zona pellucida (ZP) to help embryos hatch and improve the capacity of the embryos to implant. However, these effects remain unclear and controversial because of variation in patient characteristics, and it is critical to ascertain the indications for AH and to identify those patients who might benefit from AH. Here, this study aimed to assess the effect of laser-assisted zona thinning hatching technology (LAH) during the frozen-thawed D3 embryos on pregnancy outcomes in patients with previous repeated failures in vitro fertilization-embryo transfer (IVF-ET). To the best of our knowledge, these relationships have not been previously investigated. A retrospective cohort analysis was carried out. Infertility patients with previous repeated failure who underwent assisted reproductive therapy at our in vitro fertilization (IVF) center from May 2014 to May 2016 were enrolled. A total of 415 cleavage FET cycles (225 in the LAH group and 190 in the control group) were analyzed. Clinical outcomes including clinical pregnancy, implantation, live birth, miscarriage, and multiple gestation rates after transfer were compared between the LAH and control groups. The clinical pregnancy (49.3% versus 38.9%) and implantation rates (31.2% versus 24.6%) were significantly higher for the LAH group than the control group ($P < 0.05$). The live birth (44.8% versus 35.8%), multiple pregnancy (32.4% versus 31.0%), preterm birth (22.8% versus 17.1%), miscarriage (7.2% versus 5.4%), and ectopic rates (1.9% versus 0%) did not differ significantly between the two groups ($P > 0.05$). This study showed that LAH via zona pellucida (ZP) thinning significantly improves clinical outcomes, particularly clinical pregnancy and implantation rates, associated with FET cycles among patients with previous repeated failure.

Keywords Frozen-thawed embryo transfer (FET) · Laser-assisted hatching (LAH) · D3 cleavage embryo · Previous repeated failure

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Introduction

With the discovery of follicular developmental mechanisms, the use of hormones, the technology, of intracytoplasmic sperm injection technology (ICSI), embryo freezing/warming and transplantation of frozen embryo, assisted reproductive technologies (ART) have shown rapid development over the past 30 years. The average clinical pregnancy has reached 30 to 50% [1]. However, the utilization rate of embryos is still very low, the implantation rate of individual embryos is only 20–30% [2].

The success of embryo implantation is affected by numerous factors, including embryo quality, uterine endometrium, and intricate relationship between embryo transferred and endometrium [2–4]. However, even if a good quality and normal chromosome embryos was selected for transferring to the uterus with good endometrial receptivity, it may be unable to

implant successfully [5]. Recently, it has been believed that in vitro culture and freezing/thawing of embryos generally makes the zona pellucida (ZP) harden. And the low implantation rate has been likely to result from hardening of the ZP, which is detrimental to hatching process [6]. Failure at the stage that the embryo breaks out of the ZP can prevent implantation. Therefore, the successful hatching of embryos is likely a key event in the implantation process.

Assisted hatching (AH) is defined as an artificial manipulation to help embryos exit of ZP. AH was first attempted (the artificial rupture of ZP) to improve the capacity of the embryos to implant in clinical reproductive medicine in 1989 [3] by Cohen et al. Since then, different AH techniques have been developed, such as mechanical zona dissection with glass pipettes or chemical and enzymatic digestion methods. A comparison of four different techniques of AH in some reports suggested that laser AH may results in better clinical outcomes than three other techniques [7–9]. In recent years, most reproductive centers have used laser AH because it is fast, safe, and accurate. One method for the laser-assisted hatching was used to create a single full-thickness hole in the ZP with the inner membrane broken, which is called zona drilling. However, with such treatment, the inner membrane of the ZP was broken to represent a full-thickness opening. The possible changes in the embryos are the following: (i) the breaching of the inner membrane and rapid exposure of the embryos result in sudden changing in biochemical environmental without time for adaptive changes. (ii) Embryos might be at greater risk of bacterial infection or immunologic aggression. (iii) Blastomere loss through the hole or to separate and result in splitting that has been described as a cause of monozygotic twinning [10]. In contrast, another method for the laser-assisted hatching was used to thin an extensive area of the ZP with the inner layer kept intact and the outer layer dissolved, which is called zona thinning. With such treatment, embryos appears unlikely to have a risk. There was no hole for blastomeres to potentially escape from or become entrapped. It was thought to mimic the physiological process of hatching but not have bad effect on continued normal development of embryos by improving implantation rate. Thus, some investigators have recommended the implementation of the zona thinning technique for assisted hatching [11, 12]. The use of laser-assisted hatching (LAH) via zona thinning was already developed to a well-known technique to provide a precise and controlled method for rapidly and safely performing the procedure, with fewer dish changes and less time outside the incubators. Clinical studies have presented data indicating that AH is related to increased favorable clinical outcomes. However, the success rates following the use of assisted hatching in different ART programs remain unclear and controversial. Some studies have reported that AH does not increase the rates of implantation and clinical pregnancy. Other studies have reported that AH can improve embryo implantation potential [13]. As evidence accumulates, not all the patients will benefit from AH [14, 15]. Assisted hatching slightly improves clinical pregnancy rate in patients with poor prognoses, high basal follicle-stimulating hormone (FSH) levels, multiple IVF failures, advanced age, frozen-thawed embryo transfer (FET), and thick embryo ZP [16]. Nadir reported that endometriosis does not indicate AH based on trials with patients with endometriosis [15]. Because of variation in patient characteristics, selection criteria, and AH techniques, AH is not considered a routine procedure for all patients undergoing IVF at most reproductive centers. Therefore, it is critical to ascertain the indications for AH and to identify those patients who might benefit from AH.

Embryo implantation might be impaired when the ZP is too thick or too hard. Zona hardening likely occurs during in vitro culture conditions or after cryopreservation, which is a process that prevents the natural hatching of blastocysts [16]. Recently, the effects of AH on the clinical outcomes of FET have attracted much attention. Although some studies have demonstrated that AH is associated with a significant improvement in the clinical pregnancy rate among infertility patients with unfavorable comorbid conditions, such as previous repeated failure [4, 17], no robust conclusions regarding FET cycles can presently be drawn. Therefore, this study conducted a preliminary investigation of effect of laser-assisted zona thinning during the frozen-thawed D3 embryos on pregnancy outcomes in patients with previous repeated failures in vitro fertilization-embryo transfer (IVF-ET). The following outcomes were analyzed: implantation, clinical pregnancy, live birth, miscarriage, multiple gestation, and abortion rates. To the best of our knowledge, these relationships have not been previously investigated.

Methods

Patients

This study was conducted at the reproductive medicine center of Zhongshan Hospital, Fudan University. Data from the center's IVF database were retrospectively analyzed. To minimize the influences of various subfertility factors, patients were invited to participate only if they met the following inclusion criteria: (i) aged < 36 years, (ii) had failed to achieve a pregnancy following more than one IVF treatment in which embryos were transferred, and (iii) had an endometrial thickness ≥ 8 mm on the day of embryo transfer. Patients with hydrosalpinx, endometriosis, or uterine fibroids were excluded. From May 2014 to May 2016, a total of 495 frozen-thaw cycles were performed on patients that met the above criteria at our reproductive medicine center. Of these cycles, blastocysts were transferred in 72 cycles, and one blastocyst cycle had no surviving embryos for transfer; four cleavage-stage

cycles had no surviving embryos for transfer after warming; and three cleavage-stage embryos at D3 were used to extend blastocyst culture after thawing by patient request but were later discarded because no blastocysts formed at D5–D6. Finally, a total of 415 cleavage-stage embryos transfer cycles with D3 cryopreserved embryo transfer were evaluated retrospectively in this study (Fig. 1). The 415 cycles were divided into a control group (D3 cryopreserved embryo transfer cycles without LAH treatment occurring between May 2012 and March 2013, $n = 190$) and an LAH group (D3 cryopreserved embryo transfer cycles with LAH treatment performed since April 2013, when the new LAH technology was introduced, $n = 225$). The outcomes were measured. The study is limited by the fact that the patients' treatments were at different times. Other procedures were similar between the two groups. The ethics committee of Zhongshan Hospital approved this study.

Ovarian stimulation protocol

Our center applies long or antagonist protocols as routine ovulation stimulation programs. The stimulation protocol performed was based on the patient's age, previous history of ovulation, history of pelvic surgery, basal FSH values, and other considerations. After pituitary suppression via a gonadotrophin-releasing hormone (GnRH) antagonist or agonist was achieved, ovarian stimulation was performed using FSH (Gonal-F; Serona). The dose was adjusted based on individual response. Follicle number and size were monitored via ovarian ultrasonography, and serum estradiol (E2) levels were measured after 3, 4, or 5 days of gonadotropin treatment and then as needed until retrieval. Human chorionic gonadotropin (HCG) was administered when three or more follicles ≥ 16 mm at the largest diameter were present. Approximately 36–38 h after HCG administration, oocytes were collected via vaginal ultrasound-guided follicular aspiration.

Embryo culture and score

Based on the quality of the spermatozoa, IVF or intracytoplasmic sperm injection (ICSI) was performed 39–41 h after HCG administration. Fertilization was assessed 16–19 h after IVF or ICSI using the presence of two pronuclei (2PN) and two polar bodies (2 PB) on D1 (the oocyte retrieval day was considered as D0). Early cleavage was evaluated on D2. Embryo quality was scored on D3 using the British Bourn Hall Clinic criteria. Grade 1 and 2 embryos were defined as fair quality. Grade 3 and 4 embryos were defined as low-grade embryos. Quinn's sequential medium (Quinn's, SAGE, USA) containing 10% serum protein substitute (SPS; SAGE, USA) was used for the culture and manipulation of the oocytes and embryos. Embryos were cultured in a tri-gas incubator at 37 °C in an environment of 6% CO_2, 5% O_2, and 89% N_2.

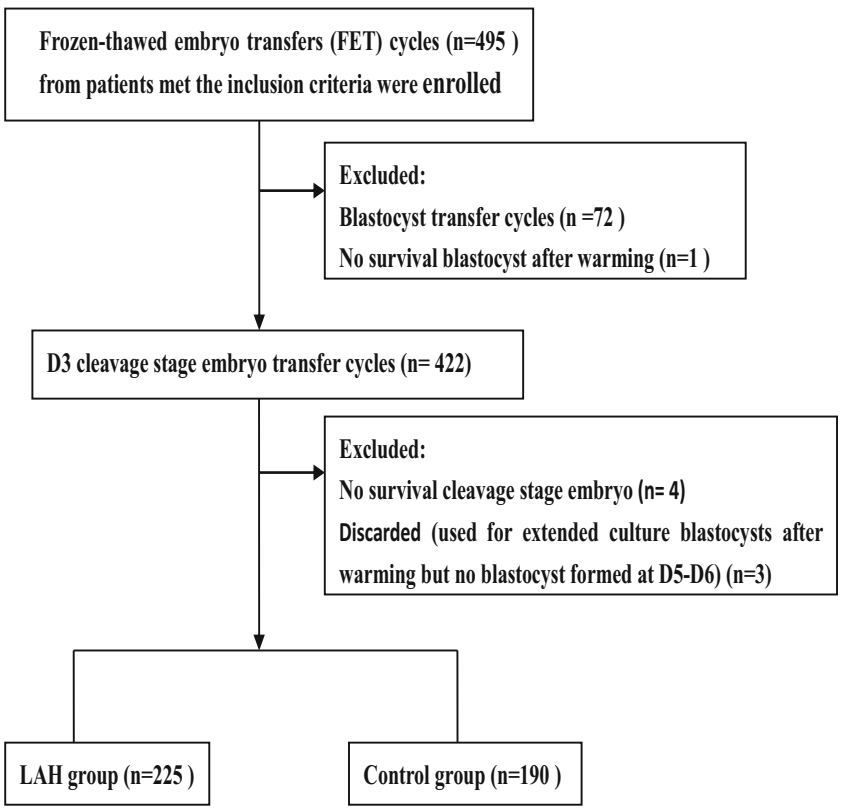

Fig. 1 Flowchart of the retrospective cohort analysis. LAH group=Laser-assisted hatching group; Control group=non-LAH group

Protocol for vitrification and warming

Two or three fair-quality D3 cleavage-stage embryos were selected for transfer in the fresh cycle, and surplus viable embryos were cryopreserved for transfer over the following vitrified-warmed cycles (Fig. 2 a1 and a2). Embryos were vitrified using a Cryotop device and vitrification solutions (KITAZATO BioPharma Co., Japan). The freezing program was performed at room temperature as follows: equilibration solution (ES) was applied for 5 min; then, vitrification solution (VS) was applied for 1 min, and the embryos were placed on the film strip of the Cryotop within a single small drop. The Cryotop was immediately submerged into liquid nitrogen and protected by the plastic cover for storage.

Embryo thawing was performed on the morning of transfer day. The warming procedure was as follows. The plastic cover was removed under the liquid nitrogen, and the film strip of the Cryotop was quickly submerged into 1 ml trawing solution (TS) for 1 min at 37 °C. Then, the embryos were transferred to 0.5 ml DS for 3 min at room temperature and washed in washing solution 1 (WS1) and washing solution 2 (WS2) individually for 5 min each at room temperature. After warming, the embryos were rinsed several times and cultured for 2 h in a fresh blastocyst culture medium (BM) droplet at 37 °C (Fig. 2 b1 and b2) until transfer.

Laser-assisted hatching procedure

The laser-assisted hatching was performed using the ZILOS-tk Zone Infrared Laser Optical System (Hamilton Thorne Instruments Biosciences, Beverly, Massachusetts).

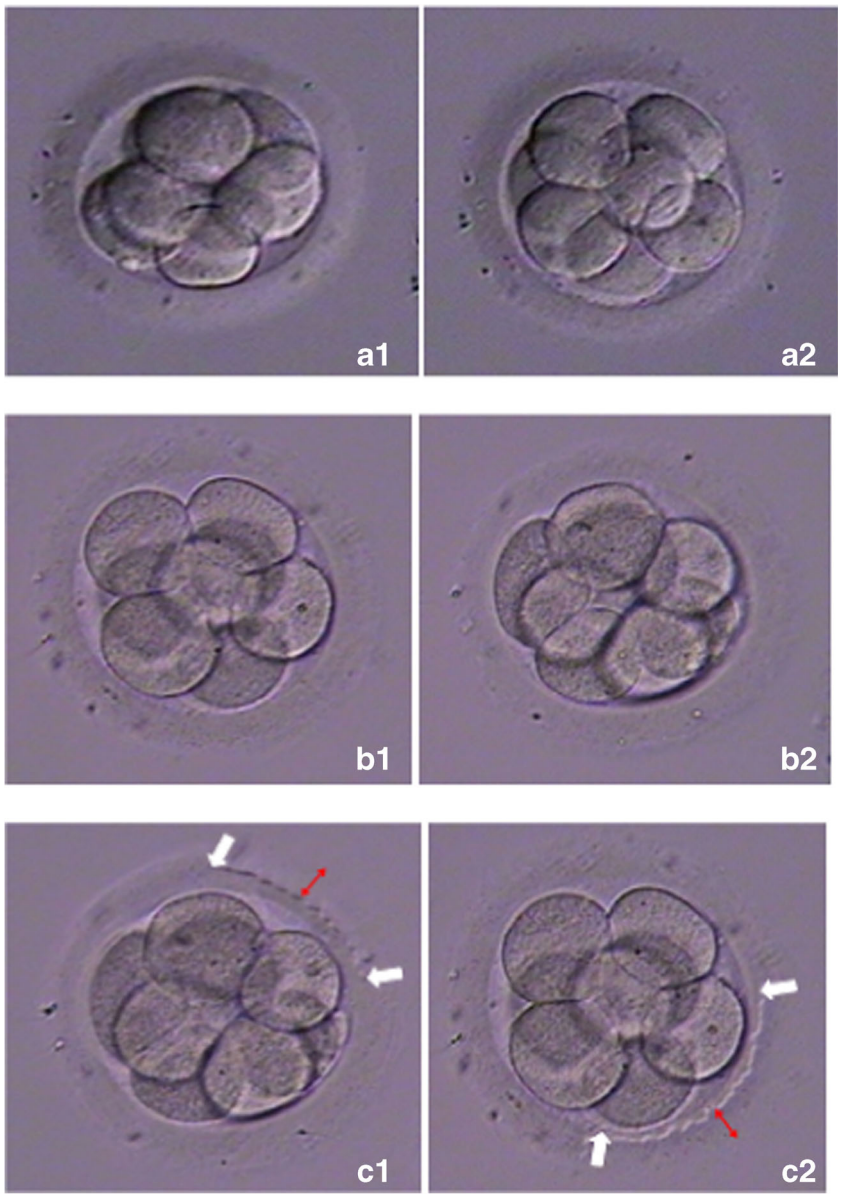

Fig. 2 LAH procedure of vitrified-thawed D3 cleavage-stage embryos. (a1, a2): D3 cleavage-stage embryos before vitrified (high quality and fair quality, respectively, score of 8C I); (b1, b2): Thawed cleavage-stage embryos; (c1, c2): ZP was treated with a laser from the outside to the inside until achieving 50–80% of the total thickness (represented by the double-ended red arrow) and spanning 25% of the circumference (represented by the double-ended white arrow).

Briefly, the invisible and non-contact laser diode beam emitting at a wavelength of 1.48 μm, which was collimated and matched with a 1-mW visible 670-nm diode laser aiming beam, was passed into an inverted microscope (Olympus IX-71, Tokyo, Japan), redirected by several mirrors and focused by the microscope's objective (× 40). The power routinely available at the image plane of the objective was 47 mW, corresponding to a maximal power density of 94 kW/cm2. The embryos treated directly in their original culture medium in embryo culture dish, which was placed onto the displacement stage of the diaphot inverted microscope. Each embryo was positioned a region of ZP into the point of aim. The aiming spot was ensured accurately to be placed between the middle and outer edge of the ZP. To minimize the detrimental effects, the embryos were positioned using a 1.48-μm-wavelength diode laser target located at an appropriate region of the ZP where there was a clear space between the inner membrane of the ZP and the blastomeres to be ensure that the blastomeres was not touched by the laser . Exposing the ZP to laser light was achieved by using a foot pedal to control the switch.

Control of the aperture size depended on the irradiation time. In the present study, one ablation of 2.6 ms or 2.8 ms duration was sufficient to create a defect 10 μm or 12 μm in diameter respectively. The power of the laser was 100%. Using five to eight ablations of 2.8 ms were made successively around the ZP at one point and continuing until 50–80% of the total thickness (the total thickness of the ZP is the distance between inner layer and the outer layer) and spanning 25% of the ZP circumference (starting at the 3 o'clock position and reaching the 6 o'clock position). The inner membrane of the ZP was not complete breached (Fig. 2 c1 and c2). The AH procedure was carried out in approximately 30 s per embryo. After the LAH procedure, in order to avoid possible toxicity of products derived from the action of laser on organic components of ZP, the embryos were then rinsed, moved to a transfer dish containing 1.0 ml BM (Quinn's 1029; SAGE, USA) with 10% serum protein substitute (SPS; Quinn's; SAGE, USA) and cultured under 6% CO_2, 2.5% O_2, and 89% N_2. All of the hatching procedures were performed on the morning of D3 at least 1 h before embryo transfer. All of the couples who received this application provided written informed consent.

Endometrial preparation

Vitrified-warmed embryo transfer was performed during natural cycles or after the artificial preparation of the endometrium with exogenous steroids (hormonal replacement treatment cycles). Natural cycles were preferred for patients with regular menstruation and normal ovulation. Embryo transfer was scheduled for 3 days after ovulation. Hormonal replacement treatment cycles were preferred for patients with irregular menstruation, ovulation disorders, or poor endometrial development. Patients received estrogen for 12 days from the beginning of menstruation; then, they were administered progesterone when their endometrial thickness reached > 8 mm. Embryo transfer was scheduled on D3 after the initiation of progesterone treatment. Luteal-phase support was achieved via a daily intramuscular injection of 40 mg progesterone (Bai Yunshan, China) for 2 weeks. For patients whose endometrial preparation failed using both of the above methods, endometrial preparations were the same as those for the natural cycle.

Assessment of pregnancy

Serum HCG was measured 2 weeks later, and clinical pregnancy was confirmed when a fetal heartbeat was identified via ultrasound 4 weeks after transfer. Monozygotic twin (MZT) pregnancy was identified when two heartbeats were recorded in one gestational sac or the number of gestational sacs exceeded that of the number of transferred embryos.

Statistical analysis

Between-group differences were tested using Student's *t* test for numerical variables and the chi-square test for categorical variables using SPSS 17.0. A P value > 0.05 was considered significant.

Results

A total of 415 cryopreserved cleavage embryo transfer cycles met the criteria and were analyzed in this study. In the LAH group, 225 FET cycles were carried out, and in the control group, 190 FET cycles were carried out (Fig. 1).

The clinical and laboratory characteristics of the patients are shown in Table 1. The two groups were similar in age (32.0 ± 3.4 years old versus 31.6 ± 3.1 years old). No significant differences were found in the survival rate of the vitrified embryos after warming (98.0% versus 98.5%), the rate of high-quality embryos (73.8% versus 74.9%) and the number of embryos transferred (2.1 ± 0.6 versus 2.2 ± 0.4) between the two groups ($P > 0.05$). In addition, the duration of infertility (3.7 ± 2.8 years versus 3.9 ± 2.7 years), primary/secondary infertility type (126/99 versus 110/80), number of previous failed cycles (2.5 ± 0.7 versus 2.6 ± 0.5), endometrial thickness (9.6 ± 1.9 mm versus 9.5 ± 1.6 mm), and other clinical characteristics did not significantly differ between the two groups ($P > 0.05$; Table 1).

The clinical outcomes for the two groups are shown in Table 2. The clinical pregnancy (49.3% versus 38.9%) and implantation rates (31.2% versus 24.6%) were significantly higher in the LAH group than in the control group ($P < 0.05$). The live birth (44.8% versus 35.8%) and multiple pregnancy rates (32.4% versus 31.0%) were also higher in the LAH group than the control group, although not significantly so ($P > 0.05$). In additional, the preterm birth (22.8% versus

Table 1 Clinical and laboratory characteristics of the patients in the LAH and Control (non-AH) groups

Characteristic	LAH	Control	*P* value
No. of cycles	225	190	–
Female age (year, mean ± SD)	32.0 ± 3.4	31.6 ± 3.1	0.237 > 0.05
Duration of infertility (year, mean ± SD)	3.7 ± 2.8	3.9 ± 2.7	0.485 > 0.05
Infertility diagnosis (%, *n*)			
Female factors	26.2 (59)	33.1 (63)	0.122 > 0.05
Male factors	20.0 (45)	13.2 (25)	0.064 > 0.05
Mixed factors	45.8 (103)	47.9 (91)	0.667 > 0.05
Unexplained infertility	8.0 (18)	5.8 (11)	0.379 > 0.05
Primary/secondary infertility	126/99	110/80	0.698 > 0.05
Treatment of transfer (%, *n*)			
Hormonal replacement cycle	74.7 (168)	78.4 (149)	0.370 > 0.05
Ovulation cycle	12.0 (27)	13.7 (26)	0.609 > 0.05
Natural cycle	13.3 (30)	7.90 (15)	0.076 > 0.05
No. of previous failed cycles	2.5 ± 0.7	2.6 ± 0.5	0.078 > 0.05
Endometrial thickness (mm)	9.6 ± 1.9	9.5 ± 1.6	0.658 > 0.05
No. of embryos thawed	484	421	–
Embryo survival (%, *n*)	98.0 (474)	98.5 (415)	0.466 > 0.05
High-quality embryos (%, *n*)	73.8 (350)	74.9 (311)	0.708 > 0.05
No. of embryos transferred	474	415	–
Embryos transferred (*n*, mean ± SD)	2.1 ± 0.6	2.2 ± 0.4	0.128 > 0.05

Values are shown as the means ± standard deviation (SD), number, or *n*/total (%)

No differences were significant ($P > 0.05$)

LAH laser-assisted hatching

17.1%) and miscarriage (7.2% versus 5.4%) rates were higher in the LAH group than in the control group, but these differences were not significant ($P > 0.05$; Table 2).

Ectopic pregnancy was reported in two cases of abortions in the LAH group; no statistically significant difference was found between the two groups. Among the babies born, no significant differences in mean birth rate were observed between the LAH group and the control group, (3.1 ± 0.6 versus 2.9 ± 0.7) ($P > 0.05$; Table 2). Among the live births, one major (only unilateral kidney) and one minor (ear defect)

Table 2 Clinical outcomes for the LAH and Control (non-AH) groups

Outcome	LAH	Control	*P* value
No. of cycles	225	190	–
Clinical pregnancy (%, *n*)	49.3 (111/225)	38.9 (74/116)	0.034 < 0.05
Implantation (%, *n*)	31.2 (148/474)	24.6 (102/415)	0.028 < 0.05
Live birth (%, *n*)	44.8 (101/225)	35.8 (70/190)	0.097 > 0.05
Miscarriage (%, *n*)	7.2 (8/111)	5.4 (4/74)	0.626 > 0.05
Preterm birth (%, *n*)	22.8 (23/101)	17.1 (12/70)	0.370 > 0.05
Multiple pregnancy (%, *n*)	32.4 (36/111)	31.0 (23/74)	0.847 > 0.05
Ectopic pregnancy (%, *n*)	1.9 (2/101)	0 (0/70)	0.236 > 0.05
Birth defects (%, *n*)	1.9 (2/101)	0 (0/70)	0.236 > 0.05
Mean birth weight (Kg, mean ± SD)	3.1 ± 0.6	2.9 ± 0.7	0.062 > 0.05

Values are shown as *n*/total (%) or means ± standard deviations (SD)

$P < 0.05$ compared with the control group

LAH laser-assisted hatching

malformation occurred in the LAH group, and no malformation occurred in the control group. No significant difference was found in the total number of malformations between the two groups (2/101 versus 1/70) ($P > 0.05$; Table 2).

Discussion

The ZP is a glycoprotein layer that surrounds the oocyte, the outer layer is thick and easily dissolved, whereas the inner layer is difficult to dissolve. ZP prevents polyspermy and fertilization by non-acrosome-intact sperm. A potential mechanism to improve embryo implantation rate may be hatching ability and contact with the endometrium of an earlier stage of embryo. During the blastocyst stage, the embryo hatches from the ZP and begins the deployment process (i.e., implantation). Inadequate hatching of the blastocyst can lead to implantation failure under IVF. Therefore, the manner in which embryos hatch from the ZP is important for embryo implantation. AH, which involves the artificial disruption of the ZP, has been proposed to improve the chances of implantation during assisted reproduction. The mechanism by which AH promotes implantation remains unclear. The implantation window is the critical period when the endometrium reaches its ideal receptive state for implantation. Precise synchronization between the embryo and the endometrium is essential. The artificial gap or slit should allow the most molecules from the endometrium to cross the ZP, as well as increase the communication and earlier contact between embryo and endometrium results in enhancing the implantation and pregnancy rate. This procedure may lead to an increase in the rate of embryo implantation [10]. LAH is controversial and debated within the medical literature. Laser system has significant advantages over chemical, mechanical, or enzymatic methods. Firstly, LAH provide touch-free objective-delivered accessibility of laser light to the target, with minimal absorption by the embryos [18]. Secondly, the laser for human reproductive medicine is affordable and easily adapted to inverted microscope. Furthermore, the laser target is controlled accurately and is focused through the microscope objective to allow faster, easier, safer and contact-free thinning manipulation of the ZP. However, the primary concern in applying laser is a potential problem with LAH is local heating caused by the laser's beam power and pulse duration. The LAH procedure may be associated with lethal damage to the embryo and damage to individual blastomeres, with reduction of embryo viability. The energy delivered onto the embryos may have adverse effects on the embryo quality and its developmental potential [19]. In contrast, most studies have failed to demonstrate any adverse effects of LAH on embryonic development and reproductive outcome [20, 21]. In 2016, Uppangala et al. proved that laser treatment of the human embryo did not significantly alter the embryo quality and metabolism observed using high resolution NMR technology [21]. The effect of thermal damage elicited by use of lasers for zona manipulation is disputed. Nonetheless, to minimize the detrimental effects, the embryos were positioned using laser target located at an appropriate region of the ZP where there was a clear space between the inner membrane of the ZP and the blastomeres to be ensure that the blastomeres was not touched by the laser. Hiraoka reported that half zona thinning was more beneficial for embryo hatching than was quarter zona thinning in vitrified-warmed embryos [22]. In the present study, the ZILOS-tk laser system was employed, which was designed to provide intense, brief laser pulses specifically optimized for ZP penetration with minimal local effects. The zona was thinned to approximately 50% to 80% of the initial thickness over 25% of the length of the ZP to improve clinical pregnancy and implantation rates associated with IVF-ET.

Since 2003, the health of children born following LAH has been studied. Despite the longtime use of LAH procedure, there have been few clinical trials assessing the effect on perinatal and neonatal outcomes, which are as important as clinical pregnancy. The reported effects of AH on live birth and deformity rates are inconsistent. Studies have showed that laser AH has been associated with an increased risk of monozygotic or multiple pregnancies [23, 24]. Multiple pregnancies, especially monozygotic multiple births, increase the likelihoods of infant mortality and morbidity. Perinatal and infant mortality are two to three times greater among monozygotic multiple births. However, the actual cause of the increase in monozygotic multiple births following AH remains unclear. Proposed potential factors influencing multiple birth include the AH procedure [24], the number of blastocysts transferred [25], and a combination of events occurring during the IVF treatment cycle [8]. Whether AH techniques are the main factors underlying the increase in multiple births is inconclusive. One study reported an increased rate of monozygotic twinning following AH (2.9% per live birth), which was higher than the average (1.2% to 5%) [26]. Therefore, given the use of AH technology, reducing the number of embryos transferred should be considered. In addition, Kanyo and Konc [27] showed that there was no evidence of increased chromosomal abnormalities or congenital malformations in 134 children born after LAH. In 2004, Primi et al. also failed to observe an increase in these rates after studying unpublished data on 62 children born following LAH [28]. Zhou et al. reported that no adverse effects were identified in perinatal outcomes after LAH in a retrospective cohort study of a total of 699 women who delivered 392 infants [29].

In the present study, with respect to the previous implantation failure of frozen-thawed cycles, patients who underwent LAH ($n = 225$) in our study from May 2012 to May 2014 showed higher clinical pregnancy and implantation rates after LAH treatment (49.3% versus 31.2% and 38.9% versus 24.6%, respectively, $P < 0.05$), suggesting that AH contributes

to favorable clinical outcomes for vitrified-warmed embryos. However, the sample size is small and this study is a retrospective analysis, further larger sample in multicenter and prospective studies are still needed. No difference was found in mean birth weight or the number of malformations in newborns between the LAH and control groups. To date, 101 healthy infants have been born after LAH at our center; two of these infants had malformations (abortion rate = 1.9%) but no chromosomal abnormalities. A total of 70 infants were born whose mothers did not undergo LAH; none of these infants had malformations (0%) or chromosomal abnormalities. Our data revealed that the multiple pregnancy rate of the LAH group was higher than that of the control group; however, this difference was not significant (32.4% versus 31.0%, respectively, $P = 0.847$). Therefore, this study raises the question of whether LAH might increase the risks of multiple pregnancies and congenital malformations. However, the number of cases reported in this study is small. A large sample of IVF-ET outcomes is required to determine whether assisted reproductive technologies increase the number of multiple pregnancies or congenital malformations. Furthermore, long-term studies examining potential anomalies after birth are needed to assess the safety of LAH.

Conclusions

Our results suggest that LAH markedly improves the clinical outcomes associated with FET cycles. Patients with one or more failed IVF cycles might benefit more from this procedure. Strong evidence suggests that LAH is a successful technique with fast, secure, efficacious, and accurate features [30, 31]. The present study found no evidence of any harmful effect of LAH on newborns. Although many papers have been published on AH, it remains unclear whether this procedure can reduce the abortion rate or increase the live birth rate. In addition, whether AH increases the fetal malformation rate has yet to be determined. To further investigate the roles that AH might play in improving clinical outcomes, additional, a prospective randomized clinical trial designed studies with large sample sizes are required.

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Authors' contributions XML carried out diagnosis, ovarian stimulation and oocytes collection. XML and YBL participated in embryo culture, vitrification and warming. YBL and XC participated in the laser-assisted hatching procedure. YBL and XML contributed to data collection and statistical analysis. XML, YBL, and SYL contributed to manuscript writing as well as to interpretation of the results. SYL participated in the design of the study and performed part of the statistical analysis. XD contributed to guideline of this study, and participated in its design and critical revision of the manuscript. All authors participated in the data collection and read approved the final manuscript.

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Data availability The datasets supporting the results of this article are included within the article.

Compliance with ethical standards

Ethics approval and consent to participate This study was approved by the ethics committee of Zhongshan Hospital. Informed consent has been obtained.

Consent for publication Not applicable.

Competing interests The authors declare that they have no conflict interests.

Abbreviations *AH*, assisted hatching; *ZP*, Zona pellucida; *LAH*, laser-assisted hatching; *FET*, frozen-thawed embryo transfer; *IVF*, in vitro fertilization; *ART*, assisted reproductive technologies; *IVF-ET*, in vitro fertilization-embryo transfer; *FSH*, follicle-stimulating hormone; *GnRH*, gonadotrophin-releasing hormone; *E2*, estradiol; *HCG*, human chorionic gonadotropin; *ICSI*, intracytoplasmic sperm injection; *2PN*, two pronuclei; *2 PB*, two polar bodies; *ES*, equilibration solution; *VS*, vitrification solution; *TS*, trawing solution; *DS*, diluent solution; *WS1*, washing solution 1; *WS2*, washing solution 2; *BM*, blastocyst culture medium; *MZT*, monozygotic twin

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