

Study of Laser Assisted Hatching for Fertility Outcome in Intra Cytoplasmic Sperm Injection and IVF Cycles

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Abstract:

Introduction: Implantation of transferred embryos in the uterus after IVF/ICSI cycles is an important process that less information about it is available. Zona hatching method is a suggested approach for this goal. The aim of this research is about performance of zona hatching by laser.

Methods: In this study 32 patients that enrolled had IVF/ICSI cycles depend on inclusion criteria of study that they included patients who were more than 35 years old, at least one previous failure of IVF, thickness or hardness of zona pelucida, the laser hatching performed on them. Then the pregnancy test (β -subunit) was done and patients with a positive result are followed with vaginal ultra sonography.

Results: Our successful results belonged to the patients who had more than 35 years old. So 50% of pregnancy was positive and then 20% about the zona pelucida thickness and minimum results were about the previous failure of IVF/ICSI cycles.

Conclusion: Laser hatching on the embryos of women more than 35 years old with IVF/ICSI cycle will optimize pregnancy outcomes.

Keywords: ivf/icsi cycle, laser hatching, pregnancy

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Introduction

In the ICSI/IVF cycles, transfer of embryo to uterus will perform when it has 4-8 cells. After this process the embryo will prepare for implantation (1). The first process of implantation is embryo exiting of zona pelucida or a layer that the embryo is inside it. So implantation is a process that the embryo adheres on uterus wall and first penetrates in the epithelium then penetrate in blood circulation so it will create the placenta. This process has time limitation and embryo implants on the anterior-posterior layer of uterus. The implantation includes three stages; encountering, adhering and invading (2).

Complex collection of molecules such as integrins and clectins cooperate in the adhering stage. Generally, cytokines, growth factors and their receptors are found in the related tissues of implantation process; these materials are biochemical tools which the physical process of adhering and invading of trophoblasts are performed by them (3).

One of important condition of implantation is that embryo must be in the suitable stage of development and surface layer of the embryo must be mature. zona pelucida or surrounding layer of oocyte and early embryo has two important roles; first

Prevention of some sperms entry and poly-

sperme creation, second on time exiting of the embryo from this layer to adhere to endometrial epithelium (4).

Blastocyst is formed about 4 days after gonadotropine secretion surge (embryo has 30 to 200 cells before implantation). Therefore the fertilized embryo should exit of zona pelucida layer to replace in the uterus. This mentioned process is called hatching (5). Implantation process includes; entrance of blastocyst in endometrial stroma that it starts by destruction of zona pelucida 1-3 days after entrance of morulla to the uterus cavity; it means that blastocyst remains in uterine secretion and then it exits of its zona pelucida to face with epithelium of uterus. Destroying of zona pelucida by expansion and contraction of blastocyst is necessary for the opening of zona and exiting of embryo in vitro. In vivo this process is performed by uttering liquid. However cytoplasmic outgrowths are important in blastocyst movement and hatching and trophoctoderm has significant role in this process. In this time blastocyst is differentiated to the inner cell mass (embryo) and trophoctoderm (placenta) that they are necessary for implantation (6).

In this process, zona pelucida is thinned and it let to hatching. If a disordering occurs in this stage the embryo could not penetrate in uterine layer. It seems that effective factors on thinning of zona pelucida begin by embryo compartment. Hardening and thickening of zona pelucida are some defined factors in prevention of this stage (7). During in vitro fertilization (IVF/ICSI) some factors such as growth in the culture medium and out of body environment cause to thickness of zona pelucida. So its natural form will change. these factors includes; the thickness of zona pelucida, more than 20 μ m, increasing of serum FSH level, older patients, embryo transfer after freezing and thawing and previous IVF cycles failures (8). To have better results from IVF cycles many researchers were studied on hatching process. First they used of mechanical and biochemical methods.

According to this purpose Cohen and et al suggested an artificial hole in the zona pelucida before transfer of the embryos to the uterus. This method is called assisted hatching (9). This technique is used for embryo exiting of zona pelucida and implantation.

There are different methods which suggested for hatching such as mechanical and biochemical

methods. Partial zona dissection is a mechanical method. Using of tyrode solution is a biochemical method (10).

Recently, use of laser began for hatching. In this method, laser is centralized on the zona pelucida at few milliseconds so the wanted point is determined and laser act on it (11).

In this research, the results of laser hatching on the patient's embryo that were in IVF/ICSI cycles is studied.

Methods

Among the patients who were the candidate of IVF/ICSI we selected these patients for laser hatching:

- women older than 35 years old
- previous IVF/ICSI cycles failure
- Transfer of freezing –thawing embryos
- zona pelucida thicker than 20 μ m
- hardening of zona pelucida

Drug protocol for induction of ovulation was, GnRH (Buserelin) 0.5cc ten days to menstruation and then gonadotropine injection on third day of cycle and control vaginal sonography was performed on 9th day of cycle for detection of ovarian follicles and endometrial thickness. In poor responder patients or patients who had weak results of ovarian reserve test or women older than 40 years old we used micro dose flare up regimen: GnRH analogue on second day of cycle and then gonadotropine on third day and vaginal sonography was done on 9th day. When the size of follicle reached to 16-18 millimeter, HCG 10000 U injected. After 36 hours puncture of ovaries was done. After 48-72 hours 4-8 cells embryos were transfer to uterus and if the patient had indication of hatching (mentioned indications) laser hatching was done by 450 us laser in two points.

Technical features/data:

630-650 nm spot targeting solid-state diode laser-red.1480nm/400mW solid state diode laser.

Pulse length range 0.1 to 2.2ms/100-2200 microseconds.

Then embryos were transferred to the uterus by cook transfer catheter. Progesterone was started on day of ovarian puncture (100mg IM or 400mg vaginal). If the frozen – thawed embryos were transferred the patient used valerate Estradiol orally. β -HCG titer measurement was done on 10th

and 14th day after embryo transfer and if β -HCG was positive vaginal sonography to see gestational sac was done two weeks after menstrual retardation. Clinical pregnancy was defined as observation of embryo in the gestational sac.

Then pregnant patients analyzed by related factors.

Results

In this study we performed laser assisted hatching on the embryos of 32 patients (Figure 1-6). The range of their age was between 27-42 years. Indications of zona hatching included; 3 cases of previous failure of IVF, 8 cases more than 35 years old, 10 cases zona thickness, 3 cases hardening of zona and other patients had complex causes included 1 case of previous failure of IVF and the age more than 35 years, 3 cases zona thickness and age more than 35 years, 2 cases zona thickness and previous IVF failure, 1 case zona hardening and age more than 35 years, 1 case of zona hardening and previous IVF failure. (See the below table).

	Number	Positive pregnancy
Previous failure of IVF	3	-
The age more than 35 years	8	4
Zona thickness	10	2
Zona hardening	3	1
Age more than 35 years + zona thickness + previous IVF	1	-
Zona thickness+ age more than 35 years	3	1
Zona thickness+ previous failure of IVF	2	-
Zona hardening+ age more than 35 years	1	-
Previous failure of IVF+ zona hardening	1	-



Figure 1. Old age & pervious failure IVF

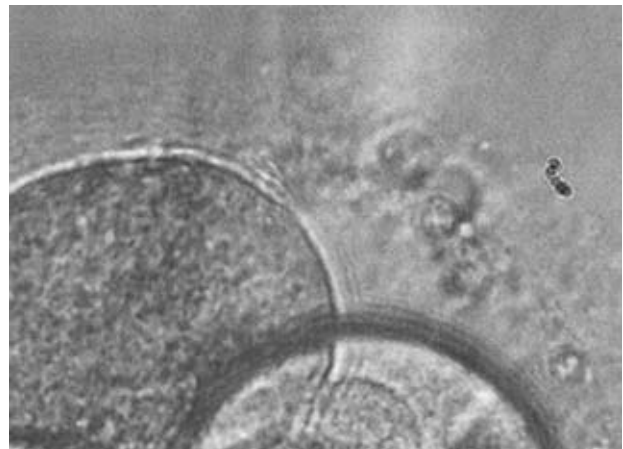


Figure 2. Woman age older than 35 years old

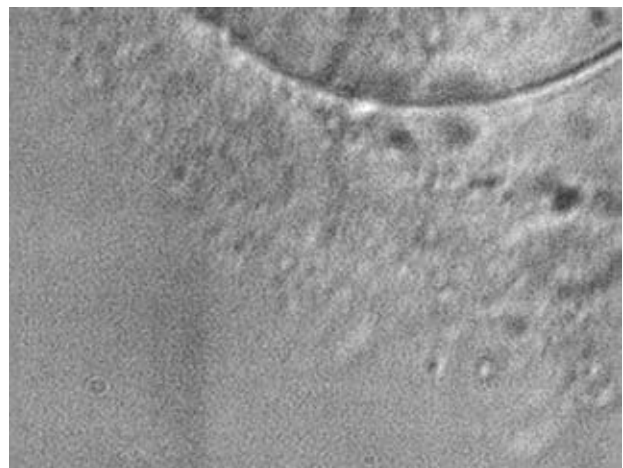


Figure 3. Zona hardening (after laser)

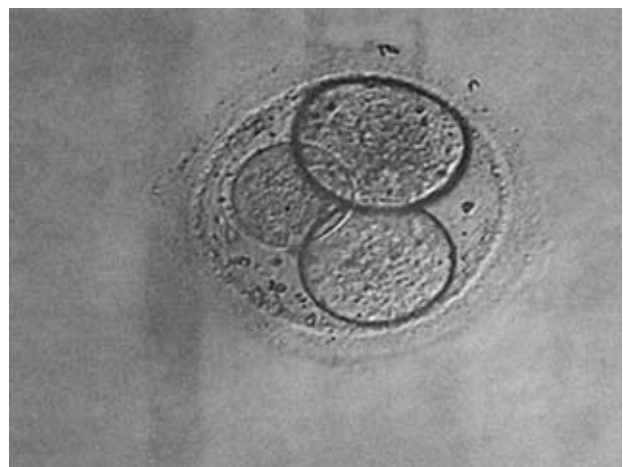


Figure 4. Zona hardening (before laser)

Discussion

Performance of Assisted Zona Hatching (AZH) is suggested to the embryo quick exiting and implantation in uterus. This technique were profitable

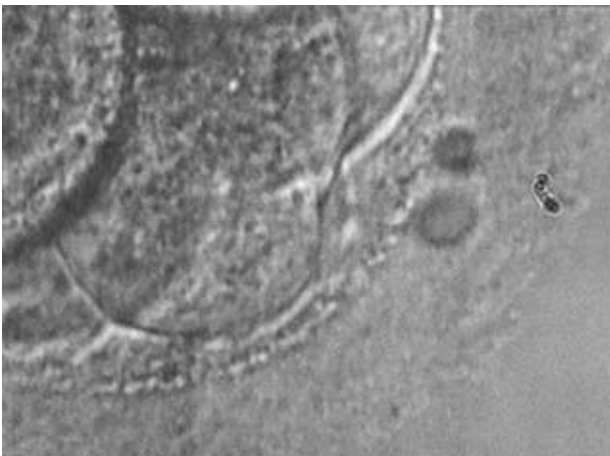


Figure 5. Zona thickening (after laser)

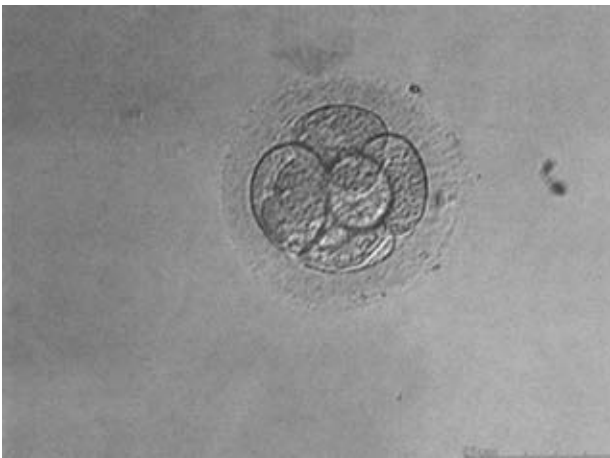


Figure 6. Zona thickening (before laser)

in many researches and it was ineffective in other studies. In this study the patients who had at least one previous failure of IVF did not achieve pregnancy by AZH. Therefore it seems that other factors interfere in these patients except of zona condition. These factors are included receptors and molecule contact between embryo and endometrium. Most positive results were related to women older than 35 years as the single factor. In the study of Markus Montag and et al a research was performed on the size and the number of puncture on the zona pelusida. They founded that if the size of puncture be too small the possibility of blastocyst capturing while exiting of zona plusida is more so they suggested medium punctures (12-15 μm). Also they recommended one puncture on zona pelusida because two points laser interfere with blastosyst exiting (12).

In Bassam and etal study routine AZH was not suggested for all patients. But in some cases such

as; the age more than 37 years old, increasing of basal FSH level, previous IVF failure in two cycles or more, zona thickness more than 15 μm and bad morphology of embryo and development retardation of embryo. In the point of their view is that AZH is effective (13).

In the study of Amy E. Jones and et al, they compared zona hatching by laser with acidified tyrode. They found that AZH performance do not damage the blastocyst growth and the performance of laser AZH prefer to acidified tyrode (14).

In the Fathi and et al study four cells embryos of rat from vitrified freezing after zona hatching by laser are studied and they founded that laser had not decreasing effect on the embryos cellularity or it had increasing effect on the cell numbers. More ever it caused to the increasing of blastocyst forming in the healthy rat four cells embryo from glass freezing (15)

In the Markous Montag research the effects of laser on the blastocyst was studied. It means that when periviteline space is invisible immediately after blastocyst laser, it is collapsed and it expanded 4 hours after lasering and it exited from zona at next 12-16 hours.

In the research of Markous Montag the effects of lasering on the aged women's embryos (more than 35 years old) studied so their success rate was 22% versus 5.3% and statistical difference was significant.

Many studies did not report the suitable effects of laser and other studies found it profitable (16-17).

This difference between the studies is due to comparison of laser approach with tyrod solution and mechanical methods. To achieve exact results, we need more researches on numerous patients. In embryos with zonal thickness, increased level of serum FSH, this approach is effective.

Another use of laser is immobilization of spermatozoid in ICSI cycles. In this approach spermatozoid prepares before ICSI for injection. So there is not need for Poly Vinyl Pyrrolidone (PVP) usage.

Conclusion

The use of assisted laser hatching is profitable on the embryos of women with 35 years old and more in IVF/ICSI. Using of this method is not logical in all cycles of IVF/ICSI.

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