



BMJ Open ALADDIN study: does assisted hatching of vitrified/warmed blastocysts improve live birth rate? Protocol for a multicentric randomised controlled trial

Alessandra Alteri ¹, Cristina Guarneri,² Laura Corti,¹ Liliana Restelli,² Marco Reschini,² Paolo Giardina,¹ Enrico Papaleo,^{1,3} Edgardo Somigliana,² Paola Viganò,³ Alessio Paffoni ⁴

To cite: Alteri A, Guarneri C, Corti L, *et al.* ALADDIN study: does assisted hatching of vitrified/warmed blastocysts improve live birth rate? Protocol for a multicentric randomised controlled trial. *BMJ Open* 2020;**10**:e031544. doi:10.1136/bmjopen-2019-031544

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2019-031544>).

Received 28 May 2019
Revised 28 January 2020
Accepted 05 June 2020



© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

¹Obstetrics and Gynaecology Department, IRCCS San Raffaele Scientific Institute, Milan, Italy

²Infertility Unit, Fondazione IRCCS Ca' Grande Ospedale Maggiore Policlinico, Milan, Italy

³Reproductive Sciences Laboratory, Obstetrics and Gynaecology Department, IRCCS San Raffaele Scientific Institute, Milan, Italy

⁴Infertility Unit, ASST Lariana, Cantù, Como, Italy

Correspondence to

Dr Alessandra Alteri;
alteri.alessandra@hsr.it

ABSTRACT

Introduction Recent data suggest a higher clinical pregnancy rate performing assisted hatching (AH) on previously cryopreserved embryos but fail to demonstrate significant effects on live birth rate. However, current evidence is based on studies with a small sample size and may hide a type II error. Moreover, poor attention has been given to the specific effect of AH on frozen/thawed blastocysts. To shed light on this topic, we developed the present protocol for a randomised trial to investigate the benefits of the laser-mediated partial removal of the zona pellucida in vitrified/warmed blastocysts.

Methods and analysis The pArtiaL zonaA pellucida removal by assisteD hatchINg of blastocysts (ALADDIN) study is a multicentric prospective comparative study with a parallel randomised controlled design aiming to investigate whether AH performed on warmed blastocysts before embryo transfer can improve live birth rate. Women allocated to the control group will undergo embryo transfer of blastocysts not previously subjected to AH. Two infertility units will be involved in the study. Enrolment of patients will last 18 months with quarterly monitoring and the entire study is foreseen to be closed in 36 months. Secondary outcomes include: proportion of transferred blastocysts/thawed blastocyst, morphological features of blastocysts before embryo transfer, implantation, biochemical pregnancy, clinical pregnancy (ultrasound visible gestational sac), miscarriage, multiple pregnancy, preterm birth (<37 weeks of gestation), obstetrical and neonatal complications and congenital anomaly rates.

Ethics and dissemination This protocol received a favourable ethical opinion from the Ethical Committee of IRCCS San Raffaele Scientific Institute and the Ethical Committee Area 2 Milan. Each participant will provide written consent to participate and remain encoded during the study. The trial results will be published in peer-reviewed journals and presented at conferences.

Trial registration number NCT03623659; Pre-results.

INTRODUCTION

The zona pellucida (ZP) surrounding a mammalian oocyte is a specialised extracellular matrix composed of glycoproteins that mediates the initial recognition and binding

Strengths and limitations of this study

- This study is a randomised controlled trial assessing live birth rate with a robust methodological design.
- This multicentric trial will consider a relatively large sample size.
- Large inclusion criteria will increase the reliability and generalisability of the data.
- The heterogeneity in clinical practice between two centres may be a major confounding factor in interpreting the results of these studies.
- In this study, it is not possible to blind the researchers to the group allocation of patients.

of the spermatozoon to the oocyte and plays an important role in the following activation events during the fertilisation process.¹ At the blastocyst stage, the embryo is subjected to the phenomenon called ‘hatching’ which includes expansion of the blastocoel with stretching, thinning and focal rupture of the ZP and extrusion of the embryo through the fractured zona. The hatching of the blastocyst is in fact a fundamental step for the establishment of a pregnancy: it allows the blastocyst to adhere to the endometrium and invade it. Physiologically, the hatching is thought to be mediated by: (1) a mechanical action following the increase in pressure within the blastocoel²⁻⁴ or (2) a protease-like hatching factor of embryonic origin⁵⁻⁷ or (3) lysine of uterine origin.⁸

Prolonged exposure of human oocytes and embryos to artificial conditions is thought to impair their ability to implant. Among the speculations about the causes of embryonic implantation failure after in vitro culture, the phenomenon called ‘ZP hardening’ has been suggested to be responsible for the impaired hatching ability of embryos.⁹⁻¹¹ The artificial rupture of the ZP is known as assisted

hatching (AH) and this technique was first described by Cohen and coworkers in order to improve the chances of implantation and clinical pregnancy during assisted reproduction.¹²

The AH is generally performed on day 3, 5 or 6 of embryo development using a laser mounted on an inverted microscope; however, the use of mechanical or chemical solutions has been proposed in the past.¹³ The indications most commonly advocated for the AH are advanced maternal age, recurrent implantation failure or use of previously cryopreserved embryos. This last indication is of particular interest because the cryopreservation methods can induce the hardening of the ZP making the implantation in utero more difficult.^{10 14 15}

Some initial meta-analyses evaluated the effect of AH of embryos on clinical outcomes, reporting a borderline significant improvement in clinical pregnancy while the live birth rate was still not proven to be increased by the procedure.^{16 17} These data were supported by a recent meta-analysis of randomised controlled trials (RCT) which confirmed higher clinical pregnancy and implantation rates using AH on previously cryopreserved embryos but failed to demonstrate significant effects on live birth rates.¹⁸ Unfortunately, the pool of included studies comprised a small sample size and focused on cleavage-stage embryos.

The lack of robust data on blastocysts represents a significant clinical gap considering that extended culture up to the blastocyst stage has markedly grown worldwide in concomitancy with the diffusion of single embryo transfer. In fact, available data on the effect of AH on blastocysts, especially after cryopreservation, are inconclusive. Only two RCTs addressed the clinical outcomes deriving from partial hatched blastocysts in frozen cycles compared with non-hatched controls.^{19 20} Although some benefits emerged in terms of clinical pregnancy, the heterogeneity of these studies did not permit to draw any conclusion on the real effect associated with AH. The scenario is even more complicated by the fact that AH procedures highly varied in terms of timing and mode of application. Procedures include manipulations of the ZP such as (1) making holes, slots or thinning of different sizes; (2) drilling, cutting, digesting or melting the zona mechanically, chemically or with a laser beam (3) on fresh or frozen/thawed embryos (iv) at different developmental stages (v) in different groups of patients.²¹ Therefore, it is likely that the general conclusions drawn from the evaluation of the overall effectiveness may withhold some confounding factors.²¹

In general, the rigorous evaluation of the role of the ZP manipulation in current/emerging in vitro fertilisation (IVF) approaches, such as blastocyst culture, cryopreservation or biopsy for preimplantation genetic testing (PGT), is surprisingly scanty. In order to contribute to fill of a major gap in the literature, we developed the present protocol for a randomised trial comparing laser-mediated partial removal of the ZP in vitrified/warmed blastocysts to non-AH controls.²²⁻²⁴

METHODS AND ANALYSIS

Design

The ALADDIN study is a multicentric prospective comparative study with a parallel randomised controlled design aiming to investigate whether the laser AH (LAH) performed on warmed blastocysts before embryo transfer can improve the live birth rate compared with controls (no LAH) in frozen embryo transfer. Two infertility units will be involved in the study: (1) IRCCS San Raffaele Scientific Institute and (2) IRCCS Ca' Granda Ospedale Maggiore Policlinico. Enrolment of patients will last 18 months with quarterly monitoring and the entire study is foreseen to be closed in 36 months.

Eligibility criteria

All women who undergo non-donor IVF cycles with or without intracytoplasmic sperm injection and scheduled for elective single embryo transfer with vitrified/warmed blastocysts will be considered for enrolment in each of the two units.

Inclusion criteria

The patients must meet all the following criteria at the time of randomisation to be eligible for recruitment:

1. Age of women at oocyte retrieval ≤ 40 years.
2. First or second frozen cycle using vitrified blastocysts.
3. Up to two previous oocyte retrievals.

Exclusion criteria

Patients will not be enrolled in the presence of any of the following conditions:

1. PGT cycle.
2. Body mass index (BMI) >35 kg/m².
3. Abnormal uterine cavity (ie, adenomyosis, submucous myoma, hydrosalpinx, septate uterus and endometrial polyps).
4. Severe male factor (use of surgically retrieved spermatozoa).

Patient and public involvement

Neither the patients nor the public were involved in the development of the research questions, selection of outcome measures, study design or study conduct.

Intervention

The hypothesis of the study is to test whether the application of a partial LAH to vitrified/warmed blastocysts might improve the live birth rate.

During an IVF treatment, the cryopreservation of blastocysts can occur for three main reasons: (1) supernumerary embryos; (2) high risk of ovarian hyperstimulation syndrome; and (3) impairment of endometrial receptivity (progesterone elevation during ovarian stimulation or inadequate endometrium).

Blastocysts are vitrified once expansion has been reached on day 5, 6 or 7 and after artificial shrinkage by a single laser shot on the trophoblast cells.²⁵ The vitrification protocol was previously described.²⁶

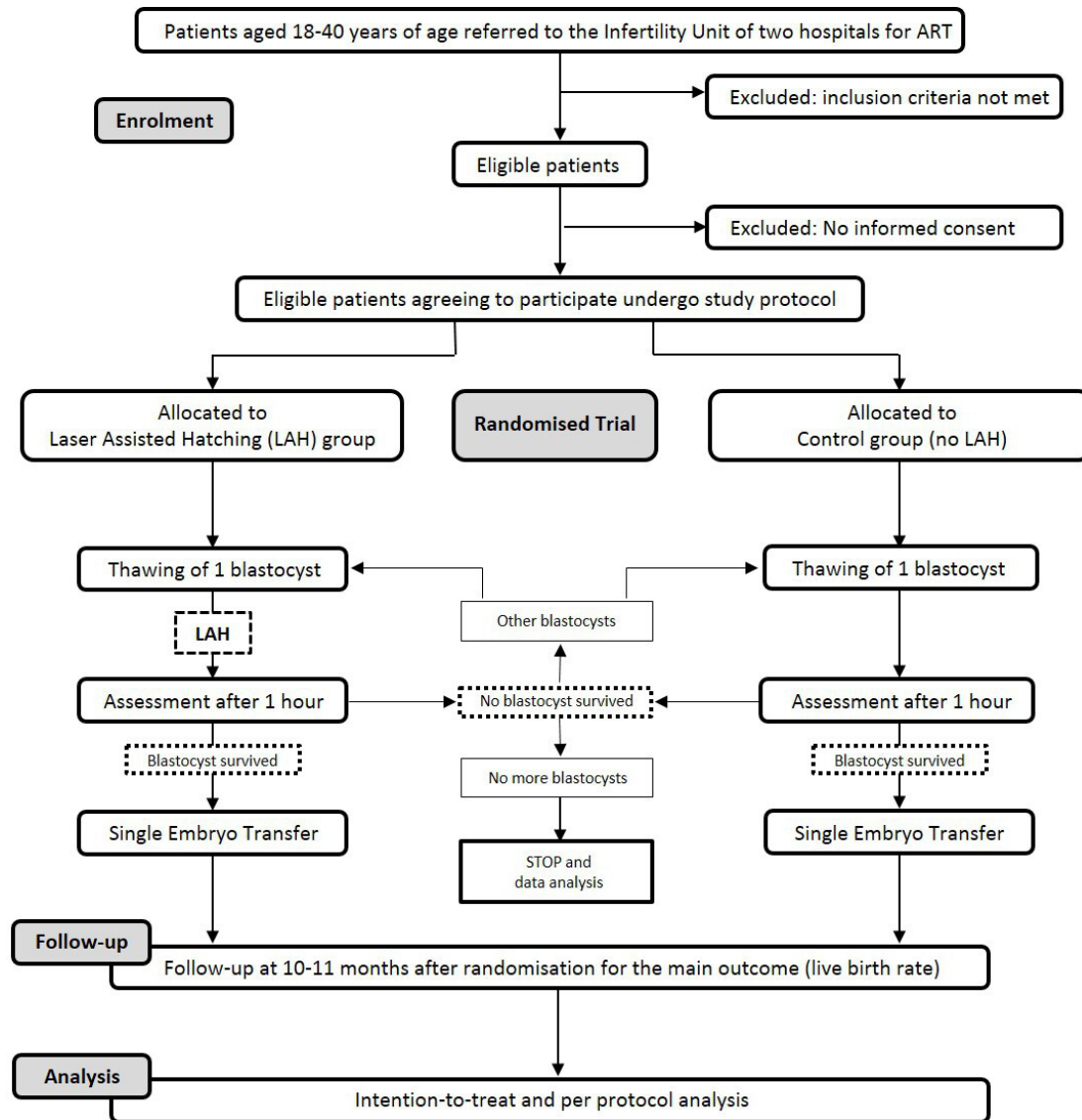


Figure 1 Flow diagram of the ALADDIN trial. ART, Assisted Reproductive Technology.

The randomisation for the study protocol will be performed at the time of warming of blastocysts.

AH procedure

Immediately after warming, LAH will be performed using a 1480 nm diode laser beam and an opening on the ZP will be initiated at the 1 o'clock position with pulse of 0.2 ms. Consecutive laser shots will be applied to reach the 5 o'clock position of the blastocyst in order to remove nearly one-third of the visualised surface of the zona. The LAH procedure will last 1 min per blastocyst. The blastocysts will then be cultured and evaluated 1 hour after warming for re-expansion and transferred at least 2 hours from thawing into the patient's uterus. Blastocysts showing complete degeneration will not be transferred. In this case, two options are foreseen: (1) in the presence of additional vitrified blastocysts, a second one will be thawed and treated according to the initially assigned randomisation arm; (2) in the absence of additional vitrified blastocysts, the patient will not undergo embryo

transfer (figure 1). The endometrium preparation will be performed in natural, modified natural cycle, or artificial cycle according to local standards of treatment.

Outcome measures

The primary outcome examined in this study will be the live birth rate, as the number of deliveries resulting in a live birth per randomised blastocyst/patient.

Secondary outcomes include proportion of transferred blastocysts/thawed blastocyst, morphological features of blastocysts before embryo transfer, implantation, biochemical pregnancy, clinical pregnancy (ultrasound visible gestational sac), miscarriage, multiple pregnancies, preterm births (<37 weeks of gestation), obstetrical and neonatal complications and congenital anomaly rates.

Sample size

The sample size was calculated based on the following assumptions and according to data pooled from the

two participating centres in the past 24 months: (1) the primary outcome is the live birth rate per randomised blastocysts/patient; (2) expected live birth rate without LAH is 28%; (3) expected live birth rate with LAH is 38%; (4) loss to follow-up is 1%; (5) type I and II errors are 0.05 and 0.20, respectively. Under these assumptions, a sample size of 350 randomised blastocysts/women per arm will allow us to detect with sufficient statistical power ($p < 0.05$) an absolute difference $\geq 10\%$ in the live birth rate between the two groups using the χ^2 test.

Recruitment

The identification of the candidates for the study will be carried out by the laboratory personnel in collaboration with the clinical staff. All of the couples scheduled for frozen embryo transfer will be considered for inclusion. Women who agree to participate will be asked to sign written informed consent, of which they will receive a copy signed by the investigator. Any wastes or exclusions due to non-satisfied inclusion criteria will be recorded. On the day of warming, the laboratory staff will evaluate the presence of the signed consent and, in the case of adhesion, will proceed to the randomisation.

Subjects involved in the study will be subjected to the same procedures except for the treatment of LAH.

Randomisation

All participants will be allocated in two groups at a ratio of 1:1 by an online software²⁷ according to the following strata:

1. Recruiting centre: (A) IRCCS Ca' Granda Ospedale Maggiore Policlinico; (B) IRCCS San Raffaele Scientific Institute.
2. Ages < 38 and ≥ 38 years.

A block randomisation will be used to reduce biases and to achieve balance in the allocation of participants to treatment arms. Investigators involved in the randomisation process will be not aware of the block size. The allocation sequence will be obtained from the personnel in charge for the thawing procedure.

Blinding

Laboratory personnel performing randomisation and treatment cannot operate blinded. The doctor who will identify patient candidates, the physician who will perform the transfer procedure, the patients and the rest of the medical staff will be blinded to the treatment performed. The laboratory operator will not reveal the assignment branch, which will be stored in the laboratory chart. The treatment will be kept blinded for both the medical staff and the patient until the cycle is closed (no pregnancy or neonatal follow-up).

The assignment arm will be revealed to the data manager at the end of data collection related to the primary outcome. There will be no foreseen circumstances where it is allowed to interrupt the blinding.

Data collection and management

For every couple, clinical and laboratory data will be systematically recorded both on paper and electronically.

After enrolment, all variables related to the study protocol will be recorded in an electronic report form and stored in a digital database. The handling of personal data will comply with the national Personal Data Protection Act.

According to standard procedures, all participants achieving a clinical pregnancy will receive a questionnaire about pregnancy outcomes and/or complications.

Data monitoring will be entrusted to personnel independent of the investigators, without any conflicts of interest. The monitoring of the recruitment process will take place every 4 months and will involve the compilation of a quantitative report including information on: (1) number of randomisations carried out compared to expected; (2) correct compilation of clinical and experimental charts; (3) correct application of the inclusion criteria; (4) possible occurrence of adverse events.

All the staff involved in the trial will provide a written statement on possible conflicts of interest. The declarations will be made available to possible participants.

A timetable of the study is reported in [table 1](#).

Statistical analysis

We will evaluate and compare the live births after the transfer of a vitrified/warmed blastocyst in women randomised to receive one of the two strategies: one in which that blastocyst is treated with partial LAH, and the second in which no LAH is performed. The effectiveness of LAH relative to untreated controls will be expressed as a relative risk and as an absolute difference, each with 95% CIs. SPSS V.20 (IBM) will be used to perform the statistical analysis.

The differences between the groups will be evaluated with the χ^2 test for the categorical variables and Student's t-tests for the continuous variables. In case of not normally distributed variables, the non-parametric Wilcoxon and Mann-Whitney tests will be used.

To evaluate the effect of possible confounding factors, a logistic regression analysis will be performed considering the main factors that, before randomisation, can influence the probability of pregnancy (preplanned): age, smoking, female BMI, parity and number of oocytes.

The result of the primary outcome will be also adjusted and analysed with the Cochran-Mantel-Haenszel test for the stratification variables (recruiting centre and female age range). Significantly different variables that will be eventually found between the two groups, such as the quality of the blastocysts, will be included in the logistic regression model as a supplementary analysis. Any missing baseline clinical data will be integrated with the multiple assignment method. The lack of data related to the primary outcome determines the dropout of the patient.

An interim analysis is planned after the randomisation of 100 blastocysts per centre by evaluating the percentage of clinical pregnancies. The Peto procedure will be used to correct the alpha error. Should there be a decrease in the percentage of clinical pregnancies with a $p \leq 0.001$, the study will be suspended.

Table 1 Schedule of enrolment and assessment of the ALADDIN trial

ACTIVITY	PLAN		MONTHS																																														
	START (Month)	DURATION (Months)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36											
Study set-up	1	1																																															
Enrolment of patients/procedure	1	18																																															
Follow-up (pregnancies/deliveries)	3	29																																															
Data analysis (interim, main outcome)	17	6																																															
Embryological/clinical outcome evaluation	19	12																																															
Data analysis (secondary outcomes)	21	10																																															
Dissemination, Publication	24	13																																															
Data analysis (main outcome)	32	4																																															

Adverse events

All adverse effects observed in the laboratory, reported by the subjects or observed by the clinical staff, will be recorded and reported to the Ethical Committees.

The occurrence of adverse events is considered highly unlikely since the proposed treatments have been applied for many years in IVF laboratories without representing, at the current state of knowledge, a possible source of adverse events or reactions.

ETHICS AND DISSEMINATION

Ethics approval

The study proposal has been approved by the Ethical Committee of IRCCS San Raffaele Scientific Institute (approval number: 16/int/2018) and by the Ethical Committee of IRCCS Fondazione Ca' Granda Ospedale Maggiore (approval number: 503_2018bis). The study has been registered in ClinicalTrials.gov.

Trial status

This trial began the recruitment and enrolment on 5 September 2018. It is anticipated to close recruitment in April 2020 and enrolment in June 2020.

Dissemination plans

The trial results will be shared at relevant conferences and published in highly cited and peer-reviewed journals.

The data sets used and analysed during the current study will be available from the corresponding author on reasonable request.

DISCUSSION

The majority of reports regarding AH in randomised clinical trials, reviews and meta-analyses have considered clinical pregnancy as the primary outcome instead of live birth.^{16 17} A limited number of studies have examined the effect of AH on live birth rates. As a consequence, there is insufficient evidence demonstrating that AH improves birth rates. Thus, the procedure is not routinely recommended in IVF cycles.^{28 29} Despite this and according to the SART CORS database, the number of patients receiving AH in the USA increased every year between 2004 and 2013 and near half of the frozen embryo transfers have been performed using an AH procedure.³⁰ Considering the economic burden and possible risks, new good-quality data are urgently required. The present trial, focusing on the live birth rate as a primary outcome in a multicentric setting, will add valuable information to the current literature and, although with limited power according to the sample size, it will contribute to the definition of secondary outcomes and associated risks. One potential limitation to some of the secondary outcomes of the study is that neonatal and follow-up data use participant self-report outcome measures, so there is the possibility that reporting biases are introduced. However, previous comparisons between data reported by the

patients and clinical data obtained from hospital charts (for those delivering in our hospitals) showed a very high concordance rate for the outcomes investigated in this study.

If this trial shows that LAH represents an efficient procedure to improve live birth rate in frozen cycles, the results may lead to evidence-based changes in current clinical practice.

Twitter Alessandra Alteri @alteri_aie

Authors' contribution AA and AP developed the study and wrote the first draft of this article with input from PV, ES and EP. LC, CG, PG and LR helped refine the protocol. MR developed the statistical analysis. AA, AP, PV and ES critically revised the manuscript and contributed to the discussion. All authors will participate in patient inclusion and outcome assessment. All authors read and approved the final manuscript.

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Alessandra Alteri <http://orcid.org/0000-0003-3092-3546>

Alessio Paffoni <http://orcid.org/0000-0003-3112-3161>

REFERENCES

- Zhao M, Dean J. The zona pellucida in folliculogenesis, fertilization and early development. *Rev Endocr Metab Disord* 2002;3:19–26.
- Bergstrom S. Delay of blastocyst implantation in the mouse by ovariectomy of lactation. A scanning electron microscope study. *Fertil Steril* 1972;23:548–61.
- Lopata A, Hay DL. The surplus human embryo: its potential for growth, blastulation, hatching, and human chorionic gonadotropin production in culture. *Fertil Steril* 1989;51:984–91.
- Surani MA. Hormonal regulation of proteins in the uterine secretion of ovariectomized rats and the implications for implantation and embryonic diapause. *J Reprod Fertil* 1975;43:411–7.
- Denker HW, Fritz H. Enzymic characterization of rabbit blastocyst proteinase with synthetic substrates of trypsin-like enzymes. *Hoppe Seylers Z Physiol Chem* 1979;360:107–14.
- Menino AR, Williams JS. Activation of plasminogen by the early bovine embryo. *Biol Reprod* 1987;36:1289–95.
- Perona RM, Wassarman PM. Mouse blastocysts hatch in vitro by using a trypsin-like proteinase associated with cells of mural trophectoderm. *Dev Biol* 1986;114:42–52.
- Gonzales DS, Bavister BD. Zona pellucida escape by hamster blastocysts in vitro is delayed and morphologically different compared with zona escape in vivo. *Biol Reprod* 1995;52:470–80.
- Cohen J, Elsner C, Kort H, et al. Impairment of the hatching process following IVF in the human and improvement of implantation by assisting hatching using micromanipulation. *Hum Reprod* 1990;5:7–13.
- Cohen J. Assisted hatching of human embryos. *J In Vitro Fert Embryo Transf* 1991;8:179–90.
- Schiewe MC, Araujo E, Asch RH, et al. Enzymatic characterization of zona pellucida hardening in human eggs and embryos. *J Assist Reprod Genet* 1995;12:2–7.
- Cohen J, Malter H, Fehilly C, et al. Implantation of embryos after partial opening of oocyte zona pellucida to facilitate sperm penetration. *Lancet* 1988;2:162.
- Balaban B, Urman B, Alatas C, et al. A comparison of four different techniques of assisted hatching. *Hum Reprod* 2002;17:1239–43.
- Ko C-S, Ding D-C, Chu T-W, et al. Changes to the meiotic spindle and zona pellucida of mature mouse oocytes following different cryopreservation methods. *Anim Reprod Sci* 2008;105:272–82.
- Vincent C, Pickering SJ, Johnson MH. The hardening effect of dimethylsulphoxide on the mouse zona pellucida requires the presence of an oocyte and is associated with a reduction in the number of cortical granules present. *J Reprod Fertil* 1990;89:253–9.
- Carney S-K, Das S, Blake D, et al. Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)). *Cochrane Database Syst Rev* 2012;12:CD001894.
- Li D, Yang D-L, An J, et al. Effect of assisted hatching on pregnancy outcomes: a systematic review and meta-analysis of randomized controlled trials. *Sci Rep* 2016;6:31228.
- Zeng M, Su S, Li L. The effect of laser-assisted hatching on pregnancy outcomes of cryopreserved-thawed embryo transfer: a meta-analysis of randomized controlled trials. *Lasers Med Sci* 2018;33:655–66.
- Debrock S, Peeraer K, Spiessens C, et al. The effect of modified quarter laser-assisted zona thinning on the implantation rate per embryo in frozen/vitrified-thawed/warmed embryo transfer cycles: a prospective randomized controlled trial. *Hum Reprod* 2011;26:1997–2007.
- Wan C-Y, Song C, Diao L-H, et al. Laser-Assisted hatching improves clinical outcomes of vitrified-warmed blastocysts developed from low-grade cleavage-stage embryos: a prospective randomized study. *Reprod Biomed Online* 2014;28:582–9.
- Alteri A, Viganò P, Maizar AA, et al. Revisiting embryo assisted hatching approaches: a systematic review of the current protocols. *J Assist Reprod Genet* 2018;35:367–91.
- Vanni VS, Somigliana E, Reschini M, et al. Top quality blastocyst formation rates in relation to progesterone levels on the day of oocyte maturation in GnRH antagonist IVF/ICSI cycles. *PLoS One* 2017;12:e0176482.
- Papaleo E, Pagliardini L, Vanni VS, et al. A direct healthcare cost analysis of the cryopreserved versus fresh transfer policy at the blastocyst stage. *Reprod Biomed Online* 2017;34:19–26.
- Alteri A, Corti L, Sanchez AM, et al. Assessment of pre-implantation genetic testing for embryo aneuploidies: a SWOT analysis. *Clin Genet* 2019;95:479–87.
- Van Landuyt L, Polyzos NP, De Munck N, et al. A prospective randomized controlled trial investigating the effect of artificial shrinkage (collapse) on the implantation potential of vitrified blastocysts. *Hum Reprod* 2015;30:2509–18.
- Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. *Theriogenology* 2007;67:73–80.
- Urbaniak GC, Plous S. Research Randomizer (Version 4.0) [Computer software], 2013. Available: <http://www.randomizer.org/>
- Practice Committee of the American Society for Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technology. Role of assisted hatching in vitro fertilization: a guideline. *Fertil Steril* 2014;102:348–51.
- Heneghan C, Spencer EA, Bobrovitz N, et al. Lack of evidence for interventions offered in UK fertility centres. *BMJ* 2016;355:i6295.
- Knudtson JF, Failor CM, Gelfond JA, et al. Assisted hatching and live births in first-cycle frozen embryo transfers. *Fertil Steril* 2017;108:628–34.