LASER ASSISTED HATCHING FOR IN-VITRO FERTILISATION (IVF)

IPT Mark

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DISCLOSURE

The author of this report has no competing interest in this subject and the preparation of this report is totally funded by the Ministry of Health, Malaysia.

EXECUTIVE SUMMARY

Background

The clinical definition of infertility used by the World Health Organization (WHO) is ‘‘a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse’’. Primary infertility is infertility in a couple who have never had a child while secondary infertility is a failure to conceive following a previous pregnancy.

According to a systematic analysis of 227 national health surveys, approximately 10.5% of women around the world experienced secondary infertility, and roughly 2% experienced primary infertility.

In-vitro fertilisation (IVF) is a type of assisted reproductive technology used for infertility treatment. It is a process of fertilisation where an egg is combined with sperm outside the body. There exist two types of IVF depending on the method used for egg insemination; conventional IVF and IVF with Intracytoplasmic sperm injection (ICSI). Generally, IVF may be used to overcome female infertility when it is due to problems with the fallopian tubes and ICSI can assist in male infertility, such as in those cases where there is a defect in sperm quality (where a sperm cell is injected directly into the egg cell).

The zona pellucida is the hard protein coat surrounding and protecting the genetic material carried within the egg. This layer is approximately 15-20 um thick and must be breached in order for the sperm to make contact with the egg. To establish a successful pregnancy, the developing embryo must break out of its shell (zona pellucida) by a process known as hatching. Once the embryo is hatched, it may implant on the endometrium and begin to grow but if it is unable to hatch, the pregnancy will not continue.

Assisted hatching involves artificial disruption of the zona pellucida using mechanical, chemical or laser. Various AH techniques have been employed including zona thinning, zona drilling (breaching by forming a hole) and complete removal of the zona. American Society for Reproductive Medicine (ASRM) review committee did not recommend routine use of AH during IVF. However, it is used in fertility clinics on patients with poor prognosis such as repeated implantation failures, advanced maternal age, poor quality embryos, and frozen-thawed/ cryopreserved-thawed/ vitrified-thawed embryos.

Laser technology has been used since 1980s in assisted reproductive therapy (ART) techniques such as assisted hatching, embryo biopsy, preimplantation genetic testing, sperm manipulation and etc. As for laser assisted hatching (LAH), it represent as an advancement of ART in enhancing procedural efficiency, reducing the exposure time of gametes and embryos to suboptimal conditions outside the incubator. It also has higher efficacy on pregnancy outcome compared to chemical assisted hatching.

In Malaysia, LAH is only offered in private fertility centre. Therefore, this technology review was requested by Senior Consultant Obstetrics & Gynaecology (reproductive medicine) from Hospital Sultanah Bahiyah in view of introducing/using the technology to increase the pregnancy outcome among IVF patients.

Objective/aim

The objective of this technology review was to evaluate the effectiveness, cost-implication, safety and organisational issues that related to laser assisted hatching for IVF

Results and conclusions

A total of 3512 records were identified through the Ovid interface and PubMed, and 13 were identified from other sources (references of retrieved articles). After removal of 524 duplicates, 3001 records were screened and 2934 were excluded. Of these, 67 relevant abstracts were retrieved in full text. After reading, appraising and applying the inclusion and exclusion criteria to the 67 full text articles, 15 full text articles were included and 52 full text articles were excluded. The articles were excluded due to irrelevant study design (n=17), irrelevant population (n=14), irrelevant outcome (n=21). The effectiveness of the included studies is as shown in Table 1.

There was fair to good level of evidenve retrieved to suggests that LAH was associated with slight increased in clinical pregnancy and implantation rate in cryopreserved/frozen-thawed embryo but not for fresh embryo except for study conducted in patient with endometriosis.Those undergoing LAH were found to have significantly higher multiple pregnancy. On the other hand, there was no significant difference for live birth and miscarriage.

In terms of safety, there was no significant different for minor and major adverse events reported for LAH compared to control. There was no evidence retrieved on the cost-effectiveness and organisational issues of LAH among IVF patients.

Methods

Electronic databases were searched through the Ovid interface: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE® Daily and Ovid MEDLINE® 1946 to Present, EBM Reviews - Cochrane Central Register of Controlled Trials - May 2019, EBM Reviews - Cochrane Database of Systematic Reviews - 2005 to May 2019, EBM Reviews - Health Technology Assessment – 2nd Quarter 2019, EBM Reviews – NHS Economic Evaluation Database 2nd Quarter 2019. Searches were also run in PubMed database and U.S. Food and Drug Administration (USFDA) website. Google and Google Scholar was also used to search for additional web-based materials and information. Additional articles were identified from reviewing the references of retrieved articles. Last search was conducted on 31st May 2019.

Table 1: Pertinent details of included studies

Author	Study design	Conception method	Sample type	Other	Clinical pregnancy			Implantation rate			Live birth			Multiple pregnancy			Miscarriage		
					LAH	No LAH	P value	LAH	No LAH	P value	LAH	No LAH	P value	LAH	No LAH	P value	LAH	No LAH	P value
Zeng 2018	SR	IVF/ICSI/both	Cryopreserved-thaw ed	-	OR=1.65 (95%CI:1.24,2.19)		<0.05	OR=1.59 (95%CI:1.06,2.38)		<0.05	OR=1.09 (95%CI:0.77,1.54)		>0.05	OR=2.30 (95%CI:1.30,4.07)		<0.05	OR=0.86 (95%CI:0.50,1.48)		>0.05
Elhanas 2017	RCT	IVF	Frozen	Overall	30/80 (37.5%)	22/80 (27.5%)	0.237	50.00%	47.30%	0.87									
				<35 years	26/56 (46.4%)	16/55 (29.1%)	0.078												
				≥35 years	4/24 (16.7%)	6/25 (24.0%)	0.725												
Lu X 2019	Retrospective cohort	IVF	Frozen	-	111/225 (49.3%)	74/116 (38.9%)	0.034	31.20%	16.95%	0.028	44.80%	35.80%	0.097	32.40%	31.00%	0.847	7.20%	5.40%	0.626
Zhou 2014	Retrospective cohort	IVF	Cryopreserved/ frozen	-	53.96%	33.43%	<0.001	31.85%	16.95%	<0.001	77.04%	70.92%	>0.05				10.81%	25.00%	<0.001
Nada 2018	RCT	ICSI with endometriosis	Fresh	-	46/158 (29.11%)	28/150 (18.67%)	0.002	17.40%	10.20%	0.002	25.32%	16.67%	0.043						
Shi 2016	RCT	IVF	Fresh	overall	40/82 (48.78%)	57/96 (59.38%)	0.157	32.45%	39.29%	0.204							15.85%	15.63%	0.967
				≥35 to 38	29/52 (55.77%)	42/54 (65.63%)	0.279												
				≥38 to 40	8/20 (40%)	11/27 (40.74%)	0.959												
				≥40	3/10 (30%)	4/5 (80%)	0.119												
Horng 2002	RCT	IVF/ICSI	Fresh	≥37 years	7/40 (17.5%)	13/82 (16.3%)	0.864	6.70%	7.30%	0.648									
Tannus 2018	Retrospective cohort	IVF/ICSI	Fresh	-	9.16%	18.44%	0.012				5.37%	12.85%	<0.01						
Li D 2016	SR	-	Mixed fresh/frozen	-	OR=1.03 (95%CI:0.81,1.30)		not mentioned				OR=1.19 (95%CI:0.77,1.83)		not mentioned	OR=1.87 (95%CI:1.33,2.63)		not mentioned	OR=1.03 (95%CI:0.56,1.90)		not mentioned
Elhusiney 2013	RCT	ICSI	Mixed Fresh/frozen	overall	35.10%	28.20%	0.324	33.30%	50.00%	0.23									
				<35 years	18.80%	29.50%	0.159												
				≥35 years	70%	25%	0.001												
				frozen	62.50%	56.30%	0.086												
				fresh	29.50%	21.70%	0.264												
				prevoius 1 trial	68.80%	30.80%	0.04												
Carney 2012	SR	IVF/ICSI	unknow n	-	OR=1.04 (95%CI:0.90,1.19)		0.63				OR=1.01 (95%CI:0.81,1.26)		0.95	OR=1.27 (95%CI:1.00,1.61)		0.053	OR=0.98 (95%CI:0.59,1.63)		0.95
Ali J et al. 2003	RCT	ICSI	unknow n	≤ 36 years	64.90%	33.30%	0.023	38.10%	17.50%	0.0039									
				≥37 years	15.00%	20.00%	1.00	6.90%	9.30%	0.911									
Antinori 1996	RCT	IVF	unknow n	Repeated failure	41/96 (42.7%)	24/104 (23.1%)	<0.05	12.20%	7.30%	<0.05									
				First time	44/111 (39.6%)	23/121 (19%)	<0.05	11.80%	7.10%	<0.05									
Ghannadi 2011	N-RCT	IVF/ICSI	unknow n	>35 years	27.71%	16.37%	<0.05							13.04%	5.26%	>0.05			
				≤35 years	50%	30.69%	<0.05							22.27%	5.94%	<0.05			

LASER ASSISTED HATCHING FOR IN-VITRO FERTILISATION (IVF)

1. BACKGROUND

The clinical definition of infertility used by the World Health Organization (WHO) is ‘‘a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse’’. Primary infertility is infertility in a couple who have never had a child while secondary infertility is a failure to conceive following a previous pregnancy.[1]

According to a systematic analysis of 227 national health surveys, approximately 10.5% of women around the world experienced secondary infertility, and roughly 2% experienced primary infertility.[2] The prevalence of secondary infertility, in particular, varies widely by region and country, ranging from < 6% to >16% of women.[3]

In-vitro fertilisation (IVF) is a type of assisted reproductive technology (ART) used for infertility treatment. It is a process of fertilisation where an egg is combined with sperm outside the body. There exist two types of IVF depending on the method used for egg insemination; conventional IVF and IVF with Intracytoplasmic sperm injection (ICSI). Generally, conventional IVF may be used to overcome female infertility when it is due to problems with the fallopian tubes and ICSI can assist in male infertility, such as in those cases where there is a defect in sperm quality (where a sperm cell is injected directly into the egg cell).[4]

The zona pellucida is the hard protein coat surrounding and protecting the genetic material carried within the egg. This layer is approximately 15-20 um thick and must be breached in order for the sperm to make contact with the egg. To establish a successful pregnancy, the developing embryo must break out of its shell (zona pellucida) by a process known as hatching. Once the embryo is hatched, it may implant on the endometrium and begin to grow but if it is unable to hatch, the pregnancy will not continue. [5]

Assisted hatching (AH) involves artificial disruption of the zona pellucida using mechanical, chemical or laser. Various AH techniques have been employed including zona thinning, zona drilling (breaching by forming a hole) and complete removal of the zona.[6] American Society for Reproductive Medicine (ASRM) review committee did not recommend routine use of AH during IVF. However, it is used in fertility clinics on patients with poor prognosis such as repeated implantation failures, advanced maternal age, poor quality embryos, and frozen-thawed/ cryopreserved-thawed/ vitrified-thawed embryos. [7]

Laser technology has been used since 1980s in ART techniques such as assisted hatching, embryo biopsy, preimplantation genetic testing, sperm manipulation and etc. As for laser assisted hatching (LAH), it represent as advancement of ART in enhancing procedural efficiency, reducing the exposure time of gametes and embryos to suboptimal conditions outside the incubator.[8] It also has higher efficacy on pregnancy outcome compared to chemical assisted hatching.[9] The procedure for LAH is illustrated in figure 1.

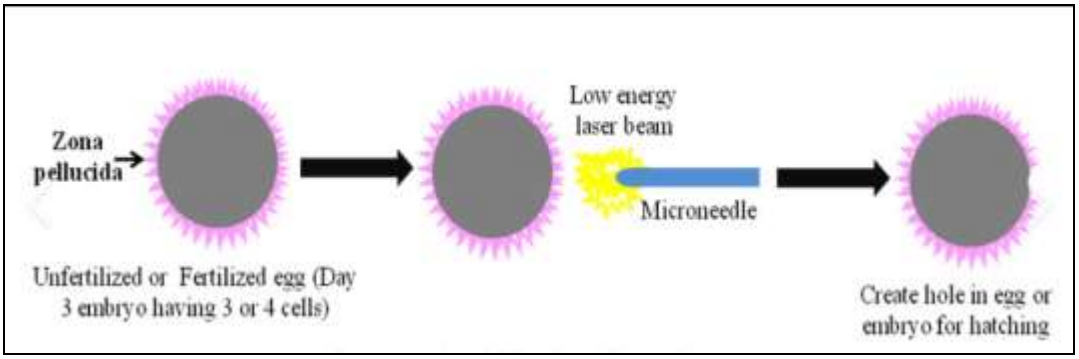

Figure 1: Laser assisted hatching

In Malaysia, LAH is only offered in private fertility centre. Therefore, this technology review was requested by Senior Consultant Obstetrics & Gynaecology (reproductive medicine) from Hospital Sultanah Bahiyah in view of introducing/using the technology to increase the pregnancy outcome among IVF patients.

2. OBJECTIVE / AIM

The objective of this technology review was to evaluate the effectiveness, cost-implication, safety and organisational issues that related to laser assisted hatching for IVF.

3. TECHNICAL FEATURES

There are two general types of laser systems exist (contact and noncontact). Noncontact lasers do not require additional physical manipulation of the embryo. Laser beams travel through the objective lenses and only microscope stage movement is required to adjust embryo position. In contrast, contact laser systems require direct contact between the laser and embryo, usually with either glass or an optical fiber.[5] However, the technical advantages of the non-contact mode and the potential of ultra-violet (UV) radiation to cause harmful mutagenic effects have led to the general preference for non-contact mode [6]

Modern lasers are constructed such that the beam travels through a specialised microscope objective of 40 × or 20 × magnification. These objectives are then fitted onto inverted microscopes with bright field,

phase contrast and Hoffman modulation optics, which are used routinely. This set-up facilitates the application of laser pulses during normal manipulations. There were several types of lasers that are commercially available as shown in Table1. Among them, four types are used in the clinical area, with wavelengths of 1480 nm or 1460 nm, and power of 300 mW or 400 mW. [8]

Table 1: Types of lases commercially available

Name	Company	Wavelength	Power	Objective	Approved work
Saturn 5	Research Instruments, UK	1480 nm	400 mW	40 ×	Clinical
Saturn 5 Active	Research Instruments, UK	1480 nm	400 mW	20 ×, 40 ×	Clinical
LYKOS	Hamilton Thorne, USA	1460 nm	300 mW	40 ×	Clinical
ZILOS-tk	Hamilton Thorne, USA	1460 nm	300 mW	40 ×	Clinical
XYKOS	Hamilton Thorne, USA	1460 nm	300 mW	20 ×, 40 ×	Research
XYClone	Hamilton Thorne, USA	1460 nm	300 mW	40 ×	Research

4. METHODS

4.1. Searching

Electronic databases were searched through the Ovid interface: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE® Daily and Ovid MEDLINE® 1946 to Present, EBM Reviews - Cochrane Central Register of Controlled Trials - May 2019, EBM Reviews - Cochrane Database of Systematic Reviews - 2005 to May 2019, EBM Reviews - Health Technology Assessment – 2nd Quarter 2019, EBM Reviews – NHS Economic Evaluation Database 2nd Quarter 2019. Searches were also run in PubMed database and U.S. Food and Drug Administration (USFDA) website. Google and Google Scholar was also used to search for additional web-based materials and information. Additional articles were identified from reviewing the references of retrieved articles. Last search was conducted on 31st May 2019.

Appendix 1 showed the detailed search strategies.

4.2. Selection

A reviewer screened the titles and abstracts against the inclusion and exclusion criteria and then appraise the full text articles for final article selection.

The inclusion and exclusion criteria were:

Inclusion criteria

Population	IVF patients either conventional IVF or ICSI-IVF
Interventions	Laser assisted hatching (LAH)
Comparators	Current practice (No hatching)
Outcomes	• Effectiveness: Clinical pregnancy rate, implantation rate, live birth, multiple pregnancy, miscarriage • Economic implication • Safety • Organisational issue
Study design	Systematic Review (SR), Randomised Controlled Trial (RCT) or non-randomised controlled trial (Non-RCT), Health Technology Assessment (HTA), economic evaluation study, cohort study
	English full text articles

Exclusion criteria

Study design	Pre- and post-intervention study , diagnostic accuracy study, cross sectional study, case series, case report, studies conducted in animals, narrative reviews
	Non English full text articles

Relevant articles were critically appraised using Critical Appraisal Skills Programme (CASP) and graded according to the US/Canadian preventive services task force (Appendix 2). Data were extracted and summarised in evidence table as in Appendix 3.

5. RESULTS AND DISCUSSION

A total of 3512 records were identified through the Ovid interface and PubMed, and 13 were identified from other sources (references of retrieved articles). After removal of 524 duplicates, 3001 records were screened and 2934 were excluded. Of these, 67 relevant abstracts were retrieved in full text. After reading, appraising and applying the inclusion and exclusion criteria to the 67 full text articles, 15 full text articles were included and 52 full text articles were excluded. The articles were excluded due to irrelevant study design (n=17), irrelevant population (n=14), irrelevant outcome (n=21). Flow chart of study selection is shown in figure 2.

There were 15 full text articles which comprised of three SRs, seven RCTs, one non-RCT, three retrospective cohort studies and one prospective cohort study finally selected for this review. The studies were conducted in China, Egypt, Taipei, Canada, United Kingdom, Qatar, Italy, Iran and Hungary.

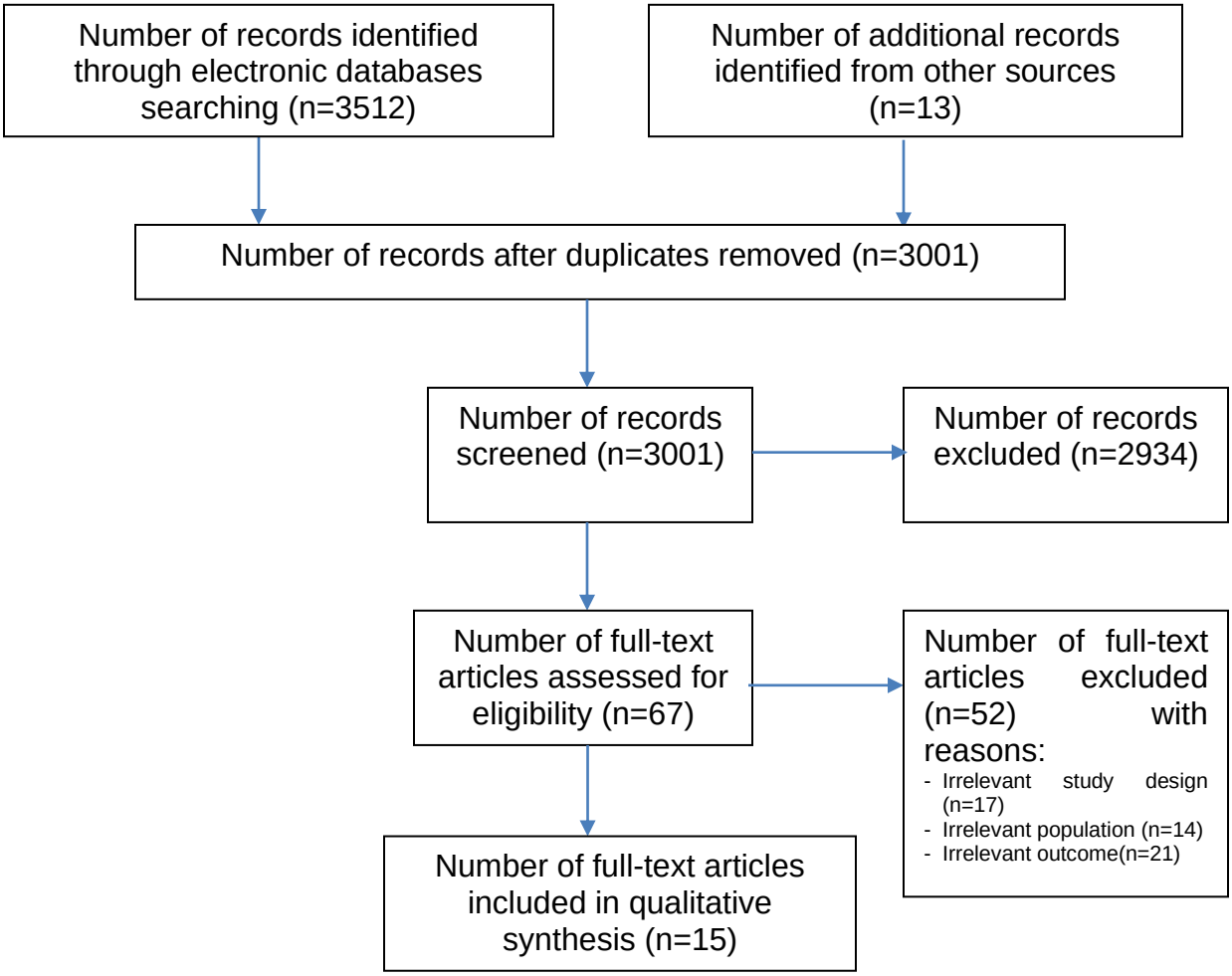

Figure 2: Flow chart of study selection

Risk of bias

One of the tools that are being used by MaHTAS to assess the risk of bias is the CASP checklist which consists of eight critical appraisal tools designed for SR, RCT, cohort studies, case control studies, economic evaluations, diagnostic accuracy studies, qualitative studies and clinical prediction rule. This is achieved by answering a pre-specified question of those criteria assessed and assigning a judgement relating to the risk of bias as either “Yes” indicates low risk of bias, “No” indicates high risk of bias, and “Can’t tell” indicates unclear or unknown risk of bias.

+	Indicates YES (low risk of bias)
?	indicates UNKNOWN risk of bias
-	Indicates NO (high risk of bias)

Figure 3 shows risk of bias of the three systematic review studies included. One study were combined with high heterogeneity.

Criteria assessed	Authors look for the right type of papers?	Selection of studies (all relevant studies included?)	Assessment of quality of included studies?	If the results of the review have been combined, is it reasonable to do so (heterogeneity)?
Zeng MF et al.	+	+	+	?
Li D et al.	+	+	+	+
Carney CK et al.	+	+	+	+

Figure 3: Assessment of risk of bias of systematic review (CASP)

Figure 4 shows risk of bias of the seven randomised controlled trials included. Majority of the studies were unclear regarding randomisation and allocation concealment of the patients.

Criteria assessed	Adequate sequence generation	Allocation concealment	Blinding of participants and personnel	Incomplete outcome data addressed	Free of selective reporting	Free of other bias
LAH versus No LAH						
Elhanas et al.	?	+	?	+	+	+
Nada AM et al.	+	+	+	?	+	+
Shi W et al.	?	?	?	+	+	+
Horng SG et al.	?	?	?	+	+	+
Elhuseiny et al.	?	?	+	+	?	+
Ali J et al.	?	?	?	?	?	?
Antinori S et al.	?	?	?	?	+	+

Figure 4: Assessment of risk of bias of RCT (Cochrane)

Figure 5 shows risk of bias of the one non-randomised controlled trials included.

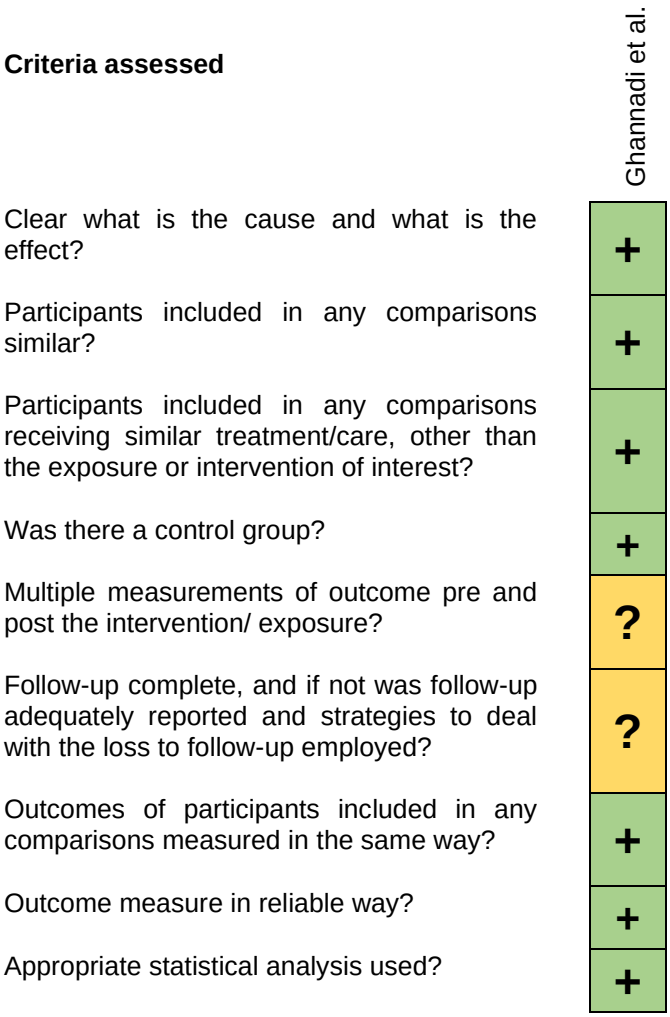

Figure 5: Assessment of risk of bias of quasi experimental studies (non-RCT) (JBI)

Figure 6 shows risk of bias of the three cohort studies included. Three studies were not involve subject follow up due to retrospective design.

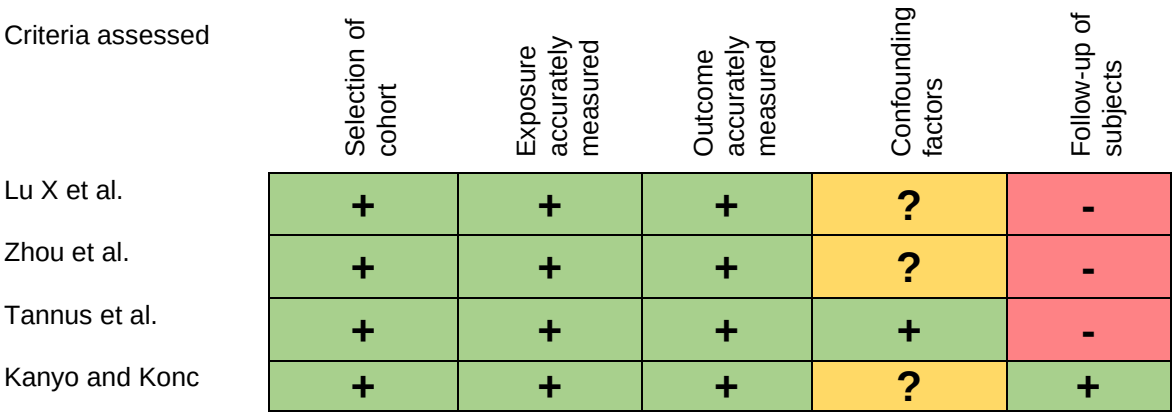

Figure 6: Assessment of risk of bias of cohort (CASP)

5.1. EFFECTIVENESS

5.1.1 CLINICAL PREGNANCY RATE

Cryopreserved/Frozen-thawed embryos

A systematic review with meta-analysis was conducted by Zeng S et al. (2018) to evaluate the effects of LAH on pregnancy outcomes of cryopreserved-thawed embryo transfer (ET). They systematically searched for RCTs with cryopreserved thawed ET that were subjected to LAH compared to those not subjected to LAH in conventional IVF or ICSI or both procedures. Patients included were aged 31 to 35 years old. A total of 12 articles were included in the review. They reported that, there was significantly higher clinical pregnancy rate per couple favouring LAH compared to control [11 RCTs, 2574 participants, OR = 1.65 [95% confidence interval (CI): 1.24, 2.19], $p< 0.05$, $I^2 = 49\%$.[10, level I]

A randomised controlled trial was conducted by Elhanas T et al. (2017) to determine if LAH improves the implantation and clinical pregnancy rate in women using transferred frozen/thawed embryos. A total of 160 women scheduled for transfer of cryopreserved embryos were randomised to LAH (n=80) and control group where zona pellucida left intact (n=80). Patients included were aged 18 to 40 years of age (either following their first IVF pregnancy, following one implantation failure or after postponing of transfer to avoid sequelae of ovarian hyperstimulation syndrome (OHSS). On the day of embryo transfer diode laser was used in the test group to include zonal microdissection. The quality and safety of embryo was assessed morphologically after hatching. On the other hand, zona of the control group were left intact. They found that, clinical pregnancy rates were higher but not statistically significant in group undergoing LAH before embryo transfer compared to those in patients in the control group [30/80 (37.5%) versus 22/80 (27.5%) respectively, p value = 0.237). In the subgroup analysis, they also found that there was no significant different in LAH and control group for both age groups [26/56 (46.4%) in LAH versus 16/55 (29.1%) in control, p=0.078 for women <35 years old and 4/24 (16.7%) in LAH versus 6/25 (24.0%) in control, p=0.725 for women ≥35 years old]. [11, level I]

Lu X et al. (2019) conducted a retrospective cohort study to assess the effect of laser-assisted zona thinning hatching technology (LAH) during the frozen-thawed D3 embryos on pregnancy outcomes in patients aged < 36 years old with history of previous IVF-embryo transfer (IVF-ET). A total of 415 cleavage-stage embryos transfer cycles (LAH=225, control= 190) were analysed from database of Zhongshan Hospital IVF centre. They reported that the clinical pregnancy rate was significantly higher in the LAH compared to control group [49.3% (111/225) versus control 38.9% (74/116), p=0.034]. [12, level II-2]

Zhou H et al. (2014) conducted a retrospective cohort study to evaluate the safety of LAH by comparing obstetric and neonatal outcome between LAH and control group in cryopreserved embryo transfer cycles. Patients included were > 35 years old and had more than one previous IVF failed cycles. A total of 843 embryo transfer cycles (480 in LAH, 335 in control) with day 3 cryopreserved embryo transfer in 699 patients were analysed from database. They reported that, there was significantly higher clinical pregnancy rate in the LAH versus control [259 (53.96%) versus 112 (33.43%), p<0.001]. [13, level II-2]

Fresh embryos

Nada AM et al. (2018) conducted a randomised controlled trial to compare the fresh ICSI embryo transfer outcomes in patients aged 18 to 39 years old with endometriosis with or without LAH zona pellucida thinning. However, history of previous IVF was not mentioned. Before randomisation, all patients received the same procedure including ICSI. A total of 308 infertile women with endometriosis were randomised into treatment (LAH) and control group where no LAH was performed. The clinical pregnancy rate per initiated cycle was defined as a serum hCG level > 20 IU/L and confirmed by observation of gestational sac with pulsating fetal pole on transvaginal ultrasound scan four weeks after transfer or six weeks post-menstrual. They reported that, the clinical pregnancy rate was significantly higher in LAH compared to control group [46/158 (29.11%) versus 28/150 (18.67%) respectively, p=0.002). [14, level I]

Shi W et al. (2016) conducted a randomised controlled trial to evaluate the effect of LAH in the advanced age patients who undergone fresh embryo transfer IVF/ ICSI in an Assisted Reproductive Centre Xi'an, China. A total of 178 patients with not more than one previous unsuccessful IVF attempt and age from 35 years to ≤42 years were randomised into treatment group with LAH (n=82) and control group (n=96). Laser assisted hatching was performed using ZILOS-tk; Hamilton Throne Instrument Bioscience. They reported that, there was no significant different in the clinical pregnancy rate in both group [LAH versus control, 40/82 (48.78%) versus 57/ 96 (59.38%), p=0.157). Similar results were found for subanalysis according to age groups ≥ 35 to 38 years, ≥38 to 40 years and ≥40 to 42 years (p>0.05)[15, level II-I]

Horng SG et al. (2002) conducted a randomised controlled trial to demonstrate the effect of LAH on pregnancy outcomes in women of advanced age who received fresh conventional IVF or ICSI embryo transfer. A total 120 women aged 37 years old or older who undergone conventional IVF or ICSI were randomised into treatment group (embryo were cultured and transferred with LAH, n=40) and control group (embryo were cultured and transferred without LAH, n=80). They reported that, there was no significant different in clinical pregnancy rate in the treatment

and control group [7/40 (17.5%) versus 13/80 (16.3%), p=0.864). There was also no significant different in sub-analysis result among women aged 37 to 39 years [5/24 (20.8%) versus 8/56 (14.2%), p=0.516] and those aged ≥ 40 years [2/16 (12.5%) versus 5/24 (20.8%), p=0.681]. [16, level II-I]

Tannus S et al. (2018) conducted a retrospective cohort study to investigate the effect of LAH on live birth rate in women aged ≥40 years old. The study retrospectively evaluated conventional IVF/ICSI cycles (681 cleavage stage, 211 blastocyst stage) that had been performed with fresh embryo among 892 women older than 40 years between the years 2011 and 2015, at a single academic reproductive centre. Embryo were cultured and transferred either on day 3 (cleavage stage) or day 5 (blastocyst stage) due to quality of embryo. Laser assisted hatching was performed using ZILOS-tk; Hamilton Throne Instrument Bioscience. Clinical pregnancy was defined as the visualization of gestational sac by vaginal sonography by 6 weeks of pregnancy. Live birth was defined as a live birth after 24 weeks of pregnancy. They reported that, there was significantly lower clinical pregnancy rate in LAH compared to control [LAH (9.16%) versus control (18.44%), (95% CI: 0.03, 0.15), p<0.01] in cleavage stage. It was remained constant after controlled for maternal age, number of MII oocytes, fertilization method, and embryo quality [odds ratio (OR) = 0.52, (95% CI: 0.31, 0.86), P=0.012].[17, level II-2]

Mixed fresh or frozen/ unknown embryo type

Li D et al. (2016) conducted another systematic review with meta-analysis to verify the effect of assisted hatching (AH) including chemical, mechanical and LAH on pregnancy outcomes. They systematically searched for RCTs that evaluated the effect of AH compared to no AH on mixed of fresh or frozen-thawed embryos. A total of 36 RCTs (N=6459) were included in the review (18 studies used LAH, 12 used chemical AH and six studies used mechanical AH). They reported that, there was no significant different in clinical pregnancy rate between LAH and control group [OR = 1.03; (95% CI: 0.81, 1.30), p value was not mentioned, I^2 = 60%, 18 RCTs].[18, level I]

A Cochrane systematic review with meta-analysis was conducted by Carney CK et al. (2012) to determine the effect of AH of embryos from assisted conception on live birth and multiple pregnancy rates. They systematically searched for the RCTs of several AH method such as mechanical, chemical and laser that used to disrupt the zona pellucida prior to embryo replacement (either in conventional IVF or ICSI) compared to no AH. A total of 31 RCTs were included in the review. However, only 15 trials were on LAH. In their subgroup analysis they reported that, there were no significant different in clinical pregnancy rate per women in LAH compared to control group [15 RCTs, 3606 women; OR= 1.04; (95% CI: 0.90, 1.19), p = 0.63, I^2 of 62%].[19, level I]

Elhuseiny A et al. (2013) conducted a RCT to determine whether LAH can improve clinical outcome of assisted reproductive techniques in both unselected patients and patients with advanced female age, with recurrent implantation failure, or who are using frozen-thawed. A total of 179 (age ranged 28 to 36 years old) consecutive women scheduled for ICSI were randomised to either LAH or no hatching group. Patients were divided into 94 test and 85 control groups. On the day of embryo transfer, the zona pellucida of the selected embryos in the test group was thinned by using an infrared optical laser system, whereas in the control group they were left intact. Clinical pregnancy rates and implantation rates were estimated. They reported that, clinical pregnancy rate was higher in LAH compared to control (35.1% versus 28.2%, p=0.32). However, the difference was not statistically significant. [20, level II-I]

However, for subgroup analysis, they reported that there was higher clinical pregnancy rate in LAH compared to control (70% versus 25%, P = 0.001) in women ≥ 35 years old. However, the difference was not significant in those <35 years old (p=0.159). The clinical pregnancy rate in LAH was also higher in women who had previous one trial compared to control (68.8% versus 30.8%, p=0.04). In contrast, the difference was not significant in those who had two, three or four previous trials. They also reported that there was higher clinical pregnancy rate in frozen compared to fresh embryo transferred (62.5% versus 29.5 %). However, p value was not mentioned. [20, level II-I]

A randomised controlled trial conducted by Ali J et al. (2003) to determine whether the pregnancy rate can be increased if the zona barrier was compromised by LAH on day two embryos in patients selected at random irrespective of indication for AH. Only embryos generated by ICSI were included in the study. However, history of previous IVF was not mentioned. A total of 107 patients were divided into LAH and control group. These two groups were further subdivided by age to a total of four subgroups, namely those ≤36 years (control, n = 30; test, n =37), and those ≥37 (control, n = 20; test, n = 20). They reported that, there was significantly higher clinical pregnancy rate in the LAH compared to control in patients ≤36 years old [LAH 64.9 % (24/37) versus control 33.3% (10/30), p=0.0203]. However, it was not significant in patients ≥37 years old [LAH 15.0 % (3/20) versus control 20.0% (4/20), p=1.00]. [21, level II-I]

Another randomised controlled trial was conducted by Antinori S et al. (1996) to report the assisted hatching in human through zona-thinning using laser. Embryos were subjected to LAH in 207 IVF patients [96 patients with repeated failure IVF (group A) and 111 patients with first time IVF (group B)]. Both groups were compared with control groups [group A' (n=104) and B' (n=121)] in which assisted hatching was not performed.

However, clinical pregnancy and implantation was not defined. They reported that, there was significantly higher clinical pregnancy rate in both groups compared to control [41/ 96 (42.7%) versus 24/104 (23.1%), p<0.05) in repeated failure IVF and [44/111 (39.6%) versus 23/121 (19%), p<0.05) in first time IVF]. [22, level II-I]

A non-randomised controlled trial was conducted by Ghannadi A et al. (2011) to investigate the benefit of LAH in patients with advanced maternal age (over 35 years old). A total of 932 cycles of conventional IVF/ICSI in females were included and were allocated into four groups. In group I and II, embryos were cultured and transferred with and without LAH in women aged ≤ 35 years old. Meanwhile, embryos of group III and IV were transferred with and without LAH in women aged >35 years old. Laser manipulation was performed using a suturn-Tm3 system using 2-3 pulses of 0.8 milisecond with 400 voltage duration. The size of the hole made in the zona was 5-10µm, depending on zona thickness of each individual embryo. However, type of embryo used was not mentioned. They reported that, there was significant increase in clinical pregnancy rate in LAH group compared to control in both age groups [(27.71% versus 16.37%, p<0.05) in >35 years old and (50% versus 30.69%, p<0.05) in ≤35 years old]. [23, level II-I]

5.1.3 IMPLANTATION RATE

Cryopreserved/Frozen-thawed embryos

Zeng S et al. (2018) in their systematic review with meta-analysis reported that, there was significantly higher implantation rate per embryo transfer favouring LAH compared to control [9 RCTs, 4975 participants, [OR = 1.59; (95% CI: 1.06, 2.38); p<0.05]. However, the heterogeneity was high (I^2 = 82%). They also reported that the implantation rate was significantly higher in LAH compared to control with regards the conception mode (conventional IVF versus ICSI) [OR=1.59 (95%CI: 1.06, 2.38), p<0.05, I^2=82%] and extent of LAH micromanipulation on zona pellucida (thinning versus drilling) [OR=1.61(95%CI: 1.06, 2.43), p<0.05, I^2=83%) but not for cryopreservation method [OR=1.45 (95% CI: 0.94, 2.23), p=0.09, I^2=83%)]. [10, level I]

Elhanas T et al. (2017) in their RCT reported that, there was no significant difference in implantation rates in group undergoing LAH compared to those in control group [50.0% versus 47.3%, p=0.87]. However, total amount of transferred embryo was not mentioned. [11, level II-I]

Lu X et al. (2019) in their retrospective cohort study reported that the implantation rate was significantly higher in the LAH compared to control group [31.2% (148/474) versus control 24.6% (102/415), p=0.028]. [12, level II-2]

Zhou H et al. (2014) in their retrospective cohort study reported that, there was significantly higher implantation rate in the LAH versus control [379/1190 (31.85%) versus 141/830 (16.95%), p<0.001]. [13, level II-2]

Fresh embryos

Nada AM et al. 2018 in their RCT reported that, the implantation rate was significantly higher in LAH compared to control group [67 fetus/385 transferred embryo (17.40%) in LAH versus 44 fetus/431 transferred embryo in control (10.2%), p =0.002]. Implantation rate was defined as the number of gestational sacs present on ultrasound scan four weeks after transfer divided by the number of embryos transferred). [14, level I]

Shi W et al. (2016) in their RCT reported that, there was no significant different in the implantation rate in both group [LAH versus control, 32.45% versus 39.29%, p=0.204). [15, level II-I]

Horng SG et al. (2002) in their RCT reported that, there was no significant different in implantation rate in both treatment and control group (6.7% versus 7.3%, p=0.648). There was also no significant different in sub-analysis result among women aged 37 to 39 years (9.0% versus 7.4%, p=0.808) and those aged ≥ 40 years (3.1% versus 7.1%, p=0.288). However, the implantation rate was not defined in the study. [16, level II-I]

Mixed fresh or frozen/ unknown embryo type

Elhuseiny A et al. (2013) in their RCT reported that, there was no significant difference in implantation rate in LAH compared to control (50% versus 33.3%, p=0.23). [20, level II-I]

Ali J et al. (2003) in their randomised controlled trial reported that, there was significantly higher implantation rate in LAH compared to control in patients ≤ 36 years old [LAH 38.1 % (40/105) versus control 17.5% (14/80), p=0.0039]. However, it was not significant in those ≥37 years old [LAH 6.9 % (4/58) versus control 9.3% (5/54), p=0.911]. [21, level II-I]

Antinori S et al. (1996) in their randomised controlled trial reported that, there was significant higher implantation rate in both groups compared to control [(12.2% versus 7.3%, p<0.05) in repeated failure IVF and (11.8% versus 7.1%, p<0.05) in first time IVF]. [22, level II-I]

5.1.4 LIVE BIRTH

Cryopreserved/Frozen-thawed embryos

Zeng S et al. (2018) in their systematic review with meta-analysis reported that, there was no significant difference between the two groups favouring LAH [4 RCTs, 1087 participants, OR = 1.09; (95%CI: 0.77, 1.54); p>0.05, I^2=0%]. [10, level I]

Zhou H et al. (2014) in their retrospective cohort study reported that, there was no significant different in live birth rate per embryo transfer in the LAH versus control [292/379 (77.04%) versus 100/141 (70.92%), p>0.05]. [13, level II-2]

Fresh embryos

Nada AM et al. 2018 in their RCT reported that, the live birth rate was significantly higher in LAH compared to control group [40/158 (25.32%) versus 25/150 (16.67%) respectively, p=0.043].[14, level I]

Lu X et al. (2019) in their retrospective cohort study reported that the live birth rate there was no significant different in the LAH compared to control group [44.8% (101/225) versus control 35.8% (70/190), p=0.097]. [12, level II-2]

Tannus S et al. in their retrospective cohort study reported that, there was significantly lower live birth rate in LAH compared to control [LAH (5.37%) versus control (12.85%), (95% CI: 0.03, 0.13), p<0.01] in cleavage stage. It was remained constant after controlled for maternal age, number of MII oocytes, fertilization method, and embryo quality [OR= 0.36, (95% CI: 0.19, 0.68), p=0.001].[17, level II-2]

Mixed fresh or frozen/ unknown embryo type

Li D et al. (2016) in their systematic review with meta-analysis reported that, there was no significance different in live birth rate between LAH and control group [OR = 1.19; (95% CI: 0.77, 1.83), p value was not mentioned, I^2 = 9.4%, 5 RCTs]. [18, level I]

Carney CK et al. (2012) in their Cochrane systematic review with meta-analysis reported that there was no significant difference in live birth between LAH and control groups [5 RCTs, 1555 women; OR = 1.01 (95% CI: 0.81, 1.26), p = 0.27, I^2 of 23%].[19, level I]

5.1.5 MULTIPLE PREGNANCY

Cryopreserved/Frozen-thawed embryos

Zeng S et al. (2018) in their systematic review with meta-analysis reported that, there was significant increase in multiple pregnancies in LAH compared to control [5 RCTs, 265 participants, OR = 2.30, (95% CI: 1.30, 4.07); p<0.05, I^2=33%].[10, level I]

Lu X et al. (2019) in their retrospective cohort study reported that for multiple pregnancy, there was no significant different in the LAH compared to control group [32.4% (36/111) versus control 31.0% (23/74), p=0.847]. [12, level II-2]

Mixed fresh or frozen/ unknown embryo type

Li D et al. (2016) in their systematic review with meta-analysis reported that, there was significantly higher multiple pregnancy rate in LAH compared to control [OR = 1.87; (95% CI: 1.33, 2.63), p value was not mentioned, I^2 = 0%, 6 RCTs]. [18, level I]

Carney CK et al. (2012) in their Cochrane systematic review with meta-analysis reported that there was significant increase in multiple pregnancy rate per woman in LAH compared to control [9 RCTs, 2869 women; OR=1.27 (95% CI: 1.00, 1.61), p = 0.006, I^2 of 63%]. However, there was significant heterogeneity reported. [19, level I]

Ghannadi A et al. (2011) in their non-randomised controlled trial reported that, there was significant increase in multiple pregnancy rate (in those pregnant women) in LAH group compared to control in women > 35 years old [22.27% in LAH versus 5.94% in control, p<0.05]. However, the different was not significant between the two groups in women ≤ 35 years old [13.04% in LAH and 5.26% in control, p>0.05]. [23, level II-I]

5.1.6 MISCARRIAGE

Cryopreserved/Frozen-thawed embryos

Zeng S et al. (2018) in their systematic review with meta-analysis reported that, there was no significant difference between the two groups for the miscarriage rate [5RCTs, 347 participants, OR = 0.86, (95% CI: 0.50, 1.48); p >0.05, I^2=0%].[10, level I]

Lu X et al. (2019) in their retrospective cohort study reported that there was no significant different in the LAH compared to control group [7.2% (8/111) versus control 5.4% (4/74), p=0.626].[12, level II-2] In contrast, Zhou H et al. (2014) in their retrospective cohort study reported that, there was significantly lower spontaneous abortion rate per clinical pregnancy in the LAH versus control [28/259 (10.81%) versus 28/112 (25.00%), p<0.001]. [13, level II-2]

Fresh embryos

Shi W et al. (2016) in their RCT reported that, there was no significant different in the miscarriage rate in both group [LAH vs control, 15.85% versus 15.63%, p=0.967). [15, level II-I]

Mixed fresh/frozen/unknown type of embryos

Li D et al. (2016) in their systematic review with meta-analysis reported that, there was no significance different in miscarriage rate between LAH and control group [OR = 1.03; (95% CI: 0.56, 1.90), p value was not mentioned, I^2 = 0%, 5 RCTs]. [18, level I]

Carney CK et al. (2012) in their Cochrane systematic review with meta-analysis reported that there was no significant difference for miscarriage rate in LAH compared to control group [8 RCTs, 1565 women; OR=0.98 (95% CI: 0.59, 1.63), p = 0.48, I^2 of 0%].[19, level I]

5.2. SAFETY

Laser for assisted hatching had received 510k approval from USFDA and classified as class II medical device. There were two studies retrieved on the adverse events of LAH.[24]

Zhou H et al. (2014) in their retrospective analysis study reported that, there were four malformations [spina bifida (three cases), congenital heart disease (one case)] which occurred in the LAH group and three malformations [lower limb malformation (one case), inguinal hernia (one case) and polydactyly (one case)] in the control group. Spina bifida, congenital heart disease and lower limb malformation were defined as major malformation. No statistically significant difference was found in the total malformations between two groups. Major malformation rate between LAH and control group was also not differ [1.37% (4/292) versus 1% (1/100)]. [13, level II-2]

Kanyo K and Konc J (2003) conducted a prospective cohort study to compile data on karyotypes, deliveries, congenital malformations and growth parameters to evaluate the safety of LAH. The study investigated 134 children from the first 96 deliveries obtained after LAH between the 2 December 1998 and the 31 December 1999. The data was compared with 894 children in spontaneous pregnancy. Questionnaires were used for telephone interviews after the delivery, at 12 weeks, at six months and at one year follow up. They reported that there were no significant difference in major malformation (chromosomal abnormalities) in LAH group compared to control [2.2% (3/134) versus 3.0% (27/894), p=0.64]. There was also no significant different in minor malformation [such as unilateral pes equinovalgus, atrial septal defect (four cases), cryptorchism, congenital naevus, duplicated Pyelum, Congenital hip luxation, Torticollis (two cases), Ductus arteriosus (three cases)] in LAH group compared to control [10.4% (14/134) versus 11.1% (99/894), p=0.32]. One baby with major malformation in the LAH group died at age of ten days. There were no additional anomalies found during follow-up examinations at 12 weeks (133/133 or 100%) at six months (132/133 or 99.2%) and at one year (131/133 or 98.5%). Lost of follow up rate was 1.5%. [25, level II-2]

5.3. COST-EFFECTIVENESS

There was no retrievable evidence on the cost-effectiveness of LAH for IVF. However, estimated price for laser equipment is [redacted] unit. [26]

5.4. ORGANISATIONAL ISSUE

There was no retrievable evidence on the organisational issue of LAH for IVF. However, training is needed for the embryologist to perform the procedure.

5.5. LIMITATIONS

This technology review has several limitations. The selection of studies was done by one reviewer. Although there was no restriction in language during the search but only English full text articles were included in this review.

6. CONCLUSION

There was fair to good level of evidenve retrieved to suggests that LAH was associated with slight increased in clinical pregnancy and implantation rate in cryopreserved/frozen-thawed embryo but not for fresh embryo except for study conducted in patient with endometriosis.

Those undergoing LAH were found to have significantly higher multiple pregnancy. On the other hand, there was no significant difference for live birth and miscarriage.

In terms of safety, there was no significant different for minor and major adverse events reported for LAH compared to control. There was no evidence retrieved on the cost-effectiveness and organisational issues of LAH among IVF patients.

7. REFERENCES

1. Sexual and productive health. Available at https://www.who.int/reproductivehealth/topics/infertility/multiple-definitions/en/. Acssessed on 18 July 2019

2. Mascarenhas MN, Flaxman SR, Boerma T et al. National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. PLoS Med. 2012; 9(12): e1001356. doi:10.1371/journal.pmed.1001356

3. The Burden of Infertility: Global Prevalence and Women's Voices from Around the World. Available at https://www.mhtf.org/2017/01/18/the-burden-of-infertility-globalprevalence-and-womens-voices-from-around-the-world/. Acssesed on 10 July 2019

4. In vitro fertilisation. Available at https://en.wikipedia.org/wiki/In_vitro_fertilisation. Acssessed on 10 July 2019

5. Laser Pulse Application in IVF. Available at https://www.researchgate.net/publication/221920896_Laser_Pulse_Application_in_IVF/link/0fcfd50d097c28afd3000000/download. Acssesed on 18 June 2019

6. Hammadeh ME. Assisted hatching in assisted reproduction: a state of the art. J Assist Reprod Genet. 2011; 28:119–128

7. Honguntikar SD, Uppangala S, Salian SR et al. Laser-assisted hatching of cleavage-stage embryos impairs developmental potential and increases DNA damage in blastocysts. Lasers Med Sci. 2015; 30 (1):95-101.doi: 10.1007/s10103-014-1625-1.

8. Davidson LM, Liu Y, Griffiths T et al. Laser technology in the ART laboratory: a narrative review. Reprod Biomed Online. 2019; 38 (5):725-739. doi: 10.1016/j.rbmo.2018.12.011.

9. Hsieh YY, Huang CC, Cheng TC et al. Laser-assisted hatching of embryos is better than the chemical method for enhancing the pregnancy rate in women with advanced age. Fertil Steril. 2002; 78: 179–182

10. Zeng M, Su S and 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(3):655-666

11. Elnahas T, Tawab N, Azmy O. Prospective randomized trial on the use of laser assisted hatching for transfer of frozen/ thawed embryos in human Intracytoplasmic Sperm injection. Middle East Fertil Soc J. 2017; 22: 309–312

12. Lu X, Liu Y, Cao X et al. Laser-assisted hatching and clinical outcomes in frozen-thawed cleavage-embryo transfers of patients with previous repeated failure. Lasers Med Sci. 2019. https://doi.org/10.1007/s10103-018-02702-3

13. Zhou H, Zao W, Zhang W et al. No adverse effects were identified on the perinatal outcomes after laser-assisted hatching treatment. Reprod Biomed Online. 2014; 29(6):692-698

14. Nada AM, Amr El-Noury A, Al-Inany H et al. Effect of laser-assisted zona thinning, during assisted reproduction, on pregnancy outcome in women with endometriosis: randomized controlled trial. Arch Gynecol Obstet. 2018; 297:521-528

15. Shi W, Hongwei T, Zhang W et al. A Prospective Randomized Controlled Study of Laser-Assisted Hatching on the Outcome of First Fresh IVF-ET

Cycle in Advanced Age Women. Reprod Sci. 2016; 1-5. DOI: 10.1177/1933719116641764

16. Horng SG, Chang CL, Wu HM et al. Laser-assisted hatching of embryos in women of advanced age after in vitro fertilization: a preliminary report. Chang Gung Med J. 2002; 25(8):531-537

17. Tannus S, Cohen Y, MD, Henderson S et al. The Effect of Assisted Hatching on Live Birth Rate Following Fresh Embryo Transfer in Advanced Maternal Age. Reprod Sci. 2018: 1-6; doi: 10.1177/1933719118799192

18. Li D, Yang LD, An J et al. Effect of assisted hatching on pregnancy outcomes: a systematic review and Meta-analysis of randomized controlled trials. 2016; Sci. Rep. 6, 31228; doi: 10.1038/srep31228

19. Carney SK, Das S, Blake D et al. Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)). Cochrane Database of Systematic Reviews 2012, Issue 12. Art. No.: CD001894. DOI: 10.1002/14651858.CD001894.pub5.

20. Elhussieny A, El Mandouh M, Hanafi S et al. Effect of laser assisted hatching on outcome of assisted reproductive technology. Open J Obstet Gynecol. 2013; 3: 18-23

21. Ali J, Rahbar S, Burjaq H et al. Routine Laser Assisted Hatching Results in Significantly Increased Clinical Pregnancies. J Assist Reprod Genet. 2003; 20 (5): 177-181

22. Antonori S, Panci C, Selman HA et al. Zona thinning with the use of laser: a new approach to assisted hatching in humans. Hum Reprod. 1996; 11(3):590-594

23. Ghannadi A, Kazerooni M, Jamalzadeh F et al. The effects of laser assisted hatching on pregnancy rates. Iran J Reprod Med. 2011; 9 (2): 95-98

24. Laser Assisted Hatching. Available at https://www.accessdata.fda.gov/cdrh_docs/pdf14/k141434.pdf. Acsessed on 10 June 2019

25. Kanyo K and Konc J. A follow-up study of children born after diode laser assisted hatching. Eur J Obstet Gynecol Reprod Biol. 2003; 110; 176-180

26. *Kertas kerja bagi menaiktaraf perkhidmatan IVF Hospital Sultanah Bahiyah.* Provided by requestor

8. APPENDIX

8.1. Appendix 1: LITERATURE SEARCH STRATEGY

Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE®Daily and Ovid MEDLINE®1946 to Present

1 FERTILIZATION IN VITRO/ (30645)
2 fertili* in vitro.tw. (1429)
3 in vitro fertili*.tw. (22548)
4 test tube bab*.tw. (168)
5 test tube fertili*.tw. (13)
6 test-tube bab*.tw. (168)
7 test-tube fertili*.tw. (13)
8 1 or 2 or 3 or 4 or 5 or 6 or 7 (38785)
9 REPRODUCTIVE TECHNIQUES, ASSISTED/ (8911)
10 assisted reproductive techn*.tw. (7932)
11 reproductive techn*, assisted.tw. (8)
12 Laser assisted hatching.tw. (67)
13 9 or 10 or 11 or 12 (14085)
14 8 and 13 (3900)
15 limit 14 to (english language and humans) (3146)

OTHER DATABASES	
EBM Reviews - Cochrane Central Register of Controlled Trials	Same MeSH, keywords, limits used as per MEDLINE search
EBM Reviews - Cochrane database of systematic reviews	
EBM Reviews - Health Technology Assessment	
EBM Reviews – NHS Economic Evaluation Database	
EBM Reviews – Database of Abstract of Review of Effects	

PubMeD
Search (((((((FERTILIZATION IN VITRO[Title/Abstract]) OR fertili* in vitro[Title/Abstract]) OR in vitro fertili*[Title/Abstract]) OR test tube bab*[Title/Abstract]) OR test tube fertili*[Title/Abstract]) OR test-tube bab*[Title/Abstract]) OR test-tube fertili*[Title/Abstract])) AND ((((REPRODUCTIVE TECHNIQUES, ASSISTED[Title/Abstract]) OR assisted reproductive techn*[Title/Abstract]) OR reproductive techn*, assisted[Title/Abstract]) OR Laser assisted hatching[Title/Abstract])

8.2. Appendix 2
DESIGNATION OF LEVELS OF EVIDENCE

I Evidence obtained from at least one properly designed randomized controlled trial.

II-I Evidence obtained from well-designed controlled trials without randomization.

II-2 Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group.

II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.

III Opinions or respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees.

SOURCE: US/CANADIAN PREVENTIVE SERVICES TASK FORCE (Harris 2001)

8.3. Appendix 3
Evidence Table : Effectiveness
Question : Is Laser Assisted Hatching Effective?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
1.Zeng M, Su S and 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(3):655-666	Study design Systematic review with meta-analysis Objective To evaluate the effects of laser AH on pregnancy outcomes of cryopreserved-thawed embryo transfer (ET) Methods Relevant studies searched in the PubMed, EMBASE, and Cochrane Central databases up to March 2017. Inclusion criteria: Articles of RCTs, cryopreserved-thawed ET, human laser AH embryo with control group in which embryos were not submitted to LAH, study that provided comparative data on clinical outcome after ET, and human ET following IVF or ICSI, or both Data extraction and quality assessment was carried out by two person Primary outcome: Clinical pregnancy, embryo	1	12 RCTs were included (N=2574 participants) Age range: 31 to 35.0	Laser AH embryo	No hatching		**Clinical pregnancy rate per couple** (11 RCTs; 2574 participants) •Significant difference favoring Laser AH compared to control [OR = 1.65; (95% CI: 1.24, 2.19), p< 0.05, I^2 = 49%.) **Implantation rate per embryo transfer** (9 RCTs; 4975 embryo transfer) •Significant difference favoring Laser AH compared to control [OR = 1.59; (95% CI: 1.06, 2.38); p<0.05]. •High heterogeneity (I^2 = 82%) **Live births per couple** (4 RCTs; 1087 participants) •No significant difference between the two groups favoring Laser AH [OR = 1.09; (95%CI: 0.77, 1.54); p>0.05, I^2=0%]. **Multiple pregnancy** (5 RCTs; 265 participants) •Significant increase in multiple pregnancies in Laser AH compared to control [OR = 2.30, (95% CI: 1.30, 4.07); p<0.05, I^2=33%] **Miscarriage rate** (5RCTs; 347 participants) •No significant difference between the two groups for the miscarriage rate (OR = 0.86, (95% CI: 0.50, 1.48); p >0.05, I^2=0%].	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	implantation rate Secondary outcome: Multiple pregnancy rate, miscarriage, live birth rate and miscarriage rate These results were combined for the meta-analysis using the Mantel-Haenszel model when using the random effects model and fixed effects model.							

Evidence Table : Effectiveness
Question : Is Laser Assisted Hatching Effective?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
2.Elnahas T, Tawab N, Azmy O. Prospective randomized trial on the use of laser assisted hatching for transfer of frozen/ thawed embryos in human Intracytoplasmic Sperm injection. Middle East Fertil Soc J. 2017; 22: 309–312	Study design Randomised controlled trial Objective To determine if laser assisted hatching improves both the implantation and clinical pregnancy rates in women using transferred frozen/ thawed embryos. Methods Patients were randomized to either intervention or control group using sealed envelope Patients included had cryopreserved embryos Patients included were aged 18–40 (either following their first IVF pregnancy, following one implantation failure or after postponing of transfer. More than 40 years old patients are excluded as well as patients with recurrent implantation failure Only excellent and good quality day three embryos are included Quality assessment was	II-1	160 patients (80 LAH, 80 control) Age:18-40 years old Mean age LAH:31.02±4.65 Non-LAH:31.71±4.85	LAH	No hatching	-	**Clinical Pregnancy Rate** Clinical pregnancy rates were higher in group undergoing LAH before embryo transfer 37.5% compared to those in patients in the control group 27.5% (P value = 0.237) respectively, however that was statistically insignificant. **Subgroup analysis for clinical pregnancy rate** Women aged less than 35 years and in women aged 35 years or more at time of treatment shows the highest clinical pregnancy rate was in women aged less than 35 years with laser-assisted hatching < 35 year old •LAH 46.4% vs No LAH 29.1%, p=0.078 ≥35 year old •LAH 16.7% vs No LAH 24.0%, p=0.725 **Implantation Rate** Implantation rates were higher in group undergoing laser assisted hatching before embryo transfer 50% compared to those in patients in the control group 47.27% (P value = 0.87), however that was statistically insignificant.	

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	carried out by the same senior expert embryologist in order to avoid inter-observer discordance Embryo transfer was then performed by a single senior expert gynecologist who was blinded in order not to distinguish between the test and control groups Primary outcome: **Clinical pregnancy rate (CPR)** defined as intrauterine gestational sac and fetal pulsations detection using transvaginal ultrasound on the fourth and the sixth Secondary outcome: **Implantation rate (IR)** defined as the number of gestational sacs detected by transvaginal ultrasound on the fourth week, divided by the total numbers of embryos transferred							

Evidence Table : Effectiveness
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Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
3.Lu X, Liu Y, Cao X et al. Laser-assisted hatching and clinical outcomes in frozen-thawed cleavage-embryo transfers of patients with previous repeated failure. Lasers Med Sci. 2019. https://doi.org/10.1007/s10103-018-02702-3	Study design Retrospective cohort study Objective To assess the effect of laser-assisted zona thinning hatching technology (LAH) during the frozen-thawed D3 embryos on pregnancy outcomes in patients with previous repeated failures in vitro fertilization-embryo transfer (IVF-ET) Method The study was conducted at the reproductive medicine center of Zhongshan Hospital, Fudan University. Data from the center's IVF database were retrospectively analyzed Total of 415 cleavage-stage embryos transfer cycles with D3 cryopreserved embryo transfer were evaluated retrospectively in the study and divided into: • Control group (D3 cryopreserved embryo transfer cycles without LAH treatment occurring between	II-2	415 cleavage-stage embryos transfer cycles (LAH=225, control= 190) <36 years old	LAH	No hatching		**Clinical pregnancy (%, n)** • LAH= 49.3% (111/225) • Control= 38.9% (74/116), p=0.034 **Implantation (%, n)** • LAH= 31.2% (148/474) • Control= 24.6% (102/415), p=0.028 **Live birth(%, n)** • LAH= 44.8% (101/225) • Control= 35.8% (70/190), p=0.097 **Miscarriage(%, n)** • LAH= 7.2% (8/111) • Control= 5.4% (4/74), p=0.626 **Multiple pregnancy(%, n)** • LAH= 32.4% (36/111) • Control= 31.0% (23/74), p=0.847 **Authors' conclusion** LAH via zona pellucida (ZP) thinning significantly improves clinical outcomes, particularly clinical pregnancy and implantation rates, associated with FET cycles among patients with previous repeated failure.	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	May 2012 and March 2013, n = 190) • LAH group (D3 cryopreserved embryo transfer cycles with LAH treatment performed since April 2013, when the new LAH technology was introduced, n = 225) Clinical outcomes including clinical pregnancy, implantation, live birth, miscarriage, and multiple gestation rates after transfer were compared between the LAH and control groups							

Evidence Table : Effectiveness
Question : Is Laser Assisted Hatching effective?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
4.Zhou H, Zao W, Zhang W et al. No adverse effects were identified on the perinatal outcomes after laser-assisted hatching treatment. Reprod Biomed Online. 2014; 29(6):692-698	Study design Retrospective cohort study Objective To evaluate the safety of laser-assisted hatching (LAH) by comparing obstetric and neonatal outcomes between assisted hatching and control groups in cryopreserved embryo transfer cycles Method Data from the centre's IVF database were retrospectively analysed 843 frozen–thaw cycles were carried out between January 2008 and August 2010 815 embryo transfer cycles with day 3 cryopreserved embryo transfer in 699 patients were analysed Patients were invited to undergo this treatment if they had previous failed cycles (≥1), were more than 35 years of age, and zona pellucida abnormalities were observed Assisted hatching was carried out using laser treatment	II-2	815 embryo transfer (ET) in 699 patients LAH=480 ET Control=335 ET Patients age >35 years	LAH	No hatching	-	The implantation rate, clinical pregnancy rate and live delivery rate were significantly higher statistically in the LAH group (all P < 0.001 The **implantation rate per transferred embryo** were significantly higher statistically in the LAH group [379/1190 (31.85%) vs control 141/830 (16.95%), P < 0.001] The **clinical pregnancy rate** were significantly higher statistically in the LAH group [259/480 (53.96%) vs control 112/335 (33.43%), P < 0.001] **Spontaneous abortion (miscarriage) rate per clinical pregnancy** was lower in the LAH group than the control group [28/259 (10.81%) versus 28/112 (25.00%), P < 0.001]. A total of 292/379 (77.04%) babies were born in the LAH group and 100/141 (70.92%) were delivered in the control group. In the live births, four malformations (3 spina bifida and 1 congenital heart disease) occurred in the LAH group and three malformations (1 lower limb malformation, 1 inguinal hernia and 1 polydactyly) in the control group No statistically significant difference was found in the total malformations between two groups. Major malformation rate not differ in the LAH group than the control group	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	(ZILOS-tk; Hamilton Thorne Instruments Biosciences, Beverly, MA01915, USA) Zona pellucida was thinned to more than ⅔ of its initial thickness and a distance of 30–40 mm Outcome measures were implantation, clinical pregnancy, spontaneous abortion, preterm delivery, birth rates and mean gestational age Clinical pregnancy was diagnosed by ultrasonographic visualization of one or more gestational sacs. Spontaneous abortion is defined as a natural death of fetus that takes place before 28 week in clinical pregnancy. Preterm birth is defined as a live birth or stillbirth that takes place after at least 28 but before 37 completed weeks of gestational age. The neonatal outcomes evaluated were sex ratio, birth weight, Apgar scores at 1, 5 and 10 min, and congenital birth defects (abortion and live						[4/292(1.36%) versus 1/100(1.0%)]. Spina bifida, congenital heart disease and lower limb malformation considered as major malformation.	

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	born). The outcomes were compared separately between singleton and multiple gestations, which avoided any bias owing to multiple pregnancies.							

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Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
5.Nada AM, Amr El-Noury A, Al-Inany H et al. Effect of laser-assisted zona thinning, during assisted reproduction, on pregnancy outcome in women with endometriosis: randomized controlled trial. Arch Gynecol Obstet. 2018; 297:521-528	Study design Randomised controlled trial Objective To compare the ICSI-ET outcomes in patients with endometriosis with or without laser-assisted zona pellucida thinning Method The study was conducted in Obstetrics & Gynecology Department, Cairo University Hospital unit, and two private IVF centers in Cairo and Beni Suif during the period from July 2015 to January 2017 They recruited all infertile women with endometriosis in the reproductive period, age from 18 to 39 who planned to undergo assisted reproduction A total of 326 patients were randomized into treatment and controlled group using computer-generated list of random numbers. Block randomization with a block size of 4 was used with 1:1 ratio of the study group (laser-assisted hatching) and	II-1	N=308 LAH (n=158), Control (n=150) age from 18 to 39	LAH	No hatching	-	**Clinical pregnancy rate** • significantly higher in the study group than in the control group [46/158 (29.11%) versus 28/150 (18.67%) respectively, p=0.002) **Implantation rate** • significantly higher in the study group than the control group [67 fetus/385 transferred embryo (17.40%) in LAH versus 44 fetus/ 431 transferred embryo in control (10.2%), p =0.002] **Live birth rate** • The live birth rate was significantly higher in the study group than in the control group [40/158 (25.32%) versus 25/150 (16.67%) respectively, p=0.043] **Authors' conclusion:** Laser-assisted hatching by thinning of the zona pellucida may be a suitable method to improve the ICSI-ET outcomes, in term of the implantation and the pregnancy rates, in cases of endometriosis	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	the control group (no laser assisted hatching) Researchers and patients were blinded After 18 subject were excluded after randomization (cycle cancellation), embryos of all 308 patients were transferred on day 3 after oocyte retrieval Primary outcome: • Clinical pregnancy rate (CPR) per initiated cycle (defined as a serum hCG level > 20 IU/L and confirmed by observation of gestational sac with pulsating fetal pole on transvaginal ultrasound scan 4 weeks after transfer or 6 weeks post-menstrual). Secondary outcome: • Live birth rate • Implantation rate per embryo transferred (defined as the number of gestational sacs present on ultrasound scan 4 weeks after transfer							

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	divided by the number of embryos transferred) Per-protocol analysis were carried out							

Evidence Table : Effectiveness
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<table>
<tr><th>Bibliographic citation</th><th>Study Type/Methods</th><th>LE</th><th>Number of Patients & Patient Characteristic</th><th>Intervention</th><th>Comparison</th><th>Length of Follow Up (If Applicable)</th><th>Outcome Measures/Effect Size</th><th>General Comments</th></tr>
<tr>
<td>6.Shi W, Hongwei T, Zhang W et al. A Prospective Randomized Controlled Study of Laser-Assisted Hatching on the Outcome of First Fresh IVF-ET Cycle in Advanced Age Women. Reprod Sci. 2016; 1-5. DOI: 10.1177/1933719116641764</td>
<td>Study design
Randomised controlled trial

Objective
To evaluate the effect of AH in the advanced age patients undergoing IVF

Method
The study was performed in the Assisted Reproduction Center, Northwest Women and Children’s Hospital, a public hospital in Xi’an, China

Couples undergoing IVF embryo transfer (IVF-ET) were included

Inclusion criteria:
• age 35 years to age ≤42 years
• basal follicle-stimulating hormone (FSH) value: FSH ≤ 10 IU/L
• fresh day 3 embryos transfer in IVF/intracytoplasmic sperm injection (ICSI) ET cycles</td>
<td>II-1</td>
<td>178 patients

LAH (n=82), control (n=96)

age 35 years to age ≤42 years</td>
<td>LAH</td>
<td>No hatching</td>
<td></td>
<td>Implantation rate
No difference was shown in implantation rate
(AH vs control, 32.45% vs 39.29%, p=0.204).

Clinical pregnancy
No difference was found in clinical pregnancy rate (AH vs control, 48.78% vs 59.38%, p=0.157)

<table>
<tr><th>Age group</th><th>AH</th><th>Control</th><th>P value</th></tr>
<tr><td>overall</td><td>40/82 (48.78%)</td><td>57/96 (59.38%)</td><td>p=0.157</td></tr>
<tr><td>≥35 to 38</td><td>29/52 (55.77%)</td><td>42/54 (65.63%)</td><td>p=0.279</td></tr>
<tr><td>≥38 to 40</td><td>8/20 (40%)</td><td>11/27 (40.74%)</td><td>p=0.959</td></tr>
<tr><td>≥40 to 42</td><td>3/10 (30%)</td><td>4/5 (80%)</td><td>p=0.119</td></tr>
</table>

Miscarriage rate
No difference was found in miscarriage rate (AH vs control, 15.85% vs 15.63%, p=0.967)

Authors’ conclusion
Laser AH has no benefit in improving implantation or pregnancy rates in advanced age women</td>
<td></td>
</tr>
</table>

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	Patients were randomly allocated to the AH group and control group. However, randomization method was not mentioned Assisted hatching was performed in the AH group on day 3 embryos before transfer by a laser treatment (ZILOS-tk; Hamilton Thorne Instruments Biosciences, Beverly) The ZP was thinned to more than two-thirds of its initial thickness and a distance of 30 to 40 um Outcome measures: • Implantation rate • Pregnancy rate (pregnancy diagnosed by ultrasonographic visualization of 1 or more gestational sacs, including an ectopic pregnancy) • Miscarriages rate (unexpected loss of a fetus before the 28th week of pregnancy, including a natural reduction in multiple pregnancy)							

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Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
7.Horng SG, Chang CL, Wu HM et al. Laser-assisted hatching of embryos in women of advanced age after in vitro fertilization: a preliminary report. Chang Gung Med J. 2002; 25(8):531-537.	Study design Randomised controlled trial Objective To demonstrate the effect of LAH on pregnancy outcomes in women of advanced age who received IVF/ET. Method Women aged 37 years or older undergoing IVF or intracytoplasmic sperm injection (ICSI) were included in the study They were allocated into 2 groups according to the last digit of their chart number: patients with an odd number (group I), even number (group II) In group I (N=80), embryos were cultured and transferred without LAH, whereas embryos of group II (N=40) were examined and treated with LAH just before being transferred Women with poor ovarian reserves (elevated baseline follicle- stimulating hormone	II-1	Mean ages Groups I: 38.8 ±1.8 Group II 39.5 ± 1.8 (p = 0.17) Group 1 (N=80) Group II (N=40)	LAH (group II)	No hatching (group I)		**Clinical pregnancy rate** • Group I: 13 (16.3%) • Group II: 7 (17.5%) , (p=0.864) **Implantation rate** • Group I : 7.3% • Group II : 6.7%, (p=0.648) No significant different in pregnancy rate and implantation rate when subanalysed by aged of 37-39 and more than 40 years old Clinical pregnancy rate 37-39 years • Group I: 14.2% • Group II: 20.8%, (p=0.516) Clinical pregnancy rate >40 years • Group I: 20.8% • Group II: 12.5% , (p=0.681) Implantation rate 37-39 years • Group I : 7.4% • Group II : 9.0%, (p=0.808) Implantation rate >40 years • Group I : 7.1% • Group II : 3.1%, (p=0.288)	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	(FSH), e.g., day 3 FSH > 15 mIU/ml) and non-obstructive azoospermia were excluded Openings of about 20 µm were created through the zona using diode laser (Fertilase, Medical Technologies, Montreux SA, Switzerland) Pregnancy was detected with a urinary pregnancy test, and results that were negative or equivocal were further confirmed by RIA for serum β-HCG levels. Clinical pregnancy was defined as a distinct intrauterine gestational sac seen on transvaginal ultrasound Pregnancy and implantation rates were analyzed on the basis of transfer cycles							

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Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
8.Tannus S, Cohen Y, MD, Henderson S et al. The Effect of Assisted Hatching on Live Birth Rate Following Fresh Embryo Transfer in Advanced Maternal Age. Reprod Sci. 2018: 1-6; doi: 10.1177/1933719118799192	Study design Retrospective cohort study Objective To investigate the effect of AH on live birth rate in women aged 40 years and older Method The study retrospectively evaluated all IVF cycles with fresh ET among women older than 40 years performed between the years 2011 and 2015, at a single academic reproductive center The inclusion criteria included female aged ≥40 years at the beginning of ovarian stimulation, first IVF cycle and single or double fresh ETs on day 3 or 5 Insemination of retrieved oocytes was performed by intracytoplasmic sperm injection (ICSI) or conventional IVF Laser-assisted hatching was performed on cleavage stage and blastocyst stage embryos Categorical data were	II-2	892 patients 681 cleavage stage LAH=502 No LAH=179 211 blastocyst stage) LAH=60 No LAH=151 mean age was 41.1 + 1.1 years	LAH	No hatching		**Effect of LAH on Cleavage Stage ET (681)** **Clinical pregnancy** • LAH= 9.16% (46) • Control= 18.44% (33), p<0.01 (0.03-0.15) **Live birth** • LAH= 5.37% (27) • Control =12.85% (23), p<0.01 (0.03-0.13) After controlling for maternal age, number of MII oocytes, fertilization method, and embryo quality, the clinical pregnancy rate [odds ratio (OR) = 0.52, (95% CI: 0.31, 0.86), P=0.012] and the Live birth rate [OR= 0.36, (95% CI: 0.19, 0.68), P=0.001] remained higher in the control group compared to the AH group. **Effect of AH on Blastocyst Stage ET (211)** **Clinical pregnancy** • LAH= 25% (15) • Control= 28.4% (43), p= 0.6 **Live birth** • LAH= 20% (12) • Control= 19.2% (29) p=0.8 After controlling for maternal age, number of blastocysts transferred, and embryo quality, the clinical pregnancy [OR= 1.17, (95% CI: 0.59, 2.4), P=0.64] and the LBRs [OR= 0.93, (95% CI: 0.44, 2.07), P =0.8] remained similar	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	presented with their frequency and percentage within the study group Logistic regression analysis was performed to adjust for possible confounding factors Between-group differences were assessed using the t test/ Mann-Whitney-Wilcoxon or w2 tests for continuous or categorical data, respectively The confounding factors included the following: maternal age, number of metaphase II (MII) oocytes, fertilization method (ICSI/IVF), number of embryo transferred, and embryo quality						Authors' conclusion: AH is associated with reduced pregnancy and LBRs of cleavage stage ET and does not improve the reproductive outcome following blastocyst transfer	

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Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
9.Li D, Yang LD, An J et al. Effect of assisted hatching on pregnancy outcomes: a systematic review and Meta-analysis of randomized controlled trials. 2016; Sci. Rep. 6, 31228; doi: 10.1038/srep 31228	Study design Systematic review with meta-analysis Objective To verify the effect of AH on pregnancy outcomes Methods Systematic searched was done on Pubmed, Web of Science, Cochrane library databases Included studies were RCTs that evaluated effect of AH human embryos compared with control group which embryos not submitted to AH Mixed of fresh embryos or frozen-thawed embryos Data extracted and assessment risk of bias was done **Outcome of interest were:** •Clinical pregnancy •Live birth •Multiple pregnancy •Miscarriage All outcomes were dichotomous and result were	II-1	36 RCTs on AH included (N=6459) •18 studies used Laser AH •12 studies used chemical AH •6 studies used mechanical AH	Assisted Hatching (AH) •Chemical •Laser •Mechanical	No hatching		**Clinical pregnancy rate** •Significance difference for overall AH compared to control [OR = 1.16; (95% CI: 1.00, 1.36), p value not mentioned, I^2 = 48.3%, 36 RCTs) •No significant difference Laser AH compared to control [OR = 1.03; (95% CI: 0.81, 1.30), p value not mentioned, I^2 = 60%, 18 RCTs) **Live births per couple** •No significance difference for overall AH compared to control [OR = 1.09; (95% CI: 0.92, 1.30), p value not mentioned, I^2 = 0%, 15 RCTs) •No significant difference Laser AH compared to control [OR = 1.19; (95% CI: 0.77, 1.83), p value not mentioned, I^2 = 9.4%, 5 RCTs) **Multiple pregnancy** •Significance difference for overall AH compared to control [OR = 1.50; (95% CI: 1.11, 2.01), p value not mentioned, I^2 = 44%, 20 RCTs) •Significant difference Laser AH compared to control [OR = 1.87; (95% CI: 1.33, 2.63), p value not mentioned, I^2 = 0%, 6 RCTs) **Miscarriage rate** •No significance difference for overall AH compared to control [OR = 1.03; (95% CI: 0.72, 1.48), p value not mentioned, I^2 = 0%, 17 RCTs) •No significant difference Laser AH	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	expressed for each trials as an odds ratio (OR) with 95% confidence interval (CI) Stratified analyses such as hatching method (chemical, laser or mechanical); conception mode, number of participants in the AH group, the extent of AH, embryo transfer status, previous failure history and embryo transfer status with previous failure history.						compared to control [OR = 1.03; (95% CI: 0.56, 1.90), p value not mentioned, I^2 = 0%, 5 RCTs)	

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Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
10.Carney SK, Das S, Blake D et al. Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)). Cochrane Database of Systematic Reviews 2012, Issue 12. Art. No.: CD001894. DOI: 10.1002/14651858.CD001894.pub5.	Study design Systematic review with meta-analysis Objective To determine the effect of assisted hatching (AH) of embryos from assisted conception on live birth and multiple pregnancy rates. Methods Systematic search was carried out on the Cochrane Menstrual Disorders and Subfertility Group Specialised Register (August 2012), the Cochrane Central Register of Controlled Trials (CENTRAL) (August 2012), MEDLINE (1966 to August 2012) and EMBASE (1980 to August 2012). Hand search of reference list also done. Three authors identified and independently screened trials. Included studies: Randomised controlled trials (RCTs) of AH (mechanical, chemical or laser disruption of the zona pellucida prior to embryo replacement) versus no AH that reported live	I	31 RCTs were included (N=5728 women who undergoing IVF or ICSI) AH group (n=2933) control groups (n=2795) Age 27 to 40 years 12 trials included women with poor diagnosis, 12 trials with good diagnosis, remainder not mentioned.	•11 trials on chemical AH •5 trials on mechanical AH •15 trials on laser AH	No hatching		**Primary outcome:** **1. Live birth (live offspring per woman)** •No significant difference between laser AH and control groups (5 RCTs, 1555 women; OR 1.01, 95% CI 0.81 to 1.26, P = 0.27, I^2 of 23%). **2. Multiple pregnancy rate per woman** •Significant increased multiple pregnancy rate per woman in laser AH compared to control (9 RCTs, 2869 women; OR 1.27, 95% CI 1.00 to 1.61, P = 0.006, I^2 of 63%). However, there was significant heterogeneity **Secondary outcome:** **3. Clinical pregnancy rate per woman** •No significant different in laser AH compared to control group (15 RCTs, 3606 women; OR 1.04, 95% CI 0.90 to 1.19, P = 0.63, I^2 of 62%). **4. Miscarriage per woman** •No significant difference in miscarriage rate in laser AH compared to control group,(8 RCTs, 1565 women; OR 0.98, 95% CI 0.59 to 1.63, P = 0.95, I^2 of 0%) Meta-analysis on Monozygotic twinning	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	birth or clinical pregnancy. Three authors independently performed quality assessments and data extraction. Primary outcome were: 1. Live birth (live offspring per woman) 2. Multiple pregnancy rate per woman Secondary outcome: 1.Clinical pregnancy rate per woman 2.Miscarriage per woman 3.Ectopic pregnancy 4. Monozygotic twinning 5.Congenital or chromosomal abnormalities 6.Failure to transfer any embryos per woman 7.Embryo damage 8.In vitro blastocyst development Data were meta-analysed using fixed-effect models						outcome was carried out by combining all AH methods but no subgroup analysis done for individual method No meta-analysis was carried out for Ectopic pregnancy, Congenital or chromosomal abnormalities, and Embryo damage No trials reported on Failure to transfer any embryos per woman and In vitro blastocyst development outcome	

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<table>
<tr><th>Bibliographic citation</th><th>Study Type/Methods</th><th>LE</th><th>Number of Patients & Patient Characteristic</th><th>Intervention</th><th>Comparison</th><th>Length of Follow Up (If Applicable)</th><th>Outcome Measures/Effect Size</th><th>General Comments</th></tr>
<tr>
<td>11.Elhussieny A, El Mandouh M, Hanafi S et al. Effect of laser assisted hatching on outcome of assisted reproductive technology. Open J Obstet Gynecol. 2013; 3: 18-23</td>
<td>Study design
Randomised controlled trial

Objective
To determine whether laser assisted hatching can improve clinical outcome of assisted reproductive techniques in both unselected patients and patients with advanced female age, with recurrent implantation failure, or who are using frozen-thawed embryos.

Method
The study was carried out over a period of 20 months from December 2010 to August 2012.

The patients were randomly selected and divided into 2 groups. However, the randomization method was not mentioned

The groups were further subdivided into a total of three subgroups of:
•patients with advanced female
•patients with implantation failure
•patients with frozen–thawed embryos</td>
<td>II-1</td>
<td>Control group (n=94)

Treatment group (n=85)

Subgroup division
•patients with advanced female age (≥35 y; control, n = 24; test, n = 30
•patients with implantation failure (for ≥1 cycles; control, n = 42; test, n = 42),
•patients with frozen–thawed embryos (control, n = 16; test, n = 16)</td>
<td>LAH</td>
<td>No hatching</td>
<td></td>
<td>Clinical pregnancy rate
LAH=35.1% versus Control= 28.2%, (p=0.32)

<table>
<tr><th>Study characteristic</th><th>LAH</th><th>Control</th><th>P value</th></tr>
<tr><td>overall</td><td>35.10%</td><td>28.20%</td><td>p=0.324</td></tr>
<tr><td><35 years</td><td>18.80%</td><td>29.50%</td><td>p=0.159</td></tr>
<tr><td>≥35 years</td><td>70%</td><td>25%</td><td>p=0.001</td></tr>
<tr><td>frozen</td><td>62.50%</td><td>56.30%</td><td>p=0.086</td></tr>
<tr><td>fresh</td><td>29.50%</td><td>21.70%</td><td>p=0.264</td></tr>
<tr><td>previous 1 trial</td><td>68.80%</td><td>30.80%</td><td>p=0.04</td></tr>
</table>

Implantation rate
LAH=50% versus control =33.3% (p=0.23)</td>
<td></td>
</tr>
</table>

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	The selected embryos for intrauterine transfer in the patients of the control group were intact, whereas in the test group, they were subjected to laser-assisted hatching (LAH). Embryo quality was assessed in all patients The embryo transfer was performed in situation that physician was blinded to the control and test groups The primary outcome was clinical pregnancy, defined as the presence of fetal heart activity by ultrasound at 6 to 7 weeks' gestation. Secondary outcomes included implantation rates, multiple gestations, and adverse fetal events.							

Evidence Table : Effectiveness
Question : Is Laser Assisted Hatching Effective?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
12.Ali J, Rahbar S, Burjaq H et al. Routine Laser Assisted Hatching Results in Significantly Increased Clinical Pregnancies. J Assist Reprod Genet. 2003; 20 (5): 177-181	Study design Randomised controlled trial Objective To determine whether the pregnancy rate can be increased if the zona barrier was compromised by LAH on day 2 embryos in patients selected at random irrespective of indication for AH. Method Investigation commenced in June 2001 till November 2001 Only embryos generated by ICSI were employed in the study There were two main groups, the control and the test groups These two groups were further subdivided by age to a total of four subgroups, namely those ≤36 years (control, n = 30; test, n =37) and those ≥37 (control, n = 20; test, n = 20) Only two of three embryos transferred to patients in the test group were subjected to LAH on day 2 cleavage-stage	II-1	107 patients	LAH	No hatching		**Clinical pregnancy rate** ≤ 36 years old • LAH 64.9 % (24/37) versus control 33.3% (10/30), p=0.0203 ≥37 years old • LAH 15.0 % (3/20) versus control 20.0% (4/20), p=1.00 **Implantation rate** ≤ 36 years old • LAH 38.1 % (40/105) versus control 17.5% (14/80), p=0.0039 ≥37 years old • LAH 6.9 % (4/58) versus control 9.3% (5/54), p=0.911	

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	embryos using the non-touch SaturnÔ Laser System of the Integra Model Embryos of patients in the control group was not subjected to LAH but all other procedures were identical to that of the test group However, clinical pregnancy and Implantation rate was not defined							

Evidence Table : Effectiveness
Question : Is Laser Assisted Hatching Effective?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
13.Antonori S, Panci C, Selman HA et al. Zona thinning with the use of laser: a new approach to assisted hatching in humans. Hum Reprod. 1996; 11(3):590-594	Study design Randomised controlled trial Objective To report the assisted hatching in human through zona-thinning using laser (Er:YAGA) Method Assisted hatching by laser zona-thinning was performed on 773 embryos from 207 IVF patients Of these embryos, 376 were transferred in 96 patients (group A) with repeated failures of standard IVF (two to four attempts) and 397 were transferred in 111 patients (group B) undergoing IVF for the first time. Both groups were compared to control groups (A' and B') in which assisted hatching was not performed.	II-1	**Group A** 96 patients repeated failure IVF **Group A'** 104 Control **Group B** 111 patients first time IVF **Group B'** 121 Control mean ages: 34.6 ± 5.2 for group A versus 34.8 ± 5.1 in group A' 34.4 ±5.1 for group B versus 34.6 ± 5.2 in B'.	LAH	No hatching		**Embryo transfer** Group A 396/96 patients Group A' 381/104 patients Group B 397/ 111 patients Group B' 411/ 121 patients **Clinical pregnancy rates** •41 (42.7%) in group A versus 24 (23.1%) in group A' (P < 0.053) •44 (39.6%) in group B versus 23 (19%) in group B' (P <0.05) **Implantation rate per embryo** •12.2% in group A versus 7.3% in group A' (P < 0.05) •11.8% in group B versus 7.1% in group B' (P <0.05)	

Evidence Table : Effectiveness
Question : Is Laser Assisted Hatching Effective?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
14.Ghannadi A, Kazerooni M, Jamalzadeh F et al. The effects of laser assisted hatching on pregnancy rates. Iran J Reprod Med. 2011; 9 (2): 95-98 Iran	Study design Non-randomised controlled trial Objective To investigate the benefit of laser assisted hatching in patient with advanced maternal age (over 35 years of age). Method Women who presented at, or were referred to, Shiraz infertility center undergoing IVF or Intra cytoplasmic sperm injection (ICSI) were included and allocated into 4 groups. •group I (age ≤35) embryos were cultured and transferred with LAH •group II (age ≤35) embryos were cultured and transferred without LAH •group III (age > 35) treated with LAH in women •group IV (age > 35) treated without LAH Embryos transfer was performed 2 days after retrieval (2-3 were transferred each cycle)	II-2	199 patients >35 years old LAH=83 No LAH =116 733 patients ≤ 35 years old LAH=404 No LAH =329	LAH	No hatching	-	**Clinical pregnancy rates** >35 years old (n=199) •Significantly increased in the LAH group [23/83 (27.71%)] compared to the control group 19/116 (16.37%)], p<0.05 ≤ 35 years old (n=733) •Significantly increased in the LAH group [202/404 (50%)] compared to the control group [101/329 (30.69%)], p<0.05 **Multiple pregnancy rates** >35 years old (n=199) •No significant differences were observed between the two groups [3/23 (13.04%)] in the laser group compared to control [1/19 (5.26%)] in women that pregnant, p>0.05 ≤ 35 years old (n=733) •Significant increase in the laser group [45/202 (22.27%)] compared to 6/101 (5.94%) control, p<0.05 In all 932 patients of this study (under and over 35 years old) the clinical pregnancy rates showed significant difference between LAH group (46.20%) compared to no LAH group (26.96%)	

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	Embryos with ≥50% fragmentation were not transferred. Laser AH was performed using the suturn-Tm3 system (Research Instrument. UK) The size of the hole made in the ZP was measured to be 5-10 micro meter, depending on the zona thickness of each individual embryo. Chemical pregnancy was defined by analyzing of β hCG hormone and clinical pregnancy as a distinct intrauterine gestational sac seen on transvaginal ultrasound. Statistical analysis was performed with student's t-test or chi- square test. In all cases, p<0.05 was considered statistically significant						Data as shown in table 1	

Table I. Subjects' characteristics and outcomes.

Characteristics	No. of study subjects	Chemical pregnancy rates (%)	Clinical pregnancy rates (%)	Multiple pregnancy rates (%)
LAH group (>35y)	83	25 (30.12%)*	23 (27.71%)*	3 (13.04%)
No LAH group (>35y)	116	22 (18.96%)	19 (16.37%)	1 (5.26%)
LAH group (≤35y)	404	206 (50.99%)*	202 (50%)*	45 (22.27%)*
No LAH group (≤35y)	329	104 (31.61%)	101 (30.69%)	6 (5.94%)
LAH group (total)	487	-	225 (46.20%)*	-
No LAH group (total)	445	-	120 (26.96%)	-

Summarizes subjects ' characteristics and outcomes. (*) Significant differences with no LAH group in the same age. ($p < 0.05$).

Evidence Table : Safety
Question : Is Laser Assisted Hatching Safe?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
15.Kanyo K and Konc J. A follow-up study of children born after diode laser assisted hatching. Eur J Obstet Gynecol Reprod Biol. 2003; 110; 176-180	Study design Prospective cohort study Objective To compile data on karyotypes, deliveries, congenital malformations and growth parameters to evaluate the safety of Laser assisted hatching Method The study investigated 134 children from the first 96 deliveries obtained after LAH between the 2 December 1998 and the 31 December 1999 All patients gave written informed consent for the LAH procedure and were asked to agree to the follow-up conditions of the study Data about the delivery and neonatal final reports were collected If any problem was mentioned the responsible pediatrician was contacted and asked for further detailed information of the examination and eventual treatment	II-2	134 children born in IVF with LAH (mother aged ≥35 years, > 3 IVF cycles) 894 children delivered in spontaneous pregnancy	LAH	Delivery in general population		Major malformation • 2.2% (3/134) in LAH • 3.0% (27/894) in general population, p=0.64 Minor malformation • 10.4% (14/134) in LAH (such as Unilateral pes equinovalgus, atrial septal defect (4 cases), cryptorchism, congenital naevus, duplicated Pyelum, Congenital hip luxation, Torticollis (2 cases), Ductus arteriosus (3 cases) • 11.1% (99/894) in general population p=0.32 One baby in major malformation group died at age of 10 days. There were no additional anomalies found during follow-up examinations at 12 weeks (133/133 or 100%) at six months (132/133 or 99.2%) and at one year (131/133 or 98.5%). Lost of follow up rate was 1.5%	

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	Two questionnaires were used for telephone interviews after the delivery, at 12 weeks, at 6 months and at 1 year. Information was compiled from the medical records as well as careful questioning of the parents.							