Laser assisted zona hatching: What is the evidence to justify its use?

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1. Introduction

Hatching of the human embryo is necessary for endometrial implantation. Therefore, this step necessarily has to occur in all successful pregnancies (1). Problems with the hatching process are thought to be a core reason for pregnancy failure in assisted reproductive technologies (ART) cycles (2). ART, particularly in vitro fertilization (IVF), has been thought to be associated with a thickened or dense zona that decreases the likelihood of embryo hatching (3). Specifically, advanced maternal age, repeated implantation failure, poor embryo quality, IVF culture environment, and cryopreservation have all been cited as factors that may be associated with a decreased rate of unassisted hatching (3,4).

Assisted hatching (AH) was devised in an attempt to artificially thin the zona pellucida (ZP) of cleavage embryos or blastocysts before embryo transfer, thus aiding the hatching process (1,3). In 1990, Cohen and colleagues described the use of micromanipulation to promote hatching following IVF by introducing an artificial incision in the ZP of embryos just prior to replacement in the uterus (5). This process was named ‘assisted hatching’ (5). This 1990 report was the first to show that AH confers a favorable impact on pregnancy (5). Since that time, multiple methods for AH have been explored. Protocols have been described that create a full thickness hole through the entire zona, accomplished by mechanical manipulation (often with either a glass microneedle or piezomicromanipulator), chemical application (often with acidified Tyrode’s solution), or laser (1,2). Other methods describe thinning the zona, accomplished through proteolytic enzymes, acidified Tyrode’s solution, or laser (2,4).

2. Controversies

The role of AH has been the source of much debate. Irrespective of the technical approach, AH remains a procedure of largely unproven worth, regardless of how theoretically appealing it might seem to apply to poorer prognosis IVF cases (6). While some centers have adopted the practice of routinely performing assisted hatching, others do not believe that this technology confers a significant clinical benefit (7). Two meta-analyses of studies evaluating potential benefits of AH reported significant heterogeneity among study results, suggesting that effects of AH may differ depending on patient characteristics (8). Both concluded that there is strong evidence that AH increases pregnancy rates among patients with a history of previous IVF failures (8). An in vitro observation of over 300 research embryos left in culture till day-9 of development showed a distinct reduction in both blastocyst formation and hatching relative to increased age of the patients (6). This observation suggested that a compromised ZP may play a role in restricting blastocyst hatching both in older women and in couples whose embryos develop more slowly (6). However, it remains uncertain whether AH is beneficial to other patient populations. The belief by many that AH has a role to play in improving pregnancy rates in certain patient populations has led to a number of trials evaluating the efficacy of AH in a variety of different clinical situations (2).

A recent Cochrane review evaluated 28 AH trials encompassing 1229 clinical pregnancies and 3646 women (2). The results from this review concluded that assisted hatching may not only improve clinical pregnancy rates but may also carry a risk of an increased incidence of multiple pregnancies. This review did not find a statistical advantage to one method of AH versus another. There are numerous recent studies that do indicate an advantage to assisted hatching in the properly selected clinical indications, such as patients whose embryos have a thickened zona, patients with elevated FSH, older patients, and patients using cryopreserved embryos (6,8). On the other hand, the benefit of AH in good prognosis patients is less clear (4,8). Furthermore, one could argue that the evidence used to justify the application of AH to poorer quality embryos appears circumstantial (6).
Accumulating data shows advantages to utilizing the laser method in assisted embryo hatching. One recent study randomized 316 women undergoing IVF/ICSI to either laser or mechanical AH on day-3 embryos (3). This study showed significantly higher implantation rates with increased, but not statistically so, rates of subsequent clinical pregnancy in the laser AH group (3). Another study directly comparing acidified Tyrode’s solution to laser AH found no difference in clinical or ongoing pregnancy rates with respect to the AH method used (9).

3. Laser assisted hatching

Laser-assisted hatching uses a highly focused laser beam, often an infrared diode laser, to remove or weaken the ZP of the embryos in precise increments. Application of this technology for AH became more frequent in the recent decade for a number of reasons.

3.1. Less technical demands

Other techniques, such as the use of acidified Tyrode’s solution and mechanical manipulation, have substantial practical drawbacks. The use of acidified Tyrode’s solution exposes the embryo to potentially damaging compounds and necessitates that the embryo be thoroughly washed after the procedure (10). Mechanical opening of ZP is possible, and avoids the problems encountered with potentially lingering chemicals. However, this technique can be technically demanding and inconsistent unless undertaken by very skilled hands. Mechanical dissection of ZP is also not sufficiently precise to enable partial ablation of ZP (6). In contrast, laser technology allows the operator, with the assistance of a specialized computer program, the ability to determine the desired target position and energy concentration of the laser beam with a high degree of specificity, maximizing precision and minimizing collateral damage to adjacent cells (3,9). Therefore, laser presents an ideal tool for microsurgical procedures that minimizes interoperator variation.

3.2. Time saving

Laser AH may be performed in significantly less time when compared to other methods. This time savings is of particular importance as some believe that the time an embryo spends outside the incubator may be correlated with decreased pregnancy success rates (3). Therefore, there could be a survival benefit to the embryo when the laser method is used compared to other methods of AH that expose an embryo to increased periods of time outside the optimized environment of the incubator.

3.3. Less invasive

Many feel that the laser method represents the least invasive method of accomplishing AH. Laser AH allows the operator to complete the hatching procedure with noncontact manipulation of the embryo. This minimizes mechanical injury to the embryo that is more likely with some other methods of AH (3). Additionally, the laser method of AH avoids the possibility of chemical injury which is a concern with the use of acidified Tyrode’s solution (3). Furthermore, the wavelength of light emitted from lasers used in AH is far from the absorption peak for DNA (9). Therefore, the specificity of this wavelength minimizes disruption of embryonic DNA during the AH process and decreases the likelihood of mutagenic affect on the embryo (9).

3.4. Clinical data

The most compelling reason to utilize laser for AH is the clinical data associated with its use. Conflicting data do exist regarding the optimal method of performing AH (1). However, many recent trials seem to show that the laser may be superior to other methods of performing AH. A prospective randomized controlled study documented a significant increase in implantation and pregnancy rates following laser AH and removal of degenerated blastomeres (11). Another prospective study by Makrakis et al. compared laser versus mechanical assisted hatching and found that the implantation rate was significantly higher in the laser AH group (3). Clinical and viable pregnancy rates were also higher, though not significantly so, in the laser AH group. A prospective randomized study by Valojerdi et al. concluded that laser AH improved pregnancy and implantation rates with frozen-thawed embryos (7). Multiple animal models have shown increased rates of embryo hatching and no detectible embryonic damage with the use of laser hatching (9).

The optimal size of zona thinning accomplished during laser AH has also been evaluated. A study found a higher clinical pregnancy rate when using the laser to thin an extended area of the ZP compared with producing a single full thickness hole (11). In another study, 120 vitrified-warmed cleavage-stage embryos were randomly subjected to either laser AH of one quarter or one half of the zona (10). The results of this study showed significant increases in implantation rates as well as clinical pregnancy rates in the embryos that had undergone the one half laser treatment (10).

Several drawbacks, both real and theoretical, do exist regarding the use of laser AH. Firstly, the equipment needed to reliably and accurately perform the procedure may be financially costly (3). There are also concerns regarding inherent dangers of using laser to accomplish AH. Some have postulated that the heat associated with the laser may raise the cellular temperature, leading to deleterious effects on developing embryos and induction of complications such as heat shock (9,10). To address these concerns, several modifications to the technology utilized in laser AH have been introduced. The use of short pulses of laser energy as opposed to a continuous beam has been proposed as a mechanism of minimizing the heat effect (9). It has been suggested that opening the zona with an infrared 1.48-μm diode laser beam provides a safe and rapid method for performing assisted embryo hatching without negative impacts, even in largely expanded blastocysts (9,11). The safety of the 1.48-μm diode laser beam has also been evaluated in mouse and human oocytes and zygotes (11). For similar reasons, others have suggested that the Zilos-tk laser at 300 μs be broadly used with all thawed embryos following cryopreservation (6).

Despite theoretical concerns regarding the laser technique, multiple studies have failed to demonstrate an increased rate of congenital or chromosomal abnormalities in children that were, as embryos, exposed to AH by laser (3,9). The increased rate of multiple gestations seen with IVF in general is a grow-
ing concern. It is known that there is an increased rate of twinning associated with AH (9). However, when comparing the available techniques used to accomplish AH, the rates of twinning are similar regardless of the AH techniques utilized (9).

In the appropriately selected situation, AH may confer a clinical benefit. Mechanical manipulation and the use of acidified Tyrode’s solution have been successfully shown to be effective in accomplishing AH, but there are substantial disadvantages to their use. In contrast, the use of laser AH accomplishes the same or superior results with significant time savings, minimal embryo manipulation, and less technical expertise required for the individual performing the procedure. Furthermore, data does not suggest that theoretical embryo damage that could be caused from laser manipulation translates into deleterious outcomes. Additional benefits of laser systems for ZP ablation include applications for embryo biopsy procedures, either for the purpose of removing degenerated blasomeres or for preimplantation genetic diagnosis (PGD) (6). For these situations, this hands-free technology enables a much faster and more convenient approach to opening the ZP than either chemical or mechanical means (6).

4. Conclusion

Since the inception of IVF, a series of technological and methodological improvements have resulted in a significant increase in pregnancy rates. In 2008, the American Society for Reproductive Medicine (ASRM) published a committee opinion stating that AH is not universally beneficial but may have clinical usefulness in specific, well-defined groups (1). In certain patient populations there are still significant challenges in achieving high clinical pregnancy rates. Many modalities, including AH, offer possible solutions that may positively impact some patient populations that are at increased risk for failed IVF cycles. With regard to AH, applying the techniques appropriately is of paramount importance. Of the various technologies available to accomplish AH, adopting the most user-friendly laser systems have been suggested (6). Ultimately, future trials will likely offer guidance in further defining the optimal candidates for this and other emerging technologies.

The following encompass our recommendations regarding AH:

1. AH should not be broadly performed in all embryos
2. Certain circumstances in which AH may be considered are as follows:
   a. Advanced maternal age
   b. Repeated implantation failure
   c. Poor embryo quality
   d. Embryo cryopreservation
   e. Thickened zona (> 13 µm)
   f. Other “poor prognosis” patients
3. The use of laser should be the modality of choice when performing AH.

References


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1. Introduction

Implantation rate per embryo transfer in IVF/ICSI programs is 10–15% for day 2 or day 3 transfers and 23–25% for blastocyst transfers. The ability of an embryo to develop and implant primarily relates to the intrinsic characteristics of the embryo, such as its chromosomal constitution and the quality of its cytoplasm. However, some proportions of euploid embryos with full developmental potential fail to implant because of hatching difficulties (1). Different approaches to improve the implantation rate have been proposed e.g., improving the tech-