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Original Research

Is laser-assisted hatching better than mechanical method for enhancing pregnancy rate in frozen-thawed blastocyst transfer cycles?

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Summary

Objective: To investigate the effect of laser-assisted hatching (LAH) compared with mechanical-assisted hatching (MAH) for enhancing clinical outcomes in frozen-thawed blastocysts (FTBs) transfer cycles. *Materials and Methods:* The culture of all pronucleate embryos to the blastocyst stage and the selection of blastocysts ≥ grade 3BB (Gardner and Schooleraft score), followed by FTBs transfer cycles with LAH (n=105) or with MAH (n=101). Clinical outcomes including clinical and ongoing pregnancy rates were investigated. *Results:* There were no significant differences in clinical and ongoing pregnancy rates between LAH and MAH groups in FTBs transfer cycles. *Conclusion:* LAH does not improve pregnancy outcomes significantly compared to MAH in FTBs transfer cycles.

Key words: Laser-assisted hatching; Mechanical-assisted hatching; Frozen-thawed blastocyst.

Introduction

Hatching of the human embryo from the zona pellucida (ZP) is a crucial as natural step for its successful implantation in the endometrium. Failure of the hatching process has been considered to be a cause of implantation failure in assisted reproductive technology. Furthermore, zona hardening is a process that prevents the natural hatching of human embryos, and it has been known to occur during in vitro culture, after cryopreservation, and in women with advanced age [1]. Excess in vitro culture of cryopreserved embryos, which was exacerbated by the frozen-thawed process, has been thought to induce alteration in the glycoprotein matrix leading to zona hardening [2]. Also, an additional study has revealed that zona hardening might be initiated by cryopreservation as well [3]. In order to overcome these problems, a variety of assisted hatching (AH) techniques have been introduced since mechanical-assisted hatching (MAH) was first reported by Cohen *et al.* in 1990 [4]. Then, frozen-thawed embryos have been considered as one of the prime candidates for AH, but the benefits of AH in frozen-thawed embryos still have been controversial. In a Cochrane review, it has not been proven that AH contributes the increasing of live birth rate [5]. However Check *et al.* reported that improved implantation and pregnancy rates when AH was performed in six- to eight-cell stage of frozen embryos [6]. Also, Tao *et al.* showed a successful outcome of AH was attained when used on frozen-thawed two-cell stage embryos [7]. More recently, some studies presented that clinical and ongoing pregnancy rates were increased in frozen-thawed blastocysts (FTBs) transfer cycles, when AH was performed [8, 9]. In a systemic review, AH was related to increased clinical pregnancy rate in women with frozen-thawed embryos [10, 11]. These studies suggested that AH was a reasonable method for improving clinical outcomes in FTBs transfer cycles.

Different technical procedures have been employed to create the zona opening, and these include the use of various acidified solutions, a thin microneedle to slice through the zona and laser-assisted hatching (LAH) which is the creation of an opening with a laser beam [12-16]. However, the effects of AH vary according to the kind and extents of AH, and its benefit has been still debatable.

Given the above considerations, this study conducted an investigation of the effects of LAH for enhancing clinical outcomes, aiming to discover the more favorable method, either LAH or MAH, for better clinical outcomes in FTBs transfer cycles.

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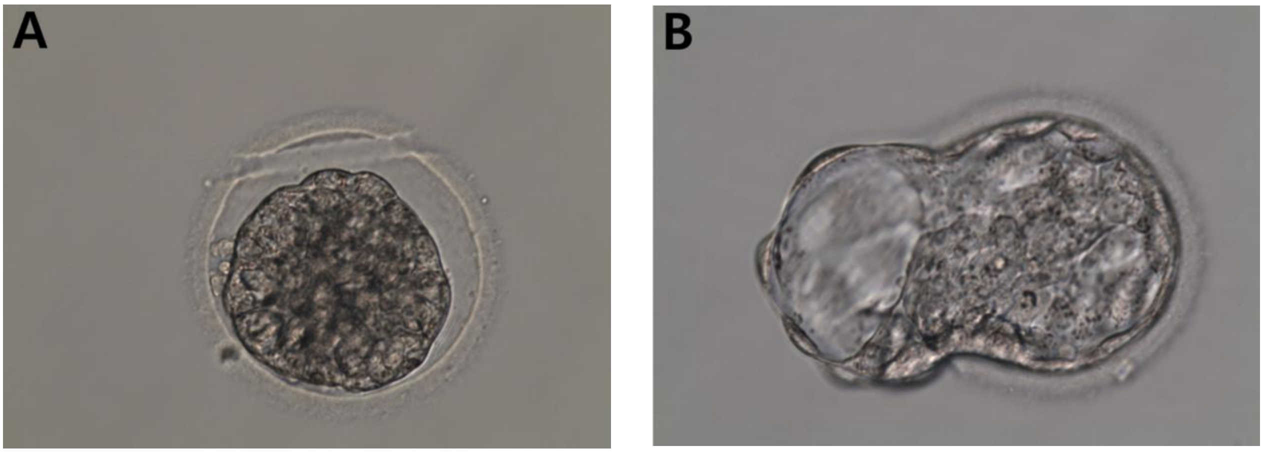

Figure 1. — Laser-assisted hatching. A) Zona pellucida showing to dissect approximately one-quarter after laser assisted hatching. B) The hatching blastocyst.

Materials and Methods

This study was retrospectively conducted from January 2014 to December 2016 at the Infertility Center, Pusan National University Hospital. A total of 206 infertile women were recruited for this study which was approved by the Institutional Review Board of Pusan National University Hospital (IRB No. : E2016024). The authors carried out controlled ovarian stimulation (COS) with oocyte retrieval and all of 206 embryo transfer cycles were undertaken by the frozen-thawed embryos. The patients who had at least one blastocyst with grade 3BB (Gardner and Schooleraft score) in morphology were included in this study. Exclusion criteria were (1) women over age 38, (2) poor responders, and (3) inappropriate endometrium for implantation which had endometrial synechiae, unresponsive thin endometrium or abnormal anatomy of uterine cavity. After infertility workups, endometrial and/or tubal corrective surgeries were performed before COS and FTBs transfers in necessary cases. All patients were divided into the LAH (n=106) and MAH (n=101) groups, and clinical variables were compared between the two groups.

The present authors adopted the routine protocol of their clinics for COS, the blastocyst culture, and the endometrium preparation, as previously published [17, 18]. Briefly, infertile women individually were received gonadotropin releasing hormone (GnRH) antagonist, mid-luteal long GnRH agonist, ultra-long or ultra-short GnRH agonist protocols. Ovarian stimulation was carried out with recombinant FSH alone or in a mixed protocol with hMG. The dosages of gonadotropin were calculated by previous COS response, age, anti-Müllerian hormone (AMH), body mass index (BMI), and basal FSH levels. When two or more follicles had attained a minimum mean diameter of 18 mm, final follicular maturation was achieved with recombinant hCG 250 mg SC or GnRH agonist 0.2 mg. Transvaginal ultrasound-guided oocyte retrieval was performed at 36 hours after hCG injection. All fertilized embryos were cultured to the blastocyst stage in sequential G1/G2 media. Embryos with good quality, which had appropriate morphology of trophectoderm, inner cell mass, and less fragmentation of blastomeres, were selected and vitrified for elective FET. Two or three months after oocyte pick-up, oral estradiol valerate was administered in gradually increasing quantities for endometrial preparation after preliminary GnRH agonist (0.1 mg/day) for pituitary luteal downregulation. At menstrual cycle day 15 endometrial thickness (EMT) was evaluated, and, if over 7-8 mm, vaginal progesterone gel (90 mg/day) and oral estradiol valerate were applied for inducing secretory phase of the endometrium. If EMT did not reach 7 mm, oral estradiol valerate was administered continuously, EMT was rechecked later, and vaginal progesterone was administered after confirming suitable EMT. Blastocysts were warmed and treated by LAH with the OCTAX NaviLaser SYSTEM or MAH with intracytoplasmic sperm injection (ICSI) pipette. These blastocysts were transferred under transabdominal ultrasound guidance by soft ET catheter. LAH was performed immediately after thawing procedure using the OCTAX NaviLaser SYSTEM. The laser was activated to dissect approximately one-quarter of the circumference of ZP (Figures 1A, B).

The authors applied the routine protocol of their clinics for MAH as previously mentioned [2]. MAH was performed using ICSI pipette after thawing procedure. Briefly, FTB was held by holding pipette to 9 o'clock direction and then ZP was completely penetrated by ICSI pipette from 3 o'clock direction to 9 o'clock direction. As holding and ICSI pipette were split, partial zona dissection was created (Figures 2A-D). About 3-4 hours after thawing, the blastocysts were examined by inverted microscope at ×400 magnification, and survival status was assessed.

Ten days after FTBs, serum beta-hCG level was checked for evaluation of pregnancy. Progesterone support was continued until ten weeks of gestation in women who had conceived. Variables of age, duration of infertility, BMI, level of AMH, basal FSH, and LH and COS protocols were analyzed between LAH and MAH groups (Table 1). Also, average number of frozen blastocysts per COS cycle and number of transferred FTBs per ET were calculated, and endometrial thickness at ET, implantation rate, and clinical and ongoing pregnancy rates were investigated between LAH and MAH groups. Implantation rate was defined as the percentages of transferred embryos that implanted and developed to the stage of documented fetal heartbeat by ultrasound. Clinical pregnancy rate was defined as the presence of gestational sac at six weeks after last menstrual period (LMP). Ongoing pregnancy was

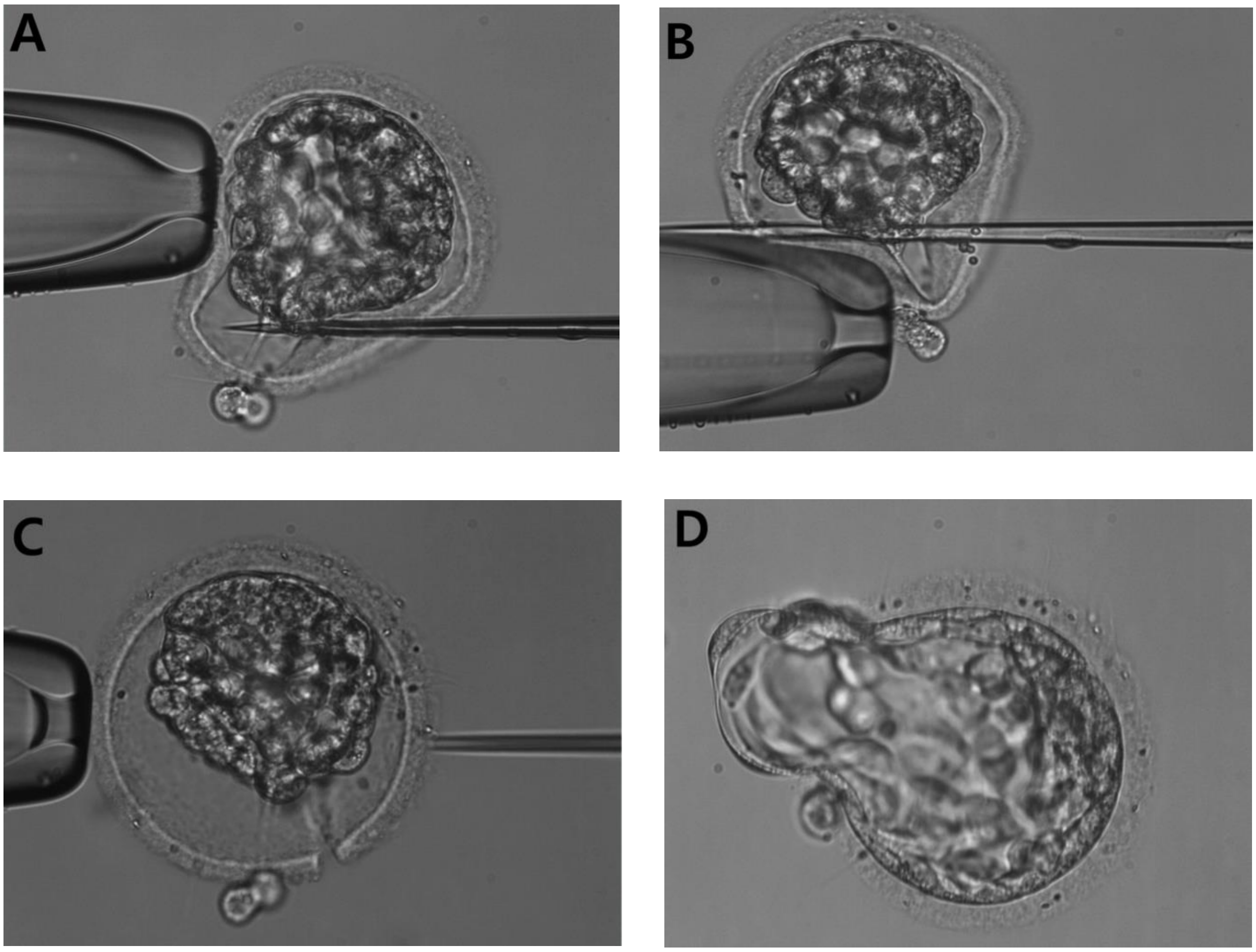

Figure 2. — Mechanical-assisted hatching. A) The fixation by the assisted hatching pipette at a 9 o' clock direction after locating a shrunken blastocyst at a 12 o' clock direction, and the penetration by the injection pipette from a 4 o' clock direction. B) The penetration by the injection pipette to a 8 or 9 o' clock direction, and the rubbing and dissecting procedures using the edge of the holding pipette and the middle part of the infection pipette. C) Zona pellucida with a split after assisted hatching (partial zona dissection). D) The hatching blastocyst.

defined as the presence of a fetus with cardiac activity at ten weeks after LMP. All data were analyzed by using the SAS® version 9.3 software with significance of two-tailed tests set at < 5%. The comparisons of statistically significant differences between groups were made using the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables.

Results

There were no significant differences in age, duration of infertility, BMI, level of AMH, basal FSH and LH, and COS protocols between the two groups (Table 1). Laboratory variables and clinical outcomes between the two groups are presented in Table 2. There were no significant differences in average number of frozen blastocyst per COS cycle and number of transferred FTBs per ET, endometrial thickness at ET. The implantation rate was higher in the MAH group, but the clinical pregnancy rate was lower than the LAH group, respectively. The ongoing pregnancy rate was lower in the LAH group. However all of these variables did not have statistical differences.

Discussion

This study focused on comparing clinical outcomes of LAH and MAH on day 5 FTBs transfer cycles. There is good evidence that AH slightly improves clinical pregnancy rate in poor prognosis patients including those with prior failed IVF cycles [19]. Recent Cochrane review has presented that AH could be attractive method for in increased chance of achieving a clinical pregnancy, but it had minimal statistical significance. In fact, AH did not increased live birth rate in this research. However these trials provided insufficient data to investigate the impact of AH on several important outcomes [5].

Also there is still a debate on the need for AH of frozen-

Table 1. — *Demographic results of patients.*

Variables	LAH (n=105)	MAH (n=101)	*p*-value
Age (years)	35.0 [33.0-37.0]	33.0 [33.0-37.0]	0.08
Duration of infertility (years)	4.0 [2.0-5.0]	3.0 [2.0-4.0]	0.14
Infertility, n (%)			
Primary	57 (54.3)	61 (60.4)	
Secondary	48 (45.7)	40 (39.6)	
Infertility diagnosis, n (%)			
Male	21 (20.0)	34 (33.7)	
Tubal	25 (23.8)	26 (25.7)	
Ovulation	16 (15.2)	14 (13.9)	
Unexplained	18 (17.1)	10 (9.9)	
Endometriosis	6 (5.7)	6 (5.9)	
Diminished ovarian reserve	2 (1.9)	2 (2.0)	
Mixed	1 (1.0)	1 (1.0)	
Others	16 (15.2)	8 (7.9)	
BMI (kg/m^2)	22.0 [20.3-24.2]	23.4 [21.2-25.0]	0.23
AMH (ng/mL)	4.2 [2.4-6.1]	4.1 [2.4-6.4]	0.70
Basal FSH (mIU/mL)	6.0 [4.4-8.1]	6.0 [4.2-8.2]	0.79
Basal LH (mIU/mL)	4.0 [2.5-6.9]	4.8 [3.0-8.9]	0.10
Stimulation protocol, n (%)			
Antagonist	61 (58.1)	58 (57.4)	
Long	35 (33.3)	40 (39.6)	
Ultra-long	4 (3.8)	2 (2.0)	
Ultra-short	5 (4.8)	1 (1.0)	

Data are presented as the median [interquartile range]. AMH: anti-Müllerian hormone; BMI: body mass index; FSH: follicle stimulating hormone; LAH: laser-assisted hatching; LH: luteinizing hormone; MAH: mechanical-assisted hatching.

Table 2. — *Comparison of laboratory variables and clinical outcomes between LAH and MAH groups*

Variables	LAH (n=105)	MAH (n=101)	P-value
FTBs per COS cycle	1.78±0.62	1.90±0.64	0.17
Transferred FTBs per ET	1.52±0.50	1.62±0.49	0.14
Endometrial thickness at ET (mm)	8.99±1.42	9.12±1.55	0.53
Implantation (%)	52(49.5)	56(55.4)	0.41
Clinical pregnancy (%)	46(43.8)	43(42.6)	0.89
Ongoing pregnancy (%)	35(33.3)	38(37.6)	0.56

Data are presented as mean±standard deviation (SD). COS: controlled ovarian stimulation; ET: embryo transfer; FTB: frozen-thawed blastocyst; LAH: laser-assisted hatching; MAH: mechanical-assisted hatching.

thawed embryo yet. Nevertheless, many studies suggested positive clinical outcomes on AH in frozen-thawed embryo transfer cycle [6-11, 20], hence AH would be considered in frozen-thawed embryo transfer cycles. The present authors usually performed AH technique in cryopreserved embryos transfer cycles with hypothesis that it would be a useful tool for improving clinical outcomes in FTBs transfer cycles [8, 9, 17, 18]. Thus, there is a need to investigate AH methods to improve clinical outcomes of frozen-thawed embryos and especially frozen-thawed blastocysts.

The technique ofAH using PZD of MAH to create an artificial opening of the ZP of early cleaved embryos was first described [4]. The mechanism of PZD is quick to perform, but it produces holes of variable sizes that may not always be optimal [21]. This methods require extensive technique skill to produce uniform, well-controlled and standardized micro-holes using micropipettes mounted on micromanipulators [22].

Initially, the LAH as AH was used to create a single full thickness hole through the ZP [15]. However, more recently, the laser to thin the ZP without creating a hole demonstrated a significant increase in hatching in vitro [23]. Laser-assisted microdissection of the ZP can be done with high precision and repeatability with no negative impact on in vitro embryo development. The technique is easy to perform and very effective with regards to the overall time requirement. This can be performed in a sterile environment without any additional micromanipulations, and also it is feasible to open the zona even in largely expanded blastocysts without visible blastocyst damage [24-27]. There is a strong evidence supporting that LAH is considered the best technique now regarding safety and efficacy [22]; however laser zona thinning can be disruptive to the hatching process based on the significant delay in completion of hatching and frequent appearance of multiple hatching sites, which may lead to incomplete hatching. Also embryo development was compromised as a result of the high amount of laser energy used [28].

In one prospective LAH versus MAH study, including women of advanced age (≥ 39 years) with primary infertility and having available embryos for transfer on day 3, LAH of embryos may result in better clinical outcome when compared to the mechanical technique in woman of advanced age undergoing IVF/ ICSI [25]. However, the day 5 FTBs did not show significant differences in clinical outcomes of LAH and MAH, in our study.

In this study, all COS protocol decisions, oocyte retrieval, and ET procedures were performed by the same physician, and AH processes were also performed by one expert. Therefore, any possible clinical and technical variations could have been greatly lowered, and it would be consequently helpful to compare clinical outcomes of AH methods. In AHs procedure of FTBs, the authors did not find LAH advantages of clinical outcome compared to MAH. The authors believe the reason why the clinical outcomes of MAH and LAH are not different from each other seems to be that all FTBs transferred with showing above grade 3BB. Moreover, because the age of the subjects was relatively younger than 35 years in both groups, no significant difference of clinical outcomes could be observed.

In conclusion, LAH was not better than the MAH for enhancing pregnancy rate in FTBs transfer cycles. Therefore, the authors postulate that when AH of FTBs is needed in clinic without laser equipment, MAH could be performed with no significant difference in clinical outcomes. This study was limited by its retrospective characteristic, a small sample size, and the use of FTBs; therefore, further case-controlled, prospective and multicenter studies are needed to evaluate these techniques. Furthermore, this study included the blastocysts of relatively good quality; hence, further research should concern blastocyst of poor quality to broaden the subject spectrum.

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