Research Article

The Effect of Quarter Laser Assisted Hatching of Human Embryos on Pregnancy Rates after Intracytoplasmic Sperm Injection

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ABSTRACT

Assisted Hatching (AH) is a technique originally created to help increase the pregnancy rates after Intra-Cytoplasmic Sperm Injection procedures (ICSI). A lot of AH techniques emerged including mechanical, chemical and Laser AH. However, Laser Assisted Hatching (LAH) was proven to be superior to other techniques due its easy integration in existing injection systems, its safety on the embryos, and the general ease of usage. LAH is hypothesised to enhance the pregnancy rates for patients undergoing ICSI cycles because it was previously found that Zona Pellucida (ZP) gets harder and firmer after ICSI, thus the growing embryo fails to hatch, due to ZP hardening, and consequently it never implants itself into the endometrium. Many meta-analyses could not conclude if AH are beneficial due to the mixed results from researches. So, there is still uncertainty. Our study was carried out to evaluate the impact of quarter LAH on the reproductive outcome of ICSI cycles in patients with poor prognosis. Quarter LAH is the process of thinning quarter the circumference of ZP. Controlled ovarian stimulation was done for the patients, oocytes were collected and denudated. ICSI was done for those oocytes, fertilization was checked, and good quality embryos were isolated for the quarter LAH process before being transferred back into the uterus on day three. Chemical pregnancy and clinical pregnancy rate were estimated for both experiment and control groups and our study revealed that quarter LAH could significantly improve both chemical and clinical pregnancy rate of ICSI cycles for patients with poor prognosis.

Keywords: *In Vitro* Fertilization, ICSI, LAH, Pregnancy rates.

INTRODUCTION

One of the unresolved problems in In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI) is the poor implantation rates after the embryo transfer of normal looking embryos[1]. This low implantation rate is related to intrinsic embryo abnormalities, defective uterine receptivity, in addition to hatching failure. Thick Zona Pellucida (ZP) is one of the main reasons that some blastocysts fail to hatch, and this leads to impaired implantation[2], which is correlated to basal FSH levels and preovulatory estradio[3]. During *in vitro* culture secondary zona hardening may also occur[4]. Laser Assisted Hatching (LAH) is thought to enhance embryo implantation by facilitating the hatching process, and more importantly, permitting early embryo endometrium interaction.

A lot of evidence suggests that assisted hatching techniques –generally- may improve clinical pregnancy rates after doing ICSI, especially in patients with poor prognosis. But after all, there is still some uncertainty[5].

Assisted hatching techniques include manipulation of the zona either mechanically, by chemicals and acids or by laser to help the embryo in the process of hatching and to facilitate embryo implantation[5].

Producing uniform and standardized holes using acid solution with micro tools under the control of manipulators have been problematic, as it requires extensive technical skills. An alternative tool for these methods has been proposed, which is drilling of ZP by Laser[6]. Laser drilling is superior to chemical and mechanical drilling in many aspects, as it provides touch-free objective-delivered access to the target, with minimal absorption by the embryos[7]. Moreover, Lasers are cost effective and could be integrated in an existing inverted microscope. Most importantly, it causes no thermal or mutagenic side effects to the embryos and is controlled accurately[7].

Also, LAH significantly reduces the time where the process of assisted hatching is being done outside of the incubator[5].

Previous studies regarding the efficacy of LAH techniques that was done on embryos of mice showed that the best way to do LAH is the total full-thickness method as it resulted in more fully hatched blastocysts, compared to other LAH techniques[8]. This was found to be completely contrary to what was found in humans; where full-thickness LAH had the worst pregnancy results[9].

Our study found that quarter LAH significantly increased the pregnancy rates of patients undergoing ICSI cycles with poor prognosis.

MATERIALS AND METHODS

This prospective randomized controlled study was held on 130 patients divided equally into two groups, the experiment group and the control group. Each group consists of 65 patients.

Patients were randomized using sealed envelope into two groups; experiment group and control group. All patients participating in this study signed a clear and thorough informed consent.

Ethical approval for this study was obtained from the Egyptian Ministry of Health's Ethical committee.

The embryos from the experiment group underwent quarter LAH -as to be prescribed later in the method section- on day three and three hours before the embryo transfer.

The embryos from the control group did not undergo any form of assisted hatching; i.e. the ZP was intact all the time.

Inclusion Criteria

Patients participating in this study fulfilled the following criteria:

- Age: Thirty-eight years old or more.
- Patients requiring 375 I.U. of gonadotropin or more per day.
- Patients with previous failed ICSI.

Method

This study was held in a private IVF center in Cairo, Egypt. This study took place from December 2017 to November 2018. All embryos included in this study were produced by ICSI.

Embryos from 65 patients (the experiment group) that were deemed to be transferred have undergone quarter LAH (quarter of the circumference of the outer layer of the ZP is thinned).

All patients underwent Antagonist induction protocol, with a minimum of 375 I.U. of gonadotropins per day.

Ova pick up was done 34 to 36 hours after the patient received a shot of Human Chorionic Gonadotropin (HCG) (Choriomon, IBSA, Egypt).

After the oocytes were retrieved, they were denudated and incubated for two hours before injection.

ICSI was done on an inverted microscope (Olympus, Japan), equipped with a micromanipulator (Narishige, Japan), and a heated stage (Tukhi Hit, Japan).

After sperm selection was done, oocytes were put into the injection dish (Falcon, UK) with media suitable for ICSI (G-MOPS, Vitrolife, Sweden). Oocyte maturity is assessed and only oocytes showing the presence of polar body were injected (MII oocytes). Oocytes were held using a holding microneedle (Humagen, Origio, Denmark) and the polar body was directed to either the 12 O'clock or 6 O'clock position. Sperm was injected into the oocyte using a microinjection pipette (Humagen, Origio, Denmark).

On the first day after ICSI, fertilization was checked and only embryos with two prominent pronuclei were transferred from four-well culture dish containing G-IVF media (Vitrolife, Sweden) into four-well culture dish containing 0.5ml of G1 media (Vitrolife, Sweden) covered with layer of paraffin oil (Virtolife, Sweden). Every well contained anywhere from one to four embryos and was left in the incubator for another 24 hours at 37 °C and 6% concentration of CO2 in air and 5% concentration of O2.

On day two after ICSI, cleaved embryos were scored and up to three embryos were selected for LAH and embryo transfer or for vitrification.

The selected embryos were preferred to have a cumulative score of at least 12 and preferably higher, i.e. 4 cells with a grading of at least 3 out of 4, or 3 cells with 4 out of 4 grading.

The cumulative embryo score is obtained by multiplying the number of cells by the embryo grade (grade 1–4), which is based on the percentage of fragmentation of the embryo. A grade 4 embryo showed no fragmentation at all, while a grade 3 embryo showed 25% fragmentation.

Microsurgical LAH

Laser drilling was done directly on the embryos in the 4-well culture dishes (Nunc, Denmark). The dish was placed on the heated stage of inverted microscope (Olympus, Japan) equipped with Saturn 3 apparatus (Research Instruments, UK). The set up used for ZP microdrilling is similar to that described in detail by Germond, and his team[7].

The laser microsurgical system is composed of invisible laser diode beam emitting at a wavelength of 1.48 µm. The exposure to Laser shot is around 10-20 millisecond. One Laser pulse is enough to create a whole with a diameter of 5 – 7 micrometers. With the help of the moving stage of the inverted microscope, the aiming spot of the laser was positioned at the ZP where the perivitelline space is greatest. The diameter of the ZP covers ~30 to 40% of the screen monitor, thus ensuring the aiming spot could be accurately placed between the middle and outer edge of the ZP. The laser beam was shot at the ZP by using the computer software.

Quarter LAH was done for the selected embryos three hours prior to the embryo transfer into the uterus.

As mentioned above, the embryos underwent quarter LAH, i.e. the inner membrane was not reached by any means. This is done by the thinning of the ZP that was

initiated at one point and continued until 25% of the ZP was irradiated (for example, laser drilling was initiated at the 12 o'clock position and consecutive irradiations were generated until the 3 o'clock position of the embryo was reached).

Embryo Transfer

Embryo transfer was done with a Labotect catheter (Labotect, Straberg, Germany), and the physician was blinded to the control and test groups.

Luteal phase support for the endometrium

All patients received 100mg of progesterone in a form of IM injections starting from the ovum pick-up day. (Prontogest, Marcyrl, Egypt).

After fourteen days from embryo transfer, patients had a pregnancy blood test. Women with βHCG = 25 or higher were considered positive i.e. pregnant. Seven weeks later, clinical pregnancy was established with the presence of fetal heartbeat.

Statistical Analysis

Statistical analysis was performed using SPSS computer program (version 19 windows) (IBM Inc., Chicago, Illinois, USA). Results are expressed as mean± standard deviations (SD) and number (%). Comparison between categorical data [n (%)] was performed using Chi square test. Test of normality (Kolmogorov-Smirnov test) was used to study the distribution of data. Accordingly, data were found to be normally distributed, so comparison between variables was performed using independent samples t-test. A P value ≤ 0.05 was considered significant.

RESULTS

This study was conducted on 130 infertile women undergoing ICSI cycles with poor prognosis; poor responder women whose age is 38 or more, required 375 I.U. of gonadotropin or more per day to well stimulate their follicles and with history of previous failed ICSI. Table 1 shows the description of the patients in the study.

Table 1: Patients basal and cycle characteristics.

	Laser	Controls	*P* Value
Number of cases	65	65	
Age	39.2 ±1.17	39.5 ±1.18	0.152
No. of oocytes	5.6 ±3.33	5.5 ± 3.50	0.806
Fertilization rate (%)	71.2 ±20.02	67.3 ±17.84	0.559
No. of Embryos Transferred	2.7 ±0.55	2.5 ±0.69	0.178

- Results are expressed as mean ±standard deviations (SD). A P value ≤ 0.05 was considered significant.

Regarding the characteristics between both groups that can affect the pregnancy rate, like age, number of retrieved oocytes, fertilization rate and number of embryos transferred per case, was all found to show no statistical difference (Table 1).

There was a statistical difference (P<0.05) between the two groups regarding both chemical and clinical pregnancy (Table 2) (Figure 1 and 2).

Table 2: Clinical and chemical pregnancy rates in the Experiment and Control groups.

	Experiment	Control	*P* Value
Chemical Pregnancy %	27/65 (41.5%)	15/65 (23.07%)	0.024
Clinical Pregnancy %	25/65 (38.4%)	13/65 (20%)	0.021

- Comparison between categorical data [n (%)] was performed using Chi square test. A P value ≤ 0.05 was considered significant.

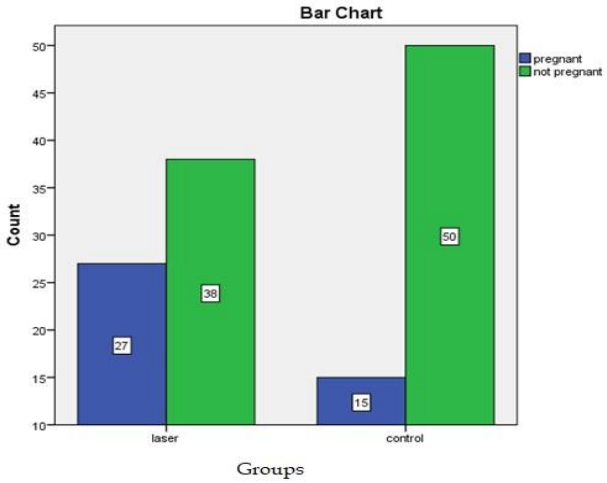

Figure 1: Chemical pregnancy rate between the two groups.

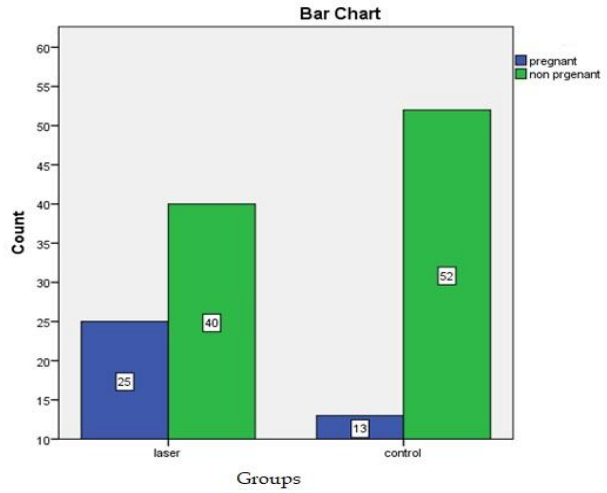

Figure 2: Clinical pregnancy rate between the two groups.

DISCUSSION AND CONCLUSION

It is still quite debatable if assisted hatching, and specifically LAH, has any benefit in increasing pregnancy rates. Some literature say that it is highly beneficial for selected infertile patients, with specific inclusion criteria[9].

Others suggest that assisted hatching generally increases the pregnancy rate in unselected and young groups of patients[10,11], and another group of researchers believe that assisted hatching techniques have no value at all in terms of increasing pregnancy rates[12], and a final group of researchers believe that assisted hatching generally increases the pregnancy rate, but it does not matter what kind of assisted hatching being used[13].

Meta-analysis done by the research team led by Da Li showed that there is still uncertainty whether assisted hatching techniques increases pregnancy rates after ICSI or not, so further research is needed[5]. Same result was also found by He and his colleagues in their systemic review and meta-analysis, where they could not draw a reasonable conclusion from their collected data[14].

There are many assisted hatching techniques, including mechanical[15,16], Chemical[2], or Laser[9]. All of those methods appeared to yield quite similar overall results but it was found that LAH techniques were the safest among all other assisted hatching techniques[12,17].

Patients in our study were offered Quarter LAH based on their selection criteria; which was if their age is > 38, and patients that were deemed to be poor responders, or those who required high dosage of gonadotropins, higher than 375 I.U., and those who had previously failed ICSI.

We did not include the total LAH in our study, because it was proven how damaging it can be to fully penetrate the ZP of the embryos, exposing the inside of the embryo to all sorts of metabolic substances produced by the embryos into the culture media, and also making the embryo very vulnerable to infection, thus drastically decreasing the pregnancy rates.

When testing this full breaching method (Mantoudis and his team) had to cut the experiment short because of how low the pregnancy rate was (5.2%), in a sign of how devastating the technique was on the vitality of embryos, and consequently the pregnancy rates[9].

In the same aforementioned study, to try and avoid the effects of the total breach of the inner membrane of the ZP, patients took doses of immunosuppressants, steroids and antibiotics. Immunosuppressants and steroids were used to avoid the immunological destruction of the embryos, and the antibiotics were used to help maintain the embryos, and the mother, healthy and to avoid any infection. Unfortunately, that did not help in increasing the pregnancy rates.

In this study, we didn't want our patients, and their embryos, to go through such an unnecessary procedure. Thus, we did not use the total breaching technique, that is why steroids and antibiotics were not used as part of our study.

In the present study, we focused on the effect of Quarter LAH on pregnancy rates, and whether if we should use Quarter LAH routinely for patients with poor prognosis.

Our results suggested that quarter LAH significantly increased chemical pregnancy rate in selected groups of infertility patients, where in the experiment group (n=65) the pregnancy rate was 41.5% (or 27/65) while it was 23.07% (or 15/65) in the control group (n=65), the difference was statistically significant (*P*= 0.024).

Clinical pregnancy rate was 38.4% (or 25/65) in the experiment group, while it was 20% (13/65) in the control group. Those results were found to be statistically significant (*P*=0.021).

Our findings were quite similar to those found by Meldrum and his colleagues, as well as Elhussieny and his team, that using Assisted Hatching techniques enhanced the pregnancy rates in patients with advanced age[18,19].

We believe the increased pregnancy rate in the experiment group is consistent with the hypothesis that quarter LAH mimics the normal hatching process of the embryo, where a series of weakened points in the ZP (due to intrinsic pressure of the growing embryo) gets thinner and then the zona opens up when the embryo is ready to hatch at the blastocyst stage.

Thus, we conclude that Quarter LAH increases both the chemical and clinical pregnancy rates in patients with poor prognosis such as advanced age (38 years old or more), previous failed ICSI and poor responders who required more than 375 I.U of gonadotropins per day to well stimulate their follicles, and we suggest that Quarter LAH should be offered routinely to patients that meet the aforementioned criteria.

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REFERENCES

[1] Fong, C.Y., Bongso, A., Sathananthan, H., Ho, J. and Ng, S.C.: Ultrastructural observations of enzymatically treated human blastocysts: Zona-free blastocyst transfer and rescue of blastocysts with hatching difficulties. Hum Reprod. 16, 2001, 540-546.

[2] Cohen, J.; Alikani, M.; Trowbridge, J. and Rosenwaks, Z.: Implantation enhancement by selective assisted hatching using zona drilling of human embryos with poor prognosis. Hum. Reprod., 7, 1992, 685–691.

[3] Loret De Mola, J. R.; Garside, W. T.; Bucci, J.; Tureck, R. W. and Heyner, S.: Analysis of the human zona pellucida during culture: correlation with diagnosis and the preovulatory hormonal environment. J Assist Reprod Genet. 14(6), 1997, 332-336.

[4] De Felici, M. and Siracusa, G.: Spontaneous hardening of the zona pellucida on mouse oocytes during in vitro culture. Gamete. Res., 6, 1982, 107–113.

[5] Da Li, Da-Lei Yang, Jing An, Jiao Jiao, Yi-Ming Zhou, Qi-Jun Wu and Xiu-Xia Wang.: Effect of assisted hatching on pregnancy outcomes: a systematic review and meta-analysis of randomized controlled trials. Sci. Rep., 6, 2016, 31228. doi: 10.1038/srep31228.

[6] Tadir, Y.; Wright, W. H.; Vafa, O.; Ord, T.; Asch, R.H. and Berns, M.W.: Micromanipulation of gametes using laser microbeams. Hum. Reprod., 6, 1991, 1011–1016.

[7] Germond, M.; Nocera, D.; Senn, A.; Rink, K.; Delacretaz, G. and Fakan, S.: Microdissection of mouse and human zona pellucida using a 1.48-microns diode laser beam: efficacy and safety of the procedure. Fertil. Steril., 64, 1995, 604–611.

[8] Chailert C.; Sanmee U.; Piromlertamorn W.; Samchimchom S. and Vutyavanich T.: Effects of partial or complete laser-assisted hatching on the hatching of mouse blastocysts and their cell numbers. Reprod Biol Endocrinol. 2013; 11, 2013, 21.

[9] Mantoudis, E.; Podsiadly, B.T.; Gorgy, A.; Venkat, G. and Craft, I.L.: A comparison between quarter, partial and total laser assisted hatching in selected infertility patients. Hum. Reprod., 16, 2001, 2182 – 2186.

[10] Ali, J.; Rahbar, S.; Burjaq, H.; Sultan, A.M.; Al Flamerzi, M. and Shahata. A. M.: Routine Laser Assisted Hatching Results in Significantly Increased Clinical Pregnancies. J Assist Reprod Genet. 20(5), 2003, 177–181.

[11] Antinori, S.; Panci, C.; Selman, H.A.; Caffa, B.; Dani, G. and Versaci, C.: Zona thinning with the use of laser: A new approach to assisted hatching in humans. Human Reprod. 11, 1996, 590-594.

[12] Elnahas, T.; Tawab N.; Azmy O.; Elnoury A.; El-Faissal Y.; Fouad T.; Elnahas A.; Abdel Rasheed M.; Aboelghar, M. and Mansour R.: Prospective randomized trial on the use of laser assisted hatching for transfer of frozen/thawed embryos in human ICSI. Middle East Fertil Soc J., 6(4), 2017, 309-312.

[13] Le, M. T.; Nguyen, T.; Nguyen, T.; Nguyen, V. T.; Le, D. D.; Nguyen, V.; Cao, N. T.; Aints, A., and Salumets, A.: Thinning and drilling laser-assisted hatching in thawed embryo transfer: A randomized controlled trial. Clin Exp Reprod Med. 45(3), 2018, 129-134.

[14] He, F.; Zhang, C.; Wang, L.; Li, S.and Hu, L.: Assisted Hatching in Couples with Advanced Maternal Age: A Systematic Review and Meta-analysis. Curr Med Sci 38, 2018, 552-557.

[15] Malter, H. E. and Cohen, J.: Blastocyst formation and hatching in vitro following zona drilling of mouse and human embryos. Gamete Res., 24, 1989, 67-80.

[16] Khalifa, E.A.M.; Tucker M.J. and Hunt P.: Cruciate thinning of the zona pellucida for more successful enhancement of blastocyst hatching in the mouse. Human Reprod., 7, 1992, 532-536.

[17] Hammadeh M.E.; Fischer-Hammadeh C.; Ali K.R.: Assisted hatching in assisted reproduction: a state of the art. J Assist Reprod Genet. 28(2), 2011, 119-128.

[18] Meldrum, D.R.; Wisot, A.; Yee, B.; Garzo, G.; Yeo, L. and Hamilton, F.: Assisted hatching reduces the age-related decline in IVF outcome in women younger than age 43 without increasing miscarriage or monozygotic twinning. J Assist Reprod Genet. 15, 1998, 418-421.

[19] Elhussieny, A.; Mandouh, M.; Hanafi, S.; Mansour, G. and El-Kotb, A.: Effect of laser assisted hatching on outcome of assisted reproductive technology. Open J Obstet Gynecol. 3, 2013, 18-23.

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