Zona Thinning with Noncontact Diode Laser in Patients Aged ≤37 Years with No Previous Failure of Implantation: A Prospective Randomized Study

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Submitted: March 2, 2000
Accepted: May 22, 2000

***Purpose**: Zona thinning (ZT) is a technique used to improve pregnancy rates among patients ≥38 years old and/or patients presenting previous implantation failure. The objective of the study was to determine whether ZT has a beneficial effect on patients younger than 37 years who are undergoing the first ICSI attempt.*

***Methods**: A total of 103 patients submitted to ICSI for the first time and those aged ≤37 years were divided in a prospective and randomized manner into two groups: group I, patients submitted to ZT (*n *= 51) (a laser diode with 1.48-μm wavelength (Fertilaser) was used for the procedure); group II, patients with no ZT (*n *= 52). In both groups, embryo transfer was performed on the second day.*

***Results**: The age of group I patients (31.8 ± 3.6) did not differ (*P *= 0.53) from that of group II patients (31.4 ± 3.6). The number of metaphase II oocytes was similar (*P *= 0.76) for the two groups (group I = 9.12 ± 5.27; group II = 8.67 ± 5.02). The average number of embryos available per transfer of group I (6.14 ± 4.02) did not differ (*P *= 0.69) from that of group II (5.75 ± 3.83). The number of embryos transferred was similar (*P *= 0.61) for the two groups (group I = 2.76 ± 0.9; group II = 2.87 ± 0.79). The thickness of the zona pellucida of group I embryos (16.6 ± 2.2 μm) did not differ (*P *= 0.08) from that of group II embryos (17.1 ± 1.7 μm). The rate of embryo implantation (20.8%) and the rate of clinical pregnancy per embryo transfer (40.3%) were higher for group II than for group I (17.7% and 33.3%, respectively), but the difference was not significant (*P *= 0.55 and *P *= 0.54).*

***Conclusions**: These results suggest that ZT in the population aged ≤37 years and with no previous failure of implantation may have no impact on intracytoplasmic sperm injection success rates.*

KEY WORDS: Assisted hatching; laser; zona thinning; intracytoplasmic sperm injection; implantation; pregnancy.

INTRODUCTION

Couples usually need assisted reproduction techniques to solve their infertility problems. However, the success rates after in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) are still low if we consider the number of embryos that do become implanted.

A set of laboratory techniques has been used since 1989 in order to improve embryo implantation in an attempt to facilitate the hatching of embryos produced in culture (1–4). The literature reports several methods for the opening of the zona pellucida (ZP) in the process of assisted hatching, i.e., mechanical (5), chemical (6), or laser (7,8) techniques.

While it seems that embryos with holes in the ZP have an increased potential for earlier and more likely hatching, complete perforation of the ZP does impose certain drawbacks. Holes in the ZP may cause loss of blastomeres or loss of the whole embryo during contractions of the reproductive tract (9); they also deprive the embryo of its protective coat, which protects against any detrimental factors in the female reproductive tract. Therefore, it seems that thinning of the ZP acts optimally to promote both early and complete hatching in the mouse (10).

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In 1996, Antinori *et al.* (11) reported that zona thinning (ZT) performed with laser (Er:YAG) produced a significant increase in implantation and pregnancy rates in a population of women with repeated embryo implantation failure and also among those who were undergoing IVF for the first time.

The objective of the present study was to evaluate the efficiency of the routine use of ZT with a 1.48-μm noncontact diode laser in patients aged $\leq$37 years with no previous failure of embryo implantation and who were undergoing ICSI for the first time.

MATERIAL AND METHODS

A total of 103 patients aged $\leq$37 years, whose husbands presented alterations of the male factor according to World Health Organization, criteria were undergoing ICSI for the first time. The patients were divided into two groups prospectively and at random, by drawing lots, using a randomization table previously elaborated for the study: I, patients undergoing ZT (n = 51); and II, patients not undergoing ZT (n = 52). The study was examined and approved by the local ethical committee and the patients were consented to the study.

For ovarian stimulation before ICSI, a second-phase blockade was used with leuprolide acetate at the dose of 0.5 mg/day (Lupron, Abbott). Fourteen days after the use of the analogue and the establishment of blockade, recombinant follicle-stimulating hormone (FSH) (Gonal F, Serono) was started at the fixed dose of 150 to 225 IU for a period of 7 days. On the eighth day of ovarian stimulation, follicle development started to be monitored by vaginal ultrasound and the doses of pure FSH were adapted according to ovarian response. When at least three follicles measuring $\geq$17 mm in diameter were observed, human chorionic gonadotropin (hCG) was administered at the dose of 10,000 IU.

Oocytes were collected from the follicles by ultrasound-guided transvaginal puncture 34–36 hr after hCG. After identification in follicular fluid, the oocytes were classified according to maturity. The cumulus–corona complex was removed by exposure to a hyaluronidase type IV solution (H-4272, Sigma Chemical Co., USA) at the concentration of 80 IU/ml. The denuded oocytes were incubated in IVF-50 medium (Scandinavian IVF Science AB, Sweden) until the time for ICSI. A discontinuous gradient of Sperm-Prep-100TM (Scandinavian IVF Science AB, Sweden) was used for separation of spermatozoa from seminal fluid in the 40% and 90% fractions.

ICSI was performed under an inverted Eclipse TE300 microscope equipped with a Hoffman lens system and coupled to automatic micromanipulators and injectors. A 10% polyvinylpyrrolidine solution (PVP-ICSI-100, Scandinavian IVF Science AB, Sweden) diluted in IVF-50 was used to immobilize the spermatozoa. The ICSI procedure was performed according to the technique described by Svalander *et al.* (12).

The oocytes are first observed for 16 to 18 hr during the procedure in order to determine the presence or absence of pronuclei. The morphological quality is then evaluated by the cumulative embryo score of Steer *et al.* (13).

ZT was performed with a laser diode (Fertilaser, Medical Technologies Montreux, Lausanne, Switzerland) with 1.48-μm wavelength operating through an objective coupled to an Eclipse TE300 inverted microscope. At the time for transfer (day 2), the embryos were placed in groups of one to four on Nunc plates containing IVF-50 culture medium. Using the stage of the microscope and no micromanipulators, the embryo was then positioned in the optic field and the ZP was focused in the direction of the laser light. ZP thinning was them performed by applying 12 ms irradiation, which is sufficient to thin the ZP between 16 and 18 μm. ZP thickness was measured with a scale present in the eyepiece of the microscope.

After the ZT procedure, the embryos were transferred to fresh culture medium in order to avoid possible toxicity to the products due to the action of laser on the organic components of the ZP. Embryo transfer was performed immediately after the procedure. The excess embryos were not hatched before frozen.

RESULTS

Patient age did not differ (P = 0.53) between group I (31.8 $\pm$ 3.6) and group II (31.4 $\pm$ 3.6). The number of metaphase II oocytes was similar (P = 0.76) for the two groups (group I = 9.12 $\pm$ 5.27; group II = 8.67 $\pm$ 5.02). The average number of embryos available per transfer of group I (6.14 $\pm$ 4.02) did not differ (P = 0.69) from that of group II (5.75 $\pm$ 3.83). The number of embryos transferred was similar (P = 0.61) for group I (2.76 $\pm$ 0.9) and group II (2.87 $\pm$ 0.79). The score of embryo morphological quality did not differ (P = 0.66) between group I (33.3 $\pm$ 15.4) and group II (34.1 $\pm$ 11.4). ZP thickness did not differ (P = 0.08) between the embryos of group

I (16.6 ± 2.2 μm) and the embryos of group II (17.1 ± 1.7 μm). The total number of embryos transferred was 141 for group I and 149 for group II. In group II, the embryo implantation rate (20.8%) and the clinical pregnancy rate per embryo transfer (40.3%) were slightly higher than those obtained for group I (17.7% and 33.3%, respectively), although the difference were not significant ($P = 0.55$ and $P = 0.54$). The abortion rates were 11.7% for group 1 and 19% for group II (Table I).

DISCUSSION

A common event occurring in embryo culture before implantation is delayed development compared to in vivo conditions (14). This may result in a decreased or blocked cleavage rate, a greater degree of fragmentation, or even alterations in the embryo implantation process. Current research is trying to reduce this effect by formulating new in vitro culture systems or by developing specific methods such as assisted hatching (AH).

The use of AH has been reported to be effective in patients whose embryos suffer chemical or physical ZP changes as a possible consequence of advanced reproductive age, high FSH concentrations, cryopreservation, or unknown causes in populations with repeated implantation failure (15–20). However, there is considerable variation in the results reported by different laboratories after the use of AH, including groups that did not observe any advantage with the use of this technique (5,21,22). This may be attributed to the type of AH used or to the stages of embryo development during which AH is performed or yet again to selection criteria and time of embryo transfer.

Table I. Results of Zona Thinning with Laser Versus Control in Patients Aged ≤37 Years with No Previous Failure of Implantation

	Group I laser	Group II no laser	*p*
Patients	51	52	
Age	31.8 ± 3.6	31.4 ± 3.6	0.53
No. of metaphase II oocytes	9.12 ± 5.27	8.67 ± 5.02	0.76
No. of embryos available	6.14 ± 4.02	5.75 ± 3.83	0.69
No. of embryos transferred	2.76 ± 0.9	2.87 ± 0.79	0.61
Embryo score	33.3 ± 15.4	34.1 ± 11.4	0.66
Zona pellucida thickness (μm)	16.6 ± 2.2	17.1 ± 1.7	0.08
Total No. of embryos transferred	141	149	
Gestations	17	21	
Implantation rate	17.7%	20.8%	0.55
Clinical pregnancy per transfer	33.3%	40.3%	0.54
Abortion rate	11.7%	19%	

On the other hand, Bertrand *et al.* (23) stated that ZP thickness is important in the analysis of the results of IVF programs, since they observed that when sperm is normal, the thickness of the ZP of fertilized oocytes (16.6 ± 3.2 μm) is significantly ($P < 0.001$) lower than that of the ZP of oocytes that were not fertilized (18.9 ± 4.0). In 1977, Garside *et al.* (24) reported that pregnancies are more frequent when embryos with a thinner ZP are transferred and that ZP measurement should be obligatory in any type of evaluation of embryo morphology. However, in our study in which ICSI was used, there were no significant differences in ZP thickness between the embryos submitted to laser treatment (16.6 ± 2.2 μm) and the controls (17.1 ± 1.7 μm).

With respect to age, Schoolcraft *et al.* (16) observed that AH dramatically increases the implantation and pregnancy rates in patients older than 40 years. Recently, Meldrum *et al.* (25) reported that the implantation rates increased with AH in patients aged 35 to 42 years, with the same incidence of spontaneous abortion or of monozygotic twins. However, Bider *et al.* (5), after performing AH in a selected group of patients aged >38 years, did not identify an increase in take-baby rate after IVF treatment. In addition, Lanzendorf *et al.* (6), in a prospective and randomized study, did not observe significant differences in implantation or pregnancy rates after the use of AH in patients aged ≥36 years compared to control.

With respect to patients with a history of recurrent implantation failure, Stein *et al.* (17) and Magli *et al.* (20) agreed that AH was an efficient method in raising the pregnancy rates in a population with a minimum of three failed implantations in previous IVF cycles.

However, with respect to the use of AH in cases with a good prognosis, i.e., young patients with no implantation failure, contradictions persist. Hurst *et al.* (22) assessed the role of AH in patients with a good prognosis for IVF in a preventive and randomized study and obtained a 23% pregnancy rate for the AH group compared to 43% for a control group and concluded that AH has no beneficial effect on this population. In contrast, Széll *et al.* (26) observed a better pregnancy rate ($P < 0.01$) in patients aged <35 years when they performed AH (60%) than in patients of the same age not submitted to AH (33%).

In the present study the ZT was performed on day 2, because in 1996, Antinori *et al.* (11) showed that

laser ZT of human embryos at 48 hr after egg retrieval significantly increases the implantation and pregnancy rate.

Our data were collected in a prospective and randomized manner for a population of women aged ≤37 years with no previous implantation failure and demonstrated higher implantation and pregnancy rates for group II (no laser) than for group I (ZT with laser), although the differences were not significant.

In conclusion, ZT with a 1.48-μm noncontact diode laser was of no benefit to a population of patients aged ≤37 years with no history of implantation failure when undergoing ICSI.

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