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Original Research Article

Studying the Effect of Laser Assisted Hatching on Clinical Pregnancy Rate of Thawed Embryos after Vitrifaction

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Abstract: The aim of the study was to evaluate the effect of laser assisted hatching on clinical pregnancy rate of thawed embryos after vitrifaction. *Inclusion criteria:* Infertility patients who had frozen embryos for transfer, Age below 40, two or more frozen embryos available for transfer. *Exclusion criteria:* Blastocysts of the patients developed to hatching stage or hatched before vitrifaction. Patients with repeated implantation failure. Patients with uterine abnormalities or previous uterine surgery. The cases will be divided into two groups (A and B) each of them consisted of 60 patients. The patients will be divided into two groups. Group (A):60 patients Day 3 embryos or blastocysts will undergo laser assisted hatching using Saturn Vlaser device (RI). Half thickness of one quarter of the circumference of the zona. One hour after thawing of the embryos and two hours before embryos transfer. Group (B):60 patients will undergo frozen - thawed embryos transfers without laser assisted hatching. Endometrial Preparation and embryos Transfers the endometrium will be prepared by administration of oral E2 and vaginal Micronized Progesterone. Age ranged between 22.0-36.0 and 23.0-37.0 years with the mean of 28.9±4.68 and 27.8±5.68 years for group A and group B respectively, with no statistical significant differences. Normal weight were found in 68.33% and 80%, over weight were found in 20.0% and 13.33% and obese were found in 11.67 and 6.67% for group A and group B respectively, there were no statistical significant differences between the two studied groups regarding weight. Total number of ET: 192 and 168 in group A and group B respectively. No. of implantation: 91 and 39 in group A and group B respectively. Implantation rate: were found in 47.4% and 32.2% in group A and group B respectively. There were statistical significant differences between group A and group B regarding total number of ET, no. of implantation and implantation rate, group A has values statistically higher than group B. Regarding pregnancy rate, total number of cases: 60 and 60 in group A and group B respectively. No. of pregnancy cases: 45 and 25 in group A and group B respectively. Pregnancy rate: were found in 75.0% and 41.6% in using group A and group B respectively. There were statistical significant differences between group A and group B regarding total number of cases, no. of pregnancy cases and pregnancy rate, group A has values statistically higher than group B. LAH increased implantation and clinical pregnancy rates in women undergoing thawed embryo transfer There were statistical significant differences between using laser and without laser groups regarding total number of cases, number of pregnancy cases and pregnancy rate, using laser group have values statistically higher than without laser.
Keywords: human oocyte , zonapellucida, laser assisted hatching, implantation.

Introduction

Zonapellucida (ZP) hatching is natural process which occurred after expansion of blastocyst and allows the embryo to implant into the uterine cavity. The blastocyst escapes from ZP with two probable mechanisms: ZP lysis by maternal or embryo (trophoectoderm) proteases and internal pressure from expanded blastocyst. Despite numerous achievements in ART, implantation rate has remained low and one of the causes of implantation failure could be failure in normal ZP hatching process[1,2].

Assisted hatching (AH), which was introduced more than two decades ago, showed the potential to increase the chance of implantation[3]. First pregnancy following AH reported in 1988 and studies regarding the impact of AH have been followed till now. Several techniques have been introduced for embryo zonahatching[4]. Laser assisted hatching (LAH), which was proposed in early 90s appears to be more safe compared to other AH techniques[4]. There are some indications for AH such as increased maternal age (≥40 years), increased FSH level, thick ZP (≥15µm), previous IVF failure (≥2), and frozen-thawed embryos. Zona hardening which is due to in vitro culture or after freeze-thaw cycle and lack of produced proteases by embryo due to suboptimal culture condition are another indications of AH. Some investigators tried to assess the impact of AH based on etiology of infertility[5].

Embryo cryopreservation

The field of cryobiology experienced increased interest in 1983 following the first successful pregnancy after transfer of a human embryo[6]. In 1984, the first live birth following embryo cryopreservation was reported in Australia, which was followed 2 years later by another such birth in the USA.

PATIENTS AND METHODS

This study included 120 patients. They were attending private IVF center. All patients consented for the study and informed of the technique

Inclusion criteria:

1. Infertility patients who had frozen embryos for transfer.
2. Age below 40.
3. Two or more frozen embryos available for transfer.

Exclusion criteria:

1. Blastocysts of the patients developed to hatching stage or hatched beforevitrifaction.
2. Patients with repeated implantation failure.
3. Patients with uterine abnormalities or previous uterine surgery.

Methods

The study design was explained to subjects, patients' questions were answered and written informed consent was obtained from them.

The study design: prospective cohort study.

The cases were divided into two groups (A and B) each of them consisted of 60 patients.

Group (A):60 patients

Day 3 embryos or blastocysts wereundergone laser assisted hatching using Saturn 5 laser device (RI). Half thickness of one quarter of the circumference of the zona. One hour after thawing of the embryos and two hours before embryos transfer[7].

Group (B):60 patients

Undergone frozen - thawed embryos transfers without laser assisted hatching.

Endometrial Preparation and embryo Transfers

The endometrium was prepared by administration of oral E2 and vaginal Micronized Progesterone.

The women received increasing doses (2 mg for four days, 4 mg for 4 days and 6 mg thereafter) of estradiol (E2) valerate tablets (Cyclo-Progynova, Schering AG, Istanbul, Turkey) following down-regulation with a GnRH agonist. When the endometrium reached or exceeded 8 mm and a triple-line echo (Lucrin, Abbott, Issy, France) (three-line appearance of the endometrium as shown in the sagittal section of transvaginal ultrasound) was evident, vaginal progesterone 800 mg progesterone (Crinone vaginal gel, Serono, Bedfordshire, UK) was started once a day, and the patient was asked to come for embryo transfer in the morning of the fourth day[8].

Vitrification

Embryos were vitrified according to the protocol developed by Kuwayama *et al.* [9] using a cryotop (Kitazato Supply Co. Fujinomiya, Japan).

The procedures of vitrification were performed at room temperature (22–26 °C).

The embryos were firstly equilibrated in 1 ml equilibration solution for 5 min, then transferred to 1 ml vitrification solution and loaded onto the cryotop within 1 min. Then the cryotop was submerged into liquid nitrogen immediately and then protected by the plastic cover.

Thawing

The plastic cover was removed in liquid nitrogen and the end of the cryotop was immersed directly into 1 ml of 37 °C TS (1.0 mol/l sucrose solution) for 1 min. The embryos were then transferred into 1 ml of DS (0.5 mol/l sucrose solution) for 3 min at room temperature and washed twice in the base medium for 5 min at room temperature. After warming, the embryos were rinsed several times and cultured in fresh G2 droplet at 37 °C.

Assisted hatching and embryo transfer

Lasar assisted hatching procedure was performed one hour after thawing and two hours before transfer. LAH was performed only on embryos that survived following thawing. The assessment of cryosurvival was performed as described by Rienzi *et al*. [10] Frozen–thawed embryos were considered to have survived if ≥50% of the blastomeres were intact or had at least three viable cells or one cleaving blastomere after thawing.

Patients were randomized into LAH versus no AH using a computer-generated list of random numbers. Informed consent was obtained from all couples.

A 1480-nm diode laser in a computer-controlled non-contact mode was used for laser hatching Assessment of pregnancy

Serum HCG was measured 2 weeks later and a clinical pregnancy was confirmed when a fetal heart beat was identified via ultrasound 4 weeks after transfer.

RESULTS

Age

Most of the studied cases (45.0% and 41.67%) for A and B groups between 25-34 years, with no statistically significant differences. Age ranged between 22.0-36.0 and 23.0-37.0 years with the mean of 28.9±4.68 and 27.8±5.68 years for A and B groups respectively, with no statistically significant differences. (P=0.249)

Weight

Normal weight were found in 68.33% and 80%, over weight were found in 20.0% and 13.33% and obese were found in 11.67 and 6.67% for A and B groups respectively, there were no statistically significant differences between the two studied groups regarding weight. (P=0.338)

Total number of ET and number of implantation

Comparison between the studied groups according to total number of E.T and number of implantation were presented in table -1, it showed that,

Total number of ET: 154 and 168 in group A and group B respectively.

No. of implantation: 73 and 39 in group A and group B respectively.

Implantation rate: were found in 47.4% and 23.2% in group A and group B respectively.

There were statistical significant differences between group A and group regarding total number of ET, no. of implantation and implantation rate, group A has values statistically higher than group B. (P=0.001)

Table-1: Comparison between the two studied groups regarding the total number of E.T and number of implantation

	Group A (n=60)	**Group B (n=60)**
Total Number of E.T	154	168
No. of Implantation	73	39
Implantation Rate	47.4 %	23.2 %
p	**<0.001***	

p: p value for Chi square test for comparing between the two studied group
*: Statistically significant at p ≤ 0.05

Pregnancy rate

Table -2 shows comparison between the studied groups regarding the pregnancy rate, it illustrated that,

Total number of cases: 60 and 60 in group A and group B respectively.

No. of pregnancy cases: 45 and 25in group A and group B respectively.

Pregnancy rate: were found in 75.0% and 41.6% in group A and group B respectively.

There were statistically significant differences between group A and group B regarding no. of pregnancy cases and pregnancy rate, group A has values statistically higher than group B. (P=0.001)

Table-2: Comparison between the two groups regarding the pregnancy rate

	Group A (n=60)	**Group B (n=60)**
Total Number of case	60	60
No. of Pregnancy cases	45	25
Pregnancy Rate	75.0%	41.6%
p	**<0.001***	

p: p value for Chi square test for comparing between the two studied group
*: Statistically significant at p ≤ 0.05

DISCUSSION

Hundred and twenty patients were included in this study, among the attendants of private IVF center. All patients consented for the study and informed of the technique. The cases were divided into two groups (A and B) each of them consisted of 60 patients. Group (A): Day 3 embryos or blastocysts were undergone laser assisted hatching using Saturn 5 laser device (RI). Half thickness of one quarter of the circumference of the zona. One hour after thawing of the embryos and two hours before embryos transfer. Group (B): Embryos were undergone frozen - thawed embryos transfers without laser assisted hatching.

Statistically significant differences between using laser and without laser groups regarding no. of implantation and implantation rate, using laser group have values statistically higher than without laser.

There were statistically significant differences between using laser and without laser groups regarding no. of pregnancy cases and pregnancy rate, using laser group have values statistically higher than without laser.

In agreement with our study, a prospective randomized study published in 2008 by Ge *et al*.[11] they evaluated the impact of assisted hatching on fresh and frozen-thawed embryos. A total of 760 fresh embryo transfer cycles and 200 frozen-thawed embryo transfer cycles were randomly assigned to either receive or not receive assisted hatching. In fresh embryo cycles, there was not a significant difference with assisted hatching. However, in the frozen embryo groups, clinical pregnancy and implantation rates were significantly greater in the assisted hatching group.

Recent studies demonstrated that partial thinning of the outer layer of the zona might be beneficial, either in enhancing the hatching rate of blastocysts[12] or in improving the implantation and pregnancy rates in women older than 37 years[13]. Characteristics of the inner surface of the zona and the role of the variability of zona thickness remain to be clarified.

Balaban, *et al*.[13]indicate that LAH before the transfer of frozen–thawed embryos increases implantation and pregnancy rates. The patient population is quite homogeneous, as all were subjected to ICSI and embryos from fresh cycles were generated within a relatively short period of time, thus decreasing the likelihood of being subjected to different culture media and laboratory environment. Gabrielsen in a pseudorandomized study (allocation based on odd–even dates) showed that acidic Tyrode's solution increased the implantation rate of cryopreserved–thawed embryos[14]. The difference in clinical pregnancy rate although increased by AH did not reach statistical significance most likely due to inadequate number of cycles included in the study.

However, Ng *et al*. [15] failed to show any beneficial effect of LAH on implantation and pregnancy rates following the transfer of thawed embryos. Patients of advanced age similarly did not benefit from LAH. The authors only noticed a trend towards increased implantation rates when the zona thickness was >16 µm. The major difference between our study and the study by Ng *et al*.[15] is that we performed LAH approximately 1hafter thawing and only on embryos that showed evidence of cleavage. Ng *et al*. [15] also indicated that their results might have differed if embryos were allowed to cleave in *vitro*.

In a European multicentre prospective randomized study, Primi *et al*. [16] were unable to show any benefit of AH in frozen–thawed embryo transfer cycles. Furthermore, AH appeared to be detrimental in the absence of immunosuppressive treatment. None of the patients in our study received immunosuppressive treatment.

CONCLUSIONS

In conclusion, our study clearly showed that

- LAH increased implantation and clinical pregnancy rates in women undergoing thawed embryo transfer.
- The strengths of this study are that it includes sufficient number of subjects, it is properly randomized and is undertaken in one centre. Participants were homogeneous, as only ICSI cases were included.
- Only embryos that showed evidence of cleavage were subjected to AH; thus, embryos with less than optimal viability were excluded.
- There were statistically significant differences between using laser and without laser groups regarding no. of pregnancy cases and pregnancy rate, using laser group have values statistically higher than without laser.

REFERENCES

1. Edwards R; Clinical approaches to increasing uterine receptivity during human implantation. Hum Reprod 1995; 10 (Suppl.): 60-6.
2. Huisman GJ, Fauser BC, Eijkemans MJ, Pieters MH; Implantation rates after in vitro fertilization and transfer of a maximum of two embryos that have undergone three to five days of culture. FertilSteril 2000; 73: 117-22.
3. Cohen J. Assisted hatching of human embryos. J Assist Reprod Genet 1991; 8: 179-90.
4. Hammadeh ME, Fischer-Hammadeh C, Ali KR;Assisted hatching in assisted reproduction: a state of the art. J Assist Reprod Genet 2011; 28: 119-28.
5. Çıray HN, Bener F, Karagenç L, Ulug U, Bahçeci M; Impact of assisted hatching on ART outcome in women with endometriosis. Hum Reprod 2005; 20: 2546-9.
6. VerMilyea MD, Liebermann J, Tucker M; Embryo cryopreservation. In: Ginsburg ES, Racowsky C (eds). In vitro fertilization: a comprehensive guide. New York: Springer; 2012. 144-61
7. Schimmel T, Cohen J, Saunders H, Alikani M; Laser-assisted zonapellucida thinning does not facilitate hatching and may disrupt the in vitro hatching process: a morphokinetic study in the mouse. Hum Reprod 2014;29(12):2670-9
8. O'Neill C; Evidence for the requirement of autocrine growth factors for development of mouse preimplantation embryos in vitro. BiolReprod 1997; 56: 229-37.
9. Kuwayama M, Vajta G, Kato O, Leibo SP; Highly efficient vitrification method for

cryopreservation of human oocytes. Reprod Biomed Online 2005; 11:300–8.

10. Rienzi L, Nagy ZP, Ubaldi P, Iacobelli M, Anniballo R, Tesarik J, et al; Laser-assisted removal of necrotic blastomeres from the cryopreserved embryos that were partially damaged. FertilSteril 2002;77:1196-201.
11. Ge HS, Zhou W, Zhang W, Lin JJ; Impact of assisted hatching on fresh and frozen-thawed embryo transfer cycles: a prospective, randomized study. Reprod Biomed Online. 2008; 16(4):589-96.
12. Blake DA, Forsberg AS, Johansson BR, Wikland M; Laser zonapellucida thinning-an alternative approach to assisted hatching. Hum Reprod 2001; 16:1959-64.
13. Balaban B, Urman B, Yakin K, Isiklar A; Laser-assisted hatching increases pregnancy and implantation rates in cryopreserved embryos that were allowed to cleave in vitro after thawing: a prospective randomized study. Hum Reprod 2006; 21(8):2136-40.
14. Gabrielsen A, Agerholm I, Toft B, Hald F, Petersen K, Aagaard J, et al; Assisted hatching improves implantation rates on cryopreserved–thawed embryos. A randomized prospective study. Hum Reprod 2004; 19:2258-62.
15. Ng E, Naveed F, Lau R, Yeung WS, Chan CC, Tang OC, et al; A randomized double blind controlled study of the efficacy of laser assisted hatching on implantation and pregnancy rates of frozen–thawed embryo transfer at the cleavage stage. Hum Reprod 2005; 20: 979-85.
16. Primi M, Senn A, Montag M, Van der Ven H, Mandelbaum J, Veiga 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; 19:2325-33.