Impact of assisted hatching on ART outcome in women with endometriosis

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BACKGROUND: Assisted hatching can improve the implantation rate in cycles with poor outcome. The impact of assisted hatching in embryos from women with endometriosis is not known. Therefore, the hypothesis that the implantation potential of embryos obtained from women with endometriosis can be improved with assisted hatching was tested. METHODS: In a prospective randomized study, transfer embryos obtained from 60 women with endometriosis were hatched using a laser system and compared to embryos obtained from patients with the same diagnosis which were left intact (n = 30). RESULTS: The characteristics of cycles were similar between groups. The pregnancy (40% zona intact, 28.3% assisted hatching), and implantation rates (19.4% zona intact, 17.8% assisted hatching) did not differ in endometriosis cycles regardless of assisted hatching. CONCLUSION: Assisted hatching does not improve outcome in women with endometriosis undergoing assisted reproduction.

Key words: assisted hatching/endometriosis/implantation/IVF outcomes

Introduction
Zona pellucida is a product of the oocyte, and breaching of the embryo from the zona is necessary for implantation. Higher pregnancy and implantation rates have been reported in cycles with poor prognosis when assisted hatching was applied to transfer embryos (Sallam et al., 2003), although other studies claimed no difference in the outcome (Edirisinghe et al., 1999; Mercader et al., 2001). One study recommended routine application of assisted hatching to increase the implantation potential of embryos (Ali et al., 2003). The effects of assisted hatching have been studied in cycles of aged women (Meldrum et al., 1998; Hsieh et al., 2002) with increased basal FSH levels (Schoolcraft et al., 1994), in cycles with previous failed attempts (Scott, 2002; Rufas-Sapir et al., 2004), or in those in which embryos displayed thick zona (Cohen et al., 1992). Whether assisted hatching has any effect on assisted reproduction cycles with other indications has not been studied.

In spite of a great deal of effort over many decades, the mechanisms that lead to infertility in women with endometriosis remain unknown. Patients with endometriosis have been reported to yield fewer oocytes, and have fewer embryos reaching 48 h, which displayed fewer blastomeres (Brizk et al., 1995; Pellicer et al., 1995). During development, a higher rate of arrested embryos is accompanied by a diminished quality, which consecutively yielded low pregnancy and implantation rates (Simon et al., 1994). Pregnancies have also been reported to result in higher early loss in women with endometriosis (Yanuschkovsky et al., 1998). However, others claimed no difference in the extent and presence of endometriosis on clinical pregnancy and implantation rates (Bükülmez et al., 2001; Khamisi et al., 2001). In these studies, fertilization, early development and embryo quality have been demonstrated not to differ in endometriosis compared to cases without known endometriosis. Normal endometrial receptivity has been reported in women with moderate to severe endometriosis who received donor oocytes from women without endometriosis (Simon et al., 1994; Sung et al., 1997; Garcia-Velasco and Arici, 1999; Garrido et al., 2000, 2002). In contrast, oocytes donated by women with moderate and severe endometriosis to women with a normal pelvis seem to have reduced quality, resulting in reduced embryo quality and reduced implantation rates (Pellicer et al., 1994, 2001). These data suggest that endometriosis-associated subfertility is related to oocyte quality, not to endometrial receptivity.

The hypothesis of this study is based on the observation that women with endometriosis have normal endometrial receptivity. It is then suggested that a potential adverse effect of endometriosis on the oocyte and the embryo affects the zona pellucida to the same extent, and that application of assisted hatching may improve the implantation rate as has been reported in cycles with poor outcome.

Materials and methods
This study was designed prospectively in the Assisted Conception Unit of the German Hospital in Istanbul over a period of 6 months.

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The study population consisted of women aged <40 years in whom endometriosis had been diagnosed as the sole infertility aetiology without any coexisting factor. Diagnosis of endometriosis was based on American Society for Reproductive Medicine (1997) criteria and only those exhibiting stages 3–4 were assessed. All endometriosis patients had been operated by laparoscopy ≥3 months before admittance to the infertility treatment. None of the patients had a previous failed trial in our clinic. The study design is given in Figure 1. Women were randomly allocated into two groups in which assisted hatching was applied to all or none of the transfer embryos. The ethics committee of the hospital approved the study and written consent was obtained from the patients. In order to complete the study within a certain time-period, randomization was done at a 2:1 ratio favouring the study group. The admittance number of the patient to the embryology laboratory was randomized with the aid of a computer program. Cycles in which transfer embryos displayed thick zona (≥15 μm) were excluded from the survey.

All patients were stimulated with a GnRH analogue according to the long protocol regime, which has been described previously (Ulug et al., 2004). The embryology procedures have been performed as described previously (Çiray et al., 2004) and ICSI was the universal method of insemination regardless of underlying infertility aetiology, as this was authors’ normal practice. Briefly, embryos were cultured individually in separate 30 μl drops of G1.3 medium (Vitrolife, Sweden) under mineral oil. Transfer embryo selection was performed according to the criteria introduced by Steer et al. (1992) and one to four embryos with the highest scores were transferred. In the presence of good quality embryos, women aged <35 years received two and between 35 and 39 years three embryos. In the presence of poor quality embryos, one more embryo was transferred.

In the ‘assisted hatching’ group (n = 60), a quarter of the zona was thinned (Mantoudis et al., 2001) in all transfer embryos by the same embryologist (F.B.) using a laser system (Fertilase; Medical Technologies SA, Montreux, Switzerland). In the ‘zona intact’ group (n = 30), none of the transfer embryos was hatched.

Embryo transfers were performed on day 3, ~2–3 h after assisted hatching procedure. All patients were prescribed prednisolone (16 mg/day) and tetracycline (100 mg/day) for 5 days, initiating at oocyte retrieval regardless of application of assisted hatching to their embryos. Clinical pregnancy was defined as presence of a gestational sac with accompanying fetal heart beat under ultrasound 4 weeks after embryo transfer. Implantation rate was defined as the ratio of gestational sacs to the number of embryo transfers.

Differences between the groups were analysed by unpaired t-test. Fisher’s exact test was used to compare differences between pregnancy and implantation rates. P < 0.05 was considered statistically significant.

**Results**

A total of 128 pure endometriosis cases (grade 3–4 according to ASRM classification) were admitted to the embryology laboratory during the study period. Of these, 14 were excluded from the survey due to advanced age of the women (≥40 years). In 12 cycles, transfer embryos displayed thick zona (≥15 μm) and 10 cycles did not result in a transfer (Figure 1). As a result, the cycle characteristics of 90 cases were evaluated.

The characteristics of the zona intact (n = 30) and the assisted hatching (n = 60) cycles are given in Table I. The following characteristics of cycles were studied: mean age of women, mean basal FSH, LH and E2 levels, mean ampoules of gonadotrophins consumed for cycles, mean peak E2 levels, and mean endometrial thickness measured at day of embryo transfer. These parameters did not differ between assisted hatching and zona intact groups. The mean basal sperm count of the male partner also remained similar.

The outcome measures of the study were: mean number of collected oocytes, mean ratio of mature and fertilized oocytes, mean number and quality of transferred embryos. None of these parameters differed between groups. The clinical pregnancy and implantation rates are given in Figure 2. The pregnancy rate of zona intact and assisted hatching groups were similar [40% (n = 12) and 28.3% (n = 17) respectively]. Likewise, the implantation rates did not differ between groups [19.4% (n = 14) zona intact and 17.8% (n = 26) assisted hatching].

![Figure 1. Study design.](https://academic.oup.com/humrep/article-abstract/20/9/2546/2356718/2547)
**Table I.** Cycle characteristics and outcome of the present study

<table>
<thead>
<tr>
<th>Zona intact</th>
<th>Assisted hatching</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n (cycles)</strong></td>
<td>30</td>
</tr>
<tr>
<td><strong>Mean age (years, range)</strong></td>
<td>34.0 ± 3.7 (26–39)</td>
</tr>
<tr>
<td><strong>Mean day 3 FSH (mIU/ml)</strong></td>
<td>9.0 ± 5.1</td>
</tr>
<tr>
<td><strong>Mean day 3 LH (mIU/ml)</strong></td>
<td>5.7 ± 2.2</td>
</tr>
<tr>
<td><strong>Mean no. of oocytes retrieved</strong></td>
<td>8.8 ± 6.2</td>
</tr>
<tr>
<td><strong>Mean no. of metaphase II/oocytes retrieved (%)</strong></td>
<td>83 ± 19</td>
</tr>
<tr>
<td><strong>Mean fertilization rate (%)</strong></td>
<td>79 ± 20</td>
</tr>
<tr>
<td><strong>Mean endometrial thickness at embryo transfer (mm)</strong></td>
<td>10.5 ± 1.9</td>
</tr>
<tr>
<td><strong>Mean peak estradiol (pg/ml)</strong></td>
<td>1958 ± 1251</td>
</tr>
<tr>
<td><strong>Mean no. of embryos transferred</strong></td>
<td>2.4 ± 1.0 (1–4)</td>
</tr>
<tr>
<td><strong>Total no. of embryos transferred</strong></td>
<td>72</td>
</tr>
<tr>
<td><strong>Mean transfer embryo score</strong></td>
<td>18.8 ± 6.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
No differences were statistically significant.


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**Figure 2.** The pregnancy (PR) and implantation (IR) rates obtained from the cycles in which embryos were transferred with (AH +) or without (AH −) application of assisted hatching. The values are percentages.

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**Discussion**

The results of the present study showed that the implantation rate is similar in endometriosis cycles when zona pellucida is hatched or left intact. Therefore the hypothesis that endometriosis is an indication for assisted hatching is rejected, and no benefit on the implantation potential is obtained when assisted hatching is applied to embryos of pure endometriosis cases.

Whether application of assisted hatching is of benefit in assisted conception is debatable (Edi-Osagie et al., 2003). However, some studies have shown that assisted hatching improves the pregnancy and implantation rates in cycles with poor prognosis (Sallam et al., 2003), in cycles of aged women (Meldrum et al., 1998; Hsieh et al., 2002), with increased basal FSH levels (Schoolcraft et al., 1994), with previous failed attempts (Scott, 2002; Rufas-Sapir et al., 2004), or in those in which embryos displayed thick zona (Cohen et al., 1992). The assessed cycles of the present study excluded most of these parameters; the effect of women’s age on the outcome was eliminated, as cycles from women aged <39 years were included in the current study. All cycles were first trial patients and had a mean basal FSH value of <9 mIU/ml. With regard to quality of the zona, few studies drew attention to its status in endometriosis cycles. In one study, peritoneal fluid from endometriosis patients decreased sperm binding to the zona pellucida in the hemizona assay (Coddington et al., 1992), which indicated that an impaired fertilization could be observed when IVF is chosen as the method of insemination. The present study employed ICSI and acceptable fertilization rates were obtained. Another study reported a mean zona thickness in endometriosis cases similar to that in cases of tubal or male-factor infertility, and which was thinner than in cases of unexplained infertility (De Mola et al., 1997). The present study excluded endometriosis cycles in which embryos displaying thick zona (≥15 μm) were obtained. Therefore any effect of endometriosis on the quality of zona is eliminated in the present study. On the other hand, regarding endometriosis patients as poor prognosis cycles is debatable. Some studies claimed a poor outcome in cycles with endometriosis (Simon et al., 1994; Brizek et al., 1995; Pellicer et al., 1995; Yanushpolsky et al., 1998), whereas others reported no difference compared to those of patients without known endometriosis (Bükülmeliz et al., 2001; Khamsi et al., 2001). The present study found an implantation rate of 18–20% in women with endometriosis with a mean age of 33–34 years. It may be said that endometriosis cannot be regarded as being associated with poor prognosis cycles according to the results of the present study. The results of our study indicate that the implantation capacity of embryos in endometriosis cases may not be altered by manipulations of the zona pellucida. This finding indicates that breaching of the embryo may not be a problem in endometriosis. Embryo quality and/or endometrial factors are likely to be effective on implantation. However, as the present study obtained similar embryo quality and endometrial thickness from cycles regardless of application of assisted hatching, any effect(s) of endometriosis on embryo and/or endometrium quality on the results is justified.

Finally, it should be stated that the statistical power of the present study is low due to small number of cycles. Hence, to obtain more reliable results, clinical trials with sufficient power are necessary in order to estimate the difference between groups, if one exists.

In conclusion, endometriosis is not an indication for assisted hatching to overcome the possible hazardous effect of the condition on the zona pellucida. Further studies are required to explore the mechanisms that impair the implantation of embryos in endometriosis cases.

**References**


Submitted on January 28, 2005; resubmitted on March 30, 2005; accepted on April 11, 2005