Clinical Value of Assisted Hatching in IVF/ICSI Programme

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Abstract

The failure of embryonic zona pellucida to hatch following blastocyst expansion has been considered as a contributing factor in implantation failure. Assisted hatching, a commonly performed micromanupulation of zona pellucida, aims to improve implantation and pregnancy rates in assisted reproduction by helping natural hatching process. The techniques used for assisted hatching, the indications of the procedure, the association of the procedure with monozygotic twinning, and the use of this method in combination with blastocyst transfer is clearly discussed in this review. The outcome of IVF with the use of this method in fresh and frozen thawed cycles is clarified. In our opinion, further research with prospective randomized studies is needed for this technique to be used in assisted reproduction.

Keywords: assisted hatching, implantation, micromanipulation, zona pellucida

Özet

"Assisted Hatching" Tekniğinin IVF/ICSI Programındaki Klinik Değeri

Embriyonik zona pellusidanın blastokistin büyümesi döneminde çatlayamamasının implantasyon esnasında başarısızlığa neden olduğu düşünülmektedir. Sık yapılan bir zona pellusida mikromanipülasyonu olan "assisted hatching" (yardımla çatlatma) yardımla üreme teknikleri uygulamalarında implantasyona ve doğal çatlamaya yardımcı olarak gebelik oranlarının artmasını sağlamaktadır. "Assisted hatching" için kullanılan teknikler, endikasyonlar, prosedürün monozigotik ikizler ile ilişkisi ve bu metodun blastokist transferi ile birlikte kombine kullanımı bu makalede geniş olarak tartışılmıştır. Bu prosedür ile ilgili ikna edici yayımlanmış birçok veri bulunmaktadır. Bu tekniğin yardımcı üreme teknikleri arasında kullanılabilmesi için prospektif randomize çalışmalara ihtiyaç vardır.

Anahtar sözcükler: "assisted hatching", implantasyon, mikromanipülasyon, zona pellusida

Introduction

The implantation of the embryo into the uterus requires hatching from its zona pellucida. The inability of the zygote to break its zona pellucida is considered as a factor for implantation failure (1-2). Assisted hatching (AH) is performed to make it easier for natural hatching to occur, also providing early embryo-endometrium contact, which favours the embryos implantation into the uterus (3). In 1989 Cohen et al. (4) first observed a higher implantation per embryo after partial zona dissection. Malter and Cohen (5) demonstrated that hatching in vitro occurs one day earlier after performing AH in 2-cell mouse embryos. Today AH is a commonly performed micromanupulation. The argument in this paper is to clarify the impact of AH concerning the studies performed on different patient populations and methods. The AH considering the indications, which benefit from this procedure, will be clearly discussed in this review.

Zona pellucida

Zona pellucida is a glycoprotein matrix, which surrounds the oocyte. It is synthesied and secreted by the growing oocyte. There are two major roles of it during fertilization. First it binds the sperm and initiates the acrosomal reaction after the sperm is bound (6-7). Secondly, it allows only one sperm to fuse with the egg. The exocytosis of the content of cortical granules of the ooctye alters the zona pellucida glycoproteins, called zona reaction, then zona pellucida becomes a physical barrier to block polyspermy (8-9). The biochemical changes occuring in the zona pellucida during fertilization are not completely understood.

Besides, zona pellucida protects the integrity of the embryo during early development and oviductal transport. When the embryo reaches the uterus, in order to implant it has to escape from its zona pellucida, a process called hatching. Through cycles of contractions and expansions at blastocyst

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stage, the thickness of zona pellucida decreases (10). The expansion of the blastocyst, thinning of the zona pellucida and dissolving of it by a proteolytic enzyme is prerequisite for natural hatching process.

Assisted Hatching Techniques

The failure of embryonic zona pellucida to hatch following blastocyst expansion has been considered as a contributing factor in implantation failure. In order to assist natural hatching process, different methods have been developed to create an opening in the zona pellucida. Openings have been created by acid tyrode solution that chemically digests zona pellucida called zona drilling (11-15). Partial zona dissection (PZD) is the mechnical technique in which zona pellucida is cut with a glass microneedle (16-23). Laser beam is also used for drilling (24-26). Also, AH have been performed using piezo-micromanipulation (27) and enzymatic (28) methods. In zona thinning, zona pellucida is made thinner in a certain area without creating a hole or slit. The mentioned different methods of AH all have advantages and disadvantages. When using mechanical hatching of zona pellucida the difficulty is about creating a hole of consistent size, and when acid Tyrode solution is used the embryotoxicity is of concern (29). Assisted hatching using diode laser is easy to handle and time saving (30).

The study conducted to evaluate the efficacy of laser and chemical-assisted hatching in terms of implantation, delivery and pregnancy rates concluded that laser-assisted hatching of embryos is more effective than the chemical method in enhancing the implantation and pregnancy rates in women aged ≥38 years (31). The comparison of four different techniques of assisted hatching (partial zona dissection, acid tyrode, diode laser, pronase thinning of zona pellucida) performed prior to day 3 embryo transfer showed that the mean number of embryos transferred, implantation rate, clinical pregnancy rate and abortion rates are likewise similar in four techniques (29). There is not a consensus in the literature about the tecnique to be preferred for AH. Besides, these two mentioned studies comparing the methods used for zona micromanipulation are not satisfactory to make a conclusion about the beneficial effect of one technique over another one. As the skill and experience of the operator performing the micromanupulation is also important for the method chosen for AH, it is better to perform the method in which the operator is most experienced.

The most commonly performed methods for AH are acid Tyrode solution or laser to create a hole of ~20-40mm perforating the entire zona pellucida. Zona-drilling by acid Tyrode solution produces a round hole. Mechanical technique, PZD, produces a slit opening. Although, conventional PZD with a slit opening of 30-40 mm has been proven beneficial (32), the size and the opening created by PZD has been a concern. The slit opening in the zona pellucida might cause entrapment of the embryo that leads to embryo degeneration or by subsequent splitting to monozygotic twinning (MZT). These problems are avoided by a more extensive ablation (~70mm) (33). Zona thinning has a beneficial effect for hatching and implantation (34-36). Among different IVF laboratories, zona-thinning dimensions varies from 20 to 80mm. Mantoudis et al (37) reported dissappointing results by comparing a single laser-created hole for assisted hatching with partial zona pellucida thinning. The reported pregnancy rates in total laser AH group was 14.6% per transfer as compared with 20.9% in the partial laser AH and 29.0% in the quarter laser AH group (37). Laser breaching of one-quarter of zona pellucida seems to be optimal method of AH in fresh embryo transfer (37-38).

A noncontact, diode laser was reported as useful to create a hole in the zona pellucida to faciliate ICSI and for AH (39). Laser assisted ICSI using a 5-10 µm opening in the zona pellucida for injection faciliates penetration of all anatomical structures (40). However lack of identification of the laser-generated hole at later development stages and need for an additional opening might impair hatching process in these embryos (41). In order to prevent this, Moser at al (42) developed laser-assisted zona pellucida thinning prior to routine ICSI. Using diode laser thinning of zona pellucida to ~50% of its original thickness in sibling oocytes and injection through this area, resulted in increased pregnancy and hatching rate, in day 3 transfers (42). Laser assisted ICSI and laser assisted zona pellucida thinning are new techniques and the ICSI outcome and hatching rates are futher to be evaluated by the use of these methods.

Indications of Assisted Hatching

Advanced maternal age and repeated implantation failures

The two of the reported indications of assisted hatching are age factor (≥38 years), and multiple failed IVF attemps (4,21). In patients with advanced maternal age, the success of assisted reproduction (ART) is decreased. This is associated with the endometrial and oocyte related factors (43). The altered hormonal status decreased uterine blood flow and insufficient endometrial proliferation lowers endometrial receptivity causing implantation failure. The ageing of the oocytes and increased chromosomal abnormalities are other contributing factors to low rates of implantation and pregnancy achieved by ART in these patients. The transfer of chromosomally normal embryos, detected by preimplantation genetic diagnosis (PGD) using fluorescence in-situ hybridization (FISH), yields to higher implantation and pregnancy rates (44) which shows that the functional and structural decline of the oocyte in advanced maternal age is more profound than endometrial receptivity in age related decrease in fertility. The study comparing AH with preimplantation genetic diagnosis showed that the implantation rates are similar in these procedures (45). The PGD for aneuploidy screening in patients with maternal age ≥36 resulted in lower number of embryos replaced with increased implantation rates but clinical pregnancy rate was similar with controls, which underwent assisted zona hatching (45).

Advanced age of women was reported to be associated with thick zona pellucida (46). As this changes in zona pellucida associated with advanced age might be contributing to implantation failure, the procedure of assisted hatching was suggested as a treatment option in the management of implantation failure. In women >40 years, with elevated FSH levels, and previous failures of IVF, assisted hatching revealed an implantation rate of 64% compared with 19% in no-AH group (47). In another study, improved implantation and pregnancy rates were observed by AH in women over >40 years of age (48). Tucker et al. (49) reported significantly higher rates of clinical pregnancy in patients >35 years (45%) compared with controls (16%). The AH using a piezo-micromanipulator improved the pregnancy and implantation rates in poor prognosis patients with good quality embryos but had no effect in patients with low quality embryo transfer (27). The results of other reported studies support the beneficial effect of assisted hatching in women of advanced maternal age (22,50-52) and repeated IVF failure (22,36,52-53).

On the contrary, De Vos et al (2) mentioned that no evidence exists showing the beneficial effect of AH in advanced age group. It was suggested that AH might have no significant impact on IVF success rates in advanced aged women as no differences in the rates of implantation and clinical pregnancy between hatched and nonhatched embryos from women over ≥ 36 years undergoing IVF were observed (54). Besides, it was also demonstrated that delivery rates are not increased in women aged >38 years when the embryos are manipulated by chemical assisted hatching (55). In a recent study with women who underwent IVF after at least three failed IVF-ET attemps, no difference on pregnancy rate was noted when patients receiving nonhatched embryos and embryos, which underwent PZD, were compared (56). The data obtained from a prospective randomized study indicates that AH does not lead to a higher implantation rate in older patients, but when AH is performed on fresh embryos after repeated implantation failure, the implantation and clinical pregnancy rates are both improved although not significantly (57).

Advanced maternal age itself is the major negative influencing factor of ART outcome and the role of AH to improve the success rates in these patients is yet to be evaluated. The data in the literature is not convincing that all women of advanced age will benefit from this procedure. The age related decline in fertility cannot be overcomed by AH alone. The combined use of alternative methods, like PGD with AH, might improve the outcome in these patients as mentioned by Demirel et al. (58) previously.

A recent meta analysis of randomized controlled studies showed that AH increases the pregnancy (OR 2.51), implantation (OR 2.38) and ongoing pregnancy (OR 2.65) rates significantly in poor prognosis patients undergoing ART (59). The data from the meta analysis showed that for patients with repeated implantation failure the OR were 2.84 for pregnancy, 2.53 for implantation and 3.51 for ongoing pregnancy rates, in favor of AH (59). The impact of AH on live birth rates and outcomes of assisted conception was evaluted in a systematic review of randomized controlled trials (60). The reported analysis showed that AH does not significantly improve the take-home baby rate of assisted conception but in patients with repeated IVF failure, and probably in older women, clinical pregnancy rates are improved (60). The results of some of the studies performed to evaluate the impact of AH is shown in Table 1.

However, in good prognosis patients AH yielded lower pregnancy rates than in controls (23% and 43%, respectively) (61). No difference in implantation and pregnancy rates were observed after AH by laser in patients of <37 years and no previous IVF failure (62). In the study by Mansour et al. (63) patient population of women under the age of 40 years undergoing their first ICSI attempt and women over 40 years with at least two-failed IVF/ICSI attemps (poor prognosis) were randomized to receive zona-free and zona-intact embryos. Acid Tyrode solution was used to remove zona pellucida before embryo transfer on day 3 and zona removal resulted in significantly higher rate of pregnancy when compared with the controls in poor prognosis patients. However, zona removal did not improve pregnancy rates in the women under the age of 40 years undergoing their first ICSI attempt group (63). It was mentioned that in young group of patients (<34 years) with repeated implantation failure AH is harmful as it causes significantly decreasing pregnancy rates and should be avoided (56). From the data presented we conclude that in young patients and patients without a history of previous failures of ART, AH is of no benefit.

Thick or Hardened Zona Pellucida And Elevated FSH Levels

The two major causes of impeded hatching are thick or hardened zona pellucida (2,14). The cross-linking of the glycoproteins present in the zona pellucida causes hardening that results in reduced hatching rates (20). Although the hardening of zona pellucida is difficult to assess, the thickness of it can be measured under an inverted microscope. Zona thickness is influenced by age of the women, FSH levels, and cause of infertility and thick zona is correlated with lower implantation rates (64). In addition, the zona pellucida becomes thicker and harder when the embryos are cultivated externally in an artificial environment (21,65).

The zona pellucida thicker than 15 mm was observed in 15% of IVF embryos (21). The selective application of AH to embryos with zona pellucida thicker than 15 µm resulted in better implantation rates when compared with no AH controls (4). On the contrary, the implantation rate of the embryos was reduced when zona drilling with acid Tyrode solution was performed on embryos whose zona pellucida was <13 µm thick. Ebner et al. (66) investigated whether a special subgroup of patients with oocytes where penetration of the oolemma is difficult during ICSI may benefit from AH. The authors prospectively randomized the mentioned patients into AH with diode laser and non-hatching group and they found significantly higher rates of implantation and pregnancy in AH group (66).

Table 1. The results of some of the studies performed to evaluate the impact of AH							
	Patients selection n= cycles	**AH**	**ET**	**Mean age**	**Mean no embryos transferred**	**PR (%)**	**IR (%)**
Chao et al. 1997(23) -AH group -No AH group	≥2 IVF failure -n=31• -n=33•	PZD, Day 2	Day 2	-36.5 -34.0	-4.7 -4.3	-42.4* -16.1	-11* -3.7
Bider et al. 1997 (55) -AH group -No AH group	>38 years -n=312 -n=274	Acid tyrode, Day 3	Day 3	-41.9 -41.1	-3.48 -2.58	-8.9** -5.1	-3.7** -3.5
Magli et al. 1998 (52) *≥38 years* -AH group -No AH group *≥3 IVF failure* - AH group -No AH group ≥3 IVF failure+ ≥38 years -AH group -No AH group	≥3 IVF failure, ≥38 years -n=45 -n=42 -n=70 -n=53 -n=20 -n=18	Acid tyrode, Day 3	Day 3	-40.5 -39.5 -32.7 -32.2 -39.6 -40.5	-3.7 -3.8 -3.8 -3.6 -3.7 -3.7	-31.0* -10.0 -36.0* -17.0 -30.0** -6.0	-11.5* -4.0 -15.0* -6.3 -11.0** -1.5
Lanzendorf et al. 1998 (54) -AH group -No AH group	≥36 years -n=42• -n=52•	Acid tyrode, Day 2	Day 3	-38.3 -38.5	-4.4 -4.4	-39.0** -41.7	-11.1** -11.3
Nakayama et al. 1999 (27) *AH group* -good quality embryos -low quality embryos *No AH group* -good quality embryos -low quality embryos	≥2 IVF failure -n=67 -n=59 -n=68 -n=54	Piezo Manipulator Day 2-3	Day 2-3	-35.4 -37.3 -35.9 -37.5	-2.8 -2.1 -2.8 -2.2	-22.4* -3.4 -7.4** -3.7	-10.1* -1.6 -2.6** -1.7
Mansour et al. 2000 (62) <40 years, 1st ICSI -zona removed -zona intact *≥2 IVF failure±≥40 years* -zona removed -zona intact	good and poor prognosis -n=27 -n=25 -n=30 -n=41	Acid tyrode, complete zonaremoval Day 3	Day 3	-32.1 -33.2 -37.3 -36.3	-3.2 -3.0 -3.9 -3.8	-44.0** -40.0 -23.0* -7.3	Not mentioned
Graham et al. 2000 (71) *Day 3* -AH group -No AH group *Day 5* -AH group -No AH group	-n=20♣ -n=15♣ -n=16♣ -n=35♣	Acid tyrode, Day 3	Day 3 or 5	-36.4 -32.7 -35.2 -31.5	-3.4 -2.9 -2.9 -2.3	-10♦** -10♦ -14♦** -19♦	-22** -30 -41** -36
Vanderzwalmen et al. 2003 (76) -AH group -No AH group	Vitrified warmed blastocysts -n=39 -n=54	PZD, post warming	Day 1 After warming	Ranged 27-41 years	-2.0 -2.0	-38.0* -19.0	-22.0* -13.0
Gabrielsen et al. 2004 (75)	Cryopreserved Thawed embryos -n=165 -n=140	Acid tyrode, post thawing	Day 1 after thawing	-33.1 -32.8	-1.94 -1.91	-17.6** -11.1	-11.4* -5.8
•no of patients, ♣no of transfers, ♦no of pregnancies *statistically significant **not significant							

The thickening of zona pellucida is also correlated with basal FSH and preovulatory oestradiol levels (67). Women with elevated basal FSH concentrations benefit from AH (4). On the other hand, no significant differences were observed in the rates of pregnancy and implantation between the 137 women with normal FSH concentrations randomized to have either hatched or nonhatched embryos (4). Other studies supports the positive effect of AH in this group of patients (21-22,47).

Assisted Hatching and Blastocyst Transfer

The transfer of selected blastocysts in ART programme results in favoured rates of pregnancy and is beneficial in abstaining the risk for multiple pregnancies (68). However, in large population studies, the implantation rate of good quality blastocysts is rarely 50% (68-70). In vitro embryo development revealed that 20% of human blastocysts have hatching problems (71) and AH affects blastocyst hatching (5, 34). In women of 40-43 years blastocyst transfer was compared with day 3 transfer with assisted hatching and the viable pregnancy rate was similar in both groups (29.2% and 26.3%, respectively) (72). These authors suggested that blastocyst transfer appears to be effective as day 3 embryo transfer with assisted hatching in older patients with good embryos (72). To enhance implantation, AH on embryos at clevage stage and then culturing to blastocyst stage has been performed and improved implantation and pregnancy rates were observed after transfer of zona pellucida manipulated blastocysts (73). In patients with two or more cycles of IVF failure, pronase solution used for enzymatic assisted hatching on blastocysts yielded a 53% pregnancy and 33% implantation rate for zona free blastocyst transfer (74).

The co-culture of the embryos is another option in the management of poor prognosis patients. However, the mentioned studies above are too few to allow for a meaningful result for the use of blastocyst culture with AH.

Assisted Hatching and Cryopreservation/Vitrification of Embryos

The pregnancy rates after frozen-thawed embryo cycyles is lower than fresh embryo cycles (75). The cryo-thawing process exacerbates hardening of zona pellucida induced by artificial culturing (21). In the mouse, the decrease in the rate of fertilization was observed to be due to freezing thawing induced zona pellucida changes (76). In the prospective randomized study on cryopreserved-thawed embryos it was found that assisted hatching using acidified Tyrode's solution improves implantation rates (11.4% in assisted hatching and 5.8% in control group) (77). In another study, assisted hatching using the partial dissection technique on vitrified-thawed blastocysts resulted in an implantation rate of 22%, where this rate was 13% in no-AH blastocysts (78). Two other reports also analysed the effect of AH on human frozen-thawed embryos, one using zona drilling on frozen day 3 embryos (79), the other using mechanical hatching on day 2 embryos (75). Both studies revealed a tendency toward improved outcome, higher rates of clinical pregnancy when compared with the controls, after performing an artificial opening on the zona pellucida. Kung et al (38) reported an acceptable rate of pregnancy (31.4%) from transfer of thawed blastocysts that underwent laser AH along one quarter of the zona pellucida on clevage stage (38). The implantation rates for AH-fresh and AH-thawed blastocysts were 28.9% and 16.9% respectively (38). All the studies performed in frozen thawed cycles, in our knowledge, implies a positive effect of micromanipulation by AH which might improve the impaired outcome in these cycles.

Assisted Hatching and Monozygotic Twinning, Ectopic Pregnancy

The incidence of monozygotic twinning (MZT) is increased with the use of assisted reproduction technologies, with or without zona manupulation (80-85). Although the factors affecting the frequency of MZT is poorly characterized, a significantly increased frequency of MZT was reported with use of ovulation induction drugs (clomiphene citrate and gonadotropins) in assisted reproduction (86-87). Intracytoplasmic sperm injection and AH are both zona breaching procedures and MZT in case of zona manipulation has been studied by different authors (81-83). The manipulation of the zona pellucida is another contributing factor to high frequency of MZT associted with ART (82-83,88-90). There are two hypotheses explaining the higher incidence of MZT following AH. The first explanation was entrapment of the hatching blastocyst leading to subdivision and monozygotic twins (83). Secondly, the development of another identical embryo might be due to premature hatching of blastomeres (91). In a retrospective analysis by Schacter et al (92), the incidence of monozygotic twins was found as 0.86% after IVF-ICSI/AH where the overall rate of MZT was 0.95%. The odds ratio of MZT after AH in ART pregnancies was found as 3.2-3.8 by Schieve et al (88) who proposed that AH posed an increased risk for MZT above the risk that have already been present from treatment with ovulation medications. On contrary, no correlation between zona pellucida manipulation and MZT was found in the study by Sills et al (85) who conducted a study to assess the association of zona pellucida micromanupulation and MZT after intracytoplasmic sperm injection and AH. These authors (85) concluded that at least three factors influence the occurrence of MZT after assisted reproduction. These are namely, ovulation induction, culture conditions, zona architecture/manipulation the exact roles of which are yet to be defined.

The retrospective analysis of the clinical pregnancies conceived after IVF of a center where AH was performed before day 3 embryo transfer on embryos of patients of advanced maternal age, decreased ovarian reserve and repeated previous IVF failures showed that the ectopic pregnancy rate was 5.4% in patients where assisted hatching was performed and 2.2% in cases without assisted hatching (93). Regarding that the patient population in which assisted hatching is indicated has decreased ovarian reserve, these authors suggested that the replacement of embryos which underwent AH might increase the risk for ectopic pregnancy as abnormal embryogenesis may also contribute to ectopic pregnancy.

Conclusion

A successful implantation in vivo requires hatching of the zona pellucida at blastocyst stage. Natural mechanism of hatching is not clear and an artificial hole created in the zona pellucida is believed to favour hatching in vivo and improve implantation. There is not a concensus in the literature and it is difficult to correlate the results of the studies performed to evaluate the impact of AH in IVF/ICSI programme, as these studies are performed on a different patient population and with different AH technique.

The data in the literature presented in this paper shows that advanced maternal age and repeated implantation failure patients, especially when the two both exits, most benefit from AH to overcome zona pellucida barrier. In our opinion, if AH is preferred in the management of poor prognosis patients in an ART unit, than patient selection should be done considering all the special features of the patients, e.g. embryo quality, zona thickness, wishes for cryopreservation, FSH concentrations.

Positive effects of this method depend on several factors and it is only beneficial in selected conditions and patients. As the clinical value of this programme is still controversial, the advantage of this procedure during counselling in ART programme needs prospective randomized studies for different indications. The few prospective randomized studies show that no benefit exists with this procedure in an ART programme. We therefore recommend withholding AH from ART programmes until a true benefit with prospective randomized studies showing increased implantation and pregnancy rates is shown.

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