Laser-assisted Hatching Improves Live Birth Rate of Vitrified-warmed Blastocyst Transfer Cycles: a Case Control Study

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Research

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Abstract

Background Assisted hatching is a widely accepted technique in assisted reproductive technology, however, it's efficiency remains controversial and lack of data on safety. The aim of this study was to assess whether laser-assisted hatching improves clinical pregnancy results of vitrified-warmed blastocyst transfer cycles.

Methods This was a retrospective cohort study of 4143 vitrified-warmed blastocyst transfer cycles from October 2014 to December 2015 at a single, university-based hospital. Cases involving blastocysts that survived after warming were divided into the assisted hatching (AH) group (n=1975) and non-AH group (n=2168). In the AH group, laser AH was performed for the warmed blastocysts before transfer. In the non-AH group, the warmed blastocysts were transferred without AH. The primary outcome was live birth rate after survived vitrified-warmed blastocyst transfers.

Results No significant differences in age, endometrial preparation regimen, number of embryos transferred, or blastocyst developmental stage were found between the two groups (P>0.05). The biochemical pregnancy (67.0% vs. 63.6%; P=0.023; odds ratio [OR], 1.177; 95% confidence interval [CI], 1.032–1.344), clinical pregnancy (59.2% vs. 56.0%; P=0.041; OR, 1.163; 95% CI, 1.024–1.321), live birth (48.6% vs. 45.4%; P=0.041; OR, 1.160; 95% CI, 1.022–1.316), and implantation (52.1% vs. 49.3%; P=0.039) rates of the AH group were significantly higher than those of the non-AH group. The early miscarriage (17.1% vs. 17.8%; P=0.674), monozygotic twin (1.5% vs. 0.90%; P=0.214), and birth defect (3.1% vs. 3.6%; P=0.571) rates were similar in both groups.

Conclusions In vitrified-warmed blastocyst transfer cycles, laser AH is associated with high clinical pregnancy and live birth rates. The benefits of AH outweigh its drawbacks, which include prolonged in vitro procedure, thermal damage, and higher workload in the in vitro fertilization laboratory.

Trial registration: retrospectively registered

Background

Since Cohen proposed assisted hatching (AH) in 1988 Cohen J, Malter H, Fehilly C, Wright G, Elsner C, Kort H and Massey J [1], it has become a widely accepted technique in assisted reproductive technology; however, its efficiency remains controversial [2–4]. In 2014, the Practice Committees of the American Society for Reproductive Medicine and of the Society for Assisted Reproductive Technology concluded that the evidence suggesting that AH may improve the live birth rate is insufficient based on a Cochrane meta-analysis including 31 randomized controlled trials and 5728 women [5]. However, a recent meta-analysis that involved 36 randomized studies, and 6459 participants reported a significant improvement in clinical pregnancy rates [6].

Cryopreservation procedures may impair successful embryonic hatching out of the zona pellucida (ZP) due to embryonic damage during freezing and zona hardening during thawing [7]. In theory, AH

performed in the IVF laboratory after freezing-thawing may assist the natural hatching process in vivo and therefore may have the potential to facilitate embryonic implantation. In recent years, AH was widely used in frozen embryo transfer (FET) cycles [4, 8–10]. A systematic review and meta-analysis of the medical literature showed that AH is associated with a significantly increased clinical pregnancy rate for women undergoing frozen-thawed embryo transfer [11, 12]. However, several scholars demonstrated that for patients undergoing frozen-thawed embryo transfer, the pregnancy and live birth rates, as well as blastocyst stage and early cleavage stage, were not significantly different between the AH and non-AH groups [13]. The results of meta-analyses and clinical studies of AH have been inconsistent [2–4, 13], and the early cleavage stage embryo has significantly attracted the attention of scholars [8, 9].

With refinement of the techniques for blastocyst culture and in compliance with the guidelines aiming to reduce the risk of multiple pregnancies, blastocyst transfer has been extensively used recently. Vitrified-warmed blastocyst from low-grade cleavage-stage embryos could result in significantly higher clinical pregnancy and implantation rates with laser-assisted hatching [14]. Valojerdi and coworkers have demonstrated that laser AH by ZP breaching could improve the clinical pregnancy and implantation rates in patients with frozen/thawed embryos[4]. Laser represents an ideal tool for assisted hatching, as the energy is easily focused on the targeted ZP producing a controlled and precise hole, and consistent between operators. There was no ultrastructural degenerative alterations of ZP following laser-mediated assisted zona drilling[15]. Laser AH was an efficient method to increase the clinical result of vitrified-warmed blastocyst transfer till now. However, it is necessary to consider that the sample size of the various groups was very small. In view of insufficient clinical evidence demonstrating the value of AH at the start of our study, especially lack of data on deliveries and safety[16], this study was performed and aimed to evaluate the efficacy and safety of laser AH during vitrified-warmed blastocyst transfer cycles with comparable clinical data.

Methods

Study design and patient selection

A total of 4143 women who underwent vitrified-warmed blastocyst transfer cycles from October 2014 to December 2015 were included in this study. The blastocyst transfer cycles of women who underwent preimplantation genetic screening were excluded from this study.

Patients who underwent FET cycles were divided into AH group and non-AH group. Assisted hatching was performed on the warmed blastocyst every other day on the basis of even dates for warming [17]. The study was double-blinded. In the AH group, all blastocysts that survived after warming underwent laser AH (or laser partial zona dissection). In the non-AH group, all the warmed blastocysts were cultured in G2 blastocyst culture media (Vitrolife, Göteborg, Sweden) without AH before the transfer. AH was performed by several expert embryologists. All AH blastocysts and non-AH blastocyst were transferred in this study.

This retrospective study was approved by the Ethics Committee of Reproductive Hospital Affiliated to Shandong University (Jinan, China). All patients signed written informed consent forms.

In vitro fertilization and embryo culture

All patients underwent in vitro fertilization (IVF) treatment. During IVF treatment, ovarian stimulation and natural cycles were performed according to the patients' baseline data. Ovarian stimulation protocols included controlled ovarian hyperstimulation after gonadotropin-releasing hormone (GnRH) agonist down-regulation or antagonist protocol. Recombinant follicle-stimulating hormone (rFSH, PUREGON; MSD Organon, Oss, Netherlands) was started on day 1–3 of menstrual cycle. The dose adjustment of gonadotropin, monitoring of the ovarian response, and the timing for triggering the final oocyte maturation during ovarian stimulation was performed at the discretion of the supervising physician. Oocyte retrieval was performed 34–36 h after the administration of human chorionic gonadotropin (hCG) at a dose of 4000–10 000 IU. Based on the sperm quality, oocytes were inseminated approximately 3–6 h after follicular aspiration using a conventional insemination method or intracytoplasmic sperm injection. Embryos were cultured separately in pre-equlibrated culture media overaid with mineral oil. The culture dish were housed in 37°C tri-gas table top incubators (K-system, Denmark) containing 5% O2 and 6% CO2, balanced with N2. Two high-quality embryos were picked out for fresh transfer on day 3. For the patients who can only accept a single embryo transfer, a single blastocyst was selected and transferred on day 5. Supernumerary embryos were cultured for blastocyst cryopreservation. On day 3, the embryos were removed from the cleavage media and placed in blastocyst media. Morphologic criteria were used for day 3 embryo scoring based on the amount of anucleate fragments expelled during early cleavage and on developmental speed[18]. Embryo scores on days 5 to 7 were assessed according to Gardner morphological criteria [19] and based on the degree of expansion and the development of the inner cell mass and trophectoderm. The selection of the vitrification blastocyst gave priority to the score of the inner cell mass, and the score of trophectoderm was also considered—ie, the rank of blastocyst grade from top to high quality was AA, AB, BA, BB, AC, and BC. All the virtrified blastocysts had developed to the stage of thinning zona pellucida. Concerns that the high pressure to which blastocysts are exposed during pipetting might rupture the trophectoderm and induce blastocoelic fluid leakage have been raised. A fully hatched embryo is expected to be more fragile. The time for vitrification performed on day 6 or day 7 was at morning time as early as possible to avoid the hatching of the blastocyst.

Vitrifying and warming procedures

Vitrifying and warming procedures for blastocysts were performed at 37°C. All blastocysts were collapsed by laser-assisted artificial shinkage before vitrification to avoid ice crystal formation by reducing the fluid content of the blastocoel [20]. Vitrification was performed using the Mukaida protocol with a CryoLoop [21]. The blastocysts were rinsed using a base solution that contained 5 mg/mL human serum albumin in HEPES-buffered modified human tubal fluid medium (Irvine Scientific, CA, USA) and subsequently placed in an equilibration solution containing 7.5% (v/v) dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) and 7.5% (v/v) ethylene glycol (EG, Sigma-Aldrich, St. Louis, MO, USA). After about 2 min, the blastocysts were transferred to the vitrification solution containing 15% (v/v) DMSO, 15% (v/v) EG, 10 mg/mL Ficoll 70 (Pharmacia Biotech Inc., Sweden), and 0.65 mol/L sucrose and vitrified for <30 seconds.

Finally, the vitrified blastocysts were placed on a CryoLoop (Hampton Research Corp., Laguna Niguel, CA, USA) and immediately immersed in liquid nitrogen.

Warming was performed in a four-well multi-dish based on the Mukaida protocol. Briefly, blastocysts were incubated in warming solution I containing 0.33 mol/L sucrose; warming solution II containing 0.2 mol/L sucrose; and base solution at 37°C for 2, 3, and 5 min, respectively. Survival of blastocysts was assessed based on the integrity of the inner cell mass and trophectoderm cells, with absent or partial degeneration and fully or partial re-expansion. After warming, the blastocysts were kept in blastocyst culture media and incubated at 37°C with 6% CO_2 before they were transferred to the uterus. Warming was performed in the morning and transferred in the afternoon at regular time everyday. The delay between warming and transfer was no less than 4 hours and no more than 6 hours. The warmed blastocyst would be evaluated before transfer, and all the survived blastocysts would be transferred.

AH procedure

In the AH group, the survived blastocysts underwent laser AH (or laser partial zona dissection) immediately after warming. Laser AH was performed under a Nikon inverted microscope (Nikon, Tokyo, Japan) that was equipped with an RI laser system (Saturn Laser System Research Instruments Ltd., Basel, Switzerland). Laser dissection was performed at the largest perivitelline space, and exposure time and the number of consecutive irradiations were dependent on the thickness of the ZP and perivitelline space. Laser power, exposure time, number of laser shots were also different between blastocysts with different degrees of expansion. The size of the laser hole should not exceed the thickness of the ZP. AH was usually performed at a position away from the inner cell mass and the trophoblastic cells to minimize the risk of thermal damage to the embryos. For most of the blastocysts warmed, the perivitelline space was large enough to perform laser AH easily. Usually, the Laser was set as follows. Hole size was from 4.0 μm to 6.9 μm in diameter. Pulse width was from 0.38 ms to 0.574 ms or so. Approximately one-eighth to one-sixth of the blastocyst ZP circumference was cut before the transfer to G2 blastocyst culture media (Fig. 1).

Preparation for FET cycles and embryo transfer

Endometrial preparation for vitrified-warmed blastocyst transfer was performed during natural cycles, hormonal replacement therapy (HRT) cycles, controlled ovarian hyperstimulation cycles, or hormonal replacement. During HRT cycles, the endometrium was prepared with 4 mg oral estradiol valerate (Progynova; Bayer AG, Leverkusen, Germany) supplementation daily for 5 days starting on day 2 or 3 of the menstrual cycle, which was followed by 6 mg estradiol for an additional 5 days. If necessary, the dose of estradiol was increased to 8 mg for another 4–5 days. When endometrial thickness exceeded 8 mm, dydrogesterone (Duphaston 20 mg; Abbott Biologicals, Amsterdam, the Netherlands) twice daily and vaginal micronized progesterone (200 mg) once daily were initiated. During natural cycles, ovulation was assessed and documented based on the disappearance or typical change in the shape of the dominant follicle. Ovulation was induced by a bolus of 8000–10,000 IU of hCG when at least one dominant follicle

reached ≥18 mm in diameter, and the thickness of the endometrium was at least 8 mm. Dydrogesterone (10 mg) was initiated twice daily. Blastocysts were warmed and transferred on day 5 after the initiation of progesterone treatment. Survived blastocyst was transferred after being cultured for 4~6 hours in blastocyst culture media with partial, fully re-expansion or different levels of hatching (Fig. 2).

Outcome measures

Pregnancy was confirmed by an increase in serum hCG concentrations (>10 mIU/mL) at 12 days after blastocyst transfer. Clinical pregnancy was determined by ultrasonographic observation of a gestational sac at week 7. Early abortion was defined as natural abortion before 12 weeks of gestation. The live birth rate was calculated as the number of live births per transfer cycle. Live birth was defined as the delivery of a live-born infant after ≥28 weeks of gestation. The monozygotic twin pregnancy rate was calculated as the number of monozygotic twin cycles per clinical pregnancy cycle. Patient ages, endometrial preparation protocols, numbers of embryos to be transferred, embryonic periods of development, clinical pregnancy rates, early abortion rates, live birth rates, and birth defect rates were recorded and compared between the AH and the non-AH groups.

Statistical analyses

All analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Statistical analyses were conducted using the t-test and chi-square test. The mean age was compared by the t test. The difference in the primary outcome between the two groups was analysed by the Pearson chi-square test. Logistic regression analyses were performed to assess the association of clinical features with blastocyst transfer results. $P < 0.05$ was considered statistically significant.

Results

A total of 4143 patients transferred with survived blastocysts were involved in this study (AH group, n = 1975; non-AH group, n = 2168). Baseline and cycle parameters of the AH and non-AH groups were comparable. No significant differences in age, endometrial preparation regimen, number of embryos to be transferred, and blastocyst stage of development were found between the two groups ($P > 0.05$) (Table 1). Biochemical pregnancy (67.0% vs. 63.6%; P = 0.023; odds ratio [OR], 1.177; 95% confidence interval [CI], 1.032–1.344), clinical pregnancy (59.2% vs. 56.0%; P = 0.041; OR, 1.163; 95% CI, 1.024–1.321), live birth (48.6% vs. 45.4%; P = 0.041; OR, 1.160; 95% CI, 1.022–1.316), and implantation (52.1% vs. 49.3%; P = 0.039) rates were significantly higher in the AH group than in the non-AH group. Moreover, the early miscarriage (17.1% vs. 17.8%; P = 0.674), monozygotic twin (1.5% vs. 0.90%; P = 0.214), and birth defect (3.1% vs. 3.6%; P = 0.571) rates were similar in both groups (Table 2).

To further understand the effects of AH, the influences of patient age and blastocyst developmental stage on the outcomes were analysed. Both in the < 35 years old patients and ≥ 35 years old patients group, AH group showed better clinical outcomes, but no significant differences were noted (Tables 3 and 4). In addition, AH remarkably increased the biochemical pregnancy (64.7% vs. 54.6%; P = 0.002), clinical

pregnancy (53.7% vs. 46.8%; P = 0.034), and live birth rates (42.2%vs. 35.7%; P = 0.042) for day 6 blastocyst cycles (Table 5); however, the differences in day 5 blastocyst cycles were not statistically significant (P > 0.05) (Table 6).

Endometrial preparation protocols might affect the results of the AH efficiency, so the effects of AH in different endometrial preparation protocols were analysed. AH group showed better clinical outcomes in both natural cycles and HRT cycles, but no significant differences were noted (Tables 7 and 8). AH increased clinical pregnancy results of the FET in COH cycles also, but only the difference in live-birth rate was significant (50.0% VS 39.6%, P < 0.05) (Table 9).

Discussion

This study showed that AH, as a method facilitating the escape of the embryo out of the ZP, could increase clinical pregnancy rates during vitrified-warmed blastocyst transfer cycles. The positive influence of AH on vitrified-warmed blastocysts was indicated by the significant increase in clinical pregnancy rates and live birth rates.

Our results are in agreement with those of previous reports [14, 22], although there are some differences. Vanderzwalmen et al. demonstrated similar effects of AH on 36 vitrified-warmed blastocyst cycles after creating a large opening in the ZP using mechanical instruments [22]. Wan et al. evaluated the effects of quarter ZP opening by laser-assisted hatching (QLAH) on clinical outcomes following the transfer of vitrified-warmed blastocysts developed from low-grade cleavage stage embryos. They showed that QLAH improves the clinical outcomes of vitrified-warmed blastocysts, especially those of day 6 vitrified blastocysts that were developed from low-grade cleavage stage embryos [14]. However, the study by Debrock et al. [2] showed no differences in the outcomes of the laser AH group (n = 302) and the control group (n = 317). The different results might derive from different developmental stages including day 2, day 3, and day 5 embryos, and the method of ZP thinning in the later study. As assessed in an in vitro model, complete ZP removal does not interfere with blastocyst viability and is advantageous for blastocyst adhesion and outgrowth. Therefore, when choosing a blastocyst for vitrified-warmed blastocyst transfer, complete ZP removal may be an essential factor because it could increase the chance of blastocyst attachment [23]. A recent systematic review led to the same conclusion, indicating that a higher degree of zona manipulation, which includes ZP removal, may lead to superior results [8].

The evaluation of clinical outcomes regarding the development stage of the vitrified blastocysts revealed that the clinical pregnancy and live birth rates of the AH group were significantly increased for day 6 vitrified blastocysts. A previous study of vitrified-warmed blastocysts developed from low-grade cleavage stage embryos also indicated that laser AH improves the clinical outcomes of vitrified-warmed blastocysts, especially day 6 vitrified blastocysts [14]. Hence, blastocyst hatching might be related to not only cryopreservation but also longer culture in vitro. It’s interesting that we found AH increased significantly the live-birth rate in COH endometrial preparation cycles, but no significant difference was found in other indicators (Table 9). The difference might be due to the endometrial status related to COH

protocols. No much attention was paid for the data was small in this research. It deserves further study in the future.

Age is a vital factor related to clinical pregnancy results. This study got the same conclusion. AH did not show a significant benefit for patients of advanced ages than these young ages (Tables 3 and 4). The findings were supported by a meta-analysis, which indicated that no reasonable conclusions could be drawn regarding reproductive outcomes after AH for patients of advanced maternal age [24]. Another study suggested that the increased monozygotic twinning rate could be an adverse outcome of AH in addition to mechanical trauma [25]. Blastocyst culture and AH have been widely reported as risk factors for monozygotic twinning [26]. In our study, we observed an increased incidence of monozygotic twins after laser AH; however, the difference between the AH (1.5%) and non-AH (0.9%) groups was not significant ($P = 0.214$). Despite the advanced embryo stage and AH application in our study, the monozygotic twin rate was not significantly increased compared to those reported in recent studies that reported monozygotic twinning rates of 1.36% after single embryo transfer [26] and 2.3% after frozen single blastocyst transfer during a standard, multicentre, randomized, controlled trial [27]. Moreover, similar conclusions could be found in some recent reports of monozygotic twinning [28–30]. However, these studies did not demonstrate the relationship between monozygotic twinning and the use of ZP manipulation techniques. In our study, we found that AH tends to increase the monozygotic twinning rate during vitrified-warmed blastocyst cycles among women aged < 35 years (1.7%) compared to those aged ≥ 35 years; however, the difference was not significant. Another study of the determinants of monozygotic twinning also reported that young maternal age might increase the monozygotic twinning rate [29]. Therefore, AH may not be the major factor that leads to the increased monozygotic twinning rate.

The safety of in vitro procedures has always been the focus of assisted reproductive technology. In this study, the birth defect rate did not increase after AH. This result was consistent with those of previous studies of assisted hatching. A retrospective cohort study suggested that AH alone does not increase the risk of a major congenital anomaly [31]. Another retrospective cohort analysis study showed that laser AH has no harmful effects on neonates, thereby suggesting that laser AH may be a safe treatment that can be used during cryopreserved embryo transfer cycles [30].

Although a higher degree of zona manipulation, including zona removal, may lead to better blastocyst outgrowth results [8, 23], embryos without ZP may be more vulnerable during routine procedures. Although no evidence of altered implantation rates or clinical outcomes with the transfer of fully hatched euploid embryos has been found [32], potential damage resulting from mechanical trauma, attachment to glass and plastic surfaces, or loss of protection from infections may be viewed as possible drawbacks of AH. As shown in this study, partial zona dissection improved clinical results and prevented the aforementioned potential damage during routine procedures. Therefore, such methods should be popularized and applied to vitrified-warmed blastocyst transfer cycles for better clinical results.

This retrospective study had a large sample size and was conducted at one IVF laboratory. All the patients in both groups received similar pre-treatment before blastocyst transfer, except the AH procedure, which allowed for an accurate estimate of the outcomes. This study showed that laser AH during vitrified-warmed blastocyst transfer cycles was associated with high clinical pregnancy and live birth rates. However, this study had some limitations. First, this was a retrospective study, and the grouping did not have well-balanced baseline clinical characteristics. Second, the blastocyst quality of the two groups was not balanced very well, which possibly affected the results. Fortunately, whether blastocysts quality is associated with live birth rate is still a controversial topics [33] [34]. Finally, the clinical data of women included in this study were not analysed comprehensively, which could have been another influencing factor. Hence, further studies, including prospective, randomized, controlled trials, are required to evaluate the clinical significance of laser AH. This study mainly focused on vitrified-warmed blastocyst transfer; therefore, future studies of cleavage stage embryos may be needed to clarify the results further.

Conclusions

In conclusion, laser AH could improve clinical pregnancy results of vitrified-warmed blastocyst transfer cycles through ZP cutting. Cutting the ZP may be conducive to the hatching and implantation of vitrified-warmed blastocysts that originated from prolonged culture or cryopreservation. The benefits of laser AH outweigh the drawbacks, which include prolonged in vitro procedure, thermal damage, and higher workload in the IVF laboratory. However, the potential effects of AH on children remain to be established.

Abbreviations

AH - assised hatching

ZP - zona pellucida

FET - frozen embryo transfer

IVF - in vitro fertilization

QLAH - quarter ZP opening by laser-assisted hatching

Declarations

Ethics approval and consent to participate

The study protocol was approved by the local ethics committee of Reproductive Hospital Affiliated to Shandong University (Jinan, China).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ML was involved in designing the study. SYM and LJL drafted the manuscript. HZ and CL were responsible for the recruitment and acquisition of data. HBZ, QL, and KX analyzed and interpreted the data. All authors were involved in writing the article and approved the final version to be published.

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Tables

Table 1
Baseline characteristics of the cycles of the AH and non-AH groups

	AH group	Non-AH group	P value
Cycles	1975	2168	
Age, years[a]	30.34 ± 4.63	30.25 ± 4.71	0.544
Endometrial preparation for FET n (%)			0.767
Natural cycle	983 (49.8%)	1093 (50.4%)	
HRT cycle	734 (37.2)	777 (35.9%)	
COH cycle	240 (12.2%)	280 (12.9%)	
Down regulation + replacement cycle	18 (0.9%)	17 (0.8%)	
Number of blastocysts transferred, n (%)	1.25 ± 0.43	1.24 ± 0.42	0.298
One blastocyst cycle	1483 (75.1%)	1658 (76.5%)	
Two blastocysts cycle	492 (24.9%)	510 (23.5%)	
Days of blastocyst development, n (%)			0.384
Day 5 blastocyst cycle	1497 (75.8%)	1682 (77.6%)	
Day 6 blastocyst cycle	467 (23.7%)	476 (22.0%)	
Day 7 blastocyst cycle	11 (0.6%)	10 (0.5%)	
The average blastocyst number			0.246
Day 5 blastocyst cycle	1.22 ± 0.41	1.21 ± 0.41	
Day 6 blastocyst cycle	1.36 ± 0.48	1.33 ± 0.47	
Day 7 blastocyst cycle	1.18 ± 0.41	1.0 ± 0.00	

[a] Maternal age at FET

Table 2
Clinical outcomes of the cycles of the AH and non-AH groups

	AH group	Non-AH group	OR (95% CI)	P value
Cycles	1975	2168		
Biochemical pregnancy rate, n (%)	1323 (67.0%)	1379 (63.6%)	1.177 (1.032–1.344)	0.023*
Clinical pregnancy rate, n (%)	1169 (59.2%)	1215 (56.0%)	1.163 (1.024–1.321)	0.041*
Live birth rate, n (%)	959 (48.6%)	984 (45.4%)	1.160 (1.022–1.316)	0.041*
Early miscarriage rate, n (%)	200 (17.1%)	216 (17.8%)	0.956 (0.773–1.181)	0.674
Implantation rate, n (%)	1286/2467 (52.1%)	1319/2678 (49.3%)	-	0.039*
Monozygotic twin rate, n (%)	17 (1.5%)	11 (0.9%)	-	0.214
Birth defect rate, n (%)	33 (3.1%)	38 (3.6%)	-	0.571

* $P < 0.05$.

Table 3
Baseline characteristics and clinical outcomes of the patients < 35 years old in AH and NAH group

	AH group	NAH group	P-value
Cycles	1589	1755	
Age, years[a]	28.60 ± 3.14	28.52 ± 3.17	0.421
Regimen of endometrial preparation for FET n (%)			0.562
Natural cycle	775(48.8%)	840(47.9%)	
HRT cycle	605(47.4)	671(38.2%)	
COH cycle	196(45.5)	235(13.4%)	
Down-regulation + replacement cycle	13(59.1%)	9(0.5%)	
Number of blastocyst transferred n (%)	1.26 ± 0.44	1.24 ± 0.43	0.388
One blastocyst cycle	1180(74.3%)	1326(75.6%)	
Two blastocysts cycle	409(25.7%)	429(24.4%)	
Days of blastocyst development n (%)			0.774
Day 5 blastocyst cycle	1237(77.8%)	1384(78.9%)	
Day 6 blastocyst cycle	346(21.8%)	365(20.8%)	
Day 7 blastocyst cycle	6(0.4%)	6(0.3%)	
The average blastocyst number			0.428
Day 5 blastocyst cycle	1.23 ± 0.42	1.22 ± 0.41	
Day 6 blastocyst cycle	1.37 ± 0.48	1.35 ± 0.48	
Day 7 blastocyst cycle	1.17 ± 0.41	1.0 ± 0.00	
Biochemical pregnancy rate n (%)	1111(69.9%)	1177(67.1%)	0.076
Clinical pregnancy rate n (%)	988(62.2%)	1048(59.7%)	0.145
Live birth rate n (%)	830(52.2%)	863(49.2%)	0.077
implantation rate n (%)	1095/1998 (54.8%)	1146/2184 (52.5%)	0.139
Monozygotic twins rate n (%)	17(1.7%)	9(0.9%)	0.083
Birth defects rate n (%)	29(3.2%)	37(4.0%)	0.361

[a] Maternal age at FET

Table 4
Baseline characteristics and clinical outcomes of the patients ≥ 35 years old in AH and NAH group

	AH group	NAH group	P-value
Cycles	386	413	
Age, years[a]	37.51 ± 2.34	37.65 ± 2.53	0.413
Regimen of endometrial preparation for FET n (%)			0.075
Natural cycle	208(53.9%)	253(61.3%)	
HRT cycle	129(33.4)	106(25.7%)	
COH cycle	44(11.4)	45(10.9%)	
Down-regulation + replacement cycle	5(1.3%)	9(2.2%)	
Number of blastocysts transferred n (%)	1.22 ± 0.41	1.20 ± 0.40	0.509
One blastocyst cycle	303(78.5%)	332(80.4%)	
Two blastocysts cycle	83(21.5%)	81(19.6%)	
Days of blastocyst development n (%)			0.330
Day 5 blastocyst cycle	260(67.4%)	298(72.2%)	
Day 6 blastocyst cycle	121(31.3%)	111(26.9%)	
Day 7 blastocyst cycle	5(1.3%)	4(1.0%)	
The average blastocyst number			0.3
Day 5 blastocyst cycle	1.17 ± 0.38	1.18 ± 0.38	
Day 6 blastocyst cycle	1.31 ± 0.47	1.25 ± 0.44	
Day 7 blastocyst cycle	1.20 ± 0.45	1.0 ± 0.00	
Biochemical pregnancy rate n (%)	212(54.9%)	202(48.9%)	0.089
Clinical pregnancy rate n (%)	181(46.9%)	167(40.4%)	0.066
Live birth rate n (%)	129(33.4%)	121(29.3%)	0.209
implantation rate n (%)	191/469 (40.7%)	173/494 (35.0%)	0.079
Monozygotic twins rate n (%)	0(0%)	2(1.2%)	0.140
Birth defects rate n (%)	4(3.0%)	1(0.8%)	0.208

[a] Maternal age at FET

Table 5
Baseline characteristics and clinical outcomes of the day 6 blastocyst cycle of the AH and non-AH groups

	AH group	Non-AH group	P-value
Cycles	467	476	
Age, years[a]	30.97 ± 4.82	30.88 ± 4.86	0.766
Regimen of endometrial preparation for FET, n (%)			0.980
Natural cycle	254 (54.4%)	254 (53.4%)	
HRT cycle	146 (31.3)	150 (31.5%)	
COH cycle	62 (13.3)	66 (13.9%)	
Downregulation + replacement cycle	5 (1.1%)	6 (1.3%)	
Number of blastocysts transferred, n (%)	1.36 ± 0.48	1.33 ± 0.47	0.301
One blastocyst cycle	300 (64.2%)	321 (67.4%)	
Two blastocysts cycle	167 (35.8%)	155 (32.6%)	
Biochemical pregnancy rate, n (%)	302 (64.7%)	260 (54.6%)	0.002**
Clinical pregnancy rate, n (%)	251 (53.7%)	223 (46.8%)	0.034*
Live birth rate, n (%)	197 (42.2%)	170 (35.7%)	0.042*
Implantation rate, n (%)	274/634 (43.2%)	242/631 (38.4%)	0.088
Monozygotic twin rate, n (%)	2 (0.8%)	1 (0.4%)	0.633
Birth defect rate, n (%)	5 (2.4%)	5 (2.8%)	0.779

*P < 0.05; ** P < 0.01

[a] Maternal age at FET

Table 6
Baseline characteristics and clinical outcomes of the day 5 blastocyst cycle of the AH and non-AH groups

	AH group	Non-AH group	P-value
Cycles	1497	1682	
Age, years[a]	30.12 ± 4.53	30.05 ± 4.62	0.691
Regimen of endometrial preparation for FET, n (%)			0.678
Natural cycle	722 (48.2%)	831 (49.4%)	
HRT cycle	584 (39.0)	626 (37.2%)	
COH cycle	178 (11.9)	213 (12.7%)	
Downregulation + replacement cycle	13 (0.9%)	12 (0.7%)	
Number of blastocysts transferred, n (%)	1.22 ± 0.41	1.21 ± 0.41	0.746
One blastocyst cycle	1174 (78.4%)	1327 (78.9%)	
Two blastocysts cycle	323 (21.6%)	355 (21.1%)	
Biochemical pregnancy rate, n (%)	1018 (68.0%)	1116 (66.3%)	0.322
Clinical pregnancy rate, n (%)	915 (61.1%)	990 (58.9%)	0.194
Live birth rate, n (%)	761 (50.8%)	812 (48.3%)	0.150
Implantation rate, n (%)	1009/1820 (55.4%)	1075/2037 (52.8%)	0.104
Monozygotic twin rate, n (%)	15 (1.6%)	10 (1.0%)	0.228
Birth defect rate, n (%)	28 (3.3%)	33 (3.8%)	0.647

[a] Maternal age at FET

Table 7
Baseline characteristics and clinical outcomes of Nature cycle of the AH and non-AH groups

	AH group	NAH group	P-value
Cycles	983	1093	
Age, years[a]	30.75 ± 4.64	30.68 ± 4.89	0.726
Number of blastocysts transferred n (%)	1.24 ± 0.43	1.21 ± 0.41	0.087
One blastocyst cycle	749(76.2%)	867(79.3%)	
Two blastocysts cycle	234(23.8%)	226(20.7%)	
Days of blastocyst development n (%)			0.388
Day 5 blastocyst cycle	722(73.4%)	831(76.0%)	
Day 6 blastocyst cycle	254(25.8%)	254(23.2%)	
Day 7 blastocyst cycle	7(0.7%)	8(0.7%)	
The average blastocyst number			0.079
Day 5 blastocyst cycle	1.19 ± 0.40	1.19 ± 0.39	
Day 6 blastocyst cycle	1.36 ± 0.48	1.27 ± 0.45	
Day 7 blastocyst cycle	1.29 ± 0.49	1.0 ± 0.00	
Biochemical pregnancy rate n (%)	667(67.9%)	698(63.9%)	0.056
Clinical pregnancy rate n (%)	602(61.2%)	630(57.6%)	0.095
Live birth rate n (%)	512(52.1%)	540(49.4%)	0.223
implantation rate n (%)	659/1217 (54.1%)	680/1319 (51.6%)	0.204
Monozygotic twins rate n (%)	6(1.0%)	3(0.5%)	0.332
Birth defects rate n (%)	19(3.4%)	22(3.8%)	0.752

[a] Maternal age at FET

Table 8
Baseline characteristics and clinical outcomes of HRT cycle of the AH and non-AH groups

	AH group	NAH group	P-value
Cycles	734	777	
Age, years[a]	29.83 ± 4.64	29.58 ± 4.41	0.290
Number of blastocysts transferred n (%)	1.25 ± 0.43	1.25 ± 0.44	0.898
One blastocyst cycle	550(74.9%)	580(74.6%)	
Two blastocysts cycle	184(25.1%)	197(25.4%)	
Days of blastocyst development n (%)			0.352
Day 5 blastocyst cycle	584(79.6%)	626(80.6%)	
Day 6 blastocyst cycle	146(19.9%)	150(19.3%)	
Day 7 blastocyst cycle	4(0.5%)	1(0.1%)	
The average blastocyst number			0.990
Day 5 blastocyst cycle	1.22 ± 0.42	1.23 ± 0.42	
Day 6 blastocyst cycle	1.36 ± 0.48	1.37 ± 0.48	
Day 7 blastocyst cycle	1.00 ± 0.00	1.0 ± 0.00	
Biochemical pregnancy rate n (%)	479(65.3%)	499(64.2%)	0.673
Clinical pregnancy rate n (%)	411(56.0%)	432(55.6%)	0.917
Live birth rate n (%)	317(43.2%)	327(42.1%)	0.677
implantation rate n (%)	450/918(49.1%)	466/974 (47.8%)	0.642
Monozygotic twins rate n (%)	4(1.0%)	5(1.2%)	0.795
Birth defects rate n (%)	10(2.9%)	10(2.9%)	0.974

[a] Maternal age at FET

Table 9
Baseline characteristics and clinical outcomes of COH cycle of the AH and non-AH groups

	AH group	NAH group	P-value
Cycles	240	280	
Age, years[a]	30.15 ± 4.32	30.20 ± 4.41	0.888
Number of blastocysts transferred n (%)	1.29 ± 0.46	1.30 ± 0.46	0.848
One blastocyst cycle	170(70.8%)	196(70.0%)	
Two blastocysts cycle	70(29.2%)	84(30.0%)	
Days of blastocyst development n (%)			0.552
Day 5 blastocyst cycle	178(74.2%)	213(76.1%)	
Day 6 blastocyst cycle	62(25.8%)	66(23.6%)	
Day 7 blastocyst cycle	0(0%)	1(0.4%)	
The average blastocyst number			0.311
Day 5 blastocyst cycle	1.28 ± 0.45	1.26 ± 0.44	
Day 6 blastocyst cycle	1.32 ± 0.47	1.44 ± 0.50	
Day 7 blastocyst cycle	1.29 ± 0.46	1.0 ± 0.00	
Biochemical pregnancy rate n (%)	162(67.5%)	175(62.5%)	0.269
Clinical pregnancy rate n (%)	144(60.0%)	147(52.5%)	0.093
Live birth rate n (%)	120(50.0%)	111(39.6%)	0.021*
implantation rate n (%)	165/310(53.2%)	167/364 (45.9%)	0.068
Monozygotic twins rate n (%)	7(4.9%)	3(2.0%)	0.215
Birth defects rate n (%)	4(2.9%)	5(4.0%)	0.740
*P < 0.05;			
[a] Maternal age at FET			

Figures

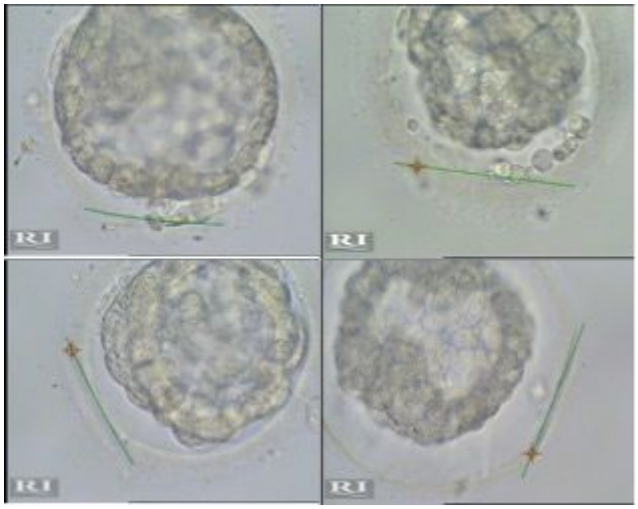

Figure 1

Blastocysts pictures photographed at the time of AH being performed. The blastocyst ZP was breached or partly cut for one-eighth to one-sixth of the ZP circumference with consecutive irradiations.

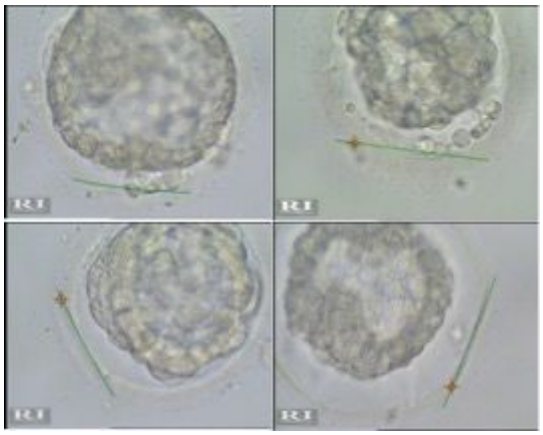

Figure 2

Blastocyst pictures photographed just after warming and after culturing for 4~6 hours in AH group (a, b) and non-AH group (c,d). Laser AH was performed on the vitrified-warmed blastocyst immediately after warming in AH group (a); Blastocyst in AH group was hatching after culturing for 4~6 hours (b); The vitrified-warmed blastocyst was not performed laser AH in non-AH group (c); Blastocyst in non-AH group was hatching after culturing for 4~6 hours (d)