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Assisted Reproductive Technologies

Assisted Hatching—A Meta-Analysis of Randomized Controlled Trials

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***Purpose*:** To conduct a meta-analysis of randomized controlled trials (RCTs) on assisted hatching.
***Methods*:** One hundred sixty-five studies were retrieved from the literature, but only 13 of them fitted our selection criteria. The meta-analysis was conducted using the RevMan software with the Peto-modified Mantel–Haenszel method.
***Results*:** Assisted hatching increases the pregnancy [OR (±95% CI) = 2.51 (1.91–3.29)], implantation [OR (±95% CI) = 2.38 (1.87–3.03)], and ongoing pregnancy rates [OR (±95% CI) = 2.65 (1.85–3.79)] significantly in poor prognosis patients undergoing IVF or ICSI. For patients with repeated IVF failures, the OR (±95% CI) were 2.84 (1.99–4.06) for pregnancy, 2.53 (1.85–3.47) for implantation, and 3.51 (2.12–5.82) for ongoing pregnancy rates, in favor of assisted hatching.
***Conclusions*:** Assisted hatching increases the pregnancy, implantation, and ongoing pregnancy rates significantly in patients with a poor prognosis undergoing IVF or ICSI, particularly those with repeated failures.

KEY WORDS: Assisted hatching; intracytoplasmic sperm injection; in vitro fertilization; meta-analysis; randomized trials.

INTRODUCTION

Despite numerous developments in IVF and ICSI, the implantation rate of the replaced embryos remains low, and it has been estimated that up to 85% of replaced embryos do not implant (1). Numerous approaches to improve the implantation rate have been proposed and practiced. These include (1) improving the technique of embryo transfer, (2) improving endometrial receptivity, and (3) improving the capacity of the embryo to implant.

Assisted hatching has been proposed as a method for improving the capacity of the embryos to implant. Assisted hatching can be achieved by thinning the zona pellucida (2,3), drilling a hole in the zona pellucida (4–13), or total removal of the zona (4). The technique can be performed chemically (using a microjet of acid Tyrode solution), (2,4,7,10–12,14), mechanically (using special tapered micropipettes) (5,6,9), or by using a LASER beam (3,8). A piezoelectric technique has also been described (13).

Assisted hatching was first described in 1990 by Cohen *et al.* (15). This pioneering work was followed by numerous publications. Some of these publications reported a significant increase in the pregnancy and/or implantation rate in all patients (3,14); other publications reported significant improvement in patients with a poor prognosis, namely women over 38 years of age, patients with thick zonae, and patients with repeated implantation failures (3,8,11,13); while a third group of publications reported a nonsignificant

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improvement in the pregnancy rate in patients with poor prognosis (4,5,7,9,14). On the contrary, some publications reported no improvement in pregnancy or implantation rates (2,6,10,12). The aim of this work was to conduct a meta-analysis of randomized controlled trials (RCTs) to evaluate the technique of assisted hatching in IVF and ICSI.

MATERIAL AND METHODS

A meticulous search of the literature was conducted for studies reporting the use of assisted hatching in IVF and ICSI. This consisted of searching the Medline database, the EMBase database, the Cochrane library as well as hand-searching relevant publications and proceedings of international congresses. A combination of the following key words were used in the search: assisted hatching, implantation, randomized controlled trial, IVF, ICSI.

A total of 165 relevant studies were retrieved from the literature. The articles were scrutinized independently by both reviewers and evaluated for inclusion in the meta-analysis using predetermined criteria. To be included in the analysis, the studies had to be prospective and randomized. Moreover, the intervention and control groups had to be similar, the patients had to be analyzed in the same group, and the follow-up had to be complete. The studies were scored independently by the first two authors, and any differences were settled in a consensus meeting with the third author. Out of the 165 studies, 13 fulfilled our predetermined criteria (2–14). A summary of the included studies is shown in Table I.

A meta-analysis of the 13 selected studies was then conducted. The primary outcome measures were the pregnancy rate, the implantation rate, and the ongoing pregnancy rate in all patients undergoing assisted hatching. The secondary outcome measures were the pregnancy, implantation, and ongoing pregnancy rates in patients with poor prognosis (namely women over 35 years of age, patients with thick zona, and patients with two or more previous IVF failures). Sensitivity analysis was also performed to determine the effect of zona thinning, zona opening, chemical-assisted hatching as well as mechanical-assisted hatching on the pregnancy, implantation, and ongoing pregnancy rates.

Pregnancy was defined as the presence of a positive serum pregnancy test (HCG >30 IU/L) 14 days after embryo transfer and/or the ultrasound visualization of at least one gestation sac at 6–8 weeks after embryo transfer. The implantation rate was defined as the number of sacs seen on ultrasound in relation to the number of embryos replaced. The ongoing pregnancy was defined as the ultrasound visualization of a living fetus with a pulsating heart beyond 10 weeks of gestation.

The chi-square test was used to compare qualitative variables, and Student's t test to compare quantitative variables, using the Microstat statistical software. The significance level was set at $P = 0.05$. The meta-analysis was conducted using the RevMan software with the Peto-modified Mantel–Haenszel method and the fixed effect model. Heterogeneity of the studies was tested by performing the Breslow–Day test.

RESULTS

The results of the meta-analysis are shown in Table II. They may be summarized as follows:

Assisted Hatching Versus No Hatching in All Patients

All 13 studies reported the pregnancy rate, 11 studies reported the implantation rate, and 10 studies reported the ongoing pregnancy rate.

1. Pregnancy rate. A total of 381 pregnancies resulted out of 1036 patients with assisted hatching (36.8%) compared to 255 out of 1075 patients with no assisted hatching (23.7%), and this difference is statistically significant ($\chi^2 = 14.956$, $P < 0.0001$). The OR (±95% CI) for the pregnancy rate was 1.59 (1.60–2.37) in favor of assisted hatching. However, because of the heterogeneity of the studies, this result cannot be accepted (Breslow–Day test, $P = 0.048$).
2. Implantation rate. A total of 442 embryos implanted out of 3096 embryos replaced in patients with assisted hatching (14.3%) compared to 288 out of 3152 embryos in patients with no assisted hatching (9.1%), and this difference is statistically significant ($\chi^2 = 38.357$, $P < 0.0001$). The OR (±95% CI) for the implantation rate was 2.38 (1.87–3.03) in favor of assisted hatching.
3. Ongoing pregnancy rate. A total of 242 ongoing pregnancies resulted out of 895 patients with assisted hatching (27.0%) compared to 154 out of 930 patients with no assisted hatching (16.6%), and this difference is statistically significant ($\chi^2 = 28.869$,

Table I. Summary of Included Studies (AH = Assisted Hatching)

Study	Method of randomization	Participants	Intervention	Main outcome
Mansour *et al.* (14)	Not specified	133 patients: 57 AH + 76 no AH including 71 patients with poor prognosis (>40 years or 2+ failed ICSI): 57 AH + 66 no AH	Complete removal of the zona pellucida using acid Tyrode's solution	Pregnancy rate and ongoing pregnancy rate
Nakayama *et al.* (13)	Not specified	248 cycles from 173 patients with >2+ previous failed IVF trials: 126 AH + 122 no AH	Zona thinning using a piezoelectric manipulator (to reduce >75% of the thickness of the ZP)	Pregnancy rate, implantation rate, and ongoing pregnancy rate
Magli *et al.* (11)	Not specified	248 cycles from patients with poor prognosis (>38 years or 3+ failed IVF trials): 135 AH + 113 no AH	Zona opening using acid Tyrode's solution	Pregnancy rate, implantation rate, and ongoing pregnancy rate
Lanzendorf *et al.* (12)	Double-blind, sealed envelops	89 patients >36 years: 41 AH + 48 no AH	Zona opening using acid Tyrode's solution	Pregnancy rate, implantation rate, and ongoing pregnancy rate
Hurst *et al.* (10)	Computer-generated tables	20 patients, first IVF trial (<35 years, normal FSH, endometrium, and semen parameters): 13 AH + 7 no AH	Zona opening using acid Tyrode's solution	Pregnancy rate, implantation rate, and ongoing pregnancy rate.
Chao *et al.* (9)	Computer-generated tables	64 patients with 2+ failed IVF trials: 33 AH + 31 no AH	Zona opening using a micro-needle	Pregnancy rate, and implantation rate
Hellebaut *et al.* (6)	Not specified	68 patients undergoing IVF or ICSI: 36 AH + 32 no AH	Zona opening using a micro-needle	Pregnancy rate, and implantation rate
Antinori *et al.* (3)	Not specified	432 patients: 207 AH + 225 no AH including 200 patients with 2+ IVF failures: 96 AH + 104 no AH	Zona thinning using Er:YAG laser	Pregnancy rate, implantation rate, and ongoing pregnancy rate
Antinori *et al.* (8)	Not specified	170 patients with 2 IVF failures: 72 AH + 98 no AH	Zona opening using a pulsed nitrogen laser	Pregnancy rate, implantation rate, and ongoing pregnancy rate
Tucker *et al.* (7)	Not specified	100 cycles: 50 AH + 50 no AH including 51 cycles in patients >35 years: 31 AH + 18 no AH	Zona opening using acid Tyrode's solution	Pregnancy rate, implantation rate, and ongoing pregnancy rate
Stein *et al.* (5)	Not specified	154 patients with 3+ failed IVF trials: 72 AH + 82 no AH)	Zona opening using a micro-needle	Pregnancy rate
Tucker *et al.* (2)	Not specified	218 patients: 110 AH + 108 no AH	Zona thinning using acid Tyrode's solution	Pregnancy rate, implantation rate, and ongoing pregnancy rate
Cohen *et al.* (4)	Not specified	167 patients: 84 AH + 83 no AH including 30 patients with FSH >15 IU/L: 15 AH + 15 no AH	Zona opening using acid Tyrode's solution	Pregnancy rate, implantation rate, and ongoing pregnancy rate

Table II. Odds Ratios for Pregnancy Rate, Implantation Rate, and Clinical Pregnancy rate for Assisted Hatching Versus no Hatching in Various Groups Studied

	OR (95% CI)	Breslow–Day test
AH vs. no AH in all patients		
PR	1.59 (1.60–2.37)	$P = 0.048$[a]
IR	2.38 (1.87–3.03)	$P = 0.065$
OPR	1.96 (1.54–2.50)	$P = 0.0028$[a]
AH vs. no AH in poor prognosis patients		
PR	2.51 (1.91–3.29)	$P = 0.36$
IR	2.38 (1.87–3.03)	$P = 0.065$
OPR	2.65 (1.85–3.79)	$P = 0.16$
AH vs. no AH in repeated IVF failures		
PR	2.84 (1.99–4.06)	$P = 0.58$
IR	2.53 (1.85–3.47)	$P = 0.35$
OPR	3.51 (2.12–5.82)	$P = 0.77$
ZO vs. no ZO in all patients		
PR	1.89 (1.46–2.44)	$P = 0.062$
IR	1.77 (1.44–2.17)	$P = 0.0002$[a]
OPR	1.80 (1.32–2.47)	$P = 0.0092$[a]
ZO vs. no ZO in poor prognosis patients		
PR	2.47 (1.81–3.37)	$P = 0.21$
IR	2.61 (1.98–3.44)	$P = 0.073$
OPR	2.66 (1.79–3.97)	$P = 0.066$
ZT versus no ZT in all patients		
PR	1.99 (1.43–2.76)	$P = 0.034$[a]
IR	1.49 (1.15–1.92)	$P = 0.15$
OPR	2.66 (1.79–3.97)	$P = 0.0057$[a]
MAH vs. no MAS in all patients		
PR	1.76 (1.01–3.05)	$P = 0.3$
IR	1.59 (0.88–2.87)	$P = 0.081$
OPR	2.11 (1.08–4.13)	$P = 0.21$
CAH vs. no CAS in all patients		
PR	1.58 (1.21–2.08)	$P = 0.075$
IR	1.41 (1.14–1.73)	$P = 0.0023$[a]
OPR	1.49 (1.13–1.97)	$P = 0.077$
CAH vs. no CAS in poor prognosis patients		
PR	2.42 (1.57–3.73)	$P = 0.12$
IR	2.21 (1.54–3.17)	$P = 0.028$[a]
OPR	2.30 (1.45–3.66)	$P = 0.07$
CAH vs. no CAS in repeated IVF failures		
PR	3.59 (1.96–6.56)	$P = 0.92$
OPR	3.23 (1.68–6.22)	$P = 0.098$

Note. Abbreviations: AH = assisted hatching; CAH = chemical-assisted hatching; MAH = mechanical-assisted hatching; PR = pregnancy rate; IR = implantation rate; OPR = ongoing pregnancy rate; ZO = zona opening; ZT = zona thinning.
[a] Results cannot be accepted because of heterogeneity of the studies.

$P < 0.0001$). The OR (±95% CI) for the ongoing pregnancy rate was 1.96 (1.54–2.50) in favor of assisted hatching. However, because of the heterogeneity of the studies, this result cannot be accepted (Breslow–Day test, $P = 0.0028$).

Assisted Hatching Versus No Hatching in Patients with Poor Prognosis

A total of 10 studies reported their results for patients with poor prognosis, namely patients over 35 years of age, patients with embryos with thick zona pellucida, and patients with two or more previous IVF failures. All 10 studies reported the pregnancy rate, 8 studies reported the implantation rate, and 8 studies reported the ongoing pregnancy rate.

1. Pregnancy rate. A total of 206 pregnancies resulted out of 651 patients with assisted hatching (31.6%) compared to 111 out of 672 patients with no assisted hatching (16.5%), and this difference is statistically significant ($\chi^2 = 40.697$, $P < 0.0001$). The OR (±95% CI) for

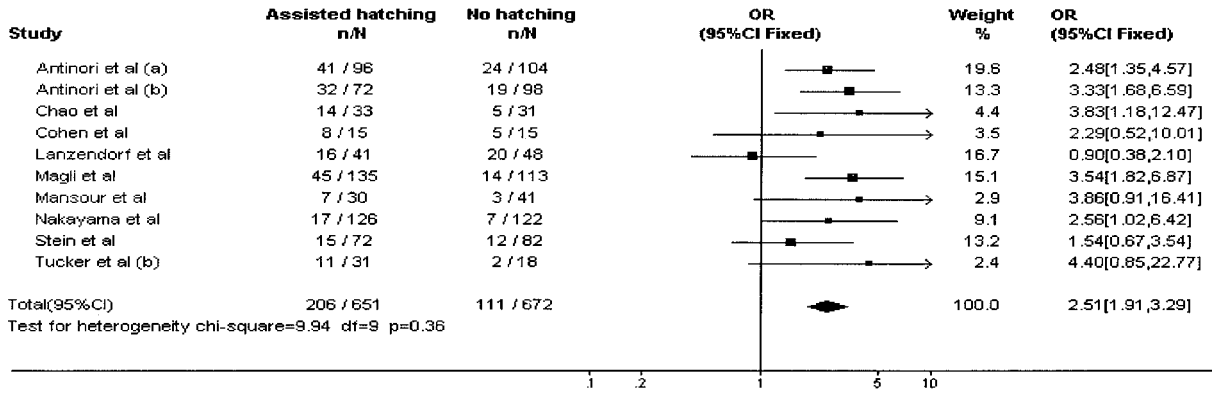

Fig. 1. Tree diagram for pregnancy rates in patients with poor prognosis undergoing assisted hatching versus those with no assisted hatching.

the pregnancy rate was 2.51 (1.91–3.29) in favor of assisted hatching (Breslow–Day test, $P = 0.36$). The results are shown in Fig. 1.

2. Implantation rate. A total of 228 embryos implanted out of 1902 embryos replaced in patients with assisted hatching (12.0%) compared to 110 out of 1966 embryos in patients with no assisted hatching (5.6%), and this difference is statistically significant ($\chi^2 = 48.735$, $P < 0.0001$). The OR (±95% CI) for the implantation rate was 2.38 (1.87–3.03) in favor of assisted hatching (Breslow–Day test, $P = 0.065$). The results are shown in Fig. 2.
3. Ongoing pregnancy rate. A total of 116 ongoing pregnancies resulted out of 546 patients with assisted hatching (21.2%) compared to 53 out of 559 patients with no assisted hatching (9.5%), and this difference is statistically significant ($\chi^2 = 28.606$, $P < 0.0001$). The OR (±95% CI) for the ongoing pregnancy rate was 2.65 (1.85–3.79) in favor of assisted hatching (Breslow–Day test, $P = 0.16$). The results are shown in Fig. 3.

Assisted Hatching Versus No Hatching in Patients with Repeated IVF Failures

A total of five studies reported their results in patients with two or more previous IVF failures and who had assisted hatching. In four studies zona opening was performed with a microject of acid Tyrode solution, while in one study, zona opening was performed with a piezoelectric technique. All five studies reported the pregnancy rate, four studies reported the implantation rate, and three studies reported the ongoing pregnancy rate.

1. Pregnancy rate. A total of 123 pregnancies resulted out of 438 patients with assisted hatching compared to 57 out of 443 patients with no

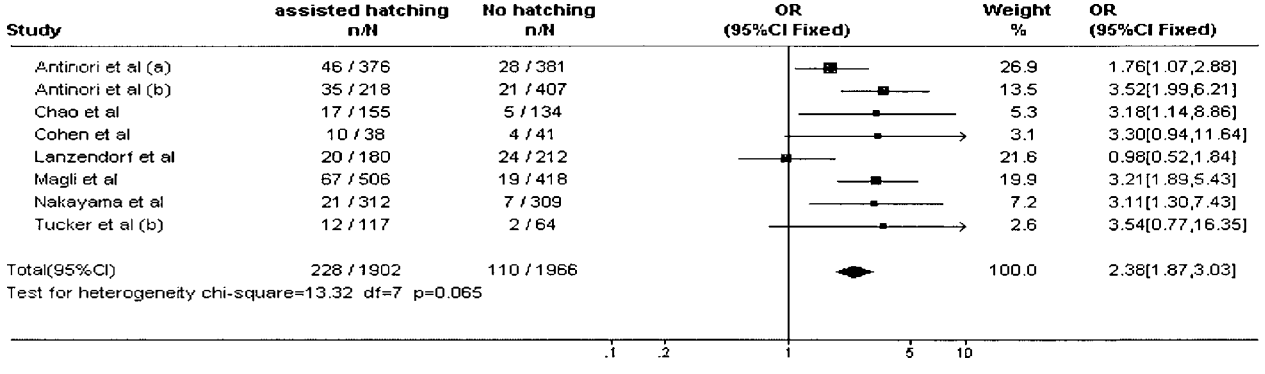

Fig. 2. Tree diagram for implantation rates in patients with poor prognosis undergoing assisted hatching versus those with no assisted hatching.

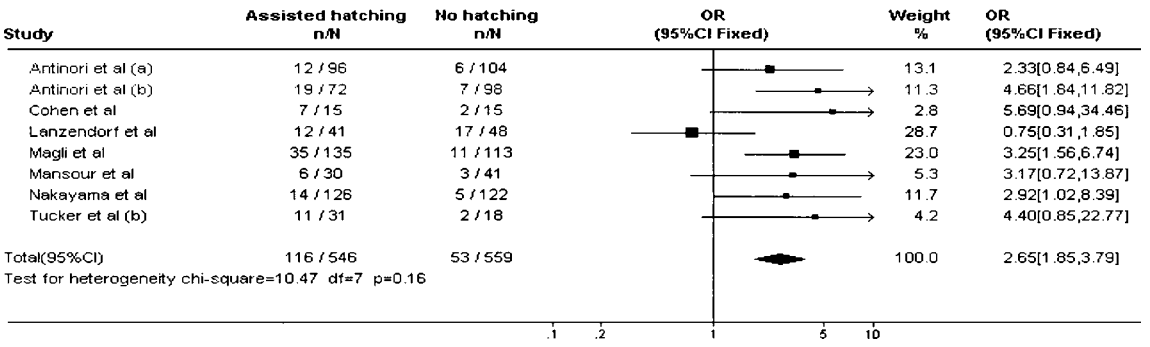

Fig. 3. Tree diagram for ongoing pregnancy rates in patients with poor prognosis undergoing assisted hatching versus those with no assisted hatching.

assisted hatching (12.9%), and this difference is statistically significant ($\chi^2 = 30.435$, $P < 0.0001$). The OR (±95% CI) for the pregnancy rate was 2.84 (1.99–4.06) in favor of assisted hatching (Breslow–Day test, $P = 0.58$). The results are shown in Fig. 4.

2. Implantation rate. A total of 151 embryos implanted out of 1349 embryos replaced in patients with assisted hatching (11.20%) compared to 59 out of 1242 embryos in patients with no assisted hatching (4.8%), and this difference is statistically significant ($\chi^2 = 35.461$, $P < 0.0001$). The OR (±95% CI) for the implantation rate was 2.53 (1.85–3.47) in favor of assisted hatching (Breslow–Day test, $P = 0.35$). The results are shown in Fig. 5.
3. Ongoing pregnancy rate. A total of 68 ongoing pregnancies resulted out of 333 patients with assisted hatching (20.4%) compared to 23 out of 333 patients with no assisted hatching (6.9%), and this difference is statistically significant ($\chi^2 = 24.642$, $P < 0.0001$). The OR (±95% CI) for the ongoing pregnancy rate was 3.51 (2.12–5.82) in favor of assisted hatching (Breslow–Day test, $P = 0.77$). The results are shown in Fig. 6.

Zona Opening Versus No Opening in All Patients

A total of 10 studies performed assisted hatching by opening the zona pellucida mechanically, chemically, using LASER or a piezoelectric technique. All 10 studies reported the pregnancy rate, 9 studies reported the implantation rate, and 7 studies reported the ongoing pregnancy rate.

1. Pregnancy rate. A total of 226 pregnancies resulted out of 662 patients with assisted hatching by zona opening (34.1%) compared to 151 out of 666 patients with no assisted hatching (22.7%), and this difference is statistically significant ($\chi^2 = 20.911$, $P < 0.0001$). The OR (±95% CI) for the clinical pregnancy rate was

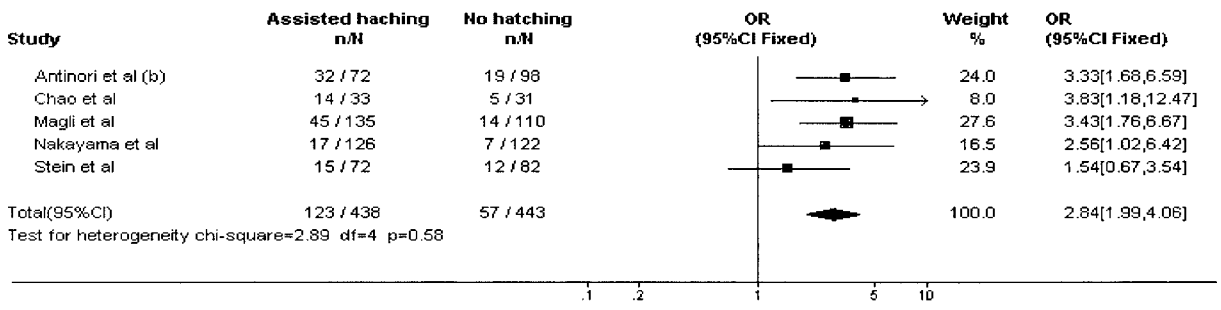

Fig. 4. Tree diagram for pregnancy rates in patients with >2 previous IVF failures undergoing assisted hatching versus those with no assisted hatching.

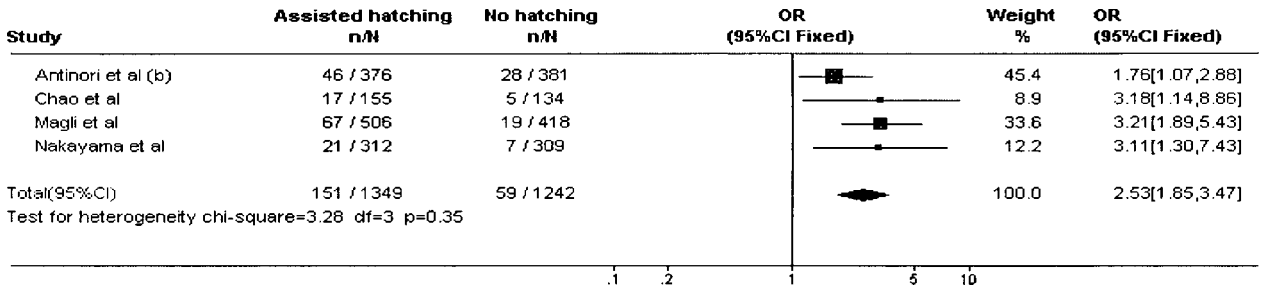

Fig. 5. Tree diagram for implantation rates in patients with >2 previous IVF failures undergoing assisted hatching versus those with no assisted hatching.

1.89 (1.46–2.44) in favor of assisted hatching (Breslow–Day test, $P = 0.062$).

2. Implantation rate. A total of 276 embryos implanted out of 1990 embryos replaced in patients with assisted hatching (13.9%) compared to 172 out of 2048 embryos in patients with no assisted hatching (8.4%), and this difference is statistically significant ($\chi^2 = 30.074$, $P < 0.0001$). The OR (±95% CI) for the clinical pregnancy rate was 1.77 (1.44–2.17) in favor of assisted hatching. However, because of the heterogeneity of the studies, this result cannot be accepted (Breslow–Day test, $P = 0.0002$).
3. Ongoing pregnancy rate. A total of 141 ongoing pregnancies resulted out of 521 patients with assisted hatching (27.1%) compared to 93 out of 521 patients with no assisted hatching (17.9%), and this difference is statistically significant ($\chi^2 = 12.174$, $P < 0.0001$). The OR (±95% CI) for the clinical pregnancy rate was 1.80 (1.32–2.47) in favor of assisted hatching. However, because of the heterogeneity of the studies, this result cannot be accepted (Breslow–Day test, $P = 0.0092$).

Zona Opening Versus No Opening in Patients with Poor Prognosis

A total of eight studies performed assisted hatching by zona opening and reported their results in patients with poor prognosis, namely patients over 35 years of age, patients with embryos with thick zona pellucida, and patients with two or more previous IVF failures. All eight studies reported the pregnancy rate, seven studies reported the implantation rate, and six studies reported the ongoing pregnancy rate.

1. Pregnancy rate. A total of 158 pregnancies resulted out of 525 poor prognosis patients with assisted hatching by zona opening (30.1%) compared to 84 out of 527 patients with no assisted hatching (15.9%), and this difference is statistically significant ($\chi^2 = 28.961$, $P < 0.0001$). The OR (±95% CI) for the pregnancy rate was 2.47 (1.81–3.37) in favor of assisted hatching (Breslow–Day test, $P = 0.21$).
2. Implantation rate. A total of 182 embryos implanted out of 1526 embryos replaced in patients with assisted hatching (11.9%) compared to 82 out of 1585 embryos in patients with no assisted hatching (5.2%), and this difference is statistically significant ($\chi^2 = 44.7914$, $P < 0.0001$). The OR (±95% CI) for

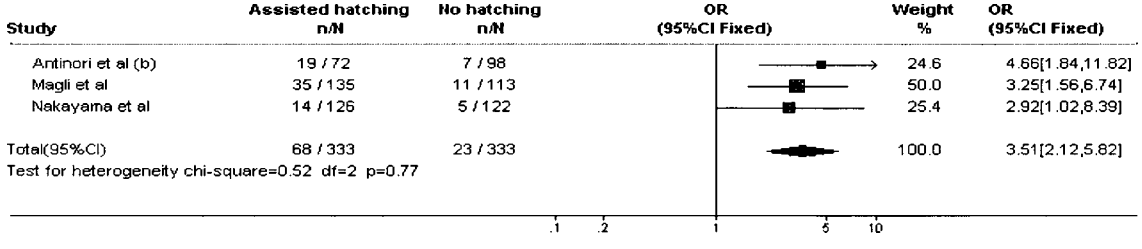

Fig. 6. Tree diagram for ongoing pregnancy rates in patients with >2 previous IVF failures undergoing assisted hatching versus those with no assisted hatching.

the implantation rate was 2.61 (1.98–3.44) in favor of assisted hatching (Breslow–Day test, $P = 0.073$).

3. Ongoing pregnancy rate. A total of 98 ongoing pregnancies resulted out of 420 patients with assisted hatching (23.3%) compared to 44 out of 414 patients with no assisted hatching (10.6%), and this difference is statistically significant ($\chi^2 = 29.444$, $P < 0.0001$). The OR (±95% CI) for the ongoing pregnancy rate was 2.66 (1.79–3.97) in favor of assisted hatching (Breslow–Day test, P = 0.066).

Zona Thinning Versus No Thinning in All Patients

Two studies performed assisted hatching by zona thinning. Both studies reported the pregnancy rate, the implantation rate, and the ongoing pregnancy rate.

1. Pregnancy rate. A total of 136 pregnancies resulted out of 317 patients with assisted hatching by zona thinning (42.9%) compared to 91 out of 333 patients with no assisted hatching (27.3%), and this difference is statistically significant ($\chi^2 = 35.061$, $P < 0.0001$). The OR (±95% CI) for the pregnancy rate was 1.99 (1.43–2.76) in favor of assisted hatching. However, because of the heterogeneity of the studies, this result cannot be accepted (Breslow–Day test, $P = 0.034$).
2. Implantation rate. A total of 166 embryos implanted out of 1106 embryos replaced in patients with assisted hatching by zona thinning (15.0%) compared to 116 out of 1104 embryos in patients with no assisted hatching (10.5%), and this difference is statistically significant ($\chi^2 = 9.658$, $P < 0.002$). The OR (±95% CI) for the implantation rate was 1.49 (1.15–1.92) in favor of assisted hatching (Breslow–Day test, $P = 0.15$).
3. Ongoing pregnancy rate. A total of 85 ongoing pregnancies resulted out of 317 patients with assisted hatching by zona thinning (26.8%) compared to 49 out of 333 patients with no assisted hatching (14.7%), and this difference is statistically significant ($\chi^2 = 13.797$, $P < 0.0001$). The OR (±95% CI) for the ongoing pregnancy rate was 2.66 (1.79–3.97) in favor of assisted hatching. However, because of the heterogeneity of the studies, this result cannot be accepted (Breslow–Day test, $P = 0.0057$).

Mechanical Assisted Hatching Versus No Hatching in All Patients

Three studies performed mechanical-assisted hatching. All three studies reported the pregnancy rate, two reported the implantation rate, and two reported the ongoing pregnancy rate.

1. Pregnancy rate. A total of 44 pregnancies resulted out of 141 patients with mechanical-assisted hatching (31.2%) compared to 29 out of 145 patients with no assisted hatching (20%), and this difference is statistically significant ($\chi^2 = 4.151$, $P = 0.042$). The OR (±95% CI) for the pregnancy rate was 1.76 (1.01–3.05) in favor of mechanical-assisted hatching (Breslow–Day test, $P = 0.3$).
2. Implantation rate. A total of 35 embryos implanted out of 256 embryos replaced in patients with mechanical-assisted hatching (13.7%) compared to 20 out of 220 embryos in patients with no assisted hatching (9.1%), and this difference is not statistically significant ($\chi^2 = 2.002$, $P = 0.157$). The OR (±95% CI) for the implantation rate was 1.59 (0.88–2.87) in favor of mechanical-assisted hatching (Breslow–Day test, $P = 0.081$).
3. Ongoing pregnancy rate. A total of 29 ongoing pregnancies resulted out of 105 patients with mechanical-assisted hatching (27.6%) compared to 17 out of 113 patients with no assisted hatching (15.0%), and this difference is statistically significant ($\chi^2 = 4.442$, $P = 0.035$). The OR (±95% CI) for the ongoing pregnancy rate was 2.11 (1.08–4.13) in favor of mechanical-assisted hatching (Breslow–Day test, $P = 0.21$).

Chemical Assisted Hatching Versus No Hatching in All Patients

Seven studies performed chemical-assisted hatching using a microjet of acid Tyrode solution. All seven studies reported the pregnancy rate, six reported the implantation rate, and seven reported the ongoing pregnancy rate in all their patients.

1. Pregnancy rate. A total of 203 pregnancies resulted out of 490 patients with chemical-assisted hatching (41.4%) compared to 153

out of 485 patients with no assisted hatching (31.5%), and this difference is statistically significant ($\chi^2 = 9.847$, $P = 0.002$). The OR (±95% CI) for the pregnancy rate was 1.58 (1.21–2.08) in favor of chemical-assisted hatching (Breslow–Day test, $P = 0.075$).

2. Implantation rate. A total of 258 embryos implanted out of 1537 embryos replaced in patients with chemical-assisted hatching (16.8%) compared to 183 out of 1424 embryos in patients with no assisted hatching (12.9%), and this difference is statistically significant ($\chi^2 = 13.783$, $P < 0.0001$). The OR (±95% CI) for the implantation rate was 1.41 (1.14–1.73) in favor of chemical-assisted hatching. However, because of the hetrogeneity of the studies, this result cannot be accepted (Breslow–Day test, $P = 0.0023$).
3. Ongoing pregnancy rate. A total of 173 ongoing pregnancies resulted out of 490 patients with chemical-assisted hatching (35.3%) compared to 133 out of 485 patients with no assisted hatching (27.4%), and this difference is statistically significant ($\chi^2 = 6.673$, $P = 0.010$). The OR (±95% CI) for the ongoing pregnancy rate was 1.49 (1.13–1.97) in favor of chemical-assisted hatching (Breslow–Day test, $P = 0.077$).

Chemical Assisted Hatching Versus No Hatching for Patients with Poor Prognosis

Five studies performed chemical assisted hatching using a microjet of acid Tyrode solution and reported their results for patients with poor prognosis, namely patients over 35 years of age, patients with embryos with thick zona, pellucida and patients with two or more previous IVF failures. In four studies, assisted hatching was performed by drilling a hole in the zona, while in one study, the zona was removed completely. All five studies reported the pregnancy rate, four reported the implantation rate, and five reported the ongoing pregnancy rate.

1. Pregnancy rate. A total of 87 pregnancies resulted out of 252 patients with chemical-assisted hatching (34.5%) compared to 44 out of 235 patients with no assisted hatching (18.7%), and this difference is statistically significant ($\chi^2 = 14.646$, $P < 0.0001$). The OR (±95% CI) for the pregnancy rate was 2.42 (1.57–3.73) in favor of chemical-assisted hatching (Breslow–Day test, $P = 0.12$).
2. Implantation rate. A total of 109 embryos implanted out of 841 embryos replaced in patients with chemical-assisted hatching (13.0%) compared to 49 out of 735 embryos in patients with no assisted hatching (6.7%), and this difference is statistically significant ($\chi^2 = 16.535$, $P < 0.0001$). The OR (±95% CI) for the implantation rate was 2.21 (1.54–3.17) in favor of chemical-assisted hatching. However, because of the heterogeneity of the studies, this result cannot be accepted (Breslow–Day test, $P = 0.028$).
3. Ongoing pregnancy rate. A total of 71 ongoing pregnancies resulted out of 252 patients with chemical-assisted hatching (28.2%) compared to 35 out of 235 patients with no assisted hatching (14.9%), and this difference is statistically significant ($\chi^2 = 11.828$, $P = 0.001$). The OR (±95% CI) for the ongoing pregnancy rate was 2.30 (1.45–3.66) in favor of chemical assisted hatching (Breslow–Day test, $P = 0.073$).

Chemical Assisted Hatching Versus No Hatching for Patients with Repeated IVF Failures

Two studies performed chemical-assisted hatching using acid Tyrode solution and reported their results for patients with two or more previous IVF failures. In one study assisted hatching was performed by drilling a hole in the zona, while in the other study, the zona was removed completely. Both studies reported the pregnancy rate and the ongoing pregnancy rate but did not report the implantation rate.

1. Pregnancy rate. A total of 52 pregnancies resulted out of 165 patients with repeated IVF failures and chemical-assisted hatching (31.5%) compared to 17 out of 154 patients with no assisted hatching (11.0%), and this difference is statistically significant ($\chi^2 = 18.512$, $P < 0.0001$). The OR (±95% CI) for the pregnancy rate was 3.59 (1.96–6.56) in favor of chemical-assisted hatching (Breslow–Day test, $P = 0.92$).
2. Ongoing pregnancy rate. A total of 41 ongoing pregnancies resulted out of 165 patients with chemical-assisted hatching (24.8%) compared to 14 out of 154 patients with no assisted hatching (9.1%), and this difference

is statistically significant ($\chi^2 = 12.779$, $P < 0.0001$). The OR (±95% CI) for the ongoing pregnancy rate was 3.23 (1.68–6.22) in favor of chemical-assisted hatching (Breslow–Day test, $P = 0.098$).

DISCUSSION

The introduction of assisted hatching in 1990 by Cohen *et al.* (15) offered an additional tool for assisting implantation in patients undergoing assisted reproduction by IVF and subsequently by ICSI. However, the results of various studies were not consistent and the aim of this work was to conduct a meta-analysis of RCTs to evaluate the technique. We have calculated that, to improve the pregnancy rate from 25 to 35%, taking 5% as the significance level and accepting a 80% probability of finding a true difference, the least number needed to study was 411 treatment cycles in each arm of the meta-analysis. Similarly, to improve the implantation rate from 10 to 15%, the least number needed to study was 822 embryos in each arm. In this meta-analysis, 1036 cycles with assisted hatching were compared to 1075 cycles with no assisted hatching, and 3096 replaced embryos subjected to assisted hatching were compared to 3152 replaced embryos not subjected to hatching.

However, when all patients were grouped together, the results of the meta-analysis could not be accepted because of the heterogeneity of the studies (Breslow–Day test, $P < 0.05$), despite the fact that the ORs were in favor of assisted hatching. This heterogeneity may be due to the different techniques used for assisted hatching or to the different populations studied (patients with good prognosis and patients with poor prognosis).

To clarify the situation, we have conducted a series of sensitivity analyses. Meta-analysis of the results of patients with poor prognosis revealed that this group of patients had a significantly higher pregnancy rate (2.5-fold), implantation rate (2.4-fold), and ongoing pregnancy rate (2.7-fold), compared to those who had no assisted hatching. When patients with repeated (two or more) IVF failures were analyzed together, the results were even better (a 2.8-fold increase in the pregnancy rate, 2.5-fold increase in the implantation rate, and 3.5-fold increase in the ongoing pregnancy rate).

As assisted hatching has been shown to be associated with an increased incidence of monozygotic twinning (16,17), some groups have performed assisted hatching by zona thinning rather than by drilling a hole in the zona pellucida (2,3). However, no RCT has been so far performed to compare both techniques directly. In an attempt to evaluate both techniques, we have analyzed the studies performing zona drilling and zona thinning separately. The results of the meta-analysis show that the results cannot again be accepted because of the heterogeneity of the studies, when all patients are grouped together. However, when patients with a poor prognosis undergoing assisted hatching by zona opening were analyzed separately, an increase of 2.5-fold in the pregnancy rate was observed as well as a 2.6-fold increase in the implantation rate and a 2.7-fold increase in the ongoing pregnancy rate.

It has been suggested that some techniques of assisted hatching may be better than other techniques. In 2002, Hsieh *et al.* compared LASER-assisted hatching to chemical-assisted hatching and reported a significant increase in pregnancy rate, implantation rate, and delivery rate (18). However, this work has not been repeated so far. Other workers have expressed various preferences regarding the various techniques used in assisted hatching on the basis of nonrandomized studies (19,20). In an attempt to evaluate the best method for performing assisted hatching, we have analyzed randomized studies performing chemical-assisted hatching and mechanical-assisted hatching separately. Meta-analysis of the studies performing mechanical-assisted hatching for all the patients showed a 1.8-fold increase in the pregnancy rate, a 1.6-fold increase in the implantation rate, and a 2.1-fold increase in the ongoing pregnancy rate. This compares to a 1.6-fold increase in pregnancy rate for studies performing chemical-assisted hatching for all the patients, as well as a 1.4-fold increase in the implantation rate, and a 1.5-fold increase in the ongoing pregnancy rate. However, proper comparison between both techniques can only be made by conducting a prospective randomized trial.

When patients with poor prognosis undergoing chemical-assisted hatching were grouped together, the increase in clinical pregnancy rate was 2.4-fold, as well as a 2.2-fold increase in implantation rate, and a 2.3-fold increase in the ongoing pregnancy rate. Better results, however, were found for patients with repeated IVF failures treated with chemical-assisted hatching. In this group of patients, the clinical pregnancy rate increased 3.6-fold and the ongoing pregnancy rate 3.2-fold.

It is concluded that assisted hatching improves the pregnancy rate, the implantation rate, and the ongoing pregnancy rate significantly for patients with poor prognosis treated with IVF and ICSI, particularly those with two or more previous failures.

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