OPINION

Assisted hatching: routine or selective application in IVF

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ABSTRACT

The implantation rate of apparently normal embryos in IVF/ICSI programs has been reported to be 10% to 15% for day 2 or day 3 transfers and 23% to 25% for blastocyst transfers. The hatching process of blastocysts is still not completely understood. In vitro culture of mammalian embryos alters the architecture of their zona pellucida; in vitro derived embryos have thicker zonas, especially the inner layer of zona pellucida. A high proportion of morphologically normal blastocysts have hatching difficulties, and 54% fail to hatch after 8 days of in vitro culture. Assisted hatching was developed as a remedy for this type of implantation failure. Several techniques for assisted hatching have been introduced over the years including; drilling a hole in the zona pellucida, three dimensional partial zona dissection, thinning the zona pellucida, or total removal of the zona. These techniques can be performed chemically, mechanically, by using a LASER beam or a piezoelectric method. Although routine performance of assisted hatching on all embryos in IVF/ICSI patients is neither scientific nor appropriate, there is convincing evidence in the literature that assisted hatching increases the implantation capability of some of the embryos. The proposed indications for assisted hatching are advanced maternal age (≥37 years), elevated basal FSH of women, 2 or more previously failed IVF attempts, embryos with thick zona pellucida (>15 μm), abnormal or poor embryo morphology and retarded developmental rate of the embryo.

Key words: Assisted hatching, Zona pellucida, implantation, LASER, Zona drilling, Tyrode's solution

The ability of an embryo to develop and implant primarily relates to the quality of originating gametes and intrinsic characteristics of the embryo, such as its chromosomal constitution and the quality of its cytoplasm. However, some proportion of euploid embryos with full developmental potential fail to implant because of hatching difficulties. It has been reported that implantation rate per embryo transfer in IVF/ICSI programs is 10% to 15% for day 2 or day 3 transfers and 23% to 25% for blastocyst transfers (1).

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The hatching process of blastocysts is still not completely understood. The zona pellucida (ZP), which is a glycoprotein coat surrounding the embryo, begins to form in early antral follicles by the contributions of both oocyte and granulosa cells. After fertilization, zona pellucida is essential for maintaining the integrity of the pre-compacted embryo and prevents dispersion of blastomeres during early cleavage before the junctional complexes between blastomeres occur prior to compaction. Before hatching, the blastocyst undergoes a series of contractions and expansions that cause a decrease in the thickness of the zona until it becomes almost invisible. In mice, the zona pellucida is digested by the synthesis of the enzyme...
trypsin in localized sites of trophectoderm, whereas a similar enzyme may be produced over the whole of its surface by human blastocysts (2-5). Expansion of the blastocyst seems not to be the predominant factor in shedding of the zona pellucida (3,4). Pulsation of the blastocyst during the hatching process was suggested to be nonphysiologic (6), and the collapse of blastocyst cavity may reflect hatching difficulties of the embryo (6,7). In successful hatching, the movement of the embryo plays a crucial role during shedding of the zona pellucida (8). Treatment of blastocysts with cytochalasin B, an inhibitor of actin filament polymerization, reversibly blocked the process of hatching.

In vitro culture of mammalian embryos alters the architecture of their zona pellucida; in vitro derived embryos have thicker zonas, especially the inner layer of zona pellucida (9). A high proportion of morphologically normal blastocysts have hatching difficulties, and 54% fail to hatch after 8 days of in vitro culture (7). Several investigators believe that the failure of hatching is due mainly to abnormal changes in the quality of the ZP, such as thickening or hardening with poor elasticity (10,11).

These conditions are observed in embryos from patients ≥ 37 years old and in those with elevated serum FSH concentrations (12,13) and are induced by a long-term exposure of embryos to suboptimal in vitro culture conditions (14,15). Recently, embryos obtained from in-vitro maturated oocytes show thickened or hardened ZP due to prolonged exposure to in vitro culture conditions.

Assisted hatching was developed as a remedy for this type of implantation failure. In 1989, Cohen and associates reported an increased implantation rate following mechanical opening partial zona dissection (PZD) of the zona pellucida in embryos resulting from IVF (16). These investigators postulated that the opening of the zona might enhance the subsequent hatching process. Cohen et al. subsequently published a randomized, prospective trial of selected assisted hatching 72 hours post-retrieval (zona drilling with acidified Tyrode’s medium), which suggested an improvement in implantation rates when the procedure was selectively applied to embryos with a poor prognosis (based on zona thickness, blastomere number, fragmentation rates and maternal age) (12).

Several techniques for assisted hatching have been introduced over the years including; drilling a hole in the zona pellucida (12,13,17-23), Three Dimensional Partial Zona Dissection (24), thinning the zona pellucida (25,26), or total removal of the zona (27,28).

These techniques can be performed chemically (using acid Tyrode's solution), (12,18,21-23,27), mechanically (using special tapered micropipettes) (13,17,20), or by using a LASER beam (19,29). A piezoelectric technique has also been described (30).

The human zona pellucida is composed of inner and outer layers (31), and superficial chemical zona thinning failed to enhance embryonic implantation (25). A full breaching or sufficient thinning of the inner layer of the ZP is necessary for efficient assisted hatching. However, natural zona thinning and expansion do not occur in blastocysts with drilling treatment, because inner pressure exerted on the ZP is released from the drilling site (32). Moreover, there is a possibility that an extrusion of the blastomere or whole embryo from a large slit may be induced by contractions of the reproductive tract. In contrast, smaller holes of the ZP trap embryos during the hatching process and prevent their implantation (32,33).

Taken together, these findings indicated that assisted hatching can exert both facilitating and deleterious effects on subsequent embryonic development depending on several factors, such as zona thickness, the area of thinning treatment, the size of the hole created, mechanical damage to the embryo by manipulation, chemical damage by acid solution, and the technical skill of the operator. For example, an inappropriately small hole may cause the embryo to become trapped during hatching, creation of trophectodermal vesicles, or strangling of the intracellular matrix, which was suggested as the possible reason for the higher rate monozygotic twinning after assisted hatching (34).

There is still no clear-cut consensus over which technique of assisted hatching will be more efficient to increase the implantation rates. Drawbacks to mechanical zona opening are its technical complexity, the inability to produce reproducible uniform openings, and the possibility of embryo
damage (35). Also the use of the acidic Tyrode's solution cannot produce standardized uniform holes. It was suggested that acidic Tyrode's solution is detrimental to human oocytes and embryos, especially to blastomeres adjacent to the opened area (32). However, by using the infrared 1.48-μm diode laser it is feasible to open the zona even in largely expanded blastocysts without visible blastocyst damage. The safety of the 1.48-μm diode laser beam has been evaluated in mouse and human oocytes and zygotes (29). 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 regard to the overall time requirement and can be performed in a sterile environment without any additional micromanipulations.

The routine or universal performance of assisted hatching on all embryos in IVF/ICSI patients is neither scientific nor appropriate. Randomized trials of assisted hatching on all embryos without any selection, revealed that there is no difference in the implantation and pregnancy rates between the treatment and control groups (17,36). In a recently published meta-analysis of randomized controlled studies on assisted hatching for all patients treated with IVF/ICSI (13 studies out of 165 fitted the selection criteria); the results of the meta-analysis could not be accepted because of the heterogeneity of the studies which may be due to the different techniques used or due to different patient populations studied (37).

On the other hand, there is convincing evidence in the literature that assisted hatching may increase the implantation capability of some of the embryos. The proposed indications for assisted hatching are advanced maternal age (≥37 years), elevated basal FSH of women and 2 or more previously failed IVF attempts, embryos with thick zona pellucida (>15 μm), abnormal or poor embryo morphology, cytoplasmic fragmentation and retarded developmental rate (12,13,38-42). Sallam et al. concluded after conducting a series of sensitivity analyses, that assisted hatching improves the pregnancy rate, implantation rate and the ongoing pregnancy rate significantly for patients with poor prognosis treated with IVF/ICSI, particularly those with two or more previous failures (37). This concurs with the recommendations of The Practice Committee of the American Society for Reproductive Medicine after reviewing the different published reports on the role of assisted hatching in IVF suggesting that assisted hatching may be clinically useful and that individual ART programs should evaluate their own patient populations in order to determine which subgroups may benefit from the procedure. The routine or universal performance of assisted hatching in the treatment of all IVF patients appears, at this point, to be unwarranted.

REFERENCES


