

Laser Assisted Embryo Hatching - A Ray of Hope for the Infertile.

Firuzi R. Parikh, Nandkishore J. Naik, Sachin K. Manjrekar, Suparna G. Nadkarni,
Sonali A. Uttamchandani, Rajesh M. Parikh.

Department of Infertility Management and Assisted Reproduction, Jaslok Hospital & Research Centre,
15, Dr. G. Deshmukh Marg, Bombay 400026.

Summary

We report on the first pregnancy in India by Laser Assisted Hatching. The procedure of Assisted Hatching using the diode laser (Fertilase, M.T.G., G.M.B.H. Germany) was carried out for 10 women undergoing I.V.F./ET. Selection criteria were-age greater than 35 years, zonal thickness greater than 15 microns or at least one previous failed cycle of in vitro fertilization.

Laser Assisted Embryo Hatching was carried out on the inverted microscope of the micromanipulator system (R.I.,U.K.). The 1.48 um laser by targeting the laser beam on the zona pellucida for 8 to 12 milliseconds, making a breach of about 20 microns in the zona, without damage to any embryos. The treated embryos were then transferred. 6/10 women conceived, (pregnancy rate 60%).

The procedure is simple, quick and advantageous over mechanical and chemical assisted hatching

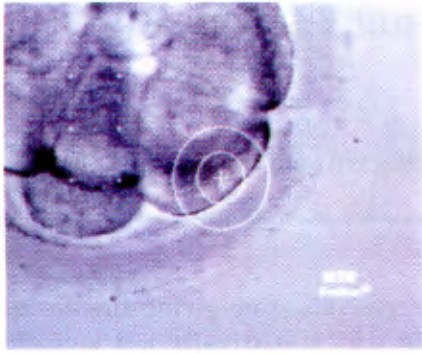
Introduction

In spite of significant advances in the laboratory methods of Assisted Reproduction, implantation rates remain low. The plausible explanation is that good quality embryos may not hatch from the zona and therefore cause failed implantation. The common causes of thickened zona are advancing maternal age (more than 35 years), sub-optimal culture conditions (Edwards et al, 1984), cryopreservation of embryos (Germond et al, 1996), and treatment with hyaluronidase which is used prior to the intracytoplasmic sperm injection procedure. Since the advent of micromanipulation, it was observed that there was an increase in implantation rates, the possible reason being that a breach was created in the zona pellucida allowing the blastocyst to expand and hatch (Cohen et al, 1990) We initiated mechanical assisted hatching in our I.V.F. programme in 1994. Initially we used the procedure for older women and those with thickened zona (Parikh, 1996). Encouraged by the improved pregnancy rates from 20% to 44% in older women, we have been routinely utilizing this technique to attain better implantation rates. More recently we have introduced the Fertilase system (MTG, Germany) which uses the 1.48 um diode laser system for laser assisted embryo hatching. Our initial experiences suggest that this technique is superior to mechanical and chemical hatching and improves pregnancy rates. We report on the first pregnancy in India by Laser Assisted Hatching and an enhanced pregnancy rate of 60% in this group of women.

Material and Methods

Ten women who underwent I.C.S.I., consented for laser assisted hatching. Couples underwent adequate counseling regarding the procedures. The stimulation protocol consisted of down regulation with GnRH agonist analogue followed by superovulation with hMG in appropriate doses. The average age of the women was 36.2 years. 6/10 women (60%) had at least 1 previous assisted reproduction cycle with implantation failures. Fifty six oocytes were retrieved (5.6 oocytes per cycle); 49 were mature (MII stage) and were subjected to the I.C.S.I. procedure. 37/49 fertilised (Fertilisation rate = 76%). 34/37 cleaved (Cleavage rate= 91%) Embryo transfer was performed at the 6 to 8 cell stage. Embryos were selected for the laser assisted hatching procedure based on any of the following criteria: age > 35 years (n=5), ii) zona thickness > 15 microns (n=7) or at least 1 previous cycle of failed I.V.F. (n=6).

The embryo was visualized on the heated stage (37.6 C.) of the RI Microscope (Research Instruments, U.K.). The Fertilase system (MTG, G.M.B.H., Germany) was activated. Markers on the screen identified the thickness of the zona to be hatched. The exposure time varied from 12 to 15 milliseconds. A 20 micron breach was thus created in the zona pellucida. (Figure I) After the laser assisted hatching the embryos were transferred to I.V.F. medium (Medicult, Denmark) prior to the embryo transfer.



A 20 mm opening is made in the Zona pellucida by the diode laser exposure time is 8 milliseconds.

On an average 3.4 embryos were transferred. Luteal phase support was with progesterone. Serum beta hCG was performed 14 days after the embryo transfer.

Results

There was no damage to the cytoplasm of the embryos. There was no significant movement or rotation of the embryos in the petridish. In one embryo the laser beam was targeted too close to one of the blastomeres and hence during the triggering of the laser beam, one of the blastomeres appeared very shiny. This has been video-recorded. However, after exposure to the diode laser, the embryo was held in culture but did not show any degenerative changes.

After the hatching the embryos were observed for extrusion of the blastomeres. None of the blastomeres extruded. Six patients conceived. Pregnancy rates were 60%.

Discussion

After extensive and thorough testing of the Fertilase system on mammalian oocytes (Rink, 1994) the system was introduced by MTG, Germany for use in assisted reproductive technology. The 1.48 μ m diode laser was mounted on the inverted microscope with micromanipulation system (R.I., U.K.). This gave a touch-free access to the zona pellucida by the laser light with minimum absorption by the culture dish and the medium.

The drilling procedure is performed usually with one laser irradiation or multiple depending on the thickness of the zona. The embryo need not be held with the holding pipette as the procedure is instantaneous and does not bring about any significant

movement of the embryos. The irradiation of the zona results in the dissipation of its tiny fragments due to photolysis. The dimensions of the opening in the zona pellucida were measured by the calibrated frame on the T.V. screen. The laser effect is strictly localized as has been earlier demonstrated by electron microscopy studies. (Germond et al, 1994)

The selective lysis is probably due to the greater susceptibility of the zona glycoprotein to disruption or due to higher absorption of the laser wavelengths by the zona pellucida components.

Besides the diode laser compares very favourably to mechanical assisted hatching, which takes more time, is not always reproducible, has a longer learning period and can sometimes damage the embryo. The diode laser presents no mutagenic risks as the radiation is in the near infrared.

The diode laser allows precision in the hatching procedure, minimizing any risk to the embryo. The reduced processing time of a few milliseconds prevents exposure of the embryo.

We report on the first pregnancy in India using Laser Assisted Hatching and an enhanced pregnancy rate of 60% in this group of women. The first babies (twins) by this technique were born in July 1999 and so far 5 babies have been born.

References

- 1) Cohen J, Elsner C, Kort H, Malter H, Massey J, Mayer MP and Weimer K Human reproduction 5, 7, 1990.
- 2) Delacretaz G, Descloux L, Rink K, Senn A, Primi MP, Germond M. Presented at the European Society for Human Reproduction and Embryology. Goteberg June 1998.
- 3) Edwards RG, Fishel SB, Cohen J, Fertilly CB, Purdy M, Slater JM, Steptoe PC and Webster JM Journal of Fertilisation and Embryo Transfer 1: 3, 1984.
- 4) Germond M, Rink K, Nocera D, Delacretaz G, Senn A, Fakan S, Fertility Sterility, 64,604,1995.
- 5) Germond M, Senn A, Nocera D, Rink K, Delacretaz G. Presented at the American Society for Reproductive Medicine Meeting, Boston. Abstract P-051, 1996.
- 6) Parikh FR, Kamat S, Nadkarni S, Arawandekar D, Parikh RM Reproduction and Fertility Supplement 50: 121, 1996.
- 7) Rink K, Delacretaz G, Salathe Rp, Senn A, Nocera D, Germond M, De Grandi P, Fakan S., Lasers Surg Med., 18: 52, 1996.