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The impact of assisted hatching on live birth rates and outcomes of assisted conception: a systematic review

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BACKGROUND: During the past decade in the UK, only one in six cycles of assisted conception has resulted successfully in a live birth. Assisted hatching (AH) has been proposed to improve outcome. This systematic review of randomized controlled trials addresses primary outcomes of live birth, clinical pregnancy and embryo implantation. METHODS: Trials on post-fertilization disruption of the zona pellucida were identified from the Cochrane Controlled Trials Register, MEDLINE, EMBASE and published bibliographies. Outcomes were analysed using random effects meta-analysis, sensitivity analysis, sub-grouping and meta-regression. RESULTS: Of 23 included trials recruiting 2572 women, only six reported live birth data. AH had no significant effect on live birth (OR 1.21, 95% CI 0.82–1.78). There was a significant benefit of AH on clinical pregnancy (OR 1.63, 95% CI 1.27–2.09), especially in the sub-group of women with previous failure of assisted conception (OR 2.33, 95% CI 1.63–3.34). Meta-regression suggested that AH might be more useful in older women. Implantation data were not considered valid for statistical analysis. The methodological quality of included trials was sub-optimal. CONCLUSIONS: AH probably enhances clinical pregnancy, especially in women with previous failure of assisted conception treatment and in older women; however, trials were of poor quality and so may be biased. Better quality trials reporting live birth are required to confirm any positive effects on the 'take-home-baby rate'.

Key words: assisted hatching/IVF/meta-analysis/randomized controlled trials/systematic review

Introduction

The World Health Organization estimates that one in six couples experience some delay in conception (World Health Organization, 1975), and an increasing number require treatment by the assisted conception procedures of IVF or ICSI. During the past decade in the UK, a total of 38 973 live births was achieved from 241 664 assisted conception cycles (16.1%), increasing only marginally from one live birth in seven cycles in 1992 to one in five today (Human Fertilisation and Embryology Authority, 2000). This has been explained by the low implantation rates (<20%) due to poor embryo quality, poor endometrial receptivity, or both (Denker, 1993; Lopata, 1996).

Cultured embryos develop slowly and poorly *in vitro*, many fail to achieve blastocyst stage or hatch, and implant at lower rates than occur naturally (Harlow and Quinn, 1982; Hsu *et al*., 1999; Mercader *et al*., 2001). Hardening of the zona pellucida resulting from cross-linking of its constituent glycoproteins has been implicated in reduced hatching rates (Cohen, 1991). Zona thickness (influenced by women's age, FSH levels and cause of infertility) has been correlated negatively with embryo implantation rates (Loret De Mola *et al*., 1997). The combination of delayed embryo hatching and advanced endometrial development in assisted conception presents a highly unfavourable environment for implantation.

Assisted hatching (AH) is achieved by zona dissection, drilling or thinning, making use of acid solutions, proteinases, piezon vibrators and lasers (Al-Nuaim and Jenkins, 2002). In general, hatched embryos implant one day earlier than unhatched embryos (Rink *et al*, 1995). The procedure is increasingly offered to older women, those with high FSH levels, higher risk of zona hardening, and following repeated implantation failure (Al-Nuaim and Jenkins, 2002). Considerable uncertainty persists regarding the impact of AH, but reports suggest that it might be associated with higher rates of embryo damage and monozygotic twinning (Hershlag *et al*., 1999). This review was undertaken to determine the impact of AH on live birth, clinical pregnancy and implantation.

Materials and methods

Searching

Relevant trials were identified from the Cochrane Menstrual Disorders and Sub-fertility Group's specialized register of controlled trials, electronic searches of The Cochrane Controlled Trials Register,

MEDLINE and EMBASE (to November 2002) using text and MeSH terms for 'zona pellucida or assisted hatching' and 'randomized or randomized controlled trial'. The searches were not limited by language or publication type (full articles or abstracts), and bibliographies of included trials were searched for further relevant studies.

Selection

All reviewers independently screened the identified abstracts for potential trials, which were retrieved and evaluated for inclusion. Criteria for inclusion were that participants were randomly allocated to AH (post-fertilization therapeutic disruption of the zona pellucida by the known method, including mechanical, chemical or laser) or no AH. The included women underwent only one cycle of treatment and had their own gametes. Trials utilizing mixed groups of hatched and un-hatched embryos were excluded. Primary outcomes included live births, clinical pregnancy and embryo implantation. The consensus definition of implantation (detection of a gestation sac on ultrasound scan) and clinical pregnancy (detection of fetal heart beats on ultrasound scan) were adopted (Human Fertilisation and Embryology Authority, 2000), and data from trials using definitions not conforming to these were excluded from the relevant analyses. Secondary outcomes included miscarriage, ectopic pregnancy, monozygotic twinning, and congenital or chromosomal abnormalities.

Validity and data extraction

Two reviewers (E.E. and L.H.) independently assessed trial quality (method of randomization, adequacy of allocation concealment, blinding, power calculation, intention to treat analysis, publication type and balanced age at baseline) and extracted trial data on forms designed for the review. Disagreements were resolved by discussion with the third reviewer (M.W.S.). Attempts were made to obtain additional information on trial methodology and outcomes from 11 principal authors of the 19 trials located before February 2002.

Quantitative data synthesis

For dichotomous data (such as live births), results for each trial were expressed as numbers of events per woman randomized, embryo transferred or clinical pregnancy, and pooled using random effects methodology (RevMan 4.1 software) where appropriate (discussed later). Heterogeneity between the results of different trials was examined using Cochran's test (assuming statistical significance at $P < 0.1$). It was intended that possible contributions of differences in participant characteristics to any heterogeneity would be investigated through the sub-grouping by age (< or ⩾37 years), serum FSH (< or ⩾8 IU/l), previous failed assisted conception cycles, and zona thickness (< or ⩾12 μm). Where possible, these sub-groupings were directly extracted from included trials; otherwise the mean trial data were used to place the whole trial in one or other subgroup.

The STATA metareg command for random effects meta-regression and a funnel plot to investigate the presence of publication bias were used. Sensitivity analyses were undertaken to examine the stability of results in relation to adequacy of allocation concealment (removing trials with unclear or inadequate allocation concealment), adequacy of randomization (removing trials with an unclear method of randomization), baseline comparability [removing trials where the 95% confidence intervals (CI) for the difference in mean age between the two arms did not include zero or where insufficient information was provided to assess this].

Results

Trial flow

The initial searches identified and screened 371 potentially relevant publications and 23 trials were eventually included in the meta-analysis (Figure 1). Six trials reported live birth, 19 clinical pregnancy and 15 implantation data. Five authors responded to a request for additional information (Hellebaut, Hurst, Lanzendorf, Magli and Olivennes; Table I). Some outcome data were not usable because of confusion over how many women were randomized to each trial group (Olivennes and Hazout, 1997) and lack of clarity about the definition of endpoints (Ryan *et al*., 1997).

Trial characteristics

The included trials were all published in English, recruited a total of 2572 women and utilized more than 8036 embryos. The characteristics of all included trials are detailed on Table I. A funnel plot showed no suggestion of asymmetry due to publication bias (Figure 2).

Methodological quality of included trials

All trials stated that randomized allocation had occurred, 10 used the wording 'randomized' and described a valid randomized method of allocation, while 13 used the wording 'randomized' but did not describe the method of allocation. No trial had adequate allocation concealment (concealment considered unclear in 17 trials and inadequate in six). Three trials employed both participant and assessor blinding, while in 20 trials blinding was either not employed or was unclear. No trial reported a power calculation, and sample sizes ranged from 20 to 225 women. No trial reported an intention-to-treat analysis, and it was generally unclear whether any participants dropped out after randomization. Seven trials were published in abstract form only (Oliveness and Hazout, 1997; Ryan *et al*., 1997; Antinori *et al*., 1999a and b; Isiklar *et al*., 1999; Laffoon *et al*., 1999; Nagy *et al*., 1999;). Eleven trials reported mean age and SD (or SE) in each group with 95% CI that included zero. Four trials presented the appropriate age data with CI values that did not include zero, and eight did not present sufficient age data to allow calculation.

The results of all meta-analyses, sub-groupings and sensitivity analyses undertaken are detailed in Table II.

Live birth

Six trials reported live birth data, with 161 live births from 523 women randomized. There was no significant difference between the AH and control groups in the odds of a live birth (OR 1.21, 95% CI 0.82–1.78, no significant heterogeneity; Figure 3). Sub-grouping was not possible because of the small number of live births reported. Sensitivity analysis on randomization and balanced age at baseline had no effect on the odds of live birth (Table II).

Clinical pregnancy

Nineteen trials reported on clinical pregnancy (722 clinical pregnancies in 2175 women). Meta-analysis demonstrated a significant positive impact of AH on clinical pregnancy (OR

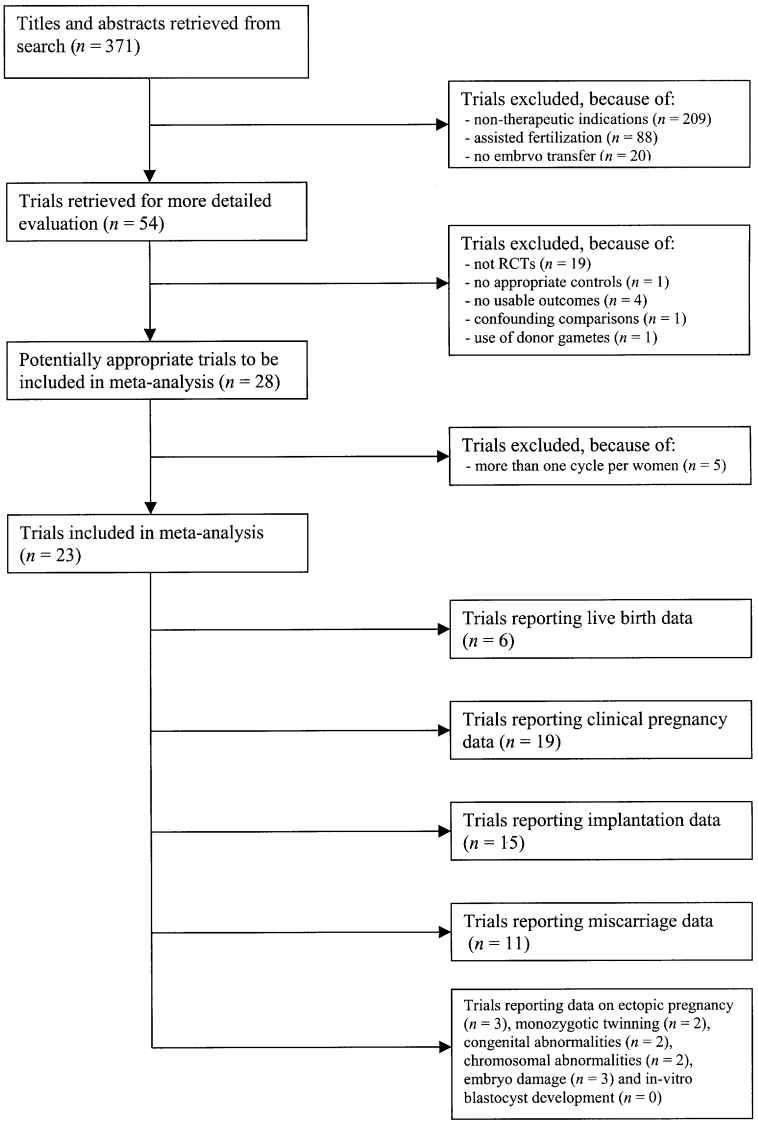

Figure 1. Trial flow diagram.

1.63, 95% CI 1.27–2.09; Figure 4), but with significant heterogeneity ($P = 0.05$). Sensitivity analysis on randomization demonstrated relatively reduced odds of pregnancy (OR 1.34, 95% CI 0.79–2.26), with loss of statistical significance and persisting heterogeneity ($P = 0.03$). Sensitivity analysis on balanced age at baseline demonstrated relatively increased odds of pregnancy (OR 1.81, 95% CI 1.23–2.66), but retained significant heterogeneity ($P = 0.02$).

Sub-group analysis of women with previous failed assisted conception attempts demonstrated a further increase in the odds of pregnancy (OR 2.33, 95% CI 1.63–3.34; Figure 5) and absence of heterogeneity ($P = 0.51$). Similar analysis of women undergoing first cycles of assisted conception demonstrated a less beneficial and non-significant effect on pregnancy (OR 1.40, 95% CI 0.77–2.53) with persisting significant heterogeneity ($P = 0.05$). Only two trials reported sub-groups of women aged >37 years, thereby preventing the exploration of heterogeneity on the basis of age sub-grouping. Meta-regression was instead used to explore the influence of age on the effects of AH on clinical pregnancy. This suggested a greater benefit of AH in older women, although falling just short of statistical significance (slope coefficient 0.05, 95% CI 0.00–0.11, $P = 0.052$). There were insufficient data to perform sub-grouping on the basis of serum FSH levels or zona pellucida thickness.

Implantation

Fifteen trials reported implantation data. Pooling provides an OR in favour of AH of 1.52 for implantation per embryo transferred, but the statistical significance and 95% CI intervals are unclear as none of the trials reported an intra-cluster correlation coefficient. Implantation per women randomized appeared to be significantly improved in women randomized to AH (OR 1.97, 95% CI 1.28–3.03), but with significant heterogeneity ($P < 0.001$) between trials. Sensitivity analyses made little difference to the OR and heterogeneity.

Table I. Characteristics of the included trials (n = 23)

Trial ID	Type	Country	AH method	WomenAH:C	EmbryosAH:C	Reported outcomes
Antinori *et al.* (1996a)	RCTb	Italy	Laser	104:104	376:381	I, CP, M, MP
Antinori *et al.* (1996b)	RCTb	Italy	Laser	104:121	397:411	I, CP, M, MP
Antinori *et al.* (1996c)	RCTa	Italy	Laser	72:98	218:407	I, CP, M, MP
Antinori *et al.* (1999a)	RCTb	Italy	Laser	96:103	221:247	CP, M, MP
Antinori *et al.* (1999b)	RCTb	Italy	Laser	73:69	321:307	CP, M, MP
Baruffi *et al.* (2000)	RCTa	Brazil	Laser	51:52	141:149	I, CP, M
Chao *et al.* (1997)	RCTa	Taiwan	Mech	33:31	155:134	I
Cohen *et al.* (1992a)	RCTa	USA	Chem	69:68	239:229	I, CP, LB, MP
Cohen *et al.* (1992b)	RCTa	USA	Chem	15:15	38:41	I, CP
Hellebaut *et al.* (1996)	RCTa	Belgium	Mech	60:60	168:162	I, CP, LB
Hurst *et al.* (1998)	RCTa	USA	Chem	13:7	52:28	I, CP, LB
Isik *et al.* (2000)	RCTa	Turkey	Chem	24:22	71:63	I
Isiklar *et al.* (1999)	RCTb	Turkey	Mech	22:22	83:78	I, CP, MP
Laffoon *et al.* (1999)	RCTb	USA	Mech	28:28	NS:NS	CP
Lanzendorf *et al.* (1998)	RCTa	USA	Chem	42:52	180:212	I, MP
Mansour *et al.* (2000a)	RCTb	Egypt	Chem	27:25	86:75	CP, LB, M, MP
Mansour *et al.* (2000b)	RCTb	Egypt	Chem	30:41	117:155	CP, LB, M, MP
Nagy *et al.* (1999)	RCTb	Italy	Laser	20:20	65:52	CP
Oliveness and Hazout (1997)	RCTa	France	Chem	NS:NS	NS:NS	CP
Ryan *et al.* (1997)	RCTb	Australia	Chem	100:100	217:217	CP
Stein *et al.* (1995)	RCTb	Israel	Mech	72:82	230:295	CP, M
Tucker *et al.* (1993)	RCTb	USA	Chem	110:108	333:312	I, CP
Tucker *et al.* (1996)	RCTb	USA	Chem	50:50	189:184	I, CP

AH = assisted hatching; C = control group; Chem = chemical means of AH; CP = clinical pregnancy data reported; EP = ectopic pregnancy data reported; I = implantation data reported; LB = live birth data reported; M = miscarriage data reported; Mech = mechanical means of AH; MP = multiple pregnancy data reported; NS = not stated; RCTa = randomized controlled trial, method of randomization clearly stated and valid; RCTb = randomized controlled trial, method of randomization not stated or unclear.

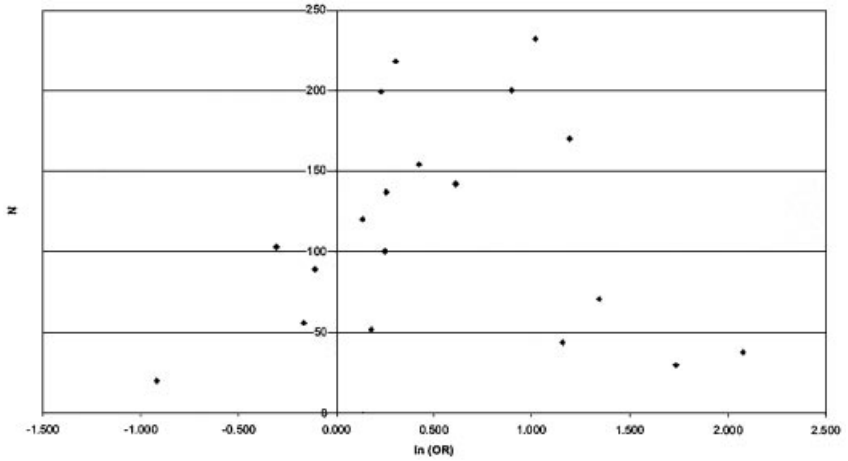

Figure 2. Funnel plot for assisted hatching review (outcome in clinical pregnancy).

Miscarriage

Miscarriage was reported by 11 trials, with 69 miscarriages complicating 453 clinical pregnancies. Meta-analysis yielded an OR of 0.70 for miscarriage per clinical pregnancy in favour of AH and 1.07 for live birth per clinical pregnancy (the converse of miscarriage rates), again in favour of AH. The validity of the 95% CI and statistical significance of these meta-analyses was, however, questionable.

Other outcomes

Data on other outcomes were sparse, with only three trials reporting ectopic pregnancies, two monozygotic twinning, and two congenital and/or chromosomal abnormalities.

Discussion

Trials located for this review collectively provided data on 2572 women and more than 8036 transferred embryos, and were reported from all five continents. The participants demonstrated a good age spread and included women with normal or high FSH levels, good or poor responders, women having first or repeat assisted conception cycles, IVF with or without ICSI, and transfer of fresh or frozen embryos. All three methods of AH were well represented.

The reviewers felt it was of primary interest to determine the overall effect of AH in assisted conception to provide the prime statistic from both provider and consumer perspectives. The review pooled data from all suitable trials irrespective of the

Table II. Results of meta-analysis, sub-groupings and sensitivity analysis

Outcomes	Type of analysis	Description of analysis[a]	No. of trials	Odds Ratios	95% CIof OR	Heterogeneity (*P*)
Live birth per woman randomized	Meta-analysis	Overall	6	1.21	0.82, 1.78	0.43
	Sensitivity	AC	0	–	–	–
	Sensitivity	RCTa	4	1.13	0.72, 1.76	0.37
	Sensitivity	Age stated	4	1.31	0.71, 2.43	0.23
Clinical pregnancy per woman randomized	Meta-analysis	Overall	19	1.63	1.27, 2.09	0.05
	Sensitivity	AC	0	–	–	–
	Sensitivity	RCTa	7	1.34	0.79, 2.26	0.03
	Sensitivity	Age stated	10	1.81	1.23, 2.66	0.02
	Sub-grouping	First AC cycle	4	1.40	0.77, 2.53	0.05
	Sub-grouping	Repeat AC cycle	4	2.33	1.63, 3.34	0.51
Implantation per woman randomized	Meta-analysis	Overall	15	1.97	1.28, 3.03(NSU)	<0.01
	Sensitivity	AC	0	–	–	–
	Sensitivity	RCTa	9	2.10	1.12, 3.92(NSU)	<0.01
	Sensitivity	Age stated	10	2.11	1.35, 3.28(NSU)	<0.01
Implantation per embryo transfer	Meta-analysis	Overall	15	1.52	1.16, 2.00(NSU)	<0.01
Miscarriage per clinical pregnancy	Meta-analysis	Overall	11	0.70	0.41, 1.19(NSU)	0.91
Live birth per clinical pregnancy	Meta-analysis	Overall	6	1.07	0.46, 2.52(NSU)	0.74
Mean age	Meta-analysis	Overall	15	WMD = 0.09 years	–0.24, 0.43	0.05

[a]'AC' indicates removing studies with inadequate or unclear allocation concealment; 'RCTa' indicates removing studies without a clearly stated and valid method of randomization; 'Age stated' indicates removing studies with baseline heterogeneity in mean age between the intervention and control arms. NSU = not statistically useful; WMD = weighted mean difference in mean age between assisted hatching and control groups.

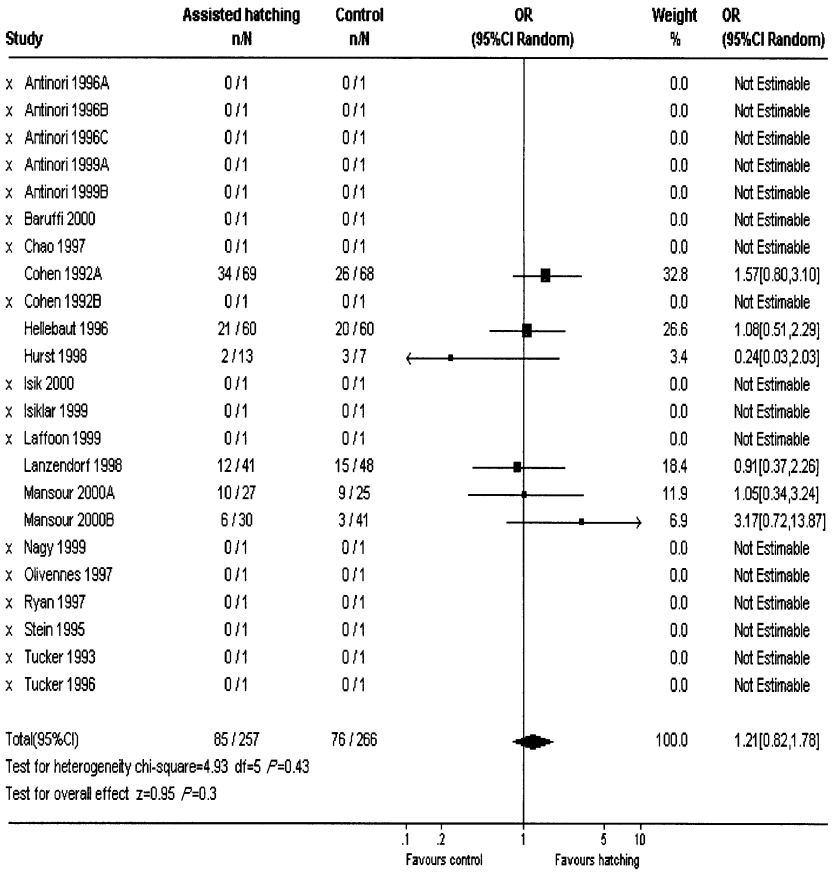

Figure 3. Meta-analysis of live births per woman randomized.

AH method employed, population of women studied or type of assisted conception procedure, before undertaking subgroup analysis in situations of particular interest. The overall methodological quality of included trials was sub-optimal as no trial met CONSORT criteria for the reporting of randomized controlled trials; no trials reported a power calculation, intention-to-treat analysis or adequate allocation concealment. This did not appear to improve with time over the 10-year span

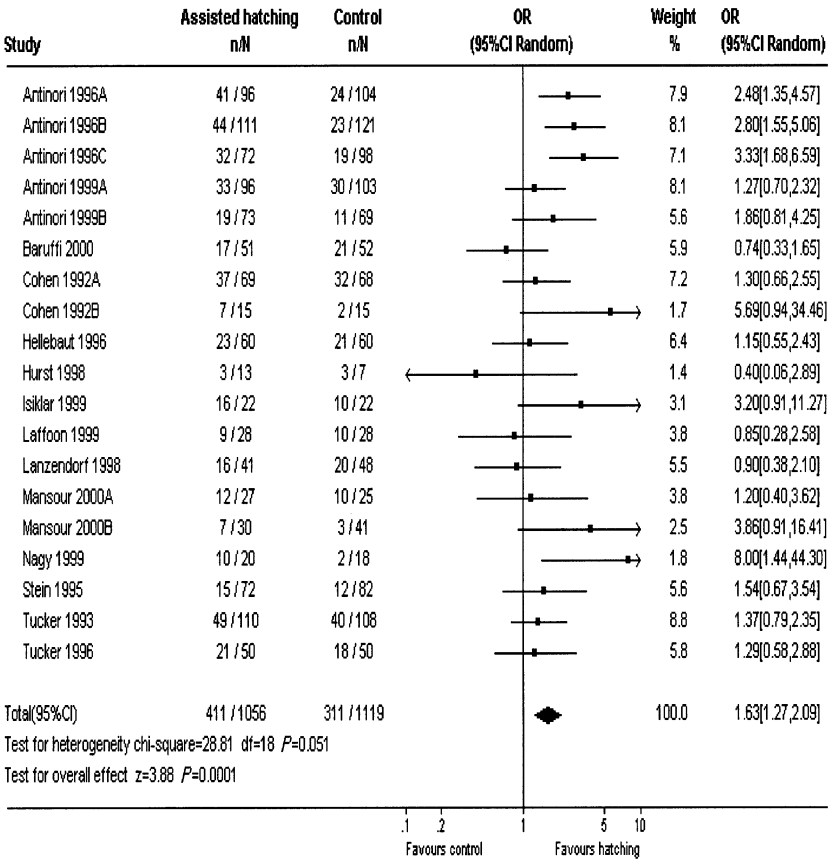

Figure 4. Meta-analysis of clinical pregnancy per woman randomized.

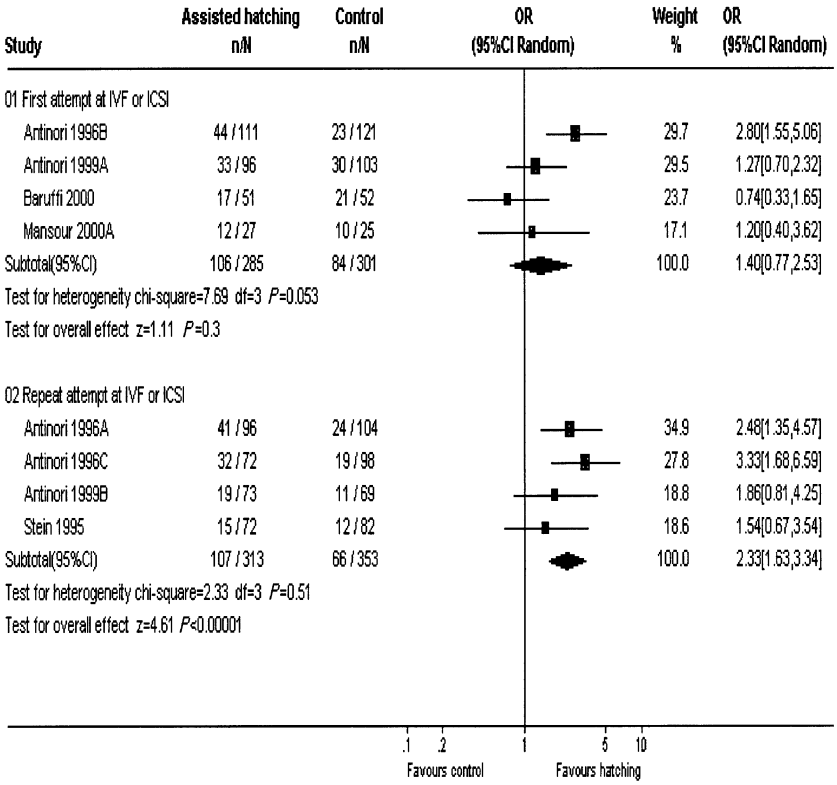

Figure 5. Meta-analysis of clinical pregnancy per woman randomized in first (01) and failed (repeated, 02) cycles of assisted conception.

of the publications (1990 to 2000). Furthermore, only 10 trials described a valid method of randomization, thereby introducing the possibility of further bias to the results.

It was disappointing that the present review was severely limited by paucity of data on the impact of AH on the most sought after outcome of assisted conception, the 'take-home-baby rate'. This probably reflects the gap that currently exists between the practice of assisted conception and clinical obstetrics, and the absence of an electronic database of records and outcomes that would facilitate follow-up of women by authorized agencies (such as the Human Fertilisation and Embryology Authority in the UK). That only six included trials reported live birth data suggested haste on the part of authors to disseminate information that was limited to short-term outcomes. For instance, none of the seven conference abstracts that was included reported live birth data. This should stimulate debate in the medical world about the criteria for reporting and publishing clinical trials of assisted conception. The small amount of existing data suggests that AH has no statistically significant impact on the odds of a live birth, increasing it by an average of 21% (the 95% CI include a reduction of 18% up to an increase of 78%), with significant heterogeneity between trials.

The odds of clinical pregnancy were significantly improved by AH, by an average of 63%, although statistical significance was lost on two of three sensitivity analyses. It was not possible to ascertain from the available evidence whether AH benefits women with high FSH levels, higher risk of zona hardening, or poor response to ovarian stimulation. Sub-group analysis suggested a stronger impact of AH on clinical pregnancy in women after failed cycle(s) of assisted conception (statistically significant OR of 2.33), compared with women undergoing first cycles (OR 1.40). Meta-regression suggested a stronger impact of AH in older women (although falling just short of statistical significance). Whilst additional trials are needed to further explore this trend, the evidence is that AH is most beneficial to women undergoing repeat cycles of assisted conception following previous treatment failure, and possibly the older ones.

Implantation is traditionally expressed 'per embryo transferred', but the pooling of these data for meta-analysis is statistically problematic. Transferring more than one embryo per woman results in an embryo cluster effect, necessitating an intra-cluster correlation coefficient to make the pooling of data meaningful. Otherwise, the larger number of transferred embryos than women randomized would narrow the 95% CI, suggesting statistical significance where there is none. A statistically more valid approach would be to report implantation 'per woman randomized'. This approach is however based on the statistical assumption that not more than one gestation sac is counted in any particular woman, and this is clearly not the case in practice. The reported trials did not provide any information about the numbers of women in whom gestation sacs were detected, neither was any standard deviation presented for the number of gestation sacs detected per woman or per embryo transferred. In analysing implantation 'per woman randomized', the review made the statistical assumption that only one gestation sac was detected per woman, omitting the one trial (Isiklar *et al*., 1999) where the number of gestation sacs detected was greater than the number of women randomized in any arm. In the absence of an ideal way to analyse implantation data, both approaches were used to highlight their pitfalls. Assisted hatching appeared to improve the chance of embryo implantation in any particular woman by 97%, but the statistical significance of this remains questionable.

It was disappointing that the sub-optimal reporting of implantation data prevented investigation of the impact of AH on implantation in different subgroups, particularly women with high FSH levels. It was perplexing that this anomaly had previously not been mentioned in the medical literature despite the very obvious shortcomings of the current method of reporting and analysing implantation data. This problem will not exist if there is universal adherence to a 'one woman-one embryo' strategy, allowing the collation and reporting of implantation data 'per woman randomized'. A temporary solution would be the reporting of intra-cluster correlation coefficients for all trials replacing more than one embryo or counting more than one gestation sac per woman, allowing the collation and reporting of implantation 'per embryo transferred'.

It is hoped that this systematic review generates debate in both provider and consumer groups to explore issues of quality and outcome recording in assisted conception treatment and research, and also leads to use of statistically valid methods of reporting and comparing implantation data.

In conclusion, the sub-optimal quality, flawed reporting and paucity of outcome data weaken the validity and strength of any recommendations from this systematic review and meta-analysis. On the available evidence, AH does not significantly improve the 'take-home-baby rate' of assisted conception. However, it does improve the odds of clinical pregnancy in women undergoing repeat cycles of assisted conception following previous treatment failure, and probably in older women. It is probably justifiable, on the basis of this evidence, to recommend AH to this population. As miscarriage rates are not affected, it can be extrapolated that with an appropriate number of trials reporting usable outcome data, a positive impact would be expected on implantation and live births. This review highlights many unresolved issues that provide potential avenues for future research, including the actual effect of AH on live births, the method of reporting implantation data, the cost implications of AH on the 'take-home-baby rate', the place of AH in women with high FSH levels, thick or hardened zona pellucida, and the long-term consequences of the procedure on embryo damage, chromosomal abnormalities and congenital malformations. Trials are needed that are of high quality, of adequate size, conform to guidelines of reporting and provide data on live births.

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References

Al-Nuaim, L.A. and Jenkins, J.M. (2002) Assisted hatching in assisted reproduction. *Br. J. Obstet. Gynecol.*, **109**, 856–862.

Antinori, S., Panci, C., Selman, H.A., Caffa, B., Dani, G. and Versaci, C. (1996a; b; c) Zona thinning with the use of laser: a new approach to assisted hatching in humans. *Hum. Reprod.*, **11**, 590–594.

Antinori, S., Versaci, C., Dani, L., Barbaro, E., Antinori, M., Cerusico, C. and Vidali, A. (1999a and b) Laser assisted hatching at the extremes of the IVF spectrum: first cycle and after 6 cycles. A randomized prospective trial. *Fertil. Steril.*, **72**, S111.

Baruffi, R.L., Mauri, A.L., Petersen, C.G., Ferreira, R.C., Coelho, J. and Franco, J.G., Jr (2000) Zona thinning with noncontact diode laser in patients aged ⩽37 years with no previous failure of implantation: a prospective randomized study. *J. Assist. Reprod. Genet.*, **17**, 557–560.

Chao, K.H., Chen, S.U., Chen, H.F., Wu, M.Y., Yang, Y.S. and Ho, H.N. (1997) Assisted hatching increases the implantation and pregnancy rate of *in vitro* fertilization (IVF)-embryo transfer (ET), but not that of IVF-tubal ET in patients with repeated IVF failures. *Fertil. Steril.*, **67**, 904–908.

Cohen, J. (1991) Assisted hatching of human embryos. *J. In-Vitro Fertil. Embryo Transfer*, **8**, 179–190.

Cohen, J., Alikani, M., Trowbridge, J. and Rosenwaks, Z. (1992) Implantation enhancement by selective assisted hatching using zona drilling of human embryos with poor prognosis. *Hum. Reprod.*, **7**, 685–691.

Denker, H.W. (1993) Implantation: a cell biological paradox. *J. Exp. Zool.*, **266**, 541–558.

Harlow, G.M. and Quinn, P. (1982) Development of pre-implantation mouse embryos *in vitro* and in vivo. *Aust. J. Biol. Sci.*, **35**, 187–193.

Hellebaut, S., De Sutter, P., Dozortsev, D., Onghena, A., Qian, C. and Dhont, M. (1996) Does assisted hatching improve implantation rates after *in vitro* fertilization or intracytoplasmic sperm injection in all patients? A prospective randomized study. *J. Assist. Reprod. Genet.*, **13**, 19–22.

Hershlag, A., Paine, T., Cooper, G.W., Scholl, G.M., Rawlinson, K. and Kvapil, G. (1999) Monozygotic twinning associated with mechanical assisted hatching. *Fertil. Steril.*, **71**, 144–146.

Hsu, M.I., Mayer, J., Aronshon, M., Lazendorf, S., Muasher, S., Kolm, P. and Oehninger, S. (1999) Embryo implantation in *in vitro* fertilization and intracytoplasmic sperm injection: impact of cleavage status, morphology grade, and number of embryos transferred. *Fertil. Steril.*, **72**, 679–685.

Human Fertilisation and Embryology Authority (2000) *Patients' guide to IVF Clinics*. The Human Fertilisation and Embryology Authority, London.

Hurst, B.S., Tucker, K.E., Awoniyi, C.A. and Schlaff, W.D. (1998) Assisted hatching does not enhance IVF success in good-prognosis patients. *J. Assist. Reprod. Genet.*, **15**, 62–64.

Isik, A.Z., Vicdan, K., Kaba, A. and Dagli, G. (2000) Comparison of zona manipulated and zona intact blastocyst transfers: a prospective randomized trial. *J. Assist. Reprod. Genet.*, **17**, 135–139.

Isiklar, A., Balaban, B., Aksoy, S., Alatas, C., Mercan, R., Nuhoglu, A. and Urman, B. (1999) The effect of mechanical assisted hatching on progression of cleavage stage embryos to the blastocyst stage. *Fertil. Steril.*, **72**, S162.

Laffoon, I.S., Sokoloski, J.E., Volk, E.A., Hughes, L., Krivinko, D.M., Sanfilippo, J.S. and Wakim, A.N. (1999) The effect of assisted hatching on the outcome of assisted reproductive technology cycles in women under 39 years of age. *Fertil. Steril.*, **72**, S243.

Lanzendorf, S.E., Nehchiri, F., Mayer, J.F., Oehninger, S. and Muasher, S.J. (1998) A prospective, randomized, double-blind study for the evaluation of assisted hatching in patients with advanced maternal age. *Hum. Reprod.*, **13**, 409–413.

Lopata, A. (1996) Implantation of the human embryo. *Hum. Reprod.*, **11**, 175–184.

Loret De Mola, J.R., Garside, W.T., Bucci, J., Tureck, R.W. and Heyner S. (1997) Analysis of the human zona pellucida during culture: correlation with diagnosis and the preovulatory hormonal environment. *J. Assist. Reprod. Genet.*, **14**, 332–337.

Mansour, R.T., Rhodes, C.A., Aboulghar, M.A., Serour, G.I. and Kamal, A. (2000) Transfer of zona-free embryos improves outcome in poor prognosis patients: a prospective randomized controlled study. *Hum. Reprod.*, **15**, 1061–1064.

Mercader, A., Simon, C., Galan, A., Herrer, R., Albert, C., Remohi, J. and Pellicer A. (2001) An analysis of spontaneous hatching in a human endometrial epithelial coculture system: is assisted hatching justified. *J. Assist. Reprod. Genet.*, **18**, 315–319.

Nagy, Z.P., Rienzi, L., Iacobelli, M., Morgia, F., Ubaldi, F., Schimberni, M. and Aragona, C. (1999) Laser-assisted hatching and removal of degenerated blastomere(s) of frozen-thawed embryos improves pregnancy rate. *Fertil. Steril.*, **72**, S4.

Olivennes, F. and Hazout, A.D. (1997) A prospective randomized study of the use of assisted hatching in IVF-ET patients with high day-3 FSH. Increased clinical pregnancy rate with assisted hatching but high rate of miscarriages. *Fertil. Steril. Abst. Am. Soc. Reprod. Med.*, S226.

Rink., K., Descloux, L., Delacretaz, G., Senn, A., Nocera, D. and Germond, M. (1995) Zona pellucida drilling by a 1.48 μm laser: influence on the biomechanics of the hatching process. Abstract SPIE Proceedings, Barcelona, 2624.

Ryan, J.P., Pike, I.L., Catt, J.W., Porter, R.N. and Saunders, D.M. (1997) Failure of assisted hatching to increase pregnancy rates following the transfer of fresh or frozen-thawed day 2 human embryos. Abstracts of 13th Annual Meeting of the ESHRE. *Hum. Reprod.*, 188.

Stein, A., Rufas, O., Amit, S., Avrech, O., Pinkas, H., Ovadia, J. and Fisch, B. (1995) Assisted hatching by partial zona dissection of human pre-embryos in patients with recurrent implantation failure after *in vitro* fertilization. *Fertil. Steril.*, **63**, 838–841.

Tucker, M.J., Luecke, N.M., Wiker, S.R. and Wright, G. (1993) Chemical removal of the outside of the zona pellucida of day 3 human embryos has no impact on implantation rate. *J. Assist. Reprod. Genet.*, **10**, 187–191.

Tucker, M.J., Morton, P.C., Wright, G., Ingargiola, P.E., Sweitzer, C.L., Elsner, C.W., Mitchell-Leef, D.E. and Massey, J.B. (1996) Enhancement of outcome from intracytoplasmic sperm injection: does co-culture or assisted hatching improve implantation rates? *Hum. Reprod.*, **11**, 2434–2437.

World Health Organization (1975) The epidemiology of infertility. Report of a WHO scientific group. *WHO Tech. Rep. Ser.*, **582**, 1–37.

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