Original Research

Retrospective study showing improved pregnancy rates with laser assisted hatching on Day 5 blastocysts in FET cycles in Indian population

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Abstract

Background: One of the common reasons for implantation failure is a thick or hard zona pellucida. This is due to biochemical changes in the zona pellucida caused by a variety of factors, including advanced maternal age, in-vitro embryo culture conditions, and cryopreservation, which may impair in-vivo hatching and implantation after thawing and transfer. As a result, laser assisted hatching (LAH) has been promoted as a method of accelerating implantation and assisting the natural hatching process. **Objective:** The primary goal of this research is to assess the effect of laser-assisted hatching (LAH) on implantation and clinical pregnancy rates in patients undergoing fertility treatment at a tertiary-level infertility clinic. The secondary goal is to assess the rate of multiple pregnancy and miscarriages following the transfer of Day 5 frozen-thawed embryos in women aged 21 to 43 years. **Materials and methods:** This study includes a total of 492 patients, ages 21 to 43, with a self-stimulating cycle who underwent an all-freeze procedure. The whole population is divided into two groups: the LAH and non-LAH groups, each containing 169 patients. The frozen Day 5 embryos of the LAH group were thawed and subjected to LAH during embryo transfer. The effect of LAH on primary outcomes such as implantation and pregnancy rates and secondary outcomes such as multiple pregnancy and miscarriage rates was compared in both the LAH and non-LAH groups. Both groups were further subdivided into two groups: patients in Group I were under the age of 35 years, while those in Group II were over the age of 35 years. **Results:** According to the study's findings, patients in the LAH group under the age of 35 have 70% implantation rates and better pregnancy outcomes than those over the age of 35. It was also discovered that LAH significantly improved clinical pregnancy rates in both young ($P < 0.001$) and older ($P < 0.03$) patients when compared to the non-LAH group. In addition, the patient's age and the number of day-5 frozen-thawed embryos transferred during embryo transfer influenced pregnancy rates ($P = 0.01$) in the LAH group versus the non-LAH group. **Conclusion:** To overcome the negative effect of zona hardening in Indian population, LAH can routinely be offered for frozen day 5 embryos after thawing in FET cycles to increase the pregnancy rate without increasing the overall rate of early miscarriages and multiple pregnancy.

Keywords: Assisted reproductive technologies, blastocysts, FET, laser-assisted-hatching, zona pellucida

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INTRODUCTION

The zona pellucida (ZP) is the outer layer of an oocyte that carries species-specific sperm receptors. It also promotes the acrosome reaction and, after fertilization, undergoes biochemical changes known as zona hardening to inhibit polyspermy.[1] The ZP of a blastocyst breaks during a process called hatching, allowing the embryo to get implanted in the inner uterine wall. This requires mechanical expansion and contraction of the blastocyst, along with ZP thinning.[2] The increasing maternal age, as well as the in *vitro* embryo culture conditions, can have a negative impact on both zona hardening and thinning.[3] A few studies have suggested that cryopreservation may also induce zona hardening.[4]

Hatching abnormalities have been put forward as a potential rationale for the lower implantation rates in some patients with advanced maternal age and in frozen-thawed IVF/ICSI-ET cycles.[3] Laser-assisted hatching (LAH) involves creating an artificial opening or making ZP thinner or altering its stability through a laser to facilitate the hatching and implantation of embryos that might not have been able to elude the intact ZP.[5] However, the effectiveness of AH in improving the outcome of fresh and frozen IVF/ICSI-ET cycles is debatable.[5] It was first used in clinical in vitro fertilization (IVF) in 1989.[6] Over the years, many studies have been conducted to explore the advantages of LAH for couples experiencing infertility, but it still requires more research to reach a rational conclusion.[7]

According to the American Society for Reproductive Medicine (ASRM) Practice Committee, AH procedures could increase the clinical pregnancy rate (CPR), but due to the limited live birth data and the increased likelihood of multiple pregnancies, they are not recommended to be used routinely for all IVF patients or patients with a poor prognosis.[8] To date, only a few of the studies conducted have reported the live birth rate (LBR). The previous two meta-analyses included 36 studies with 6459 patients and 31 studies with 5728 patients, respectively, but did not find a statistically significant correlation between LBR and LAH treatment in either study or the control group.[9,10]

Thus, the goal of our clinical study is to focus on the use of laser-induced zona thinning (also known as laser-assisted hatching, or LAH) of frozen-thawed day 5 embryos during embryo transfer with the hypothesis that it might be a useful tool for increasing implantation and clinical pregnancy rates with lower risks of multiple pregnancies and miscarriage rates, particularly in older females (>35 years) who underwent frozen-thawed ICSI-ET cycles.[11,12]

MATERIALS AND METHODS

Inclusion and exclusion criteria

The study includes 492 patients ages 21 to 43 who were treated with frozen-thawed ICSI-FET at Arihant Hospital, India, between March 2021 and December 2022. Patients with donor cycles ($n = 62$), poor endometrium ($n = 10$), and poor-quality blastocysts ($n = 15$) were excluded from the study. Finally, the evaluation of 338 patients with self-ICSI-FET cycles, good-quality vitrified blastocysts, and a triple-line endometrium with a thickness of more than 8 mm was done.

Patient identification is done on the basis of the final digits of the registration number, which was assigned to them by the administrator at the reception desk on their initial visit to our centre. The whole selected population of 405 patients was divided into two groups: the LAH group ($n = 169$) and the non-LAH group ($n = 236$). These groups were further subdivided into two groups: Group I with patients' ages <35 years and Group II with patients' ages >35 years.

Ovarian stimulation protocol

All females were subjected to controlled ovarian stimulation using an antagonist regimen with 225 IU as the average dose of gonadotropins on days 2 or 3 of periods. A fixed antagonist was added on day 6. Follicular development was monitored using vaginal ultrasonography. The gonadotropin dose was increased from 150 to 375 IU and then adjusted based on the response of the ovarian follicular development, which was monitored using vaginal ultrasonography. A trigger, either an recombinant human chorionic gonadotropin (HCG) injection or an GnRH agonist trigger, was given when most of the follicles had reached 18 mm in size. The drugs incorporated were triptorelin 0.1 mg and hCG 7500 IU. Transvaginal egg collection was performed 36 hours after the trigger administration using a 17-gauge ovum pick-up needle.

Embryo development and vitrification

The oocytes collected were then fertilized with her husband's semen sample only through intra-cytoplasmic sperm injection (ICSI) Resulting embryos underwent LAH procedure. Fertilized oocytes on Day 1 were then cultured until Day 5 for blastocyst formation. Regular checks were done to monitor embryonic development on Days 2, 3, and 5, respectively.

Embryos were frozen on Day 5 using Kitazato embryo vitrification media. Only those embryos were frozen that had shown proper blastocyst formation in 120 hours with cohesive trophectoderm and prominent and tightly packed inner cell mass (ICM).

Thawing procedures

Each straw was defrosted separately, and the entire process was carried out at room temperature. The thawing process was carried out using the Kitazato Thaw-Kit. Prior to usage, all solutions are pre-adjusted to room temperature except the thawing solution, which needs to be warmed to 37°C. Embryos were thawed for at least 2 hours before frozen embryo transfer (FET).[13]

Randomization 169 of patients and LAH

The LAH operation was done just before the embryo transfer, and embryo thawing was done at least 2 hours before the embryo transfer. Only embryos that survived after freezing underwent LAH. Cryo-survival evaluation procedures followed those outlined by Rienzi *et al.*[14] Embryos that had been frozen and then thawed were deemed to have survived if the blastocyst had coehesive tropectoderm with compact ICM without any degenerations.[15]

LAH was carried out using a 1480-nm diode laser in a computer-controlled non-contact mode (IVF Workstation and Zona Laser Treatment System, RI Saturn 5 Instruments) installed in an inverted microscope used in the IVF laboratory.

Each embryo underwent quarter laser-assisted hatching (Q-LAH), as previously mentioned.[16] Laser drilling was used to thin the zona pellucida, starting at one place and continuing until 25% of the zone was drilled. Embryos chosen for laser therapy were first transfused in a 10-µL drop of pre-equilibrated culture media supplemented with human serum albumin (HSA) in a Falcon culture dish, overlaid with paraffin oil, to finally give the laser shots. Just after the LAH treatment, the embryos were again shifted to an embryo transfer dish.

Frozen embryo transfer

Frozen embryo transfer was performed by hormonal replacement therapy. A baseline scan was done in all cases, and tablet Progynova (Bayer Zydus Pharma) was started on day 2 once the endometrial thickness was favourable (≥8 mm) with triple line echogenicity. The embryo transfer was done gently with a Labotech catheter. Luteal support was started by administering 100 mg of oil-based progesterone or vaginal progesterone (Capsule Susten, Sun Pharma) daily. Chemical pregnancy was defined by the analysis of the β-hCG hormone on the 14th day after embryo transfer, and clinical pregnancy as a distinct intrauterine gestational sac seen on transvaginal ultrasound.

Statistical analysis

The data were logistically regressed using TS-PLUS 2000. We also calculated power and reported a 95% confidence interval (95% CI) for the odds ratios (OR) associated with the model's contributing factors.[15] A *P*-values of <0.05 was considered statistically significant.

RESULTS

The study included 405 patients, after considering the exclusion criteria, who were undergoing infertility treatment at our tertiary-level fertility clinic. The method of fertilization was ICSI with the husband's semen sample, who chose self-ICSI-FET cycles. Out of 405 patients, 169 were assigned to the study group (LAH group), while the remaining 236 were assigned to the control group (non-LAH group). Both LAH and non-LAH groups were further divided into two groups: Group I (patients aged <35 years) and Group II (patients aged >35 years). Two high-quality frozen-thawed blastocysts (Day 5 embryos), subjected to LAH, were transferred into the uterine cavity of each women with the help of ultrasonography. An overview of the study is summarized in Table 1.

The results showed that the age group with less than 35 years is showing higher implantation and pregnancy rates, that is, 67.14% compared to group with age more than 35 years. One of the most prevalent disadvantages of LAH is that it can lead to twinning, specifically monozygotic twinning, which is not observed in our procedure, that is, on the transfer of a single zygote, a single pregnancy is achieved, which has been observed either by monitoring a patient's delivery pattern or through a scanning procedure. When the primary outcomes were compared between the study and control groups depending on age categories, it was discovered that the LAH group had higher implantation rates than the non-LAH group, that is, 67.14% versus 63.56% in <35 years category ($p< 0.001$) and 65.63% versus 56.82% in >35 years category ($p <0.03$). (Table 1)

However, statistical studies merely reveal a trend, and the outcomes did not differ substantially. When we analysed the pregnancy rates of the LAH+ and LAH- groups depending on the age of the patients, we discovered

Table 1: Detailed summary of the study

Study conducted on	385			
Pregnancy opted	Self			
Technique performed	LAH^{+}	LAH-	LAH^{+}	LAH-
Number of patients	169	216	169	216
Age group	<35 years	<35 years	>35 years	>35 years
No. of patient	140	129	29	87
No. of positive outcome	94	82	19	49
No. of negative outcome	41	47	10	38
Percentage of positive outcome	67%	63.56%	65.63%	56.82%
Statistical significance	$P < 0.001$	$P < 0.03$		
Total miscarriages	7	9	2	14
Percentage	7.44%	39.13%	3.28%	60.86%
Statistical significance	$P < 0.004$	$P < 0.006$		
Total multiple pregnancies observed**	Not observed	12	Not observed	13
Percentage	–	34.28%	–	37.14%
Statistical significance	$P < 0.021$			

**Statistical significance is between non-LAH groups as data were not observed in the LAH group.

Table 2: Z-score between the LAH and non-LAH group

	95% Confidence Interval	
	Min	Max
Raw percent difference	30.54%	49.46%
Variant recipe response rate	156.6	191.6
Z score		8.829
Raw difference		40.00%
Index to control		174

that LAH substantially enhanced the pregnancy rates in patients ($P < 0.001$). In the below-mentioned table, the *Z* score is score has also been tabulated as to bring more clarity to data distribution.

Zscore

The z score is tabulated on the basis of comparative analysis of positive outcomes that is drawn on the basis of population group divided into LAH and non-LAH (Table 2).

DISCUSSION

The blastocyst must hatch from the ZP before implantation. The foundation of AH is the idea that altering the human ZP could encourage the hatching and implantation of embryos that would otherwise be unable to escape from the intact ZP.[3,4] The alteration might be implemented by either getting rid of it, drilling a hole, making it thinner, or changing its stability. The adoption of various AH techniques is predicated on the idea that thinning the ZP or making an artificial hole/opening would help embryos hatch.[17]

AH promotes *in vivo* blastocyst hatching, which might increase the pregnancy rates. In groups of patients with poor prognosis and/or poor embryonic morphology, with higher FSH levels and embryos with thicker ZP, who were older and had a history of numerous IVF failures, assisted hatching was observed to improve the success of IVF-ET cycles. Poor embryo scores and advanced female age may both be related to thick ZP as well as cryopreservation.[4,5,7,8]

The findings and observations of many LAH investigations are in stark conflict with one another. Results from the research should be viewed with caution due to the variation in their outcomes.[18]

A more frequent and successful report is observed when comparing the pregnancy rates of fresh IVF cycles with patients with poor prognosis (prior IVF failures) after using different techniques of LAH.[19] However, another prospective randomized study found no difference in pregnancy rates in a population aged <35 years and with no previous implantation failure.[16] In frozen-thawed ET cycles, assisted hatching may be able to solve this issue and raise implantation and conception rates. Similar to fresh IVF cycles, frozen-ET cycles' post-AH findings are quite contradictory. Retrospective investigations have demonstrated that assisted hatching using chemical zona drilling in frozen-thawed cycles raises pregnancy rates.[20] However, no benefit of the method could be seen in cryopreserved ICSI-ET cycles in other investigations utilizing the same procedures. Patients with frozen-thawed embryos had an increase in pregnancy rates thanks to LAH, but patients of advanced female age showed no such improvement.[20] Our findings from frozen-thawed ICSI-ET cycles demonstrate that LAH raises pregnancy rates. The two groups' patient populations, embryo features, and methods for applying cryopreservation and embryo culture were all extremely identical. This eliminated any potential bias

from our findings.[25] In accordance with our findings, other studies also discovered that LAH markedly boosted pregnancy rates in cryopreserved cycles with slowly frozen and vitrified embryos in comparison to fresh cycles.[21]

The magnitude of the ZP thinning may have an impact on the outcomes, which might be the rationale for the less effective use of LAH. A study compared the impact of the laser's ZP thinning size (40 m vs. 80 m) on the results of frozen-thawed ET cycles.[22] Following the transfer of a frozen-thawed cleaved embryo, they discovered that the size of the laser thinning may affect the rates of implantation and pregnancy.[22,23] The data demonstrate how the size of the ZP thinning region by LAH during vitrified-warmed embryo transfers affects the clinical pregnancy rate and how one-half ZP thinning produces much better results than one-quarter ZP thinning.[23]

Giving birth duplet or triplets or multiples pregnancy is the term used for such condition is the most common disadvantage in case of LAH. The rates of multiple pregnancy observed is (13, 7.69% $P = 0.05$), miscarriage (22, 13.01% $P = 0.05$), and live birth (13, 7.69% $P = 0.05$) revealed comparable results for both groups. In summary, this meta-analysis demonstrates that LAH is related to a higher clinical pregnancy rate, embryo implantation rate, and multiple pregnancy rate in women with cryopreserved-thawed embryos. However, LAH is unlikely to increase live birth rates and miscarriage rates.

Limitations

The limitation of this study are retrospective study and small sample size of the patient as IVF is new to India normal and are extent unaware about it.

CONCLUSION

In conclusion, in Indian women, to overcome the negative effect of zona hardening, LAH can be offered on frozen embryos as a routine strategy before FET in frozen cycles in order to increase the possibility of pregnancy. There is no overall increase in the miscarriage rate or multiple pregnancy rate in patients without LAH.

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Nil.

Conflicts of interest

There are no conflicts of interest.

COMMENTARY

Retrospective study showing improved pregnancy rates with laser-assisted hatching on day 5 blastocysts in frozen embryo transfer cycles in Indian population

The Vienna Consensus meeting of embryologists in 2017 recommended exercising caution and care when introducing new techniques in the *in vitro* fertilization (IVF) laboratory. Although the first pregnancy using mechanical force to partially zona dissect mature oocytes was reported in 1988 by Cohen *et al.* for male-factor couples, the utilization of assisted hatching (AH) in IVF laboratories has not undergone a proper systematic evaluation. Under the "add-ons" traffic light system, implemented by the Human Fertilisation and Embryology Authority (HFEA), AH is listed in the red category, indicating that a regular cycle of proven fertility treatment is effective without any treatment add-ons. Although reproductive scientists have gathered important information on the composition, assembly, and structure of zona pellucida (ZP) and its role in human oogenesis and egg–sperm interaction, it is important to note that information on preimplantation development notoriously lags behind; hence, any potential benefits of manipulation should only be considered in this context.

Over the years, three techniques for AH have been introduced. Mechanical, chemical, or laser manipulations of ZP has been used to facilitate the creation of holes, slots, and thinning of the zona of different sizes. Tadir (1991) and Palanker *et al.* (1991) first reported laser-mediated zona drilling, a precise microsurgical breach in the ZP. Two modes available: are contact mode, laser-guided through optical fibre employing ultraviolet (UV) or infrared (IR) wavelength. In a randomised prospective study, Tucker and coworkers (1991) reported no significant improvement in clinical pregnancy in frozen/thawed embryos. Although the procedure initially led to encouraging clinical outcomes, even after 35 years after the first mechanically manipulated pregnancy, the use of AH remains controversial due to heterogeneity among published studies and the absence of a meta-analysis of randomized controlled trials (RCTs) favoring AH over no AH.

The study reported deserves attention as this is the first to assess the effect of laser AH on clinical and implantation rates using vitrified/warmed blastocysts in the Indian population. Similar studies have been reported elsewhere with promising outcomes.

In their comparative study, Sharma *et al.* reported improved clinical pregnancy rates favoring AH in the age group of <34 years compared to the group without

AH in the same age group. Although the study is promising, the number of patients included and allocated to each group is significantly low to draw any statistically significant conclusion. The stimulation protocols are consistent with those used in the European clinics; however, data on the number of oocytes collected, fertilized, blastocyst conversion rate, number vitrified, and the number that survived the warming process with their grades pre- and post-vitrification would have made this study even more informative. The methodology of the study lacks clarity on how the consistency of the quarter zona thinning was maintained. Equally, the site of ZP thinning, either near the inner cell mass (ICM) or the opposite side, is a piece of vital information (Matsubayashi *et al*., 2010). To avoid multiple pregnancies, most clinics in the West now follow elective single-embryo transfer (SET). Therefore, in this study, outcome data on multiple pregnancies and live births rates are equally important. Furthermore, any additional data on the percentage of congenital abnormalities registered at the time of delivery would confirm the safety of AH and encourage the use of AH in IVF cycles, in particular, the vitrified/warming cycles.

The commentary highlights the need for detailed methodology, data collection, presentation, discussion, and conclusion statement. The authors are encouraged to reflect on my comments.

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