THE EFFECT OF ASSISTED HATCHING AND DEFRAGMENTATION ON IVF OUTCOME IN PATIENTS WITHOUT GOOD QUALITY EMBRYOS FOR TRANSFER

Ahmet Zeki ISIK, Gamze Sinem CAGLAR, Eran SOZEN, Gorkem TUNCAY, Kubilay VICDAN

Ufuk Universitiy Faculty of Medicine, Ankara, Turkey

SUMMARY

Objective: *To evaluate the effect of assisted hatching and defragmentation applied to poor quality human embryos regarding the implantation and pregnancy rates.*

Materials and methods: *A retrospective analysis was performed in patients (n=168) of all ages with no good quality embryos for transfer (Veeck classification embryos>grade 1 and/or >10% fragmentation). The first group consisted of cycles in which mAHA was performed to all transferred grade 2 embryos. The second group included transfer cycles where all the embryos were highly fragmented (between 10-50% fragmentations, grade 3) and aAHA and microsurgical fragment removal were applied to all of them.*

Results: *In first group positive ßhCG was 33%, clinical pregnancy (fetal heart beat) rate was 27% and implantation rate was 11,28%. These rates were 37,1%; 28,8% and %16,19 respectively in the aAHA plus defragmentation group. In cases over 35 years of age in aAHA plus defragmentation group acceptable implantation (14,46%) and clinical pregnancy (31,58%) rates were achieved.*

Conclusion: *In patients with no good qualitytransferable embryos AHA combined with defragmentation can be utilised with acceptable success rates in laboratorieswhere there are experienced personnel available for this procedure otherwise only AHA can be the best option.*

Key words: *assisted hatching, defragmentation, embryo fragmentation, embryo quality, ICSI*

Journal of Turkish Society of Obstetrics and Gynecology, (J Turk Soc Obstet Gynecol), *2013; Vol: 10, Issue: 3, Pages: 138- 42*

TRANSFER EDİLECEK İYİ KALİTEDE EMBRYOSU OLMAYAN HASTALARDA ASSİSTED HATCHİNG VE DEFRAGMANTASYONUN IVF SONUÇLARINA ETKİSİ

ÖZET

Objektif: *İyi kalitede transfer edilebilir embryosu olmayan hastalarda 3. günde assisted hatching işlemine ek olarak uygulanan defragmantasyonun implantasyon ve gebelik oranlarına etkisi araştırılmıştır.*

Gereç ve yöntemler: *İyi kalitede embryosu olmayan (Veeck klasifikasyonu grade >1 ve/veya >%10 fragmantasyon) tüm yaşlardan hastalar (n=168) retrospektif olarak incelendi. Birinci grupta transfer edilen tüm embryolar grade 2 olup, mekanik assiated hatching (mAHA) uygulanmıştı. İkinci gruba ise asit Tyrodes aAHA ve defragmantasyon yapılmış ileri derecede fragmantasyonu olan (>%10-50 fragmantasyon) grade 3 embryo transfeleri dahil edildi.*

Sonuçlar: *Birinci grupta pozitif βhCG %33, klinik gebelik (fetal kalp atımı olan) oranı %27 ve implantasyon oranı %11,28 idi. Bu oranlar aAHA ve defragmentasyon grubunda sırası ile. %37, 1, % 28, 8 ve %16,19 idi. 35 yaş üstü hastalarda aAHA ve defragmentasyon grubunda kabul edilebilir implantasyon (%14,46) ve klinik gebelik (%31,58)*

Address for Correspondence: Ahmet Zeki Işık. Özel İrenbe Tüp Bebek Merkez, Talatpaşa Bulvarı 1436 sok. no. 6, 35227 Alsancak, İzmir, Turkiye
Phone: + 90 (532) 411 81 84
e-mail: azeki@irenbe.com
Received: 26 November 2008, revised: 14 March 2009, kabul tarihi: 01 May 2013, online yayın tarihi: 02 May 2013

DOI ID:10.5505/tjod.2013.52297

oranları elde edilmişti. Transfer edilecek iyi kalitede embryosu olmayan hastalarda defragmentasyon işlemi kabul edilebilir başarı oranları nedeni ile tecrübeli laboratuar ekibi olan merkezlerde AHA işlemine ilave olarak uygulanabilir. Aksi takdirde sadece AHA daha uygun bir tercihtir.

Anahtar kelimeler: *assisted hatching, defragmantasyon, embryo fragmantasyonu, embryo kalitesi, ICSI*

Türk Jinekoloji ve Obstetrik Derneği Dergisi, (J Turk Soc Obstet Gynecol), *2013; Cilt: 10, Sayı: 3, Sayfa: 138- 42*

INTRODUCTION

In assisted reproductive technologies, the success depends on embryo quality. Most of the protocols to evaluating embryo grading are based on embryo fragmentation, blastomer size and apperance. More than 10% fragmentation in embriyonic volume is associated with impairment in embryo development(1). The quote that embryo fragmentation leads embrionic development discontinuation remains unclear. The outcome of embryos with fragmentation and had no discontinuation in development is still uncertain.

The studies carried out up today indicates that embryo development disorders associated with increased fragmentation correspondingly decrease implantation rates(2-4). It has been stated that not only the grade of fragmentation but also the pattern of fragmentation is an important parameter for implantation(5).

It is suggested that removing the fragmentations from a developing embryo, is an effective method which eliminates the destructive effects(5). When the process of defragmentation is applied to human embryos with fragmentation, positive effects on embryo development and good quality blastocyst development has been observed(6). Furthermore, the clinical results after transfer of defragmentated embryos and high quality unfragmented embryos were found to be similar(7).

The goal of this study is to evaluate the pregnancy outcome of defragmentation in addition to assisted hatching in patients who has no grade 1 embryos for transfer.

MATERIALS AND METHODS

The study group consisted of 168 intracytoplasmic sperm injection (ICSI) cycles performed in a private IVF clinic, between January 2003 and September 2006. The cycles which outcomes are known and resulted with embryo transfer were included in this study. All cycles were fresh ICSI cycles and patient's own gametes were used in the procedures. Donor cycles and freeze thaw cycles were excluded. In the third day of oocyte pick up procedure, the patients who had grade >1 and/or > 10% fragmented embryos (n=168) were analyzed retrospectively. All age groups were included in the study.

Gonadotrophin releasing anologue (GnRH)(Lucrin, Abbott, Switzerland) has been used in controlled hyperstimulation (COH) cycles as long or microdose flare up protocol. Long protocol were chosen in normoresponders and microdose flare up protocol were chosen in patients in whom ovarian reserve were diminished. In mAHA group 42 (66%) patient received long protocol, 21 patients received microdose flare up protocol; in aAHA and defrag. group 69 (65.7%) patients received long protocol, 36 patients received microdose flare up protocol. In mAHA group 53 (51%) patients were in their first cycle and in aAHA and defrag. group 53 (51%) patients were in their first COH cycles. After pituiter down regulation, rFSH(Puregon, Organon, Netherlands) was started and adjusted according to patients own ovarian response (150-300 IU/day). In microdose flare up protocol after 21 day oral contraseptive use, on the third following day 40 micrograms Lucrin were applied twice a day. Afterwards on the third day of this procedure 300-450 IU/day gonadotrophins were started. Ovulation was triggered by an injection of 10000 IU hCG (Pregnyl, Organon, Netherlands) when at least 3 follicules reached 18 mm in diameter. Oocyte pick up procedure was performed after 35 hours of hCG trigger.

Assesment of embryo culture and fragmentation:

Intracytoptasmic sperm injection (ICSI) was performed to all methaphase II oocytes after hyaluronydase application following oocyte pick up procedure. Narishage micromanipulator, Olympus IX inverted microscope was used in ICSI procedure. Fertilised oocytes were cultured in 25 µl micro gut medium and analysed seperately.

All embryos were assessed and graded according to

Veeck classification[8]. The evaluation of fragmentation was performed by the chief embriologist of the laboratory. Mechanical assisted hatching (mAHA) were performed to all grade 2 embryos by partial zona dissection pipette (Conseption Technologies, San Diego). The grade 3 embryos with excessive fragmentation (>10-50 %) underwent assisted hatching by acid Tyrode's solution (Medicult, Denmark) with AHA pipette (Research Instruments, United Kingdom). Afterwards total or partial fragment removal was performed without damaging blastomeres following the suction of acidified solution from the environment (time limited to 3 minutes maximum). After the defragmentation of embryos all were incubated 2 hours additionally before the transfer. All of these procedure were performed by chief of the laboratory. All of the embryo transfers were performed on the 3rd day of oocyte pick procedure.

ICSI cycles were analysed into two groups. In first group, fragmentation was < 10 % and only AHA was performed. In the second group embryos had > 10% fragmentation and underwent AHA and defragmentation. Two groups were compared for women age, number of oocytes, number of metaphase II oocytes, number of transferred embryos, positive ßhCG, implantation and clinical pregnancy rates.

RESULTS

Two thousand six hundred and fourteen oocytes were obtained in 168 oocyte pick up procedures. There were 2075 metaphase II oocytes and 1356 were fertilised. Totally 547 embryos were transferred.

There were 63 patients in group 1 and 105 patients in group 2. Mean age of women, mean number of oocytes, metaphase II oocytes and fertilised oocytes were similar among groups (Table I). In group 2 mean number of transferred embryos were significantly higher than group 1 (3.39 vs 3.09 respectively, p=0.037).

In group 1 positive ßhCG was 33%, the rate of clinical pregnancy (fetal heart beat in ultrasound examination) was 27% and implantation rate was 11.28%. These results were 37.1%, 28.8% and 16.19% in AHA and defragmentation group, respectively. No significant difference was found in these parameters among groups (p>0.05)(Table II).

Table I: *Patient charecteristics of patients in both groups.*

	mAHA n=63	aAHA+defrag. n=105	P
Age (years)	31,79 ± 5,23	31,74 ± 4,84	NS
Number of oocytes	15,12 ± 0,35	15,81 ± 6,49	NS
Number of MII oocytes	12,55 ± 9,71	12,23 ± 5,21	NS
Number of 2PN	7,15 ± 4,91	8,62 ± 4,24	NS
Number transferred embryos	3,09 ±1,07	3,39 ± 0,78	0.037

All values are mean±standart deviation.

Table II: *Cycle outcomes of two groups.*

	mAHA N=63	aAHA+defrag N=105	P
Pregnancy	33,3%	37,1%	NS
N=	(21)	(39)	
Implantation rate	11,28%	16,19%	NS
(n=sac+)	(22)	(57)	
Clinical pregnancy	27%	28,8%	NS
N=	(17)	(30)	
Live birth			NS
Single	(9/12) 75%	(6/12) 50%	
Twins	(4/5) 80%	(10/11) 90,9%	
Triplets		(1/6) 16,6	

Both groups were reevaluated for women age. Pregnancy, clinical pregnancy, implantation rate and live birth rate were depicted on Table III. Pregnancy, clinical pregnancy and implantation rates were similar among groups in patients who were under age 35 (p>0.005). In first group there were 19 patients who were >35 years of age, and mean age in this group was 39.57±2.81 and mean transferred embryos was 2.89±1.14. In the group II there were 38 patients >35 years of age, mean age in this group was 39.45±3.05 and mean number of transferred embryos was 2.23±1.52. However there was no statistically significance in patients >35 years in mAHA group (n=19).The implantation (6.25 %) and clinical pregnancy (21.04%) rates were slightly lower than the other group. Nevertheless in aAHA and defragmentation group, among> 35 years patients(n=38) there were reasonable implantation rates (14.46), and clinical pregnancy rates (31.58%). In group I > 35 years patients, there was no live births and in group II of same age two patients had live births. A statistical analysis could not be performed due to inadequate number of patients.

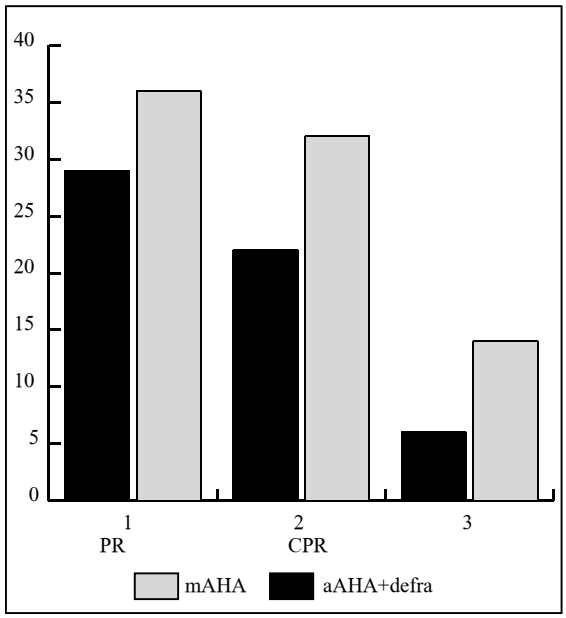
Table III: *The outcomes of patients according to age among groups.*

	PR (%)		CRP (%)		IR (%)	
	Group I	Group II	Group I	Group II	Group I	Group II
Age ≤ 35	35,68	37,97	29,2	29,1	13,10	16,72
Age > 35	28,57	36,36	21,04	31,58	6,25	14,46

PR: *pregnancy rate,* ***CPR:*** *clinical pregnancy rate,* ***IR:*** *implantation rate.*

Figure 1: *Cycle results in patients over the age of 35.*

mAHAn:19 aAHA + defrag. n:38.



PR: *pregnancy rate (+ beta HCG);* ***CPR:*** *Clinical pregnancy rate,* ***IR:*** *Implantation rate.*

DISCUSSION

This trial was planned to find out the effects of fragmentation that deteriorates embrionic morphology on ICSI cycles' outcome. According to the literature embryo development is impaired by the increase in fragmentation[3,9,10]. In in vitro trials embryo development is effected negatively by fragmentation. Fragmentation rate and pattern is also effective on blastocyst development. In addition it is notified that the embryos which have widespread fragmentation have a lower possibility to develop blastocysts[6,10].

There is very few information about defragmentation procedure and whether it is efficient in impaired embryo development or not. In recent years a trial mentioned that defragmentation was encouraging in enhancement of fragmented embryo. The authors declared an increase in blastocyst formation also they observed an improvement in blastocyst grade[6]. Furthermore, Eftekhari -Yazdi et al stated that defragmentation process decreases number of dead and apoptotic cells [6].

The positive effects of defragmentation process was emphasized previously. The low grade embryos which underwent defragmentation process have similar implantation and live birth rates like grade I embryos [3,11]. In an other study[7] there was a similarity in implantation rates between mild fragmanted embryos which underwent assisted hatching and defragmentation when compared to grade I embryos which only underwent assisted hatching. In our study the patients who has no good quality embryos for transfer (embryo quality >grade 1 and/or >10% fragmentation) there was no significant difference between AHA and defragmentation and only AHA groups in implantation and pregnancy rates.

There was a decrease in implantation (6.25%) and clinical pregnancy (21.4%) in cases over 35 years in mAHA group. Nevertheless in cases over 35 years in group with aAHA and defragmentation a reasonable implantation (14.46%) and clinical pregnancy (31.58%) was achieved. This difference might be related to the high number of embryos transfered in group II however it should be keep in mind that these patients had significantly poor quality embryos.

Assisted hatching procedure is not a routine process recommended in general IVF population. On the other hand it is preferable in patients who has got >2 unsuccessful IVF treatment, low quality embryos and in older ages (≥38years)[12]. In patients who has no good embryos for transfer, defragmentation process could be carried out by experienced embryologists. By the way, reasonable pregnancy outcomes could be achieved with poor quality embryos. Nonetheless mAHA is more preferable in case of unexperienced laboratory staff to avoid embryo damage.

REFERENCES

1. Alikani M, Van Blerkom J. Temporal and spatial aspects of fragmentation in early human embryos: possible effects on developmental competence and association with differentail elimination of regulatory proteins from polarized domains. Human Reproduction 1999; 14: 429- 47.
2. Staessen C, Jansssenwillen C, Van Den Abbeel E, Devroey P, Van Steirteghem AC. Avoidence of triplet pregnanacies

by elective transfer of two good quality embryos. Hum Reprod 1993; 8: 1650- 3.

3. Giorgetti C, Terriou P, Auquier P, Hans E, Spach JL, Salzmann J, et al., Embryo score to predict implantation after in vitro fertilization: based on 957 single embryo transfers. Hum Reprod 1995; 10: 2427- 31.
4. Ziebe S, Petersen K, Lindenberg S, Andersen AG, Gabrielsen A, Andersen AN. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in vitro fertilization. Hum Reprod 1997: 12: 1545- 9.
5. Alikani M, Cohen J, Tomkin G, Garrisi J, Mack C, Scott RT. Human embryo fragmentation in vitro and its implications for pregnancy and implantation. Feril Steril 1999; 71: 836- 42.
6. Eftekhari-Yazdi P, Valojerdi MR, Ashtiani SK, Eslaminejad MB, Karimian L. Effect of fragment removal on blastocyst formation and quality of human embryos. RBM online 2006; 13(6): 823- 32.
7. Keltz MD, Skorupski JC, Bradley K, Stein D. Predictors of embryo fragmentation and outcome after fragment removal in in vitro fertilization Fertil Steril 2006; 86: 321- 4.
8. Veeck LL. An Atlas of Human Gametes and Conceptuses: An Illustrated Reference for Assisted Reproductive Technology. 1999 Parthenon Publishing, New York.
9. Alikani M, Calderon G, Tomkin G et al. Cleavage anomalies in early human embryos and survival after prolonged culture in vitro. Human Reprod 2000 15; 2634- 43.
10. Hardy K, Stark J, Winston RML. Maintenance of inner cell mass in human blastocysts from fragmented embryos. Biol of Reprod 2003 68; 1165- 9.
11. Jurisicova A, Varmuza S, Casper RF. Programmed cell death and human fragmentation. Mol Hum Reprod 1996; 2: 293- 8.
12. The Practice Committee of the Society for Assisted Reproductive Technology and The Practice Committee of American Society for Reproductive Medicine. The role of assisted hatching in in vitro fertilization. A review of the literature. A Committee opinion. Fertil Steril 2008; 90: S196- 8.