A randomized double-blind controlled study on the efficacy of laser zona pellucida thinning on live birth rates in cases of advanced female age

N. Frydman¹,³, S. Madoux¹, L. Hesters¹, C. Duvernoy¹, E. Feyereisen², A. Le Du¹, G. Tachdjian¹, R. Frydman² and R. Fanchin²

¹Department of Genetic and Reproduction and ²Department of Gynaecology and Obstetric, Hospital Antoine Béclère, Clamart, France
³To whom correspondence should be addressed at: Service de Génétique et Reproduction, Hôpital Antoine Béclère, 157 rue de la Porte de Trivaux, 92140 Clamart, France. E-mail: nelly.frydman@abc.ap-hop-paris.fr

BACKGROUND: It is conceivable that defective embryo hatching plays a part in the mechanisms involved in the decrease of embryo implantation rates with advancing age. In an effort to test this hypothesis, we tested the effectiveness of assisted hatching (AH) in women ≥37 years of age. METHODS: We prospectively studied 103 IVF-embryo transfer patients undergoing 103 embryo transfers. All of them were ≥37 years of age and had <3 previous IVF-embryo transfer attempts. Laser-AH of transferred embryos was either performed (AH group, n = 49) or not (control group, n = 54) according to randomized and double-blind methodology. Primary outcome was live birth rate. RESULTS: Population characteristics were comparable in AH and control groups as well as the mean number of embryos transferred (2.7 ± 0.6 versus 2.7 ± 0.6) and the prevalence of top quality embryos transferred (65 versus 59%, respectively). We failed to find any statistically significant difference between AH and control groups with regard to implantation (16.1 versus 16.7%, respectively) and live birth rates (22.4 versus 29.6%, respectively). CONCLUSION: The present study indicates that AH does not improve IVF-embryo transfer outcome in women aged ≥37 years.

Key words: advanced female age/assisted hatching/implantation rates/live birth rates

Introduction

The zona pellucida (ZP) is an envelope secreted by the oocyte at the secondary follicle stage. It contains a species-dependent sperm receptor, triggers the acrosome reaction and undergoes biochemical modifications called zona hardening that aims to avoid polyspermy (Schiewe et al., 1995a). After fertilization, the ZP protects the blastomeres from external aggression, and it is ruptured at the blastocyst stage to allow embryo implantation in a process called hatching. Hatching is achieved through mechanical expansion and contraction of the blastocyst and the thinning of the ZP (Schiewe et al., 1995b).

In IVF-embryo transfer, abnormalities in the hatching process have been suggested as a possible explanation for the low implantation rates observed in some patients (Malter and Cohen, 1989; Cohen et al., 1992), although this hypothesis remains ill documented. The artificial rupture of the ZP before embryo transfer [assisted hatching (AH)] was proposed to foster spontaneous hatching and improve embryo implantation rates. The AH technique has been used in different indications (Tucker et al., 1991; Olivennes et al., 1994; Stein et al., 1995; Chao et al., 1997; Gabrielsen et al., 2004). One of these is to compensate for the reduction in embryo implantation rates observed in aging women. Yet, the effectiveness of AH to improve IVF-embryo transfer outcome in this group of patients remains controversial (Tucker et al., 1996; Magli et al., 1998; Petersen et al., 2002). Therefore, to clarify these conflicting views, we conducted a prospective, randomized clinical trial to test the efficacy of AH. We have used a quarter laser zona thinning method, with the hypothesis that it may represent a tool for improving live birth rates in cases of advanced female age.

Materials and methods

Patients

We studied prospectively 103 IVF-embryo transfer patients. All patients met the following inclusion criteria: (i) ≥37 years of age; (ii) less than three previous IVF-embryo transfer attempts and (iii) having reached embryo transfer process. Indications for IVF-embryo transfer were male factor (43%), tubal factor (31%), endometriosis (17%) or unexplained infertility (9%). An informed consent was obtained from all women, and this investigation received the approval of our internal Institutional Review Board.
Controlled ovarian hyperstimulation protocol

Women received a time-release gonadotrophin-releasing hormone (GnRH) agonist, (3 mg i.m., Decapeptyl, Beaufour Ipsen Pharma, Paris, France) on cycle day 2. Three weeks later, complete pituitary desensitization was confirmed, and hMG (225 IU/day, s.c.) therapy (Menopur, Ferring Pharmaceuticals, Gentilly, France) was initiated. Daily hMG doses and timing of hCG administration were further adjusted according to the usual criteria of follicular maturation. Administration of hCG (Gonadotrophine Chorionique ‘Endo’), Organon Pharmaceuticals, Saint-Denis, France; 10 000 IU, i.m.) was performed when at least four follicles exceeded 17 mm in diameter and estradiol (E₂) levels per mature follicle (≥17 mm in diameter) were higher than 200 pg/ml. Oocyte retrieval (OR) was performed approximately 36 h after hCG administration by transvaginal ultrasound-guided aspiration.

Fertilization and embryo culture

Oocytes were rinsed and preserved in 3 ml of IVF culture medium (MediCult, Lyon, France) until sperm preparation. According to usual sperm parameters, as described elsewhere (Frydman et al., 2004), we performed conventional IVF or ICSI when necessary. After fertilization, zygotes were cultured in microdrops of 35 µl ISM1 culture medium (MediCult, Lyon, France) at 37°C under 5% CO₂ until the second day of embryo development. Embryos were then graded as A, B, C and D based on their morphology. Embryos with four blastomeres of similar size and no fragmentation were scored A. Those with four blastomeres of similar size, but including 10–20% fragmentation, or showing appropriate developmental stage (three or five blastomeres) were scored B. Embryos with 20–30% fragmentation were labelled grade C, whatever the number of blastomeres. Those having more than 30% fragmentation and those with at least one multinucleated blastomere were scored D. Embryos with grade A and B were considered ‘top quality embryos’.

Assisted hatching

On day 2 after OR, just before embryo transfer, the embryos that were selected for embryo transfer underwent AH (AH group, 49 embryo transfers) or not (control group, 54 embryo transfers) according to a randomization list based on sequentially numbered sealed envelopes. In case of AH, embryos were treated directly in their culture medium (microdrops under oil). For AH, ZP was thinned using a 1.48 µm wavelength (infrared) diode laser (Fertilase™ system; Medical Technologies, ALTDORF, Germany). Each exposure was for 10ms and was initiated at one point and continued along the ZP until 25% was dissolved using a maximum of seven adjacent pulses. Irrespective of AH, before embryo transfer, embryos were placed in a medium containing glycosaminoglycan hyaluronate (UTM MediCult, Lyon, France). Embryo transfers were performed using a Frydman catheter (CCD, France). Patients and clinicians were not aware of study groups (AH or control). Embryo transfers were performed using a sound-guided aspiration.

Statistics

The measure of central tendency used was the mean, and the measure of variability was the standard deviation. Medians and ranges were used when normality of data distribution could not be ascertained. Comparisons between continuous variables from the AH and the control groups were performed using the Student’s t-test when data distribution was normal or the Mann–Whitney U-test when normality could not be confirmed. Qualitative variables were compared using the chi-square test. The present study was powered to detect anticipated differences of 30% in live birth rates at >80% power at 0.05 significance level. This calculation was based on the difference in live birth rate between young (40%) and aged patients (10%) at our centre. A P value <0.05 was considered statistically significant.

Results

Patient characteristics

Patient characteristics in AH and control groups are summarized in Table I. As shown, patient ages, indications for IVF-embryo transfer, day 3 ovarian reserve assessment and the rank of the current IVF-embryo transfer attempt were similar in both the groups. Furthermore, the stimulation regimen and ovarian response were similar in the two groups.

Embryology data and embryo implantation results

Embryology data and embryo implantation results are summarized in Table II. Both groups were comparable with regard to the total number of oocytes retrieved, fertilization rates and embryos obtained. In line with this, the prevalence of top embryos obtained, the number of transferred embryos and the prevalence of top embryos transferred were similar in AH and control groups.

Of 49 attempts included in AH group, 24 were submitted to classical IVF and 25 to ICSI; fertilization rates (160/227, 60 versus 173/244, 71%), clinical pregnancy rates (8/24, 33 versus 9/25, 36%) and implantation rates (10/66, 15 versus 11/64, 17%) were comparable. In the control group, classical IVF was used in 25 attempts and ICSI in 29 cases. Fertilization rates (203/283, 71 versus 143/205, 69%), clinical pregnancy rates (12/25, 41 versus 9/25, 36%) and implantation rates (13/85, 15 versus 12/64, 18%) were comparable. In each group, there was no significant difference in IVF-embryo transfer outcome whether ICSI or classical IVF was used.

Clinical pregnancy rates per OR (gestational sac observed at ultrasound scans at around 7 weeks of amenorrhea) were comparable in the AH and the control groups (34.7 versus 38.8%).

Table I. Population characteristics in the assisted hatching (AH) and control groups

<table>
<thead>
<tr>
<th></th>
<th>AH (n = 49)</th>
<th>Control (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of couples</td>
<td>49</td>
<td>54</td>
</tr>
<tr>
<td>Age (years)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.0 (37.0–42.3)</td>
<td>38.5 (37.0–41.6)</td>
</tr>
<tr>
<td>Basal FSH (UI/l)</td>
<td>6.3 ± 1.7</td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td>Basal inhibin (pg/ml)</td>
<td>85 ± 40</td>
<td>87 ± 39</td>
</tr>
<tr>
<td>Rank of IVF-ET attempt</td>
<td>2.0 ± 1.0</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>Male factor</td>
<td>20 (41%)</td>
<td>25 (46%)</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>16 (33%)</td>
<td>15 (28%)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>8 (16%)</td>
<td>9 (17%)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>5 (10%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>hMG requirement (UI)</td>
<td>2670 ± 684</td>
<td>2817 ± 689</td>
</tr>
<tr>
<td>Day of hCG administration</td>
<td>11.7 ± 1</td>
<td>11.9 ± 1.4</td>
</tr>
<tr>
<td>E2 level (pg/ml)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2920 ± 1230</td>
<td>2492 ± 1100</td>
</tr>
<tr>
<td>Number of ≥17 mm follicles&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9 ± 3</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>Endometrial thickness (mm)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.5 ± 2.1</td>
<td>10.4 ± 2.1</td>
</tr>
</tbody>
</table>

ET, embryo transfer.
P values are not significant.
<sup>a</sup>Medians (range).
<sup>b</sup>On the day of hCG administration.
TABLE II. IVF-embryo transfer outcome in the assisted hatching (AH) and control groups

<table>
<thead>
<tr>
<th></th>
<th>AH (n = 49)</th>
<th>Control (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of oocytes inseminated</td>
<td>9.6 ± 4.4</td>
<td>9.0 ± 4.3</td>
</tr>
<tr>
<td>Prevalence of ICSI</td>
<td>51%</td>
<td>46.3%</td>
</tr>
<tr>
<td>Fertilization rates (95 CI)</td>
<td>70.4% (64.2–76.7)</td>
<td>73.6% (68.6–78.7)</td>
</tr>
<tr>
<td>Number of embryos obtained</td>
<td>6.8 ± 3.8</td>
<td>6.4 ± 3.4</td>
</tr>
<tr>
<td>Prevalence of top quality embryos (95 CI)</td>
<td>37.4% (28.8–46.3)</td>
<td>35.1% (24.9–41.4)</td>
</tr>
<tr>
<td>Number of transferred embryos</td>
<td>2.7 ± 0.6</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>Prevalence of top quality embryos transferred (95 CI)</td>
<td>65.1% (51.9–75.3)</td>
<td>59.2% (45.9–69.1)</td>
</tr>
<tr>
<td>Clinical pregnancy rate per OR* (95 CI)</td>
<td>34.7% (20.8–48.5)</td>
<td>38.8% (25.5–52.3)</td>
</tr>
<tr>
<td>Ongoing pregnancy rate per OR (95 CI)</td>
<td>22.4% (10.3–34.5)</td>
<td>29.6% (17.0–42.2)</td>
</tr>
<tr>
<td>Implantation rate (95 CI)</td>
<td>16.1% (8.6–22.6)</td>
<td>16.7% (10.5–23.1)</td>
</tr>
<tr>
<td>Deliveries</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Live birth per OR (95 CI)</td>
<td>22.4% (10.3–34.5)</td>
<td>29.6% (17.0–42.2)</td>
</tr>
<tr>
<td>Take home babies</td>
<td>15</td>
<td>18</td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, oocyte retrieval.
P values are not significant.
*aPresence of gestational sac with positive heart beat.
b≥12 weeks of amenorrhea.

Implantation rates obtained in the AH and the control groups were also not statistically different (16.1 versus 16.7%, respectively). Accordingly, ongoing pregnancy (>12 weeks of amenorrhea) and live birth rates per OR were comparable in the AH and the control groups (22.4 versus 29.6%, respectively).

Moreover, we compared IVF-embryo transfer outcome between AH and control groups in a subset of patients aged more than 39 years (26 patients in AH and 20 in control). Given that this additional analysis was not powered to compare implantation rates, they were identical (5/72, 6.9 versus 4/57, 7.0%).

Discussion

The present investigation was designed to test the hypothesis that AH might overcome the decline in implantation rates and live birth rates associated with advanced female age. Its methodological characteristics included a case/control, double-blind randomization performed just before embryo transfer of selected women aged ≥37 years. Our results failed to show any beneficial effect of laser ZP thinning as compared to controls in terms of implantation rates (16.1 versus 16.7%, respectively) and live birth rates (22.4 versus 29.6%, respectively).

This prospective randomized trial was spurred by the controversy on the effectiveness of AH for this indication. The benefit of AH for advanced age was first put forth by Tucker et al. (1996). In a prospective study (subgroup analysis result), for a subset of 49 women ≥35 years (31 versus 18 embryo transfer cycles), these authors observed a three-fold increment of embryo implantation rates (12/117, 10.3 versus 2/64, 3.1%; P < 0.01) in women undergoing AH as compared with controls and concluded that AH marginally improves IVF-embryo transfer outcome in older women. Unfortunately, in their study, adequate randomization between women undergoing AH or serving as controls was not performed. This probably unbalanced the population size between AH and control groups and prevented the necessary control of confounding variables. In a subsequent prospective study (subgroup analysis result), Magli et al. (1998) included 87 cases (45 versus 42 embryo transfer cycles) performed in women who were more than 38 years of age. In line with Tucker et al. (1996) data, these authors observed roughly three times as high-implantation rates in the AH group as in controls (11.5 versus 4% P < 0.02). Incidentally, average ages of participants included in this latter study was comparable to those from our present series, although age cut-offs differed slightly. Conversely a third study included 100 cases (50 versus 50 embryo transfer cycles), aiming at testing the effectiveness of AH for advancing age (more than 38 years of age) failed to find any improvement in implantation rates (Petersen et al., 2002). In their study, these authors performed one or two shots of laser to obtain four different holes in the ZP, whereas in our present study, we merely elected to thin the ZP. A meta-analysis of randomized controlled trials was conducted by Sallam et al. (2003). They included the studies of Magli et al. (1998) and Tucker et al. (1996) with other studies aiming at testing the efficacy of AH in cases of thickened ZP and repeated IVF failures. They reported a beneficial effect of AH in patients with poor prognosis, in particular, those with previous IVF failures. These results were confirmed in a systematic review conducted by Edi-Osagie et al. (2003), in which the trials were identified from the Cochrane Controlled Trials Register. Finally, Primi et al. (2004) in a European multicentre prospective randomized study reported a stronger impact of AH in cases of implantation failure after several embryo transfer of high-quality embryos.

The controversy between studies having shown a positive effect of AH for aging women (Tucker et al., 1996; Magli et al., 1998) and those failing to evidence the same effect (Petersen et al., 2002; present data) may be explained by several points. First, whereas Petersen et al. (2002) and our own trial used laser for assisting hatching, both studies which showed a beneficial effect of AH (Tucker et al., 1996; Magli et al., 1998) employed acid tyrode solution to dissolve the ZP. Yet, the effectiveness of laser has been confirmed not only by several teams (Germond et al., 1995; Antinori et al., 1996; Rink et al., 1996) but also by some investigators (Hsieh et al., 2002) who found that laser is even more effective than the acid tyrode method. Unfortunately, by design, our present study was not able to clarify this issue.

The second point, is the possible difference between ZP thinning instead of ZP breachng. The concept of AH using a quarter thinning of the ZP was introduced by Mantoudis et al. (1996). These authors suggested that the methodology for ZP thinning instead of ZP breaching may influence the potential risk for embryo trapping through ZP, embryonic infection, and blastomere loss. Eventually, Blake et al. (2001) demonstrated a significant increase of complete blastocyst hatching rate as compared with control embryos using quarter thinning of the ZP on in vitro cultured human embryos.

Another point that may contribute to explain the lack of efficacy of AH observed in the present study could be the surprisingly high pregnancy and implantation rates obtained in both the groups, although it included only women ≥37 years of age.
It was therefore possible to incriminate the preserved ovarian follicular status in these patients, as a factor that may attenuate the expected beneficial effects of AH. Nevertheless, ongoing pregnancy and live birth rates (22.4 versus 29.6%, respectively) remained modest in AH and control groups as would be expected in a poor prognosis population. Finally, we have compared a subset of patients aged more than 39 years (26 patients in AH and 20 in control); implantation rates between both groups were identical (7.7 versus 7.1%).

It is noteworthy that, although the present study was inadequately powered to detect differences in live birth rates of less than 30%, the close similarity of both the groups regarding this outcome measure supports the hypothesis that AH is ineffective at improving IVF-embryo transfer outcome. However, whereas our results rule out a major beneficial effect of AH in ≥37 years of age, complementary studies powered to detect milder improvements in IVF-embryo transfer outcome with AH in a similar population are formally needed to clarify this issue.

In line with the results of the present study, the only morphological ZP characteristic reported to affect either fertilization (Bertrand et al., 1995) or implantation rates (Garside et al., 1997; Palmstierna et al., 1998; Gabrielsen et al., 2001) is ZP thickness variation (ZPTV). However, ZPTV as well as ZP thickness appear not to be affected by advancing age (Gabrielsen et al., 2001), which suggests that declining embryo implantation rates in this group of women are probably due to additional embryonic factors, as aneuploidy rate that varies from 40 to 63.2% (Kahraman et al., 2000; ESHRE PGD Consortium Steering Committee, 2002; Staessen et al., 2004).

In conclusion, the present study showed similar IVF-embryo transfer outcome in women ≥37 years of age undergoing AH or serving as controls. These results indicate that the putative decline in embryo implantation and live birth rates with advancing age is not circumvented merely by ZP thinning before embryo transfer but probably involves additional embryonic factors.

Further investigations could help to assess effectiveness of AH in aged patients, with more detailed ZP characterization, such as ZPTV measurements or ZP texture assessment (Shen et al., 2005). Finally, whether the combination of advancing female age with other detrimental parameters, such as previous repeated implantation failures (Edi-Osagie et al., 2003; Sallam et al., 2003; Primi et al., 2004), or poor morphologic embryo characteristics should constitute reasonable indications for AH deserves to be demonstrated in additional clinical trials.

References
transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. Hum Reprod 19,2849–2858.


Submitted on July 7, 2005; resubmitted on January 5, 2006, February 27, 2006; accepted on March 24, 2006