

Assisted Hatching for In Vitro Fertilization-Embryo Transfer: An Update

Shahryar K Kavoussi*

Austin Fertility & Reproductive Medicine/Westlake IVF, 300 Beardsley Lane, Bldg B, Suite 200, Austin, Texas, USA

*Corresponding author: Shahryar K Kavoussi, M.D., M.P.H., Austin Fertility & Reproductive Medicine/Westlake IVF, 300 Beardsley Lane, Bldg B, Suite 200, Austin, Texas, USA, Tel: 001-(512) 444-1414; Fax: 001-(512) 444-5621; E-mail: austinfertility@gmail.com

Received date: 06-02-2014; Accepted date: 13-03-2014; Published date: 17-03-2014

Copyright: © 2014 Kavoussi SK. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Assisted Hatching (AH) is a technique performed after In Vitro Fertilization (IVF) and involves the artificial thinning or opening of the Zona Pellucida (ZP) prior to Embryo Transfer (ET) as an attempt to improve the probability of embryo implantation. AH can be performed by embryologists via mechanical, chemical, or laser-assisted means. A few studies suggest that a larger size of ZP opening/thinning as well as a site near the ICM may be associated with a greater probability for complete hatching. It is not recommended to apply AH to all IVF cycles universally. Subgroups of patients that may benefit from AH include those with prior implantation failure, those undergoing Frozen-Thawed Embryo Transfer (FET) cycles, and women who are 38 years of age or older. IVF programs should identify subgroups of women within their patient population who may benefit clinically from AH.

Keywords: Embryo; Blastocyst; Assisted hatching; Zona pellucida

Introduction

In utero, as an embryo at the blastocyst stage expands and thins its surrounding glycoprotein covering, the Zona Pellucida (ZP), rupture of the ZP eventually occurs with the aid of proteases known as lysins. The embryo hatches and begins the implantation process via interaction with endometrial cells. The inefficiency of Implantation Rates (IR) after Embryo Transfer (ET) may be attributed to various factors including the potential failure of a euploid blastocyst to hatch from the ZP. Assisted Hatching is a technique performed after in vitro fertilization and involves the artificial thinning or opening of the zona pellucida by the embryologist prior to ET as an attempt to improve the probability of embryo implantation [1].

Methods of AH

AH has been in use since the first report by Cohen in 1988 [2] and has since evolved in terms of modality by which the ZP is artificially thinned or opened. AH can be performed by mechanical, chemical, or laser-assisted means. When AH is performed to create an opening in the ZP, the procedure may be performed via the use of acidified Tyrode's solution, partial zona dissection with a glass microneedle, laser photoablation or piezomicromanipulation. When AH is performed to thin and not breach the ZP, acidified Tyrode's solution, proteolytic enzymes, or laser-assistance may be used [1].

The effect of the size of ZP breaching or thinning on AH outcomes

Several studies have suggested that the larger size of ZP breaching or thinning may confer a clinical benefit. In a study by Hiraoka et al., 101 consecutive frozen-thawed embryo transfer procedures were grouped into no Laser-Assisted Hatching (LAH) (n=30), LAH with 40 µm ZP opening (n=40), and LAH with 50% circumferential ZP opening (n=31). Cleavage stage embryos were thawed and grown to blastocyst stage in patients who had failed both fresh and frozen

cleavage stage ET; LAH was performed at the expanded blastocyst stage. The Pregnancy Rates (PR), Implantation Rates (IR), and Live Birth Rates (LBR) were higher in the group with 50% ZP opening (74%, 52%, 65%) when compared with the control group (17%, 10%, 13%; $P < 0.01$) and the group with 40 µm ZP opening (43%, 27%, 38%; $P < 0.04$) [3]. The same authors conducted a study in order to examine the effect of the size of ZP thinning on clinical outcomes in vitrified-warmed cleavage stage FET. Random assignment of 120 cases was made to 50% ZP thinning and 25% ZP thinning groups. Clinical Pregnancy Rates (CPR) (46.7 vs 25.0%; $P = 0.0218$) and IR (32.0 vs 16.2%, $P = 0.0090$) were significantly increased in the 50% ZP thinning group [4]. Zhang et al. studied the effect of the size of ZP thinning by LAH on the clinical outcome of cleavage stage FET [5]. Among 122 consecutive procedures, 31 were in the control group (no AH), 34 were in the AH group with 40 µm ZP thinning, and 57 were in the AH group with 80 µm ZP thinning. PR and IR were significantly higher in the 80 µm AH group as compared with control (40.3 vs 16.1%, $P = 0.03$; 21.5 vs 7.5%, $P = 0.007$, respectively). IR was significantly higher in the 80 µm AH than the 40 µm AH group (21.5 vs 9.4%, $P = 0.024$). The authors concluded that the greater size of ZP thinning may matter for PR and IR in cleavage stage FET [5].

The effect of the site of AH on outcomes

Since two observational studies had shown that the natural hatching site of the human blastocyst is near the inner cell mass (ICM) whereas that of the mouse is at the side opposite to the ICM [6,7], a prospective randomized study was conducted in order to study whether or not the choice of AH site is important to complete hatching. The authors of this preliminary report demonstrated a significantly higher rate of complete hatching when LAH was performed on thawed blastocysts near the ICM as compared to the side opposite to the ICM [8]. Ren et al. evaluated the effect of LAH site in vitrified-warmed blasts on clinical outcome of FET. A total of 16 women were randomized to a group with AH near site of ICM or to a group with AH at the site opposite to the ICM. LAH was performed within 20-30 min after blastocyst thaw once the ICM was detected. There was no difference in IR, PR, LBR, or monozygotic twin rate between the two groups [9].

The effects of AH on clinical outcomes

Unselected patients

The American Society for Reproductive Medicine (ASRM) committee opinion states that the existing literature does not support the universal application of AH to all IVF cycles [1]. The majority of studies that have evaluated the effect of AH on the IR and PR of unselected IVF patients have shown no increase in these outcome measures [10-16] and a recent systematic review and meta-analysis by Martins et al concluded that AH was unlikely to improve CPR in unselected fresh ET cycles whereas AH in unselected FET cycles was efficacious [17].

Advanced maternal age

It has been theorized that ZP hardening may occur due to endocrine changes or the absence of lysins as a function of oocyte aging in women of advanced maternal age. Although some studies suggest benefit of AH in women of advanced maternal age, some show no difference in IR and CPR [17-22]. Subgroup analysis of Advanced Maternal Age (AMA) patients in the review by Martins showed no benefit of AH in women of AMA who underwent fresh ET [16].

Thick ZP

A prospective, randomized, double-blinded, crossover study sought to determine whether AH impacts clinical outcomes in women younger than 38 years whose embryos have a thickened ZP, defined as $> 13 \mu\text{m}$ (n=121). Patients were randomized to a control group (no AH) or group that had AH performed by acidic Tyrode's solution. There was no difference in IR, CPR, or LBR between groups; therefore, this study suggests that AH does not appear to provide benefit to women under age 38 who undergo IVF [23,24].

Prior implantation failure

In women with prior implantation failure, AH has been shown to significantly increase the CPR [16,25]. LBR was not proven to be increased; however, because most trials did not report LBR, there may have been insufficient data to draw conclusions regarding the effects of AH on LBR. The ASRM Committee Opinion found that AH may be of benefit to women with > 2 failed IVF cycles [1].

Frozen-thawed embryo transfer (FET)

It has been suggested that the processes of embryo cryopreservation and thawing may lead to changes in the microarchitecture of the ZP with associated ZP hardening. If such changes theoretically impair the chance of the embryo rupturing from the ZP, it has been thought that AH may be indicated. The recent systematic review and meta-analysis of randomized clinical trials by Martins et al showed that AH was associated with a significant increase in IR and PR in unselected or poor-prognosis women with frozen-thawed ET [16,26-33].

Endometriosis

There has been controversy about whether oocyte/embryo quality or endometrial receptivity is predominantly impaired in women with endometriosis, some studies have suggested that the former is related to endometriosis-associated subfertility [34]. In order to determine if AH improves IR in women with endometriosis, Nadir Ciray et al., conducted a prospective randomized study of 60 women with

endometriosis who had LAH performed for their embryos and 30 women with endometriosis who did not have LAH. There was no difference in PR (28.3% LAH group, 40% control group) or IR (17.8% LAH group, 19.4% control group) between groups. The authors concluded that AH does not improve outcome in women with endometriosis [35].

Multiple gestation

Monozygotic twinning has been found to be more common in IVF cycles and the incidence is increased in pregnancies following AH [36,37]. The recent review by Martins showed that AH increased multiple gestation rates [16]. The 2012 Cochrane Review found that there was low quality evidence showing a significant increase in multiple gestation rates per woman [25].

Conclusions

AH is a technique that has been used in IVF laboratories for over 25 years. Several studies suggest that a larger size of artificial ZP opening/thinning as well as performing AH at a site close to the ICM may be associated with a greater propensity for complete hatching. Universal application of AH to all fresh IVF cycles is not recommended in accordance since the existing literature shows no difference in outcomes. Although LBR data may be insufficient at this time, AH seems to increase IR and CPR in cases of prior implantation failure as well as in FET cycles. Although there is some conflicting data about improved outcomes among women of advanced maternal age, data exists that supports AH in women 38 years of age or older. As recommended by ASRM, each IVF program should assess their own patient characteristics and determine whether or not AH may provide benefit to certain subgroups of their patients.

References

1. Practice Committee of Society for Assisted Reproductive Technology (1988) The role of assisted hatching in in vitro fertilization: a review of the literature. A Committee opinion. *Fertil Steril* 90: S196-S198.
2. Cohen J, Malter H, Fehilly C, Wright G, Elsner C, et al. (1988) Implantation of embryos after partial opening of oocyte zona pellucida to facilitate sperm penetration. *Lancet* 2: 162.
3. Hiraoka K, Fuchiwaki M, Hiraoka K, Horiuchi T, Murakami T, et al. (2008) Effect of the size of zona pellucida opening by laser assisted hatching on clinical outcome of frozen cleaved embryos that were cultured to blastocyst after thawing in women with multiple implantation failures of embryo transfer: a retrospective study. *J Assist Reprod Genet* 25: 129-135.
4. Hiraoka K, Hiraoka K, Horiuchi T, Kusuda T, Okano S, et al. (2009) Impact of the size of zona pellucida thinning area on vitrified-warmed cleavage-stage embryo transfers: a prospective, randomized study. *J Assist Reprod Genet* 26: 515-521.
5. Zhang XJ, Yang YZ, Lv Q, Min LH, Li XL, et al. (2009) Effect of the size of zona pellucida thinning by laser assisted hatching on clinical outcome of human frozen-thawed embryo transfers. *Cryo Letters* 30: 455-461.
6. Veeck LL, Zaninovic N (2003) Blastocyst hatching. In: Veeck LL, Zaninovic N, editors. *An atlas of human blastocysts*. London: Informa Healthcare 159-171.
7. Gonzales DS, Jones JM, Pinyopummintr T, Carnevale EM, Ginther OJ, et al. (1996) Trophoctoderm projections: a potential means for locomotion, attachment and implantation of bovine, equine and human blastocysts. *Hum Reprod* 11: 2739-2745.
8. Miyata H, Matsubayashi H, Fukutomi N, Matsuba J, Koizumi A, et al. (2010) Relevance of the site of assisted hatching in thawed human blastocysts: a preliminary report. *Fertil Steril* 94: 2444-2447.

9. Ren X, Liu Q, Chen W, Zhu G, Zhang H (2013) Effect of the site of assisted hatching on vitrified-warmed blastocyst transfer cycles: a prospective randomized study. *J Assist Reprod Genet* 30: 691-697.
10. Hellebaut S, De Sutter P, Dozortsev D, Onghena A, Qian C, et al. (1996) Does assisted hatching improve implantation rates after in vitro fertilization or intracytoplasmic sperm injection in all patients? A prospective randomized study. *J Assist Reprod Genet* 13: 19-22.
11. Tucker MJ, Luecke NM, Wiker SR, Wright G (1993) Chemical removal of the outside of the zona pellucida of day 3 human embryos has no impact on implantation rate. *J Assist Reprod Genet* 10: 187-191.
12. Antinori S, Panci C, Selman HA, Caffa B, Dani G, et al. (1996) Zona thinning with the use of laser: a new approach to assisted hatching in humans. *Hum Reprod* 11: 590-594.
13. Mansour RT, Rhodes CA, Aboulghar MA, Serour GI, Kamal A (2000) Transfer of zona-free embryos improves outcome in poor prognosis patients: a prospective randomized controlled study. *Hum Reprod* 15: 1061-1064.
14. Cohen J, Alikani M, Trowbridge J, Rosenwaks Z (1992) Implantation enhancement by selective assisted hatching using zona drilling of human embryos with poor prognosis. *Hum Reprod* 7: 685-691.
15. Hurst BS, Tucker KE, Awoniyi CA, Schlaff WD (1998) Assisted hatching does not enhance IVF success in good-prognosis patients. *J Assist Reprod Genet* 15: 62-64.
16. Martins WP, Rocha IA, Ferriani RA, Natri CO (2011) Assisted hatching of human embryos: a systematic review and meta-analysis of randomized controlled trials. *Hum Reprod Update* 17: 438-453.
17. Valojerdi MR, Eftekhari-Yazdi P, Karimian L, Ashtiani SK (2008) Effect of laser zona pellucida opening on clinical outcome of assisted reproduction technology in patients with advanced female age, recurrent implantation failure, or frozen-thawed embryos. *Fertil Steril* 90: 84-91.
18. Kutlu P, Atvar O, Vanlioglu OF (2010) Laser assisted zona thinning technique has no beneficial effect on the ART outcomes of two different maternal age groups. *J Assist Reprod Genet* 27: 457-461.
19. Antinori S, Selman HA, Caffa B, Panci C, Dani GL, et al. (1996) Zona opening of human embryos using a non-contact UV laser for assisted hatching in patients with poor prognosis of pregnancy. *Hum Reprod* 11: 2488-2492.
20. Magli MC, Gianaroli L, Ferraretti AP, Fortini D, Aicardi G, et al. (1998) Rescue of implantation potential in embryos with poor prognosis by assisted zona hatching. *Hum Reprod* 13: 1331-1335.
21. Nakayama T, Fujiwara H, Yamada S, Tastumi K, Honda T, et al. (1999) Clinical application of a new assisted hatching method using a piezo-micromanipulator for morphologically low-quality embryos in poor-prognosis infertile patients. *Fertil Steril* 71: 1014-1018.
22. Chao KH, Chen SU, Chen HF, Wu MY, Yang YS, et al. (1997) Assisted hatching increases the implantation and pregnancy rate of in vitro fertilization (IVF)-embryo transfer (ET), but not that of IVF-tubal ET in patients with repeated IVF failures. *Fertil Steril* 67: 904-908.
23. Hagemann AR, Lanzendorf SE, Jungheim ES, Chang AS, Ratts VS, et al. (2010) A prospective, randomized, double-blinded study of assisted hatching in women younger than 38 years undergoing in vitro fertilization. *Fertil Steril* 93: 586-591.
24. Carroll J, Depypere H, Matthews CD (1990) Freeze-thaw-induced changes of the zona pellucida explains decreased rates of fertilization in frozen-thawed mouse oocytes. *J Reprod Fertil* 90: 547-553.
25. Carney SK, Das S, Blake D, Farquhar C, Seif MM, et al. (2012) Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)). *Cochrane Database Syst Rev* 12: CD001894.
26. Balaban B, Urman B, Yakin K, Isiklar A (2006) Laser-assisted hatching increases pregnancy and implantation rates in cryopreserved embryos that were allowed to cleave in vitro after thawing: a prospective randomized study. *Hum Reprod* 21: 2136-2140.
27. Fang C, Li T, Miao BY, Zhuang GL, Zhou C (2010) Mechanically expanding the zona pellucida of human frozen thawed embryos: a new method of assisted hatching. *Fertil Steril* 94: 1302-1307.
28. Ge HS, Zhou W, Zhang W, Lin JJ (2008) Impact of assisted hatching on fresh and frozen-thawed embryo transfer cycles: a prospective, randomized study. *Reprod Biomed Online* 16: 589-596.
29. Ng EH, Naveed F, Lau EY, Yeung WS, Chan CC, et al. (2005) A randomized double-blind controlled study of the efficacy of laser-assisted hatching on implantation and pregnancy rates of frozen-thawed embryo transfer at the cleavage stage. *Hum Reprod* 20: 979-985.
30. Petersen CG, Mauri AL, Baruffi RL, Oliveira JB, Felipe V, et al. (2006) Laser-assisted hatching of cryopreserved-thawed embryos by thinning one quarter of the zona. *Reprod Biomed Online* 13: 668-675.
31. Sifer C, Sellami A, Poncelet C, Kulski P, Martin-Pont B, et al. (2006) A prospective randomized study to assess the benefit of partial zona pellucida digestion before frozen-thawed embryo transfers. *Hum Reprod* 21: 2384-2389.
32. Gabrielsen A, Agerholm I, Toft B, Hald F, Petersen K, et al. (2004) Assisted hatching improves implantation rates on cryopreserved-thawed embryos. A randomized prospective study. *Hum Reprod* 19: 2258-2262.
33. Kung FT, Lin YC, Tseng YJ, Huang FJ, Tsai MY, et al. (2003) Transfer of frozen-thawed blastocysts that underwent quarter laser-assisted hatching at the day 3 cleaving stage before freezing. *Fertil Steril* 79: 893-899.
34. Simón C, Gutiérrez A, Vidal A, de los Santos MJ, Tarín JJ, et al. (1994) Outcome of patients with endometriosis in assisted reproduction: results from in-vitro fertilization and oocyte donation. *Hum Reprod* 9: 725-729.
35. Nadir Ciray H, Bener F, Karagenc L, Ulug U, Bahçeci M (2005) Impact of assisted hatching on ART outcome in women with endometriosis. *Hum Reprod* 20: 2546-2549.
36. Hershlag A, Paine T, Cooper GW, Scholl GM, Rawlinson K, et al. (1999) Monozygotic twinning associated with mechanical assisted hatching. *Fertil Steril* 71: 144-146.
37. Schieve LA, Meikle SF, Peterson HB, Jeng G, Burnett NM, et al. (2000) Does assisted hatching pose a risk for monozygotic twinning in pregnancies conceived through in vitro fertilization? *Fertil Steril* 74: 288-294.