



Day 3 Vs Day 5 Quarter Laser Zona Thinning-Assisted Hatching in Frozen Embryo-Transfer

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Abstract

Objective: *The objective of this study is to check efficacy of quarter laser zona thinning-assisted hatching (qLZT-AH) in FET cycles and to compare pregnancy and implantation outcomes of day 3 and day 5 embryos receiving qLZT-AH.*

Materials and methods: *It is a retrospective-study in which a total of 139 women were randomly divided into 2 groups: test (in which patients received qLZT-AH before ET, n=60) and control (transfer was done without qLZT-AH, n=79), in both day 3 and 5 FET.*

Results: *In day 3 control vs test group, significant difference ($p=0.02$) was observed on day 3 PR. No significant difference was observed in IR ($p=0.15$). Similarly, in day 5 control vs test group, significant difference ($p=0.03$) was observed in PR. No significant difference was observed in IR ($p=0.38$). No significant difference was observed in either PR ($p=0.25$) or IR ($p=0.63$) when day 3 and 5 test groups were compared.*

Conclusion: *From the above results, it can be concluded that qLZT-AH improves PR in patients receiving day 3 and 5 FET, while the IR remains similar, whether qLZT-AH was performed or not. On comparing day 3 and 5, no significant difference was observed in patients receiving qLZT-AH.*

Keywords: *Laser assisted hatching, frozen embryo transfer, intracytoplasmic sperm injection, pregnancy outcomes, implantation outcomes.*

Introduction

Zona assisted laser hatching is a process in which thickness of zona pellucida is reduced using laser shots. Quarter laser zona thinning assisted hatching (qLZT-AH) is a method in which thinning of ZP is initiated at one point and continued until 25% of ZP is irradiated (e.g. if drilling is initiated at 12 o'clock position, consecutive radiations will be generated until 3 o'clock position of embryo) prior to embryo transfer.

Laser thinning of ZP helps in exchanging metabolites between the developing embryo and endometrium(1). It should ideally be performed in embryos with ZP thickness exceeding 20µm. ZP thickness more than 20µm is observed when FSH levels are higher in women with advanced maternal age. qLZT-AH is also done when embryos formed are of poor quality or when there is no secretion of hatching factors, (or deficiency in lysine production). Zona hardening occurs as a result of vitrification which could hamper implantation by affecting hatching. Elasticity and thinning of the zona pellucida are essential for the hatching process, both of which can be adversely influenced by advancing maternal age and in vitro culture conditions.

Zona pellucida (ZP) must be shed within a time period when the uterus is receptive in order for a pregnancy to occur. Assisted hatching (AH) has been proposed to increase the pregnancy and embryo implantation rate, probably by enhancing early communication between the embryo and endometrium.(2) An infrared laser system appears to be the most suitable method for assisted hatching, as it has good repeatability and consistency between operators and is simple to perform.

During cryopreservation zona hardening may occur which compromises the in-vivo hatching and implantation following thawing and transfer(3). In this case assisted hatching may troubleshoot the problem viz. acidified Tyrode's medium (also known as zona drilling), micro-manipulator, laser etc. Assisted hatching methodologies are designed to facilitate the embryo's escape from the zona pellucida, and this strategy has been suggested as means of improving pregnancy rates in patients with previous implantation failure. Laser assisted hatching is the least subversive technique to achieve this. Assisted hatching is recommended for:

- Women with advanced maternity age
 - Poor oocyte quality or poor ovarian reserve (high FSH levels or low AMH)
 - Poor embryo quality; cases with embryos harder and/or thicker zona pellucida; high cell fragmentation; cases using frozen eggs or embryos.
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- Women who have 1 or more failed IVF cycles
 - Recurrent implantation failure

The aim and objective of the study is to check efficacy of quarter laser thinning zona assisted hatching in frozen-thawed embryo transfer (FET) cycles and to compare their reproductive outcomes (pregnancy and implantation rates) of day 3 and day 5 quarter laser zona thinning assisted hatching.(4)

With the advent of laser assisted hatching (LAH), this complication could be overcome, as LAH uses 1.48 μ m highly focused Infrared diode laser beam which is touch-free, objective delivered focused laser light to produce opening in ZP with a single pulse of few millisecond, with no mechanical, thermal or mutagenic side effects, (Germond et.al 1995). (5)

Materials and Methods

It is a retrospective-study carried out in a private IVF lab setup from December 2018 to December 2019; in which a total of 139 women were divided into 2 groups: test (in which patients received qLZT-AH before ET, n=60) and control (transfer was done without qLZT-AH, n=79), in both day 3 and 5 FET.

Inclusion criteria:

- Women aged between 23-38 years with BMI ranging between 20-38.
- Patients with good ovarian reserve
- ICSI for all
- Frozen embryo transfer
- Normo-responders.
- Previous failure of ART Cycle (recurrent implantation-failure)
- Patients with good endometrial thickness (> 7mm)

Exclusion criteria:

- First ART cycle
- Poor responders
- Poor ovarian reserve (low-AMH)
- Patients with un-corrected Asherman syndrome, submucosal-polyp or fibroid and congenital-uterine anomaly.

Embryo grading

As it is a frozen embryo-transfer study of day 3 and 5 embryos, the embryos were thawed and transferred same day after incubating for at least 90 mins post thawing, depending on the ability of embryos to regain their shape from their collapsed stage. Day before thawing, a center well dish is prepared containing 1ml of Blast media. The plate is kept in bench-top incubator for overnight set at 37°C and 6% CO₂.

On the next day a plate is made using Kitazato thawing media and their thawing protocols were performed to retrieve embryos from tip of embryo vitrification device. Embryos were graded using SART grading system. Embryos graded as A (symmetrical blastomeres with no fragmentation), B (even blastomeres with minor fragmentation) and C (uneven blastomeres with few fragmentation) were used for transfer on day 3. Blastocyst were graded according to size of blastocele cavity as early, expanding, expanded and hatching ; ICM and TE distribution. Grade A, B, C blastocyst was used for transfer considering best grade transfer first.

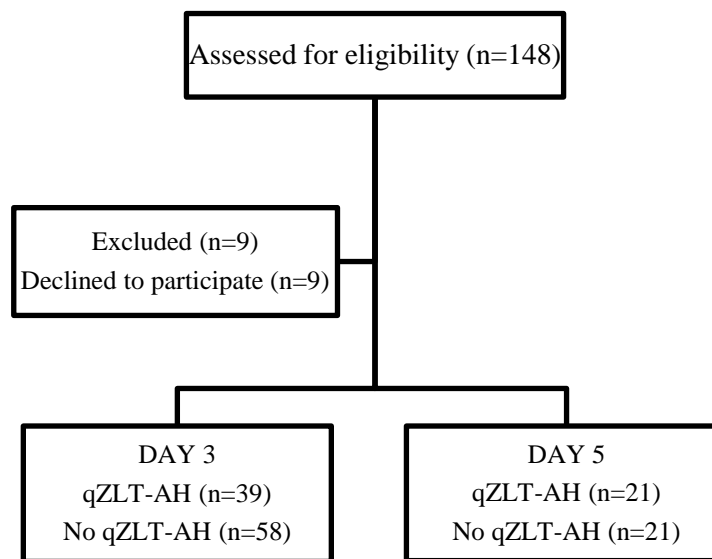


Figure 1. Flow diagram

Assisted hatching

Hatching was done using OCTAX NaviLase Laser Shot system. For quarter laser zona thinning assisted hatching, one fourth of the zona pellucida was thinned (for example from 12 o'clock position to 3 o'clock position) using laser by the action of zona thinning(6). Other actions like hole or hatching can also be used depending on skill of the embryologist.

Embryo transfer

One to three top-grade embryos from both groups (control and test), were transferred in day 3 and day 5 ET. During transfer, embryos are drawn into ET catheter using sandwich method, in a fashion where the embryos with media is sandwiched between air bubbles, hence one can easily detect the release of embryos in uterus on ultrasound.

Statistical Analysis

Statistical analysis was performed by the SPSS program for Windows, version 17.0 (SPSS, Chicago, Illinois). Continuous variables are presented as mean \pm SD, and categorical variables are presented as absolute numbers and percentage. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired t test, whereas the Mann-Whitney U test was used for those variables that were not normally distributed. Categorical variables were analyzed using either the Chi square test or Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

A total of 60 patients underwent qLZT-AH in both day 3 and 5 group (43.16%), in day 3 group 39 patients had qLZT-AH (40.2%) and 21 patients in day 5 had qLZT-AH (50%).(7) Table 1 indicates different clinical characteristics of day 3 ET group patients. The table shows that there is no significant difference in any clinical characteristics between the control and the test group. Similarly, table 2 indicates different clinical characteristics of day 5 ET patients. The table shows that there is no significant difference in any clinical characteristics between the control and the test group.

CHARACTERISTICS	TEST	CONTROL	P VALUE
NO. OF PATIENTS	39	58	NA
AGE	33.4 \pm 2.8	32.5 \pm 2.1	NA
AMH	3.32 \pm 1.27	3.64 \pm 1.31	0.31
AFC	8.91 \pm 2.38	9.03 \pm 2.52	0.84
ENDOMETRIAL THICKNESS	8.91 \pm 0.61	9.06 \pm 0.70	0.25
DURATION OF INFERTILITY	6.04 \pm 2.03	6.74 \pm 1.93	0.15
NO. OF EMBRYOS TRANSFERRED	1.32 \pm 0.47	1.30 \pm 0.46	0.88

Table1: Clinical characteristics of day 3 group:

CHARACTERISTICS	TEST	CONTROL	P VALUE
NO. OF PATIENTS	21	21	NA
AGE	31.1±3.25	32.2±2.79	0.34
AMH	3.43±1.31	3.50±1.26	0.86
AFC	9.02±2.20	9.03±2.01	0.98
ENDOMETRIAL THICKNESS	8.78±0.24	8.93±0.53	0.25
DURATION OF INFERTILITY	5.84±2.39	6.21±2.20	0.60
NO. OF EMBRYOS TRANSFERRED	1.34±0.48	1.57±0.33	0.08

Table 2: Clinical characteristics of day 5 group:

Control group			
NO. OF TRANSFERS	NO. OF EMBRYOS	PR	IR
58	122	29.31%	21.31%
Test group			
NO. OF TRANSFERS	NO. OF EMBRYOS	PR	IR
39	97	51.28%	29.9%
Outcomes			P value
Pregnancy rate			0.02
Implantation rate			0.15

Table 3: Day 3 control vs test group

Table 3 indicates comparison between test and control group of day 3 embryo transfer patients. The parameters used to compare test and control group are pregnancy and implantation rate. The effectiveness of quarter laser zona thinning assisted hatching is observed in test group.(9)In terms of pregnancy rate, there is almost 2 times increase in the values. The PR of control group was 29.31%, while PR of test group was 51.28%. The P value of pregnancy rate, $p=0.02$, is significant (<0.05), which indicates appreciable outcome in patients whose embryos were treated with qLZT-AH before transfer. On comparing implantation rate of control and test group, remarkable difference is seen in values. The IR of control group was 21.31%, while IR of test group was 29.9%.

Although, the P value of implantation rate, $p=0.15$, is not significant, still there is a good increase in IR just with the use of qLZT-AH. So, the use of qLZT-AH significantly increases the pregnancy rate. Hence, qLZT-AH is effective in terms of PR. No such effectiveness was observed in terms of implantation rate.

Control group			
NO. OF TRANSFERS	NO. OF EMBRYOS	PR	IR
21	53	33.33%	22.64%
Test group			
NO. OF TRANSFERS	NO. OF EMBRYOS	PR	IR
21	51	66.67%	35.3%
Outcomes		P value	
Pregnancy rate		0.03	
Implantation rate		0.38	

Table 4: Day 5 control vs test group

Table 4 indicates comparison between test and control group of day 5 embryo transfer patients. The parameters used to compare test and control group are pregnancy and implantation rate. The effectiveness of qLZT-AH is observed in test group.

In terms of pregnancy rate, there is twice increase in the percentage value. The PR of control group was 33.33%, while PR of test group was 66.67%. The P value of pregnancy rate, 0.03, is significant, which indicates appreciable outcome in patients whose embryos (blastocyst) were treated with qLZT-AH before transfer. Even if we compare implantation rate of control and test group, we can see remarkable difference in values. The IR of control group was 22.64%, while IR of test group was 35.3%.

Although, the P value of implantation rate ($p=0.38$), is not significant, still there is a good increase in percentage of IR just with the use of qLZT-AH.(10) So, the use of qLZT-AH significantly increases the pregnancy rate in patients of test group, i.e; patients whose embryo were treated with quarter laser zona thinning assisted hatching. Hence, qLZT-AH is effective in terms of PR. No such effectiveness was observed in terms of implantation rate.

OUTCOME	DAY 3	DAY 5	P VALUE
PREGNANCY RATE	51.28%	66.67%	0.25
IMPLANTATION RATE	29.89%	35.29%	0.63

Table 5: Day 3 vs day 5 test group

Table 5 indicates difference in outcomes of day 3 and day 5 test groups. On comparing day 3 and day 5 test group, no significant difference was observed in patients receiving qLZT-AH in both PR and IR.

Discussion

According to a study conducted by Mojtaba Rezazadeh Valojerdi, PoopakEftekhari-Yazdi, Leila Karimian, Fatemeh Hassani, BaharMovaghar (2010) on Effect of Laser Zona Thinning on Vitrified-Warmed Embryo Transfer at the Cleavage Stage. The aim of this study was to determine if laser zona thinning could improve the rates of pregnancy and implantation for vitrified-warmed embryo transfer at the cleavage stage. The results of this investigation show that laser zona thinning may have an unexpected effect on the rates of clinical pregnancy (28.5 versus 43.0, $p=0.02$; control group vs test group)and implantation (11.2 vs 16.7, $P=0.04$;control group vs test group)of vitrified-warmed embryos at the cleavage stage(11).

Similarly, another randomised double-blind controlled trial was performed by Ernest Hung Yu Ng, Estella Yee Lan Lau, William Shu Biu Yeung, Tak Ming Cheung, Oi Shan Tang, Pak Chung Ho (2008) on Comparison of Laser Zona Pellucida Thinning and Breaching in Frozen-Thawed Embryo Transfer at the Cleavage Stage. The trial concluded that Laser ZP thinning is associated with significantly higher implantation and ongoing pregnancy rates in FET cycles compared with the case of laser ZP breaching. (12)

From the previous studies that were conducted on effect of zona thinning using laser assisted hatching on frozen-thawed embryos, it can be concluded that significant difference was observed in pregnancy as well as implantation rate. Thus zona thinning using LAH is effective and improves rates for pregnancy and implantation for vitrified- warmed embryo transfer.(13). Although these studies failed to prove the effectiveness of zona thinning laser assisted hatching on day 5 groups.

And hence through this study, comparison of day 3 and day 5 quarter laser zona assisted hatching in frozen embryo transfer, we tried to evaluate and compare:

- Day 3 control (not receiving qLZT-AH) vs day 3 test (receiving qLZT-AH) group.
- Day 5 control (not receiving qLZT-AH) vs day 5 test (receiving qLZT-AH)
- Day 3 test (receiving qLZT-AH) vs day 5 test (receiving qLZT-AH) group.

From the results of this study, it is evident that the use of quarter laser zona thinning assisted hatching in frozen embryo transfer has significant difference on pregnancy rate of patients receiving it. Although, implantation rate remains similar, irrespective of qLZT-AH being done or not.

There was a significant difference ($p = 0.02$), (refer table 3) observed on day 3 Pregnancy rate of patients of test group. The PR of day 3 control group was 29.31% and that of the test group was 51.28%. An increase of almost 22% was observed in PR of test group compared to that of PR of control group. This clearly shows that the use of qLZT- AH increases pregnancy rate in patients receiving it.

However, there was no significant difference observed ($p = 0.15$) (refer table 3) on day 3 Implantation rate of patients of test group. The IR of day 3 control group was 21.31% and that of the test group was 29.9%. Although, an increase of 8.58% was observed in the IR of test group, the value was still similar. It was thus concluded that, no significance difference was observed in terms of Implantation rate, the irrespective of quarter laser zona assisted hatching being done or not.

Likewise, significant difference was observed ($p= 0.03$) (refer table 4) on day 5 Pregnancy rate of patients of test group. The PR of day 5 control group was 33.33% and that of the test group was 66.67%. An increase of almost double the value of control group (33.34% increase) was observed in PR of test group. This clearly shows that the use of qLZT-AH increases pregnancy rate in patients receiving it be it day 3 or day 5. (14)

Nevertheless, there was no significant difference observed ($p= 0.38$) (refer table 4) on day 5 Implantation rate of patients of test group. The IR of day 5 control group was 22.64% and that of the test group was 35.29%. Although, an increase of 12.65% was observed in the IR of test group, the value was still similar. It was thus concluded that, no significance difference was observed in terms of implantation rate, the values remain similar in both test and control group, irrespective of quarter laser zona assisted hatching being done or not.(15)

Even if we compare test group of day 3 and test group of day 5, no significant difference was observed in terms of either Pregnancy rate ($p = 0.25$) or Implantation rate ($p = 0.63$).

If we compare pregnancy rate of day 3 (PR = 51.28%) embryo transfer from day 5 (PR = 66.67%) embryo transfer, an increase of 15.4% is observed but it is not significant.

Also, if we compare implantation rate of day 3 (IR = 29.89%) embryo transfer from day 5 (IR = 35.29%) embryo transfer, an increase of 5.4% is observed but it is not significant.

Finally on comparing day 3 and day 5, no significant difference was observed in patients receiving qLZT-AH. The above statement sums up the answers to the study of comparing day 3 and day 5 qLZT-AH in FET.

Conclusion

From the result of the present study, it can be concluded that qLZT-AH improves pregnancy rate in patients receiving Day 3 as well as Day 5 frozen embryo transfers. While the implantation rate remains similar, irrespective of quarter laser zona assisted hatching being done or not. However, it was observed that with quarter laser zona assisted hatching, implantation rate was slightly higher, although it wasn't that significant.

Since the study size was small, a larger sample size is required to assess the actual effect of quarter laser zona thinning assisted hatching of embryos on implantation rate of both day 3 and day 5 embryos.

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