ZONA PELLUCIDA THINNING VERSUS DRILLING DURING LASER-ASSISTED HATCHING OF FROZEN/THAWED EMBRYOS IN HUMAN ICSI

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Abstract

Objective: In frozen and thawed embryos, hardening can harm the zona pellucida (ZP). In this study, the ZP was thinned or drilled prior to frozen embryo transfer (FET). **Methods:** Patients were divided into two groups at random to receive LAH using either thinning or drilling after thawing. At a depth of 60-80% of the ZP thickness, 2-3 holes were made to thin the tissue using a laser. The inner membrane was left unharmed. The laser opening in LAD was created from the ZP's outside to the interior. **Results:** A total of 200 IVF/ICSI FET cycles were performed, comprising 100 cycles with thinning LAH and 100 cycles with drilling LAH. Human chorionic gonadotropin positivity rates (59% vs. 57%), implantation rates (39.21% vs. 35.48%), clinical pregnancy rates (57.0% vs. 55.0%), and miscarriage rates (1.75% vs. 9.09%) were comparable between the thinning LAH group and the drilling group. According to the LAH approach, there were no appreciable variations in pregnancy outcomes across subgroups formed based on age (older or younger than 35 years). **Conclusion:** This study showed that regardless of the age of the women, partial ZP thinning or drilling had equal results in terms of implantation and pregnancy rates utilizing thawed embryos.

Keywords: Assisted reproductive techniques; Laser assisted hatching; Zona pellucida

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INTRODUCTION

Frozen embryo transfer (FET) is a procedure used more frequently in assisted reproductive methods. In in vitro fertilization (IVF) cycles, the cumulative pregnancy rate is increased with the use of embryo cryopreservation [1]. However, zona pellucida (ZP) stiffening brought on by embryo cryopreservation can hinder embryo hatching and diminish the implantation rate [2,3].

The ZP of frozen-thawed embryos is perforated or thinned by AH, which is thought to assist embryonic implantation by making it simpler for the embryo to escape the surrounding ZP [4,5].

Since the 1990s, AH has been applied to humans [6]. For AH, there are four primary methods: mechanical partial ZP dissection with glass pipettes, chemical ZP opening with acidic Tyrode solution, enzymatic ZP thinning with pronase, and diode laser-assisted hatching (LAH) [4].

The first three techniques need the use of manual or motorized micromanipulators and qualified embryologists, and they run the danger of causing toxicity or direct mechanical harm. According to research, LAH is more efficient and less complicated [7].

Due to its benefits, which include quick exposure times, easy operation, precise placement, indirect touch, safety, and effectiveness [8], laser-assisted hatching (LAH) is a popular AH technique. The two LAH techniques that are most frequently utilized in clinical practice are zona drilling and zona thinning. On the one hand, zona drilling breaches the inner membrane by making a single full-thickness hole in the ZP. However, zona thinning preserves the inner ZP layer while significantly thinning the outer ZP layer [9].

Despite several studies comparing the advantages of the two approaches, it is still unclear which procedure yields the best therapeutic results. According to research, T-LAH may outperform D-LAH in terms of pregnancy outcomes in human embryos [10].

PATIENTS AND METHODS

This study was a prospective, randomized, controlled study that took place in a private IVF center in Cairo from October 2020 to December

2022 with the help of the national research center. The work was authorized in November 2019 by the National Research Center's Ethical Committee. 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.

Two hundred frozen-thawed ET cycles in total were examined and randomly split into two groups, yielding 100 cycles each for T-LAH and D-LAH. Prior to the trial, each participant signed a written informed consent form. All of the patients matched the requirements: (A) Inclusion criteria: Patients who had a previous ICSI failure, unexplained infertility, or male infertility (B) Exclusion criteria: Patients with hydrosalpinx, pyosalpinx, endometriosis, or uterine fibroids were excluded.

All study participants were undergone controlled ovarian stimulation by GnRH agonist long protocol starting from the 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 the 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 were cultured in 30-µL micro-drops with a 1-step medium and overlaid with paraffin oil in an atmosphere of 6% CO2, 5%O2, 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.

Embryos' freezing was attained via vitrification following the standard protocols. All cryopreserved embryos were thawed according to lab standard protocol and were assessed for quality, and only those with excellent and good quality; were transferred to the uterus. Quality assessment was always carried out by the same senior expert embryologist in order to avoid inter-observer discordance. After thawing, Laser Assisted Hatching was performed 2 hours before embryo transfer using a 1480 ηm infrared diode laser. The Zona Laser Hatching system (OCTAX LASER SYSTEM) was mounted to IVF Workstation, and OLYMPUS inverted microscope was used. The laser thinning will be performed without reaching the inner membrane at a depth of 60-80% of the ZP thickness. In LAD, the laser opening will be made from the outside to the inside of the ZP.

To prepare the endometrium, all women received estradiol valerate 2 mg three times a day, starting day one of the cycle nonstop. Assessment of endometrial thickness by ultrasound was done on the 14th day of the cycle and repeated until it reached or exceeded 8 mm with the appearance of a triple line echo. Then we added progesterone 400 mg, once daily. Embryo transfer was performed by a single senior expert gynecologist who was blinded in order not to distinguish between the two groups. All patients received progesterone 400 mg twice daily for luteal phase support. A quantitative BHCG assay was performed fifteen days after embryo transfer. Once the pregnancy test was positive, transvaginal ultrasound was performed on the fourth and sixth weeks to detect intrauterine gestational sac and fetal pulsations. The primary outcome was the implantation rate (IR), which was defined as the number of early gestational sacs detected by transvaginal ultrasound on the fourth to the sixth week divided by the total number of transferred embryos. The secondary outcome was the clinical pregnancy rate (CPR), defined as the number of pregnancy cycles divided by the total number of transfer cycles.

Data analysis: Statistical Product and Service Solutions (SPSS) was the software utilized for all statistical analyses. Data were described using the mean, standard deviation (SD), or frequencies and percentages when appropriate. The Student t-test was used to compare numerical variables between the research groups. To compare categorical data, the Chi-Square (X2) test was used. P values of 0.05 or below were regarded as statistically significant.

RESULTS

Two hundred IVF/ICSI cycles comprising 278 and 248 embryos, respectively, were randomly separated into the T-LAH and D-LAH groups for the purpose of data collection. Regarding the age of the female spouse, BMI, period of infertility, reason for infertility, number of prior unsuccessful cycles, or miscarriage rate, there was no statistically significant difference between the groups. Two hundred seventy-eight embryos were transferred, and 109 (39.21%) were implanted in the T-LAH group. Two hundred forty-eight embryos were transferred, and 88 (35.48%) were implanted in the D-LAH group. No distinction between the two groups was seen in the rate of -hCG positivity, as reported in Table 1. In the T-LAH group, implantation rates and clinical pregnancy rates were (39.21%, 57%) similar to those in the D-LAH group

(35.48% and 55%) (P > 0.05). The patients were classified according to age in Tables 2 and 3.

DISCUSSION

The zona pellucida (ZP) becomes harder throughout the vitrification process, which makes hatching more challenging [11]. Implantation of an embryo is adversely correlated with zona thickness [12]. In assisted reproduction, aided hatching increases implantation rates [13]. Implantation and rates of clinical pregnancy are greatly increased by laser-assisted hatching through zona drilling or thinning [11,14]. In this study, we contrasted the two main LAH techniques of drilling and thinning. No distinction between the two groups was found in the rate of -hCG positivity. In the T-LAH group, implantation rates and clinical pregnancy rates were (39.21%, 57%) similar to those in the D-LAH group (35.48% and 55%) (P > 0.05). Thinning was found to be superior to drilling in early LAH research. The disparity between those results and ours can be attributed to the drilling group's extremely low success rates (clinical pregnancy rate: D-LAH, 5.2% vs. T-LAH, 22.1%) [15]. According to subsequent reports, drilling is more technically challenging, involves a higher level of operator competence, and may harm the embryo [16].

The success rate in our study was higher than the early results in both groups (clinical pregnancy rate: T-LAH, 57% vs. D-LAH, 55%). Although T-LAH exhibited slightly superior outcomes, the difference was not significant. Numerous studies have shown that maternal age significantly influences the genetic composition and viability of the embryo. The results of ICSI-ET may be impacted by unsuccessful hatching. The treatment outcome for older patients is thought to be enhanced by AH approaches [17]. Many researchers have looked into aided hatching in cases when the zona pellucida might have hardened, such as advanced maternal age, recurrent implantation failure, and cryopreserved embryos. In a research published by Schoolcraft, patients > 40 years of age receiving assisted hatching had significantly higher implantation and clinical pregnancy rates [18]. However, Bider and colleagues were unable to demonstrate any benefit in women over 37 who underwent laser-assisted hatching [19]. When patients with repeated implantation failure for more than two cycles had their embryos treated to LAH, Edirisinghe et al. found no improvement in terms of previous implantation failure [20]. That was refuted by two meta-analyses of various assisted hatching procedures conducted by Edi-Osagie et al. and Sallam et al., which showed elevated implantation and clinical pregnancy rates in patients who had experienced repeated implantation failure [21,22].

This study examined the effectiveness of two AH techniques in patient subgroups aged ≤ 34 and ≥ 35 years. The differences were not statistically significant. The rates of multiple pregnancies and miscarriages were similar between the two methods. Patients who had previously experienced unsuccessful implantation during frozen-thawed cycles demonstrated similar implantation and clinical pregnancy rates with T-LAH treatment compared to D-LAH treatment. Additionally, there were no appreciable variations in the outcomes of the two strategies for individuals experiencing their first rounds of embryo transfer.

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

Frozen embryo transfer		**Thinning**			**Drilling**			**Total**			**Chi-Square/ t-test**	
											X^2 or T	**P-value**
Patient Age (Years)	**Range**	20	-	45	20	-	43	20	-	45	-1.683	0.094
	Mean ±SD	30.920	±	5.886	32.260	±	5.359	31.590	±	5.654		
Weight	**Range**	54	-	108	57	-	112	54	-	112	0.664	0.507
	Mean ±SD	78.420	±	11.998	77.340	±	10.965	77.880	±	11.477		
Height	**Range**	1.52	-	1.71	1.51	-	1.72	1.51	-	1.72	-1.275	0.204
	Mean ±SD	1.606	±	0.048	1.614	±	0.047	1.610	±	0.048		
BMI	**Range**	22.19	-	39.74	23.34	-	37.86	22.19	-	39.74	1.382	0.169
	Mean ±SD	30.369	±	4.150	29.618	±	3.501	29.994	±	3.848		
Duration of infertility (Years)	**Range**	1	-	18	1	-	18	1	-	18	0.810	0.419
	Mean ±SD	5.980	±	3.682	5.600	±	2.909	5.790	±	3.315		
Cause of infertility	**Male**	37		37.00	45		45.00	82		41.00	1.334	0.721
	Unexplained	38		38.00	33		33.00	71		35.50		
	Tubal	14		14.00	12		12.00	26		13.00		
	Combined	11		11.00	10		10.00	21		10.50		
N. of failed cycles	**No**	56		56.00	61		61.00	117		58.50	4.864	0.302
	One	29		29.00	31		31.00	60		30.00		
	Two	9		9.00	7		7.00	16		8.00		
	Three	4		4.00	0		0.00	4		2.00		
	Four	2		2.00	1		1.00	3		1.50		
Oocyte Number	**Range**	1	-	30	1	-	30	1	-	30	0.366	0.715

	Mean ±SD	11.080	±	5.626	10.760	±	6.703	10.920	±	6.174		
M2 N.	**Range**	1	-	18	1	-	15	1	-	18	0.588	0.557
	Mean ±SD	6.410	±	3.467	6.110	±	3.747	6.260	±	3.604		
N. of fertilized oocytes	**Range**	1	-	13	1	-	12	1	-	13	0.730	0.466
	Mean ±SD	4.730	±	2.518	4.460	±	2.710	4.595	±	2.612		
Implantation rate		102/256		39.84%	88/248		35.48%					0.378
Chemical pregnancy	**Negative**	41		41.00	43		43.00	84		42.00	0.082	0.774
	Positive	59		59.00	57		57.00	116		58.00		
Clinical pregnancy	**No**	43		43.00	45		45.00	88		44.00	0.081	0.776
	Yes	57		57.00	55		55.00	112		56.00		
Abortion	**No**	56		98.25	50		90.91	106		94.64	2.972	0.085
	Yes	1		1.75	5		9.09	6		5.36		

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 patients aged 20- 35 years.

Patient Age Groups (20-34 Years) Frozen embryo transfer		**Thinning**			**Drilling**			**Total**			**Chi-Square/ t-test**	
											X^2 or T	**P-value**
Duration of infertility (Years)	**Range**	1	-	12	1	-	8	1	-	12	0.750	0.455
	Mean ±SD	4.603	±	2.314	4.333	±	1.704	4.481	±	2.058		
Cause of infertility	**Male**	26		35.62	28		46.67	54		40.60	2.670	0.445
	Unexplained	26		35.62	14		23.33	40		30.08		
	Tubal	12		16.44	10		16.67	22		16.54		
	Combined	9		12.33	8		13.33	17		12.78		
N. of failed cycles	**No**	48		65.75	42		70.00	90		67.67	2.194	0.533
	One	20		27.40	17		28.33	37		27.82		
	Two	4		5.48	1		1.67	5		3.76		
	Three	1		1.37	0		0.00	1		0.75		
Oocyte Number	**Range**	2	-	30	1	-	30	1	-	30	0.250	0.803
	Mean ±SD	11.973	±	5.416	11.700	±	7.169	11.850	±	6.244		
M2 N.	**Range**	1	-	18	1	-	15	1	-	18	0.572	0.568
	Mean ±SD	7.000	±	3.460	6.633	±	3.923	6.835	±	3.666		
N. of fertilized oocytes	**Range**	1	-	13	1	-	12	1	-	13	0.725	0.470
	Mean ±SD	5.137	±	2.479	4.800	±	2.881	4.985	±	2.663		
Implantation rate		98/216		45.37 %	60/153		39.22 %					0.239
Chemical pregnancy	**Negative**	23		31.51	22		36.67	45		33.83	0.392	0.531
	Positive	50		68.49	38		63.33	88		66.17		
Clinical pregnancy	**No**	24		32.88	23		38.33	47		35.34	0.429	0.512
	Yes	49		67.12	37		61.67	86		64.66		
Abortion	**No**	49		100.00	37		100.00	86		100.00	-	-
	Yes	0		0.00	0		0.00	0		0.00		

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 patients aged 35- 45 years.

Patient Age Groups (35- 45 Years) Frozen embryo transfer		**Thinning**			**Drilling**			**Total**			**Chi-Square/ t-test**	
											X^2 or T	**P-value**
Duration of infertility (Years)	**Range**	4	-	18	4	-	18	4	-	18	2.419	0.018*
	Mean ±SD	9.704	±	4.131	7.500	±	3.305	8.388	±	3.790		
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	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		
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		
Implantation rate		11/62		17.74 %	28/95		29.47 %					0.096
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		
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.

CONCLUSION

This study demonstrated that thawed embryo transfers following T-LAH or D-LAH did not significantly alter the implantation and pregnancy rates. Various ages of populations showed these results.

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