

The role of assisted hatching in in vitro fertilization: a guideline

Practice Committee of the American Society for Reproductive Medicine

There is moderate evidence that assisted hatching does not significantly improve live birth rates in fresh assisted reproductive technology cycles and insufficient evidence for the benefit of assisted hatching in patients with poor prognosis or undergoing frozen embryo transfer cycles. This document replaces the document of the same name published in 2014. (Fertil Steril® 2022;117:1177-82. ©2022 by American Society for Reproductive Medicine.)

El resumen está disponible en Español al final del artículo.

Key Words: IVF, assisted reproduction, embryo, blastocyst, twinning

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Hatching of the blastocyst is a critical step in the sequence of physiologic events culminating in implantation. Failure to hatch due to intrinsic abnormalities in either the blastocyst or the zona pellucida (ZP) may be one of many factors limiting human reproductive efficiency. Assisted hatching (AH) involves artificial thinning or breaching of the ZP and has been proposed as one technique to improve implantation and pregnancy rates after in vitro fertilization (IVF). An increased implantation rate after mechanical opening of the ZP (partial zona dissection) was first reported in 1990 (1). A randomized trial of patients who underwent AH 72 hours after retrieval (ZP drilling with acidified Tyrode solution) suggested an improvement in implantation rates when the procedure was selectively applied to embryos with a “poor prognosis” (based on ZP thickness, blastomere number, fragmentation rates, maternal age, and other factors) (2).

Since these early reports, many assisted reproductive technology (ART) programs have incorporated AH in an effort to improve clinical outcomes.

Historically, AH was performed before embryo transfer on days 3, 5, or 6 after fertilization using various methods, including creation of an opening in the ZP by thinning with acidified Tyrode solution (3, 4), partial ZP dissection with a glass microneedle (5), laser photoablation (6), or use of a piezo micromanipulator (7). Currently, AH is most commonly performed with full-thickness, laser-AH on the day of embryo transfer.

Although there is a theoretical benefit to AH, the procedure may be associated with complications, including damage to the embryo and/or damage to individual blastomeres with reduction of embryo viability. In addition, artificial manipulation of the ZP has been associated with an increased risk of monozygotic twinning (MZT) (8, 9). There are other scenarios in which embryologists routinely breach the ZP, such as opening on day 3 or 5 for facilitation of a biopsy for preimplantation genetic testing or collapsing the embryo before freezing; this guideline document does not consider hatching in these scenarios, as it is intrinsic and required for these procedures. The aim of the

current guideline is to assess the impact of AH on the day of embryo transfer specifically on the rates of live birth, clinical pregnancy, implantation, and MZT, in both fresh and frozen embryo transfer (FET) cycles.

LIMITATIONS OF PREVIOUS STUDIES

There are multiple challenges to interpreting the results of previous studies of the effectiveness of AH. In current ART practice, most clinics use the method of full-thickness, laser-AH on the day of embryo transfer. Many of the earlier published studies included methods of hatching that are not used frequently today (e.g., acidified Tyrode solution or partial ZP dissection). This guideline includes only studies that examine the association between full-thickness, laser-AH on the day of embryo transfer and pregnancy outcome. Additional limitations of the current literature are that many investigations are underpowered and report only surrogate outcomes, such as clinical or ongoing pregnancy rather than live birth.

METHODS

For a complete description of the methodological process, including search strategy, assessment of the literature, and review, please see [Appendix 1](#).

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DOES AH IMPROVE LIVE BIRTH RATES WITH FRESH EMBRYO TRANSFER? ARE THERE SUBSETS OF PATIENTS WHO BENEFIT?

To evaluate live birth rates with and without AH for fresh embryo transfer, this guideline references one high-quality randomized controlled trial (RCT) (10), 2 moderate-quality RCTs (11, 12), 5 moderate-quality systematic reviews (13–17), and 4 intermediate-quality cohort studies (18–21).

One high-quality RCT assigned 203 patients with a good prognosis who were planning day 3 fresh embryo transfers to AH ($n = 121$) or no AH ($n = 82$) (10). The inclusion criteria were age <39 years; first or second IVF cycle with no more than one prior failed cycle; diagnosis of unexplained infertility, endometriosis, or male factor or tubal factor infertility; and good-quality cleavage-stage embryos. No differences between the 2 groups were seen in the rates of clinical pregnancy (53% vs. 54%, $P = .92$), miscarriage (13% vs. 15%, $P = .64$), or live birth (47% vs. 46%, $P = .90$).

One intermediate-quality RCT evaluated the benefits of AH in 210 women of advanced maternal age (≥ 37 years) and 796 women with recurrent implantation failure (≥ 2 cycles) (12). Patients were randomized on the day of transfer, and women who had only poor-quality embryos and those who had a ZP thickness $>16 \mu\text{m}$ were excluded. After AH, there was no difference in clinical pregnancy rates in women of advanced maternal age (15.1% vs. 21%, $P = .12$) or in women with recurrent implantation failure (27.1% vs. 26.9%, $P = .57$). The study did not assess live birth rates. One randomized trial ($n = 60$ women) showed no benefit on pregnancy rate with AH of fresh transfer embryos derived from frozen donor oocytes (43.3% and 33.3%, respectively; $P = .1967$), although this may have been related to the relatively small sample size (11).

A retrospective analysis of the Society for Assisted Reproductive Technology (SART) database from 2004–2006, with over 225,000 fresh transfer cycles, found that the use of AH was associated with an increased rate of clinical pregnancy (odds ratio [OR], 1.29; 95% confidence interval [CI], 1.27–1.32; $P < .001$). The investigators did not stratify the subjects by age or diagnosis (20). A subsequent retrospective analysis of SART data from 2004–2011 that examined outcomes of AH in initial cycles restricted to cases in which diminished ovarian reserve was the primary diagnosis found that live birth rates were actually significantly lower when AH was performed (adjusted odds ratio [aOR], 0.77; 95% CI, 0.71–0.84) (18). A large registry study from Japan examined more than 35,000 fresh cycles in all age groups and found lower live birth rates among patients in the AH group than among patients in the control group (OR, 0.87; 95% CI, 0.82–0.93) (19). When the investigators limited the subanalysis to women aged ≥ 35 years, AH continued to be associated with lower live birth rates (OR, 0.88; 95% CI, 0.81–0.95), even after controlling for age, fertilization method, duration of culture, stimulation protocol, and luteal support (aOR, 0.88; 95% CI, 0.79–0.98). The limitations of these registry studies include absence of data about technical methods of AH, how diminished ovarian reserve was diagnosed, and why AH was performed. A retrospective study of 892 women aged >39

years undergoing their first cycle of IVF found that the use of laser-AH was associated with a lower live birth rate after transfer of cleavage-stage embryos (OR, 0.36; 95% CI, 0.19–0.68) but did not have any effect on the outcome after blastocyst transfer (21).

Several meta-analyses have attempted to examine the benefits of AH, but all have limitations because they included studies with different technical methods of performing AH and studies that would have been excluded from this guideline document. In this document, we included only meta-analyses with a significant number of studies using laser for AH. A meta-analysis of 36 RCTs found a significant increase in clinical pregnancy rates with AH (OR, 1.16; 95% CI, 1.00–1.36; moderate heterogeneity [$I^2 = 48.4\%$]), but when the analysis was restricted to the 18 studies that used laser, there was no improvement (OR, 1.03; 95% CI, 0.81–1.30) (15). When the investigators evaluated only the 5 studies that reported live birth rates after laser-AH, there was no significant improvement with AH (OR, 1.19; 95% CI, 0.77–1.83). When the analysis was restricted to the 21 studies that assessed patients with a good prognosis (those who did not have a history of prior failed cycles), there was no significant improvement in clinical pregnancy rates after AH (OR, 1.18; 95% CI, 0.98–1.40; moderate heterogeneity [$I^2 = 33.9\%$]). Another meta-analysis including 28 studies with multiple technical methods of AH found that performing AH did not result in a significant increase in clinical pregnancy rates for all participants (relative risk [RR], 1.11; 95% CI, 1.00–1.24) (16). A systematic review restricted to patients aged >35 years found no significant difference in the live birth rate (RR, 0.88; 95% CI, 0.65–1.18) or the clinical pregnancy rate (RR, 0.92; 95% CI, 0.76–1.12) for the 3 studies using laser (17).

In the Cochrane Review, the initial evaluation of all included studies (28 RCTs) showed an increase in clinical pregnancy rate with AH (OR, 1.29; 95% CI, 1.12–1.49) (13). When the investigators limited the analysis to high-quality studies with more robust methodology (16 RCTs), the improvement in clinical pregnancy rate was attenuated (OR, 1.20; 95% CI, 1.00–1.45; $P = .05$). In subgroup analyses of the 12 studies that used laser for AH, the clinical pregnancy rate was slightly better in the AH group (OR, 1.27; 95% CI, 1.03–1.56). In an analysis of the studies that reported live births (7 RCTs), AH did not result in a significant improvement (OR, 1.13; 95% CI, 0.83–1.54). A 2012 updated Cochrane Review included 31 RCTs; 5 of them evaluated laser-AH and found no improvement in live birth rate in women who underwent AH (OR, 1.01; 95% CI, 0.81–1.26) (14). The investigators did not assess patients with poor prognosis who underwent laser-AH.

Summary Statement

- In studies evaluating pregnancy rates in an unselected patient population, there is moderate evidence that live birth rates are not significantly different between embryos that have undergone AH vs. those that have not. In patients with a poor prognosis, the data are mixed regarding improvement in live birth rates with laser-AH.

Recommendation

- Laser-AH should not be routinely recommended for all patients undergoing IVF. There are insufficient data to make a recommendation for selected groups, such as patients with poor prognosis. (Strength of evidence: B/C; strength of recommendation: moderate.)

DOES AH IMPROVE LIVE BIRTH RATES WITH FROZEN EMBRYO TRANSFERS? ARE THERE SUBSETS OF PATIENTS WHO BENEFIT?

To evaluate live birth rates with FETs, this guideline refers to 2 intermediate-quality RCTs (12, 22), one high-quality systematic review/meta-analysis (23), 3 intermediate-quality systematic reviews/meta-analyses (13–15), and 2 intermediate-quality large database studies (19, 24) that did not demonstrate improvement in live birth rates in women undergoing FET with AH.

One RCT found no significant difference in live birth rates after FET blastocyst transfer in the AH group ($n = 96$ patients) compared with the control group ($n = 102$ patients) (40.6% vs. 28.4%; P value not reported but calculated to be .07) in patients undergoing transfer of day 5 blastocysts, but it did demonstrate an increase in live birth rate when transfer was limited to day 6 blastocysts (AH group $n = 72$, control group $n = 75$; live birth rate 43.1% vs. 26.7%, $P < .05$) (22).

One intermediate-quality study evaluated the benefits of AH in 180 women after FET (12). Patients were randomized on the day of transfer, and women who had only poor-quality embryos (>50% partially damaged or degenerated) were excluded. The clinical pregnancy rate was improved in women who used hatched frozen embryos compared with controls (31.1% vs. 11.1%, $P = .001$). It is notable that the pregnancy rate in the control group was 11.1%, which may indicate that these results are not generalizable.

Several systematic reviews have addressed this question, although they did not perform subgroup analyses based on the AH technique. A Cochrane systematic review found no significant improvement in pregnancy rates among women undergoing AH in FET cycles (13). The Cochrane systematic review was updated in 2012 with 9 additional trials and found similar results (14). More recent meta-analyses have confirmed those findings. One meta-analysis reported an OR for live birth of 1.2 (95% CI, 0.5–2.83) in the AH vs. no AH groups of women undergoing FETs (15). Another meta-analysis evaluating the effect of AH on pregnancy outcomes of FETs also did not show a benefit to live birth rate (OR, 1.09; 95% CI, 0.77–1.54) (23).

A large database study examining more than 59,000 FET cycles in Japan did not observe a significant improvement in live birth rates among patients in the AH group vs. the control group (OR, 0.93; 95% CI, 0.84–1.03) (19). A retrospective analysis of first FET cycles from the SART database from 2004–2013, with over 151,533 cycles, found that the use of AH was associated with a slight decrease in live birth rate (34.2% vs. 35.4%, $P = .001$). Among women older than 42 years, the decrease in live birth rate with AH was more

profound, although the sample size was small for this group (14% vs. 30% with no AH; $P < .001$) (24).

Summary Statement

- In patients undergoing FET, the data are mixed regarding improvement in live birth rate with laser-AH.

Recommendation

- There are insufficient data to make a recommendation for laser-AH in FET cycles. (Strength of evidence: B; strength of recommendation: moderate.)

DOES AH INCREASE MONOZYGOTIC TWINNING?

To evaluate whether AH increases MZT, this guideline refers to 8 intermediate-quality retrospective cohort studies (25–32), 1 intermediate-quality large database study (33), 6 intermediate-quality meta-analyses (14–17, 34–36), and 2 intermediate-quality case-control studies (9, 39).

There are several intermediate-quality studies that support higher MZT rates with AH (9, 15, 16, 25, 27, 31, 35, 36). There are also several intermediate-quality studies that show no increased risk of MZT with AH.

An intermediate-quality meta-analysis (34) of 40 studies from January 2005 to July 2018 evaluated women with and without MZT after IVF. In the 16 studies that compared IVF with and without AH, there was a statistically significant association between AH and MZT after IVF (OR, 1.17; 95% CI, 1.09–1.27; $P < .0001$; moderate heterogeneity [$I^2 = 29\%$]). However, this association could not be confirmed when the analysis was limited to only high-quality cohort and case-control studies (random-effects model, OR, 1.00; 95% CI, 0.81–1.24; $P = .99$; moderate heterogeneity [$I^2 = 53\%$]). Additionally, it is important to note that the AH methods (laser, mechanical, and chemical) varied among the studies.

An analysis of the SART database from 2004–2010 evaluated 197,327 pregnancies, of which 2,924 resulted in MZT. In multivariate analysis, although MZT was more likely with day 5 or 6 embryos, the addition of AH had a nonsignificant effect (aOR, 1.77–3.29 with AH and 2.43–3.36 without AH), whereas with day 2 or 3 embryos, AH had a substantial significant effect (aOR, 2.05–2.48 with AH and 1.00 without AH) (33).

Although several other intermediate-quality studies show an increase in MZT with AH, they all have significant limitations, such as not reporting the type of twins observed (15), different methods of AH (9, 16), lack of a confirmatory ultrasound and/or placental pathology confirming MZT (36), and different days of embryo transfer (26, 31). For example, in an analysis of the 28,596 pregnancies from the US National ART Surveillance System (NASS) database from 2003–2012, among day 2 or 3 embryo transfers, AH significantly increased the risk of MZT (adjusted RR, 2.16; 95% CI, 1.53–3.06), but this finding did not persist when day 5 and 6 blastocyst embryo transfers were evaluated (26). Another

retrospective analysis of NASS data from 2000–2010 of 751,879 cycles involving a day 3 or day 5 embryo transfer revealed that AH was used in 337,109 cycles (44.8%). The risk of MZT was increased following single day 3 embryo transfer in patients who had AH: 2.0% vs. 1.3% (aOR, 1.77; 95% CI, 1.31–2.38) (31). Because of the retrospective nature of the NASS studies, it is difficult to control for many variables. In a case-control, population-based study of IVF embryo transfer cycles utilizing the SART database (n = 535,503), 11,247 pregnancies were evaluated for the risk of MZT after having AH performed (9). After adjustment for multiple variables (patient age, number of embryos transferred, number of prior cycles, diagnosis, use of intracytoplasmic sperm injection, and use of cryopreserved embryos), AH was associated with an increased risk of MZT compared with other multiple-gestation pregnancies (aOR, 3.2; 95% CI, 1.2–8.0) and compared with singleton pregnancies (aOR, 3.8; 95% CI, 1.8–9.8). A limitation of the NASS and SART datasets is that although they provide information about the AH method, it was probably primarily not laser-AH given the time frame of this study.

There are a similar number of intermediate-quality studies that suggest there is no increase in MZT with AH. However, many of the studies that do not show a significantly increased risk of MZT with AH were underpowered, had limited numbers of MZT pregnancies reported (14, 17, 29, 35), were inconsistent in the number of embryos transferred (28), had insufficient data regarding the method of AH (30), or compared hatched cleavage-stage embryos with unhatched blastocysts (32). A retrospective review of autologous and oocyte donation IVF cycles that analyzed 4,976 pregnancies from 2000–2007 found that MZT rates were not significantly different between day 3 embryo transfer cycles with or without AH (1.1% vs. 1.3%; aOR, 0.94; 95% CI, 0.26–3.44; $P = .74$) (29).

Summary Statement

- Data are mixed regarding the risk of MZT with AH. Some evidence from several studies supports higher rates of MZT with AH. However, a similar number of studies suggest there is no increase in MZT with AH.

Recommendation

- There is insufficient evidence to definitely conclude that AH is associated with MZT, as the outcome is rare and the available studies have conflicting findings. (Strength of evidence: B; strength of recommendation: moderate.)

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patient, the available resources, and institutional or clinical practice limitations. The Practice Committee and the Board of Directors of the American Society for Reproductive Medicine have approved this report.

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El papel de la eclosión asistida en la fertilización in vitro: una guía.

Hay evidencia moderada de que la eclosión asistida no mejora significativamente las tasas de nacidos vivos en los ciclos reproducción asistida en fresco y evidencia insuficiente sobre el beneficio de la eclosión asistida en pacientes con mal pronóstico o sometidos a ciclos de transferencia embrionaria con descongelados.

Este documento sustituye al documento del mismo nombre publicado en 2014.