Is assisted hatching beneficial in patients with recurrent implantation failures?

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Summary

Purpose: To assess the possible role of assisted hatching in patients with recurrent implantation failure during IVF cycles.
Design: Prospective randomized study.
Setting: IVF unit of an academic medical center.
Patients: Women who underwent IVF after at least three failed IVF-ET attempts.
Interventions: Patients were prospectively randomized to undergo assisted hatching of their embryos prior to their replacement by mechanical partial zona dissection.
Results: The study (assisted hatching) and control groups included 104 and 103 patients, respectively. There were no significant between-group differences in patient age, cause of infertility, mean number of previous IVF trials, number of oocytes retrieved, fertilization rate, or number of embryos transferred. No difference in pregnancy rate was noted on comparison of the whole study group, to the whole control group (21% and 27%, respectively). However, when the results were re-analyzed by age groups, assisted hatching was found to be harmful in the youngest group (< 34 years), significantly decreasing pregnancy rates (15% vs 35%, $p < 0.05$).
Conclusion: Repeated implantation failure alone is not an indication for assisted hatching. Although assisted hatching appears to be effective in a selected group of older patients, in younger patients it may further hamper implantation and should be avoided.

Key words: Assisted hatching; IVF; Repeated implantation failure.

Introduction

The proper nidation of embryos in the uterus depends on their successful expansion and hatching from their acellular glycoprotein coat, the zona pellucida (ZP).

Impairments in this process may be responsible for the comparatively low implantation rates attained in *in vitro* fertilization (IVF) programs [1]. Inadequate or delayed ZP rapture in IVF may be due, in part, to its hardening following prolonged exposure to an artificial environment. This affects its elasticity and susceptibility to enzymatic digestion [2, 3], and in turn, its proper thinning and opening, leading to incomplete hatching.

Several techniques initially introduced to enhance fertilization have been adopted to facilitate hatching of human embryos formed *in vitro*. These range from chemical dissolution [4] and mechanical dissection [5] to laser drilling [6]. All create a restricted breach of the ZP and all have been found to yield similar implantation and pregnancy rates [7]. Today, assisted hatching is offered as a supplementary treatment in IVF protocols to older women [4, 8] and to women with recurrent implantation failures [6, 9]. Some researchers claim it should be used for all embryos prior to transfer [10]. According to the American Society of Reproductive Medicine, however, assisted hatching may be clinically useful, but its routine or universal performance at this point is unwarranted. This conclusion was supported by a recent review of the literature which found that the benefit of assisted hatching for patients of advanced age remains unclear. Furthermore, while assisted hatching significantly increased the pregnancy/implantation rate in patients with previous implantation failures [11], it had no such effect in patients in their first IVF attempt. The aim of the present study was to prospectively compare the effect of assisted hatching on pregnancy rates in patients with recurrent implantation failure.

Patients and Methods

Protocol

The study sample consisted of all patients attending our IVF unit who had already undergone at least three consecutive failed IVF-embryo transfer (ET) treatment cycles and complied with the following criteria: regular ovulatory menstrual cycles (21-35 days), normal endocrine profile, normal uterine cavity and endometrium (evident by hysteroscopy and ultrasound) and at least three embryos for transfer. Patients with male factor infertility, habitual abortions, or clinically relevant systemic disease were excluded. Each patient was thoroughly counseled and provided informed consent prior to participating in the study. The study was approved by the Hospital Committee for Ethics in Human Research.

Ovarian stimulation

Controlled ovarian hyperstimulation in all patients was based on the early follicular-long suppression GnRH-a protocol. When two or more follicles of at least 18 mm in mean diameter were present and the hormonal profile was satisfactory, 10,000 units of hCG were administered. Oocytes were retrieved

Revised manuscript accepted for publication September 11, 2003

transvaginally 34-36 hours later under ultrasound guidance. Transvaginal embryo transfer was performed 48-72 hours after oocyte retrieval. On the day of embryo transfer the patients were randomized to undergo assisted embryo hatching or no treatment. All patients received luteal support with natural progesterone. Clinical pregnancy was defined as visualization of a gestational sac by ultrasound and elevation of serum hCG level.

Assisted hatching procedure and culture conditions:

Gametes and embryos were cultured in IVF medium (Medi-Cult, Copenhagen, Denmark). Assisted hatching was performed in Hepes buffered medium (sperm preparation medium, Medi-Cult) under liquid paraffin oil (Medi-Cult) using the partial zona dissection technique [12]. The highest morphologically graded embryos were selected for transfer, and only patients with three or four grade A or B embryos were included in this study. Prior to their replacement, manipulated embryos were further incubated for 1.5 hr. at 37°C in an atmosphere of 5% CO_2.

Statistical analysis

Statistical analysis was performed with the non-paired Student's *t*-test. Results are presented as means ± SD; $p < 0.05$ was considered statistically significant.

Results

Two hundred and seven women were included in the study, 104 in the study group and 103 in the control group. Their clinical data are shown in Table 1. There were no between-group differences in causes of infertility, patient age, number of previous IVF cycles, number of oocytes retrieved, fertilization rate and number of embryos transferred. According to the morphological assessment, none of the embryos was damaged by the procedure.

No statistically significant difference in pregnancy rate was noted on comparison of the whole group. When the results were re-analyzed by age group, we found a higher, albeit not statistically significant, clinical pregnancy rate in the older patients after assisted hatching than in their untreated counterpart. However, in the younger patients (< 35 years), assisted hatching significantly decreased the pregnancy rate (Figure 1). Opposite trends were noted between patient age and pregnancy rate in the control and study groups (Figure 1).

Table 1. — *Age distribution and treatment results of study (AH) and control groups of patients.*

	AH Group	Control Group
No. of patients	104	103
Age distribution < 35	34	38
Age distribution 35-40	40	34
Age distribution > 40	30	31
No. of embryos replaced	356	369
Mean no. of embryos transferred	3.42 ± 0.4	3.58 ± 0.5
Clinical pregnancy	22 (21%)	28 (27%)

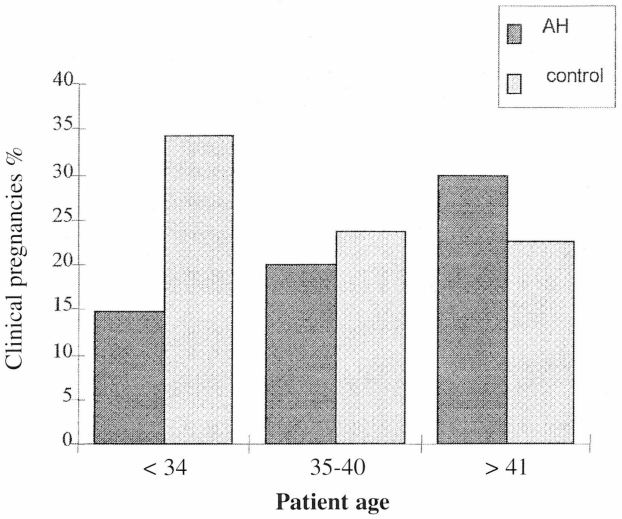

Figure 1. — Effect of assisted hatching on pregnancy rates in IVF patients of different ages.
Dark columns represent assisted hatching groups and light columns control groups. Pregnancy rates significantly decreased compared to controls in younger patients (< 35 years) after assisted hatching (* $p < 0.05$).

Discussion

Hatching is the results of mechanical and enzymatic forces exerted by the embryo and it depends on normal embryonic development. Delayed cell divisions and blastomere loss may delay hatching and cause asynchrony. One study found that murine embryos mechanically damaged by destruction of one of their four blastomeres were hatching-defective and that chemically assisted hatching restored hatching to within normal range [12]. Assisted hatching leads to earlier embryo-endometrium contact and enables earlier implantation [13]. It is a simple, safe and rapid procedure when performed by an experienced embryologist in selected cases.

In the present study, we found no effect of assisted hatching on pregnancy rates in patients with recurrent implantation failure. Our results do not support the conclusion of Veiga and Boiso [11], which was based on published studies which had variable results and whose interpretation was difficult owing to differences in design and patient selection criteria. By contrast, the strength of our study lies in its prospective design and large sample size. Therefore, we suggest that repeated implantation failure alone is not an indication for assisted hatching.

Our higher, though not statistically significant, clinical pregnancy rate in the older patients who underwent assisted hatching compared to those who did not is in agreement with previous studies. Cohen *et al.* [4] found assisted hatching (in their cases by chemical zona drilling) to be most efficient in patients aged > 38 years, and they noted a possible correlation among age, basal

FSH level and physical or chemical changes in the zona pellucida. These findings together with ours imply that during IVF, the hatching process of embryos from older women may be impaired and that this group could benefit from artificial assisted zona hatching. However, in young patients, according to our experience, assisted hatching appears to hamper implantation of good quality embryos. A similar conclusion was reached by Hurst *et al.* [14] who evaluated the contribution of AH in the treatment of IVF patients with a "good prognosis". We recommend that AH be restricted to embryos of older patients (> 40 years) and should not be offered to younger patients.

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