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

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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 piezomicromaniupulation. 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 40μm 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 40μm ZP thinning, and 57 were in the AH group with 80μm 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 80μm AH than the 40μm 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 μm (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.

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