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ASSISTED REPRODUCTION

Laser assisted zona thinning technique has no beneficial effect on the ART outcomes of two different maternal age groups

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Abstract
Purpose Laser assisted zona thinning is a technique used for facilitating embryo's hatching process and is commonly used for the embryos of poor prognosis patients with advanced maternal age, repeated implantation failures, poor embryo quality or thick zona pellucida. The aim of this study was to investigate the effect of zona thinning on both good or poor prognosis patients of two different age groups.
Methods We investigated two different age groups (group 1: <35 years and group 2: ≥35 years) and compared the effect of assisted zona thinning (groups 1A and 2A) versus not-thinned controls (groups 1B and 2B) in both groups.
Results The clinical pregnancy rates were 57% and 56% in groups 1A and 1B (p=0.86), 43% and 38% in groups 2A and 2B (p=0.59) respectively.
Conclusions Our results suggest that laser assisted zona thinning of day 3 embryos has no beneficial effect on clinical pregnancy and implantation outcomes.

Keywords ART · Maternal age · Laser assisted zona thinning · Zona pellucida

Introduction

Hatching of the zona pellucida (ZP) by growing blastocysts within the first days of the life, is one of the most important events that occurs during the implantation process. A day 5 or 6 embryo has to form a leak through the zona barrier and flow into the uterine cavity where it implants into the endometrium. If hatching is not successful, implantation can not take place.

It has been suggested that, in vitro culture conditions may impair the mechanism of blastocyst hatching [1] and that assisted hatching (AH) of the ZP in asssited reproduction techniques (ART) treatments may improve the implantation and pregnancy rates [2–5]. However, the success rates following this procedure have considerably varied according to the patient population and the AH methods used. It has been proposed that poor-prognosis patients benefit best from the AH procedure [6]. This group includes patients with an elevated follicle stimulating hormone (FSH) [7], thick zona pellucida [7], previous in-vitro fertilization (IVF) failures [8], and advanced maternal age [9–11]. Hovewer, the effect of assisted hatching on the outcomes of good prognosis patients remain unclear.

There are several methods of assisted hatching. These are acidic Tyrode's solution, mechanical partial zona dissection (PZD) and laser [12]. The principals of all the methods are the same. They are based on either a hole forming or a leak on the zona pellucida, or thinning it in order to facilitate the passage of the embryo. Although very few studies have compared these methods, in general laser drilling seems to be a less invasive and safer method compared to acidic Tyrode's solution [12–14].

Assisted hatched embryos may show earlier hatching than non-hatched embryos [1, 15]. However, whether this will cause insufficient expansion of the embryo or not is unknown. According to Cohen and Feldberg [16], mechanically performed AH procedures inhibited completion of the hatching process due to the formed hole being too small.

Assisted hatching is performed by two main applications: 1. forming a hole or a leak through zona pellucida or 2. thinning a part of it. Although these two methods of

Capsule Laser assisted zona thinning technique has no beneficial effect on the ART outcomes of two different maternal age groups: <35 years and ≥35 years.

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artificial hatching have the same aim, to facilitate the embryo's passage through the zona barrier, the former seems more close to the natural process since there is still a part of the zona left for the embryo to break and thus provides more time for expansion to occur.

In this study we aimed to analyse the effect of laser assisted "zona thinning" on the pregnancy outcomes and to see if there was a difference between the two different age groups: 34 and younger and 35 and older.

Materials and method

Subjects

252 infertile couples having ART treatments between February 2008–August 2008 at Medicana Camlica Hospital, Istanbul, Turkey were included in the study. Patients of all age groups with at least 4 metaphase II oocytes were included. Cases of severe male infertility or where testicular sperm were used, and cases with preimplantation genetic diagnosis indication were excluded. Couples to whom embryo transfer was performed on day 2 were also excluded in order to standardize embryo scoring. Randomization was performed in a computerized manner. The study was approved by the Institutional Review Board of Medicana Camlica Hospital.

Procedure

Women were given a controlled ovarian stimulation by one of the following; either the gonadotrophin-releasing hormone (GnRH) analogue supression or GnRH antagonist protocols, and human menopausal gonadotrophins or recombinant FSH. When the leading follicle reached 17 mm in size, a hCG injection was administered. Oocytes were picked-up 36 h following the injection by the guidance of transvaginal ultrasonography. The microinjection procedure was performed as described by Van Steirteghem et al. Semen parameters were analysed according to the World Health Organisation criteria (WHO, 1999) and the spermatozoa to be used for intracytoplasmic sperm injection (ICSI) procedure was prepared by the two-layer (90–45%) gradient method (Nidacon, Mölndal, Sweden). SAGE (CooperSurgical, USA) sequential media was used for the culture and manipulation of oocytes and embryos.

Assessment of embryo score

Fertilization was confirmed by the observation of two pronuclei 16–18 h after ICSI. Cleavage stage embryos were assessed under an inverted microscope with X40 magnification (Olympus IX71, Japan) on day 3 according to the criteria of Ziebe et al. [17]. He defined embryos with 6–10 cells on day 3 with even blastomeres and <10% fragmentation as grade A, embryos with uneven blastomeres and/or 10–20% fragmentation as grade B, embryos with 20–50% fragmentation as grade C and embryos with >50% fragmentation as grade D. Embryo scores for each patient were calculated by taking the sum of each embryo's score which was obtained by multiplying the number of blastomeres on day 3 by 4 for grade A, by 3 for grade B, by 2 for grade C and by 1 for grade D. For those couples who had 3 or less embryos on day 2, embryo transfer was planned on day 2 and those couples were therefore excluded from the study.

Application of the AH procedure

AH was performed by the laser method (Octax Eyeware, Germany). Zona was only thinned, and not punctured. At least 3, at most 5 shoots (4.0 ms) were performed to the proper region of zona pellucida where blastomeres were not adjacent to the inner membrane of embryos. Following the thinning process, the measured thickness of zona pellucida on the laser assisted thinned surface was not less than 5 μm. The inner membrane was never touched. Written informed consent was taken from all the couples who received this application.

Embryo transfer

Only the couples to whom day 3 embryo transfer was performed were included in statistical analysis. All the embryos to be transferred to a patient, underwent AH. Embryo transfer was performed by the guidance of abdominal ultrasound. Serum βhCG concentrations were measured 15 days following the oocyte retrieval. >15 mIU/ml was defined as a positive pregnancy. Observation of the fetal heart beat was defined as a clinically positive pregnancy.

Study design

This study investigated the effect of AH in two separate groups. Group 1: patients with <35 years of age (n=139) and group 2: patients with ≥35 years of age (n=113). No comparison was planned between these groups since the parameters at the beginning differed significantly as expected (ages, mean embryo score, etc). Those two groups were further divided into two subgroups: groups 1A and 2A: patients to whom AH was performed, groups 1B and 2B: patients to whom AH was not performed. There was no difference between the subgroups (between 1A and 2A, between 1B and 2B) regarding mean female age, mean

number of oocytes retrieved, mean embryo score and mean number of embryos transferred (Table 1).

Statistical analysis

SPSS for Windows 10.0 software package was used for statistical analysis. Rates and proportions were evaluated by chi-squared test, differences between groups were evaluated by *t*-test. The results were evaluated within 95% confidence interval, and the *P* value of <0.05 was defined as statistically significant.

Results

A total of 1,795 oocytes were obtained from 139 patients in group 1 and 1,248 oocytes were obtained from 113 patients in group 2. 395 vs. 330 embryos were transferred to patients in groups 1 and 2 respectively. The clinical pregnancy rates were 66 and 40% for groups 1 and 2 respectively.

AH was performed on 73 patients (group 1A) in group 1 and not performed on 66 patients (group 1B). Likewise 58 patients (group 2A) in group 2 were performed AH while 55 were not (group 2B). All the embryos to be transferred in group1A and 2A underwent AH according to the design of the study. Group 1A was compared to 1B and group 2A was compared to 2B with respect to clinical pregnancy and implantation rates. The parameters that could affect the results were similar between these subgroups (Table 1). These parameters were the mean number of oocytes retrieved, the mean number of embryos transferred, mean embryo scores and mean ages of the female partners.

The clinical pregnancy rates were 57% (42/73) and 56% (37/66) in groups 1A and 1B (p=0.86), 43% (25/58) and 38% (21/55) in groups 2A and 2B (p=0.59) respectively. The implantation rates were 30% and 28% (p=0.64) in group 1A and 1B; 19% and 17% (p=0.07) in group 2A and 2B respectively.

Discussion

There are many studies that have investigated the effect of AH on clinical outcome. However, the results are conflicting. Most of them suggest that it has beneficial effects on pregnancy and implantation rates of poor prognosis group such as advanced maternal age and previous implantation failures [7–10, 18, 19], others suggest that it may be a useful method for poor prognosis patients [6] and some say it has no benefit at all [20, 21]. One group indicates better results when this technique has been used routinely [5]; while the others do not [22]. Recently Sagoskin has reported that AH did not improve clinical outcomes among good prognosis patients [23].

There are many issues concerning the unsuccessful hatching of the blastocyst both in vivo and in vitro. Those issues include the zona hardening because of suboptimal culture conditions in vitro [24], natural zona thickness [7] and zona thickening following cryopreservation [25, 26]. It has been reported in literature that implantation rate per embryo transfer for blastocsyst transfers was 23–25% [27]. It has also been shown that 54% of the blastocysts fail to hatch after 8 days of culture [28].

A natural expansion does not take place in an embryo with a drilled zona and as a consequence early release may be watched through the hole. The process of hatching includes the expansion of the embryo [10], the thinning of the zona [29] and a series of contractions [30]. During these contractions a group of cells may be lost through that hole before its proper time and that part would be seperated from the whole mass since the inner pressure is not enough to push all the mass out at that time. On the other hand, if a very small hatch is formed on the zona, the hatching process may not be completed [16].

Laser assisted AH method was more accurate for our study since the aim was to make the zona thinner and not to hatch it completely. This controlled thinning was best achieved by making several shoots over the zona in order to get at most a 5 μm thickness.

Table 1 Comparison of group parameters

	<35years of age (Group 1)			≥35years of age (Group 2)		
	Group 1A	Group 1B	*P* value	Group 2A	Group 2B	*P* value
Female age (years)	29.9±2.9	28.9±3.4	0.08	38.0±2.3	37.4±2.4	0.23
No. of retrieved oocytes	13.2±5.3	12.5±4.3	0.34	10.5±3.4	11.5±3.8	0.15
No. of embryos transferred	2.8±0.3	2.8±0.3	0.83	2.9±0.5	2.8±0.5	0.24
Embryo score	64.4±13.6	61.3±12.7	0.17	52.8±14.9	49.1±15.6	0.19
Clinical pregnancy (%)	57 (42/73)	56 (37/66)	0.86	43 (25/58)	38 (21/55)	0.59
Implantation (%)	30	28	0.64	19	17	0.07

Values are mean ± SD

According to our study, laser assisted zona thinning of human embryos before transfer has no beneficial effect on clinical outcomes of the two different maternal age patient populations. This may be due to many factors such as the suggestion that the inner layer of zona pellucida should be fully breached in order to promote implantation [31]. Besides, by the introduction of more accurate devices for quality control systems in IVF laboratories, we can provide a better environment for the needs of in vitro cultured embryos, and that may be a factor eliminating the suboptimal conditions in vitro to some extent, therefore an extra zona hardening may not take place.

A similar result with our study was obtained by Hurst et al. [32] which was conducted on an unselected good prognosis patient population. That study reported a pregnancy rate of 23% for the hatching group and 43% for the control group. Likewise randomized trials without any selection, reported no differences in implantation and pregnancy rates between treatment and control groups [23]. Sagoskin's and Balakier's results correlated with our results showing no improvement in clinical outcomes among good prognosis patients [23, 33]. Besides, very recent studies of a group reported that the procedure does not improve implantation, clinical pregnancy, or live birth rates in women younger than 38 years with embryos having ZP thickness of ≥13 μm [34, 35]. Furthermore another very recent study by Valojerdi showed that clinical pregnancy rate was significantly lower with laser assisted zona thinning on vitrified-warmed cleavage stage embryos [36] although it is widely accepted that it gives beneficial results [27].

Conclusion

As a conclusion we suggest that laser assisted zona thinning before embryo transfer on day 3 has no beneficial effect on patients of the two different age groups we investigated. However, more specific studies should be conducted on different subgroup of patients using different assisted hatching methods (such as partial or total) in order to define a selective group which may benefit better from the procedure.

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