International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 2(D); February 2018; Page No. 9904-9907
DOI: http://dx.doi.org/10.24327/ijcar.2018.9907.1653

Research Article

ROLE OF LASER ASSISTED HATCHING IN FROZEN THAW ART CYCLES

Sonali Tawde., Himanshu Bavishi., Falguni Bavishi and Parth Bavishi

Department of Obstetrics & Gynaecology, University/ Institute: Bavishi Fertility Institute, Mumbai, Ahmedabad

A R T I C L E I N F O

Article History:
Received 15th November, 2017
Received in revised form 21st December, 2017
Accepted 23rd January, 2018
Published online 28th February, 2018

Key words:
Laser, hatching, frozen, implantation, pregnancy, live birth rates

A B S T R A C T

Introduction: Laser hatching improves embryo-endometrial cross talk, endometrial receptivity & implantation.
Purpose: To evaluate role of laser assisted hatching in frozen thaw ART cycles.
Material & Methods: This is a retrospective study conducted from Jan. 2015 & Dec.2016. Age group 30-40yrs, AMH 1.5-6 ng/ml were included. 178 patients in study group were compared with 134 patients in control group.
Results & Observations: For D2/3 embryos, 30-35yr group, Implantation rate(IR) between study and control group was found to be 18.9% vs 14.28%, clinical pregnancy rate (CPR) 33.8% vs 25% & live birth rate (LBR) 26.4% vs 22.91%. In 35-40yrs group, rates were 12.09% vs 11.11% IR, 20% vs 16.66% CPR, 17.7% vs 13.88% LBR. For blastocysts, in younger group, IR was 30.43% vs 27.14% in controlgroup, CPR 36.9% vs 31.42%, LBR 32.6% vs 28.57% resp. Blastocysts, in older group had 36.84% vs 23.33% implantation rates, 63% vs 26.66% CPR, 57.8% vs 20% LBR. Results were better in LH group & the difference was significant in elder age group with blastocyst transfer.
Conclusion: Laser hatching improves the outcome in FET cycles.

INTRODUCTION

Due to advances in technique of vitrification, more & more frozen thaw cycles are being done. The success of frozen cycles have been comparable to that of fresh cycles. One of the disadvantages of vitrification of embryos is hardening of zona pellucida. Hatching is very crucial step in life of the embryo, which improves the embryo-endometrial cross talk and implantation. Some euploid embryos with full developmental potential fail to implant because of hatching difficulties [1]

An emerging body of evidence suggests that AH(assisted hatching) may improve clinical pregnancy rates, particularly in poor prognosis patients[2], however, there still remains considerable uncertainty.

Failure to hatch, due to intrinsic abnormalities in either the blastocyst or zona pellucida (ZP), may be one of many factors limiting human reproductive efficiency. There are 3 types of assisted hatching: 1) Mechanical- done with glass pipettes 2) Chemical method which uses acid tyrode's solution & 3) Laser assisted hatching. After Laser hatching has been widely available, mechanical & chemical methods are not being used, as laser is the safest method, causing least damage to the embryos.

Corresponding author:* **Sonali Tawde
Department of Obstetrics & Gynaecology, University/ Institute: Bavishi Fertility Institute, Mumbai, Ahmedabad

duration, it causes minimal thermal damage to embryo in the vicinity. Possible disadvantages could be due to loss of blastomeres, more when done at cleavage stage & inhibition of natural expansion of blastocyst. In many studies, thinning of the human zona pellucida rather than a complete zona drilling is shown to increase (i) blastocyst hatching and (ii) implantation rate [3,4].

Aim

Aim of this study is to evaluate the role of Laser Assisted Hatching (LAH) in frozen thaw ART cycles.

MATERIAL & METHODS

This is a retrospective study conducted from Jan.2015 to Dec. 2016. Laser hatching was done based on patient preference. All patients were explained about LAH procedure. Total 312 consecutive cycles were studied. 178 patients consented for laser hatching (study group) & 134 patients did not opt for the technique (control group). Laboratory protocols regarding vitrification & thawing, culture media remained the same during study period. Number of embryos transferred were maximum 3 for cleavage stage and 2 for blastocyst group. Only grade1 embryos on D3, grade 4AA/4AB blastocysts were transferred. None of the embryos were damaged during hatching procedure. Primary outcome measured were Implantation rate, clinical pregnancy and live birth rates. They were analysed statistically using the Chi square-test. Age &

AMH was compared using two-sample *t*-test. A *P*-value of <0.05 was considered statistically significant.

Inclusion criterias

Patients with age group of 30-40 yrs, with AMH between1.5-6ng/ml were included in the study. All of them underwent self egg ICSI in fresh cycles, frozen cycles were down regulated with leuprolide depot injection. In blastocyst group, patients who had their embryos frozen on D5 in fresh cycle were included.

Exclusion criterias

Cases with known very thin endometrial thickness (<7mm), moderate/severe endometriosis, severe oligoasthenospermia, TESA/TESE, big fibroids, severe PCOS(AMH>15 ng/ml), anatomical causes, genetic factors were excluded to reduce confounding factors.

Technique of hatching

Embryos were stabilized by holding pipette at 9 0'click position, laser settings were adjusted. Pulse duration was 500µs. We performed thinning of zona pellucida for 25-30% of the circumference, beginning from 3 0'clock position.(Fig.1)

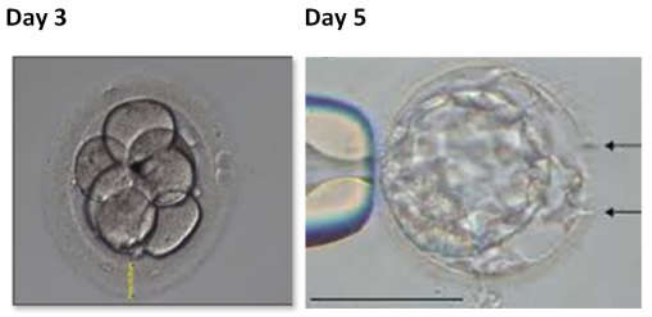

Figure 1 Laser Assisted Hatching technique

Endometrial preparation

All patients received leuprolide depot in previous cycle for down regulation. Endometrium was prepared by estradiol valerate oral preparations started on D3/4 of the cycle, progesterone was initiated when endometrial thickness was 8 mm or more. Transfer was planned on day 3/5 of progesterone.

RESULTS & OBSERVATIONS

Table 1 summarizes total number of patients in each group, depending on their age (30-35 or 35-40yrs) and day of embryo transfer(D3/D5). Average no. of previous failed cycles, causes & duration of infertility, no. of embryos transferred & cryosurvival rates were similar in 2 groups.

Table 1 Total No.Of Patients

AGE GROUP	Day of ET	LH+ (n1=178)	NO LH (n2=134)
30-35yrs	D3	68	48
	D5	46	35
35-40yrs	D3	45	36
	D5	19	15

Average age in study group was 33.26yrs, 32.7yrs in control group (P value 0.33 not sign.)(Fig.2). Average AMH in 2 groups was 2.94 vs 3.53 respectively (P value 0.36 not sign.)(Fig.3).

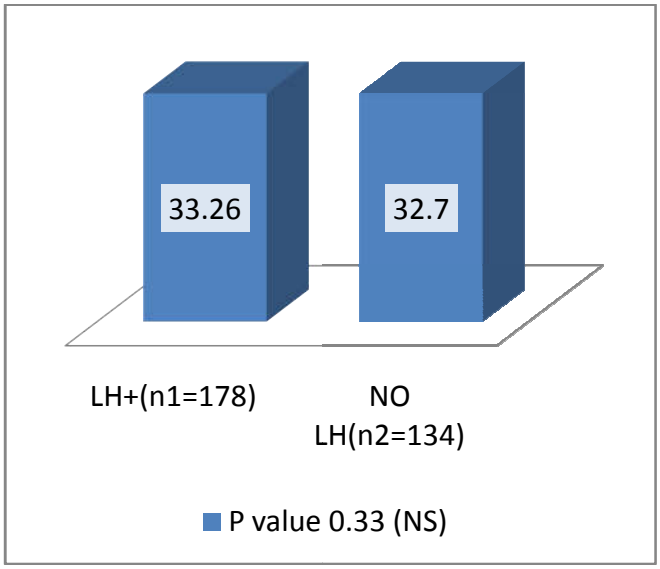

Figure 2 AVERAGE AGE in study & control group

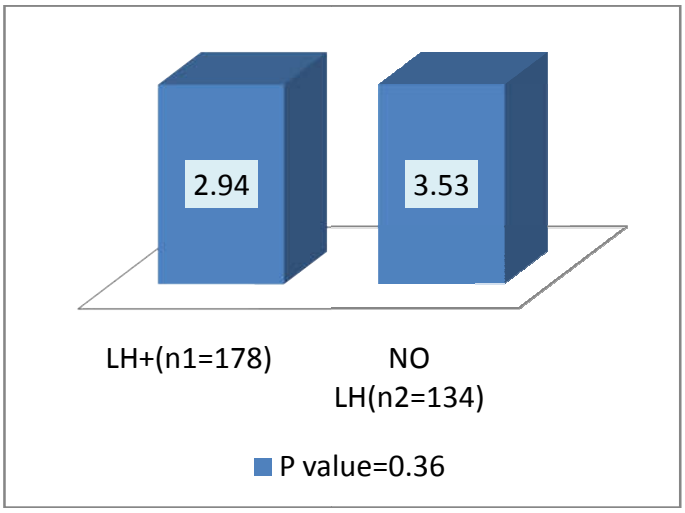

Figure 3 AVERAGE AMH in study & control group

Table 2 Implantation rates in study & control groups, 30-35 age group were, 18.9% vs 14.28% (P value 0.28, NS) for D3, 30.43% vs 27.14% (P value 0.64 NS) for blastocyst group. In older age group 35-40yrs, implantation rates were 12.09% vs 11.11%(P value 0.17 NS) for D3, 36.84% vs 23.33% (P value 0.23 NS)for D5 group. Though they were marginally better in LH group, the difference was statistically insignificant.

Table 2 implantation rates in 4 groups

AGE GROUP	Day of ET	LH+ (n1=178)	NO LH (n2=134)	P Value
30-35 yrs	D3	18.9%(36/190)	14.28%(18/126)	0.28(NS)
	D5	30.43%(28/92)	27.14%(19/70)	0.64(NS)
35-40 yrs	D3	12.09%(15/124)	11.11%(10/90)	0.17(NS)
	D5	36.84%(14/38)	23.33%(7/30)	0.23(NS)

Table 3 Clinical pregnancy rates(CPR) in study & control group for younger age group were, 33.8% vs 25% (P value 0.30 NS) for D3, 36.9% vs 31.42% (P value 0.60 NS) for D5 group. In older age group it was, 20% vs 16.66% (P value 0.70 NS) in D3, 63% vs 26.66% (P value <0.05) in D5. Pregnancy rates were better in LH group in all categories, blastocyst in 35-40 category had significantly higher pregnancy rates in LH group (P value 0.034).

Table 3: Clinical Pregnancy Rates In 4 Groups

AGE GROUP	Day of ET	LH+ (n1=178)	NO LH (n2=134)	P Value
30-35 yrs	D3	33.8%(23/68)	25%(12/48)	0.30(NS)
	D5	36.9%(17/46)	31.42%(11/35)	0.60(NS)
35-40 yrs	D3	20%(9/45)	16.66%(6/36)	0.70(NS)
	D5	63%(12/19)	26.66%(4/15)	0.034(SIGN)

Table 4 Live birth rates for younger group were 26.4% vs 22.91% (P value 0.66 NS) for D3, 32.6% vs 28.57% (P value 0.69 NS) for D5 group. For older group, it was 17.7% vs 13.88% (P value 0.63 NS) for D3, 57.8% vs 20% (P value 0.025 significant) for D5 group. Rates were better in LH group, difference was statistically significant in blastocyst group for 35-40 age category (P<0.05).

Table 4 Live Birth Rates In 4 Groups

AGE GROUP	Day of ET	LH+ (n1=178)	NO LH (n2=134)	P Value
30-35 yrs	D3	26.4%(18/68)	22.91%(11/48)	0.66(NS)
	D5	32.6%(15/46)	28.57%(10/35)	0.69(NS)
35-40 yrs	D3	17.7%(8/45)	13.88%(5/36)	0.63(NS)
	D5	57.8%(11/19)	20%(3/15)	0.025(SIGN)

Table 5 Miscarriage rates in youger age, study & control groups were 21.73% vs 8.33% for D3 (P value 0.31NS), 11.76% vs 9.09% for D5(P value 0.82NS). For age group 35-40, rates were 11.11% vs 16.66% for D3 (P value 0.75 NS), 8.33% vs 25% for D5 (P value 0.38 NS). Miscarriage rates were comparable in both groups.

Table 4 Miscarriage Rates In 4 Groups

AGE GROUP	Day of ET	LH+ (n1=178)	NO LH (n2=134)	P Value
30-35 yrs	D3	21.73%(5/23)	8.33%(1/12)	0.31(NS)
	D5	11.76%(2/17)	9.09%(1/11)	0.82(NS)
35-40 yrs	D3	11.11%(1/9)	16.66%(1/6)	0.75(NS)
	D5	8.33%(1/12)	25%(1/4)	0.38(NS)

Multiple pregnancy rates were 10.27% in LH group, as against 11% in NO LH group. All the twin pregnancies were dizygotic twins. None of the delivered babies were detected with any genetic or physical abnormalities so far.

DISCUSSION

There was no difference in terms of patient characteristics between study & control groups. Treatment and laboratory protocols, media used remained the same throughout study period. This is a retrospective, non randomised study, so some biases can not be ruled out. We tried to minimize the bias by keeping all parameters same e.g. grading of embryos, endometrial preparation methods, ET catheters, excluding poor prognostic factors like very thin ET or fibroids or endometriosis which may affect implantation rates. In this study blastocyst transfers in elderly age group seemed to benefit from Laser Assisted Hatching, improving the clinical pregnancy rates & live birth rates. Old age can be an additional causative factor for hardening of zona, other than vitrification, leading to reduced implantation rates and pregnancy rates. Such cases might get better results from Laser Hatching.

A Cochrane comprehensive review and a meta-analysis (2012) identified 31 randomized, controlled trials (RCTs) involving a total of 5,728 women undergoing IVF or intracytoplasmic sperm injection (ICSI) that compared outcomes from 2,933 women in the assisted hatching group to 2,795 women in the control group[5] . The odds ratio for clinical pregnancy per woman randomized was, slightly but significantly in favour of assisted hatching. It also states that, AH plays major role in improving CPR for those with prior failed cycles, poor prognosis and in frozen transfers, but LBR were not significantly different. The incidence of monozygotic twins was statistically not different between 2 groups.

ASRM (American Society of Reproductive Medicine) 2014[6], concluded that due to limited number of studies, there is insufficient data to prove role of AH in improving live birth rates. It stated that, AH improves CPR for poor prognosis patients, including those with previous failed cycles, but AH should not be routinely recommended for all IVF patients.

Recent ESHRE guidelines for good practice in IVF laboratories suggests, AH to be considered as experimental technique due to conflicting reports[7].The most up-to-date meta-analysis, including 36 RCTs with 6,459 participants, suggested that women who underwent AH were associated with a significant increase in clinical pregnancy and multiple pregnancy rate. However, non-significant results were observed in terms of live birth and miscarriage [8]. Some studies have even reported lesser pregnancy and implantation rates with AH [9]. We can say that the studies so far have been inconclusive regarding the role of Laser hatching in improving the outcome of ART cycles. Due to conflicting results in various studies, role of Laser Assisted Hatching is not clear. It helps in selective conditions like previous failed cycles or frozen thaw cycles, but whether it should be used routinely, remains unanswered.

CONCLUSION

In our study, outcome in terms of Implantation rate, Clinical pregnancy rate & Live birth rate was better in LH (study) group for all categories. Group with blastocyst transfer in 35-40 age group, the difference in CPR &LBR was statistically significant. Miscarriage rates in both groups were comparable. Incidence of multiple pregnancy rate or monozygotic twins was not higher in LH category, none of the pregnancies reported any abnormality in the offspring. This is in accordance with American Society of Reproductive Medicine (ASRM) recommendations, which states, individual assisted reproductive technology programmes should evaluate their own unique patient populations in order to determine which subgroups may benefit from AH. Prospective high quality RCTs, are needed to investigate the effect of AH on live birth, miscarriage, and other long-term outcome.

Compliance with Ethical Standards

Conflict of interest: The authors declare that there is no conflict of interest.

Ethical Approval: All the procedures in the study were in accordance with the ethical standards of the institution.

Informed consent Informed consent was obtained from all the individual participants in the study.

References

1. G.J. Huisman, B.C. Fauser, *et al* Implantation rates after in vitro fertilization and transfer of a maximum of two embryos that have undergone three to five days in culture. *Fert Steril, 73 (2000),* pp. 117-122

2. Pfeifer S. *et al*. Role of assisted hatching in in vitro fertilization: a guideline. *Fertil Steril* 102, 348-351 (2014)
3. Blake, D. A., Forsberg, A. S *et al*. Laser zona pellucida thinning–an alternative approach to assisted hatching. *Hum Reprod* 16, 1959-1964 (2001)
4. Antinori, S., Selman, H. A., *et al* Zona opening of human embryos using a non-contact UV laser for assisted hatching in patients with poor prognosis of pregnancy. *Hum Reprod* 11, 2488-2492 (1996)
5. Carney SK, Das S, Blake D, *et al*. Assisted hatching on assisted conception in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). *Cochrane Database Syst Rev 2012:CD001894.Fertil Steril 2014*; 102:348-51 By American Society For reproductive medicine
6. M. Cristina Magli, Etienne Van den Abbeel, *et al* Revised guidelines for good practice in IVF laboratories. *Human Reproduction* Vol.23, No.6 pp. 1253–1262, 2008
7. Da Li, Da-Lei Yang, *et al* Effect of assisted hatching on pregnancy outcomes: a systematic review and meta-analysis of randomized controlled trials *Scientific Reports* 6, Article number: 31228 (Aug 2016)
8. Primi MP, Senn A, *et al*. A European multicentre prospective randomized study to assess the use of assisted hatching with a diode laser and the benefit of an immunosuppressive/antibiotic treatment in different patient populations. *Hum Reprod*. 2004;19:2325-3

How to cite this article:

Sonali Tawde *et al* (2018) 'Role of Laser Assisted hatching in Frozen Thaw Art Cycles', *International Journal of Current Advanced Research,* 07(2), pp. 9904-9907. DOI: http://dx.doi.org/10.24327/ijcar.2018.9907.1653

****