LASER ASSISTED HATCHING (THINNING VERSUS DRILLING) IN FRESH AND FROZEN EMBRYO TRANSFER IN ADVANCED MATERNAL AGE

Ahmed Ayoub[1*] , Amr El noury[2] , Osama Azmy[1], Sameh Salama[1] , Mazen Abdel-Rasheed[1] , Tamer Elnahas[1], Sherif Dahab[3] , Emadeldin R matar[4] , Ehab Salama[1]

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Abstract:

Background: Laser assisted hatching (LAH) is a technique used in assisted reproductive technology to increase the chances of successful pregnancy in couples struggling with infertility. Advanced maternal age has been identified as a risk factor for decreased fertility and increased rates of chromosomal abnormalities in embryos, leading to a decrease in the success rate of in vitro fertilization (IVF) procedures. Our study focused on the use of LAH in couples with advanced maternal age by either drilling or thinning techniques.

Methods: we included 126 women divided into 2 groups : group 1 LAH thinning (T-LAH) (53) had 26 fresh embryo transfer and 27 frozen embryo transfer while group 2 LAH drilling (D-LAH) (73) had 33 fresh embryo transfer and 40 frozen embryo transfer. LAH thinning (T-LAH) was done for group 1 while LAH drilling (D-LAH) of zona pellucida was done for group 2. Chemical and clinical pregnancies were observed.

Results: group 1 LAH thinning (T-LAH) had a similar β-human chorionic gonadotropin-positive rate (37.74% vs. 45.21%), implantation rate (21.37% vs. 28.83%), clinical pregnancy rate (35.85% vs. 41.10%), miscarriage rate (15.79% vs. 20.00%), to group 2 LAH drilling (D-LAH). Also there was no any significant difference in either clinical pregnancy or implantation rates when comparing thinning and drilling in each fresh and frozen embryo transfers cycles.

Conclusion: Overall, the available data in our study suggested that no significant differences between both techniques of LAH (thinning versus drilling) in improving IVF outcomes in advanced maternal age, but further research is needed to determine its safety and efficacy in this population.

Keywords: Laser assisted hatching, Intracytoplasmic sperm injection, invitro fertilization, Zona pellucida, advanced maternal age.

1 Reproductive Health and Family Planning Department, National Research Centre, Giza, Egypt
2 National Institute of Laser Enhanced Science, Cairo University, Cairo, Egypt
3 Obstetrics and Gynecology Department, Kasr Aini Hospital, Cairo University, Cairo, Egypt
4 Department of Pathology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
*** Corresponding author**: Ahmed Ayoub, MSC **E-mail :** dr_ayoub2080@yahoo.com

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Background:

Hatching is a natural process that occurs when an embryo emerges from the zona pellucida (ZP) surrounding it before implantation. When hatching fails to occur, it can lead to implantation failure [1]. Assisted hatching (AH) is used in in vitro fertilization (IVF) and embryo transfer (ET) to improve implantation by disrupting the ZP. This is typically done during the cleavage or blastocyst stage of embryo development using techniques like chemical or laser-assisted hatching (LAH) [2]. LAH is currently the most widely used technique due to its shorter procedure time, greater precision, and reduced variability between technicians. However, the effectiveness of AH is unclear due to differences in clinical indications, study populations, and AH techniques used. The American Society of Reproductive Medicine recommends against routine use of AH for all patients due to limited data from previous trials [3].

AH is often used for patients with advanced maternal age or decreased oocyte yield after controlled ovarian stimulation, as older women's oocytes may have structural abnormalities, such as a hardened ZP, that lower success rates [4]. However, studies on the benefits of AH in advanced maternal age have been limited and have included broad age ranges, limiting their applicability to this age group [5]. However, the benefits of assisted hatching specifically for women of advanced maternal age is still unclear, many studies having been conducted with small sample sizes and a wide range of ages, including those under 35, which may limit the applicability of their findings to this particular group [6,7].

Thinning (T-LAH) and drilling (D-LAH) are two different methods of AH by creating an opening in the ZP. Thinning involves using the laser to partially thin the ZP, while drilling involves creating a small hole or opening in the zona pellucida. The choice of technique depends on a variety of factors, including the age of the patient, the quality of the embryos, and the experience of the embryologist and both techniques still a subject to study [8].

T-LAH is considered to be a gentler and more conservative approach to LAH. It involves using the laser to partially thin the ZP, which creates a weakened area that is more likely to rupture during the hatching process. T-LAH is often used in cases where the zona pellucida is abnormally thick or where the embryo has a low hatching potential. T-LAH can also be useful in cases where the embryo is at risk of damage during the hatching process, as it can help to reduce the force required for hatching [9]. D-LAH, on the other hand, involves creating a small hole or opening in the zona pellucida. This technique is typically used in cases where the embryo is at high risk of damage during the hatching process [10]. D-LAH allows for a more precise opening to be created in the zona pellucida, which can reduce the risk of damage to the embryo. However, D-LAH can also increase the risk of damage to the embryo if not done properly, as it requires more skill and experience [11].

Aim of the study:

The goal of this study is to assess the effects of AH either thinning or drilling on the reproductive outcomes on women aged 35 and above.

Patients and Methods:

This study was a prospective randomized controlled study that had been held in a private IVF center in Cairo with collaboration of the national research center over a period of 2 years.

A hundred twenty-six cycles were done (53 T-LAH and 73 D-LAH) and were enrolled according to the following criteria: We Included women above age of 35 and below 45 years old with a previous ICSI failure, unexplained infertility or male infertility. Women with hydrosalpinx, pyosalpinx, endometriosis, or uterine fibroids were excluded.

Ethical approval and Consent:

This study is registered in clinical trials under number NCT05311553 The scientific ethical committee approval of our institute for this study was 19021 Informed written consent was taken from all participants before recruitment in the study, and after explaining the purpose and procedures of the study.

All study participants were undergone controlled ovarian stimulation by GnRH agonist long protocol starting from mid luteal phase. The complete pituitary suppression was confirmed by serumE2 level less than 30 pg/ml and serum LH level less than 2 mIU/ml, then gonadotropin therapy was started from day 2 to the day of maturation confirmed by presence of 3 mature follicles or more, one of them 18 mm; at this time 10000 IU of hCG was administered then ovum pick up was done 36 hours after hCG administration under transvaginal ultrasound guidance Cumulus cell masses around the oocytes was removed using pull-and-cut denudation pipettes in a 5-well culture dish (MTG, Bruckberg, Germany) containing 27 IU/mL of hyaluronidase.

All oocytes that were mature at 4-6 h after oocyte collection had been inseminated according to the quality of the spermatozoa and oocytes and the patient's previous IVF history. Fertilization was confirmed 17-18 h after insemination by the presence of two distinct pronuclei. Zygotes had been cultured in 30-µL micro-drops with a 1-step medium and overlaid with paraffin oil in an atmosphere of 6% CO_2, 5%O_2, and 95% humidity at 37 °C. The available embryos were assessed in all patients according to the criteria of equal and regular blastomeres, a viable blastomere number, and fragmentation ratio.

LAH was conducted using a Laser System with a 1.48-µm diode-laser at 300 mW through thinning or drilling. The embryos will undergo either T-LAH or D-LAH in 10- to 20-µL micro-drops consisting of G-MOPS plus medium. The embryos were fixed in the micro-drops on the inverted microscope. The size of one laser shot was 5µm in the ZP using the same total number of 3000-µs pulses. In T-LAH, the goal of the thinning method is to make the outer portion of the outer protective glycoprotein layer thinner. The laser thinning will be performed by making 2-3 holes without reaching the inner membrane at a depth of 60–80% of the ZP thickness. In D-LAH, the laser opening will be made from the outside to the inside of the ZP.

The laser beam shot towards the ZP above the perivitelline space between two blastomeres to minimize the risk of harming the embryo, under careful control. The embryos in the micro-drop were washed several times after LAH and then transferred to a 1-well dish (Falcon 353,653, USA) containing G-2 culture medium. LAH was performed 2 to 3 h before embryo transfer. The embryos were cultured in an atmosphere of 6% CO_2, 5% O_2, and 95% humidity at 37 °C.

Embryo transfer

Our policy is not to transfer poor quality embryos, and these embryos are discarded. Supernumerary embryos were cultured to the blastocyst stage and at least average embryo blastocysts (3BB) were cryopreserved on day 5 or 6.

Luteal support in fresh cycles included estradiol valerate 6 mg and micronized vaginal progesterone300 mg daily. Luteal support was continued until 10 weeks of gestation

Pregnancy was considered as positive if serum levels of hCG were >10 IU/L when performed 2 weeks after oocyte retrieval. Clinical pregnancy was defined as the visualization of gestational sac by vaginal sonography by 6 weeks of pregnancy.

Results:

In this study, 126 women were enrolled and split into 2 groups: A total of 53 women were in group 1 LAH thinning (T-LAH), of whom 26 received fresh embryos transfer and 27 received frozen embryos transfer, whereas 73 women were in group 2 LAH drilling (D-LAH), of whom 33 received fresh embryos transfer and 40 received frozen embryos transfer. LAH thinning (T-LAH) was done for group 1 while LAH drilling (D-LAH) of zona pellucida was done for group 2.

Regarding the females' age, duration of infertility, reason of infertility, number of collected and fertilized oocytes, or miscarriage rate, there was no statistically significant difference between the groups (Table 1).

Group 1 LAH thinning (T-LAH) had a similar β-human chorionic gonadotropin-positive rate (37.74% vs. 45.21%), implantation rate (21.37% vs. 28.83%), clinical pregnancy rate (35.85% vs. 41.10%), miscarriage rate (15.79% vs. 20.00%), to group 2 LAH drilling (D-LAH). Also there was no any significant difference in either clinical pregnancy or implantation rates when comparing thinning and drilling in each fresh and frozen embryo transfers cycles ($P < 0.05$; Table 1).

Also when comparing thinning and drilling in each fresh and frozen embryo transfers cycles, there was no statistically significant difference regarding the cause of infertility, number of prior unsuccessful cycles, age, weight and height of the females. In fresh embryos transfer cycles in patients aged more than or equal 35 years, thinning LAH was done in 26 cycles and drilling LAH was done in 33 cycles. Implantation rates and the rates of chemical and clinical pregnancies were not significantly different in the T-LAH group (24.64%, 42.31%, 42.31%) compared to those in the D-LAH group (27.94%, 42.42%, 36.36%) respectively ($P < 0.05$; Table 2).

In frozen embryos transfer cycles in patients aged more than or equal 35 years, thinning LAH was done in 27 cycles and drilling LAH was done in 40 cycles. Implantation rates and the rates of chemical and clinical pregnancies were not significantly different in the T-LAH group (17.74%, 33.33%, 29.63%) compared to those in the D-LAH group (29.47%, 47.50%, 45.00%) respectively ($P < 0.05$; Table 3).

Table 1: Clinical and laboratory characteristics of the patients in the T-LAH and D-LAH

Patient Age Groups 35-45 Years		Laser										Chi-Square or T-Test	
		Thinning			Drilling			Total					
		N		%	N		%	N		%		X^2 or T	P-value
Duration of infertility (Years)	Range	4	-	18	4	-	18	4	-	18		1.792	0.076
	Mean ±SD	9.830	±	3.540	8.630	±	3.832	9.135	±	3.745			
Cause of infertility	Male	21		39.62	31		42.47	52		41.27		4.516	0.211
	Unexplained	17		32.08	29		39.73	46		36.51			
	Tubal	6		11.32	9		12.33	15		11.90			
	Combined (M&T)	9		16.98	4		5.48	13		10.32			
Oocyte Number	Range	1	-	25	1	-	25	1	-	25		-0.574	0.567
	Mean ±SD	7.736	±	5.332	8.288	±	5.324	8.056	±	5.313			
M2 N.	Range	1	-	12	1	-	14	1	-	14		-0.236	0.814
	Mean ±SD	4.491	±	2.750	4.616	±	3.090	4.563	±	2.941			
N. of fertilized oocytes	Range	1	-	9	1	-	10	1	-	10		-0.264	0.793
	Mean ±SD	3.434	±	1.976	3.534	±	2.199	3.492	±	2.100			
At day	Range	3	-	5	3	-	5	3	-	5		0.408	0.685
	Mean ±SD	4.154	±	0.881	4.061	±	0.864	4.102	±	0.865			
Embryos transferred	Range	1	-	4	1	-	4	1	-	4		1.355	0.178
	Mean ±SD	2.472	±	0.953	2.233	±	0.993	2.333	±	0.980			
Chemical pregnancy	Negative	33		62.26	40		54.79	73		57.94		0.703	0.402
	Positive	20		37.74	33		45.21	53		42.06			
Clinical pregnancy	No	34		64.15	43		58.90	77		61.11		0.356	0.551
	Yes	19		35.85	30		41.10	49		38.89			
Implantation rate		28/13		21.37%	47/163		28.83%						0.145
N. of sacs	Range	1	-	3	1	-	3	1	-	3		-0.485	0.630
	Mean ±SD	1.474	±	0.612	1.567	±	0.679	1.531	±	0.649			
Abortion	No	16		84.21	24		80.00	40		81.63		0.138	0.711
	Yes	3		15.79	6		20.00	9		18.37			

Values are presented as mean ± standard deviation or number/total (%). No differences were significant (P > 0.05) T-LAH, thinning laser-assisted hatching; D-LAH, drilling laser-assisted hatching

Table 2: Clinical and laboratory characteristics of the patients in the T-LAH and D-LAH in Fresh embryo transfer

Patient Age Groups 35-45 Years Fresh embryo transfer		Laser						Chi-Square or T-Test	
		Thinning		Drilling		Total			
		N	%	N	%	N	%	X^2 or T	P-value
Cause of infertility	**Male**	10	38.46	14	42.42	24	40.68	5.172	0.160
	Unexplained	5	19.23	10	30.30	15	25.42		
	Tubal	4	15.38	7	21.21	11	18.64		
	Combined (M&T)	7	26.92	2	6.06	9	15.25		
N. of failed cycles	**No**	3	11.54	6	18.18	9	15.25	7.972	0.093
	One	3	11.54	11	33.33	14	23.73		
	Two	7	26.92	10	30.30	17	28.81		
	Three	9	34.62	4	12.12	13	22.03		
	Four	4	15.38	2	6.06	6	10.17		
Weight	**Range**	61 - 99		59 - 101		59 - 101		1.366	0.177
	Mean ±SD	80.577 ± 9.052		76.606 ± 12.440		78.356 ± 11.165			
Height	**Range**	1.52 - 1.67		1.51 - 1.71		1.51 - 1.71		0.043	0.966
	Mean ±SD	1.607 ± 0.041		1.606 ± 0.043		1.606 ± 0.042			
Oocyte Number	**Range**	2 - 25		1 - 18		1 - 25		-0.186	0.853
	Mean ±SD	6.769 ± 4.998		7.000 ± 4.535		6.898 ± 4.704			
M2 N.	**Range**	1 - 12		1 - 10		1 - 12		0.605	0.547
	Mean ±SD	4.154 ± 2.477		3.758 ± 2.513		3.932 ± 2.483			
N. of fertilized oocytes	**Range**	1 - 7		1 - 8		1 - 8		0.438	0.663
	Mean ±SD	3.231 ± 1.557		3.030 ± 1.879		3.119 ± 1.733			
At day	**Range**	3 - 5		3 - 5		3 - 5		0.408	0.685
	Mean ±SD	4.154 ± 0.881		4.061 ± 0.864		4.102 ± 0.865			
Chemical pregnancy	**Negative**	15	57.69	19	57.58	34	57.63	0.000	0.993
	Positive	11	42.31	14	42.42	25	42.37		
Clinical pregnancy	**No**	15	57.69	21	63.64	36	61.02	0.216	0.642
	Yes	11	42.31	12	36.36	23	38.98		
Implantation rate		17/69	24.64%	19/68	27.94%				0.660
N. of sacs	**Range**	1 - 3		1 - 3		1 - 3		-0.134	0.895
	Mean ±SD	1.545 ± 0.688		1.583 ± 0.669		1.565 ± 0.662			
Abortion	**No**	9	81.82	11	91.67	20	86.96	0.491	0.484
	Yes	2	18.18	1	8.33	3	13.04		
	Mean ±SD	1.607 ± 0.041		1.606 ± 0.043		1.606 ± 0.042			

Values are presented as mean ± standard deviation or number/total (%). No differences were significant (P > 0.05) T-LAH, thinning laser-assisted hatching; D-LAH, drilling laser-assisted hatching

Table 3: Clinical and laboratory characteristics of the patients in the T-LAH and D-LAH in Frozen embryo transfer

Patient Age Groups 35-45 Years Frozen embryo transfer		Laser						Chi-Square or T-Test	
		Thinning		Drilling		Total			
		N	%	N	%	N	%	X^2 or T	P-value
Cause of infertility	Male	11	40.74	17	42.50	28	41.79	0.357	0.949
	Unexplained	12	44.44	19	47.50	31	46.27		
	Tubal	2	7.41	2	5.00	4	5.97		
	Combined (M&T)	2	7.41	2	5.00	4	5.97		
N. of failed cycles	No	8	29.63	19	47.50	27	40.30	6.723	0.151
	One	9	33.33	14	35.00	23	34.33		
	Two	5	18.52	6	15.00	11	16.42		
	Three	3	11.11	0	0.00	3	4.48		
	Four	2	7.41	1	2.50	3	4.48		
Weight	Range	59 - 100		57 - 112		57 - 112		1.699	0.094
	Mean ±SD	80.630 ± 10.467		75.700 ± 12.375		77.687 ± 11.814			
Height	Range	1.52 - 1.69		1.52 - 1.72		1.52 - 1.72		-0.824	0.413
	Mean ±SD	1.603 ± 0.046		1.613 ± 0.047		1.609 ± 0.046			
Oocyte Number	Range	1 - 25		1 - 25		1 - 25		-0.484	0.630
	Mean ±SD	8.667 ± 5.568		9.350 ± 5.736		9.075 ± 5.636			
M2 N.	Range	1 - 11		1 - 14		1 - 14		-0.636	0.527
	Mean ±SD	4.815 ± 3.000		5.325 ± 3.362		5.119 ± 3.208			
N. of fertilized oocytes	Range	1 - 9		1 - 10		1 - 10		-0.546	0.587
	Mean ±SD	3.630 ± 2.323		3.950 ± 2.375		3.821 ± 2.341			
Chemical pregnancy	Negative	18	66.67	21	52.50	39	58.21	1.330	0.249
	Positive	9	33.33	19	47.50	28	41.79		
Clinical pregnancy	No	19	70.37	22	55.00	41	61.19	1.604	0.205
	Yes	8	29.63	18	45.00	26	38.81		
Implantation rate		11/62	17.74%	28/95	29.47%				0.096
N. of sacs	Range	1 - 2		1 - 3		1 - 3		-0.648	0.523
	Mean ±SD	1.375 ± 0.518		1.556 ± 0.705		1.500 ± 0.648			
Abortion	No	7	87.50	13	72.22	20	76.92	0.728	0.393
	Yes	1	12.50	5	27.78	6	23.08		

Values are presented as mean ± standard deviation or number/total (%). No differences were significant (P > 0.05) T-LAH, thinning laser-assisted hatching; D-LAH, drilling laser-assisted hatching

DISCUSSION:

Assisted hatching is a technique used in IVF to help embryos to hatch from their ZP. This procedure involves creating a small hole or thinning the zona pellucida to facilitate the embryo's escaping from it and allow implantation in the uterus. Advanced maternal age is a term used to describe women who are 35 years or older at the time of conception. Women of advanced maternal age have a higher risk of infertility, pregnancy complications, and chromosomal abnormalities in the developing embryo. Assisted hatching has been proposed as a potential solution to some of these challenges.

In our study we performed laser assisted hatching thinning versus drilling in women above the age of 35 to 45 years in both fresh and frozen embryo transfer cycles. We could not detect any significant difference in neither chemical nor clinical pregnancy rates in both fresh and frozen embryo transfers.

Several studies have investigated the role of assisted hatching in advanced maternal age. A study group published research in 2019 examining the impact of D-LAH on fresh embryo transfer blastocysts on pregnancy outcomes in women aged 35-40 years. The study found that the clinical pregnancy rate was not significant in the assisted hatching group compared to the control group [12].

One meta-analysis published in 2018 found that it is difficult to draw definitive conclusions about the reproductive outcomes of AH in women of advanced maternal age due to limited sample sizes included in the studies. Therefore, they recommended to conduct studies with high methodological quality and adequate power to investigate the potential benefits of AH in assisted conception for this patient population [13]. It is still a challenge to have a big sample study due to lack of patients involved in assisted reproduction, low acceptance rate to try a procedure with no evidence based recommendations and finally due to the cost of this procedures that are not covered by insurance in most of the countries.

In a 2019 study on patients with recurrent implantation failure, it was found that both D-LAH and T-LAH techniques had comparable clinical outcomes irrespective of maternal age up to 40 years old. Furthermore, there was no evidence to suggest that T-LAH was superior to D-LAH [14]. Also, Lee and his research group found that there were no significant differences in the implantation and pregnancy rates when transferring thawed embryos after T-LAH or D-LAH procedures. ZP thickness and the women's age had no effect on the implantation and clinical pregnancy rates in women who underwent ICSI-FET and AH treatment by these two methods [15].

Knudston et al. analyzed frozen embryo transfer cycles between 2004 and 2013 using the Society for Assisted Reproductive Technology (SART) database. They found that AH was associated with a significantly reduced LBR compared to the control group. Women of advancing age were particularly affected, with a greater reduction in LBRs compared to younger women [16]. Another study by Butts et al. examined the effect of AH on live birth in women with diminished ovarian reserve who underwent fresh ET. They found that AH was associated with a significant reduction in the LBR compared to the control group [17].

Several studies have compared the pregnancy rates of embryos that underwent thinning or drilling during laser assisted hatching (LAH) procedures. While the results of these studies are not always consistent, some general trends can be observed. One study published comparing the pregnancy rates in women performed LAH on their embryos using either thinning or drilling. The study found that the clinical pregnancy rate was higher in the thinning group compared to the drilling group [18]. Another study in 2016 compared the pregnancy outcomes of 142 women who underwent LAH using either thinning or drilling. This study found no significant difference in the clinical pregnancy rate between the two groups (39.7% for thinning versus 37.0% for drilling). However, the ongoing pregnancy rate was significantly higher in the drilling group (33.6%) compared to the thinning group (19.7%) [19].

However, more research is needed to confirm these findings and to determine which technique is more effective in different clinical situations. It is important to note that the success of LAH procedures depends on a variety of factors, including the age and fertility status of the patient, the quality of the embryos, and the experience of the embryologist performing the procedure.

In terms of success rates, some studies have shown that LAH in general can improve the chances of successful embryo implantation [20,21]. However, the success rates can vary depending on a variety of factors, including the age of the patient, the quality of the embryos, and the experience of the embryologist. Some studies have suggested that drilling may be more effective than thinning in improving implantation rates [22], and others proved the opposite [23] but more research is needed to confirm this and especially concentrating on advanced maternal age group.

Our study has some limitations, including its prospective design and small sample size as it was conducted at a single center and also because of the financial issue as assisted reproductive techniques are not covered with insurance in a low income country. Some of our results did not reach statistical significance due to the limited number of patients. To further enhance our understanding of the impact of laser-assisted hatching (LAH) on fresh and frozen embryo transfers (ETs) in advanced maternal age, larger prospective studies conducted across multiple centers are needed.

In conclusion, the available data in our study suggested that no significant differences between both techniques of LAH (thinning versus drilling) in improving IVF outcomes in advanced maternal age. However, further research is needed to determine which groups of women are most likely to benefit from this procedure and to identify any potential risks associated with its use. It is important for women considering assisted hatching to discuss the risks and benefits of the procedure with their healthcare provider.

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