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A comparison of four different techniques of assisted hatching

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BACKGROUND: Assisted hatching (AH) has been proposed as a means to increase the implantation rate in patients with poor prognosis for pregnancy. The procedure appears to be effective when used selectively. Several different methods for AH have been introduced over the years but comparative studies are lacking. The aim of the current study was to compare retrospectively the efficacy of AH performed with four different methods in patients undergoing IVF or ICSI. METHODS: AH was performed prior to day 3 embryo transfer in 794 IVF/ICSI cycles. Indications for AH were females aged >35 years and/or elevated follicular phase FSH levels, previous failed IVF/ICSI cycles, poor embryo quality, and thick zona pellucida (>15 μm). Assignment to one of the four methods of AH was according to the availability of the particular method during the study period. The study was not randomized. RESULTS: Partial zona dissection was used in 239, acid Tyrode in 191, diode laser in 219 and pronase thinning of the zona pellucida in 145. Mean female age, mean number of previous failed IVF/ICSI cycles, number of oocytes retrieved, fertilization and cleavage rates, good quality embryos and zona thickness on day 3 did not differ between groups. Mean number of embryos transferred, implantation rate, clinical pregnancy rate, and abortion rates were likewise similar. CONCLUSIONS: Selective AH using four different methods yields similar implantation and pregnancy rates.

Key words: assisted hatching/implantation/IVF

Introduction

The success of assisted reproduction depends upon the intricate relationship between the transferred embryo and the endometrium. Failure of the embryo to implant may be due to several factors most of which have not been delineated. In-vivo hatching of the embryo at the blastocyst stage is an essential step in the events culminating in successful implantation. It has been speculated that failure of the embryo to hatch following its fertilization *in vitro* and subsequent transfer may be one of the reasons for the low efficiency of this process.

The loss of uniformity and thinning of the zona pellucida appears to precede in-vivo hatching. It has been shown that implantation rates varied from 10 to 29% for embryos with a uniform and thick versus irregular and thin zona pellucida (Cohen *et al*., 1989). Exposure of the embryo to the in-vitro environment may result in hardening of the zona that is resistant to thinning and subsequently impair the hatching process. Although it is difficult to assess and quantify the hardness of the zona, its thickness can be measured under an inverted microscope. It was reported that 15% of IVF embryos displayed a zona pellucida thicker than 15 μm (Cohen *et al*., 1990). The same group, in a prospective randomized study, showed improved implantation rates when zona drilling with acidified Tyrode's medium was applied selectively to poor prognosis embryos (Cohen *et al*., 1992). The method has been defined as assisted hatching (AH). Many studies have been performed since then with variable results (Tucker *et al*., 1993; Antinori *et al*., 1996; Hellebaut *et al*., 1996; Mandelbaum, 1996; Chao *et al*., 1997; Fong *et al*., 1998; Hurst *et al*., 1998; Lanzendorf *et al*., 1998; Edirisinghe *et al*., 1999; Gabrielsen *et al*., 2000; Graham *et al*., 2000). It is difficult to correlate the findings of these studies due to different design, patient selection criteria, and technique of AH.

Several techniques for AH have been introduced over the years. Mechanical AH by partial zona dissection was followed by zona thinning with acidified Tyrode's solution or pronase and finally by laser. Each method has its advantages and shortcomings. However, studies comparing the relative effectiveness of each method are lacking. The aim of this study was to evaluate the results of four different methods of AH performed in our institution over a period of 2 years.

Materials and methods

A total of 794 IVF/ICSI cycles where the embryos were transferred on day 3 following AH were studied. Only first cycles of

AH were included. The study was retrospective and the method of AH that was selected was dependent upon the availability of that particular method and the preference of the embryologist. Indications for AH did not change over the study period and were as follows: (i) female age >35 years and/or elevated follicular phase FSH levels (>10 mIU/ml between days 2 and 4); (ii) previous failed implantation; (iii) thick zona pellucida (>15 μm); (iv) the presence of poor cleavage stage embryos (grades 3 and 4).

Chronologically in our clinic, hatching was initially performed by partial zona dissection followed by acid Tyrode, and subsequently by pronase zona thinning and laser. The patients were divided into four groups: group 1: mechanical AH (i.e. partial zona dissection) (n = 239); group 2: acid Tyrode AH (n = 191); group 3: laser AH (n = 219); group 4: pronase zona thinning (n = 145). A control group was selected among couples undergoing IVF or ICSI during the same time period. The control group (group 5) was formed by selecting every fourth couple who underwent IVF or ICSI without AH during the same time period.

Patient selection criteria, ovarian stimulation, oocyte retrieval and embryo transfer

Patients were selected for IVF or ICSI according to standard accepted indications. Ovarian stimulation was undertaken using a long GnRH analogue protocol combined with pure or recombinant FSH. In some cycles, flare protocol was used. Oocyte retrieval was performed ~36 h after the injection of 10 000 IU of HCG. Embryo transfer was performed on day 3 following AH. Wallace or Frydman catheters were used for embryo transfer.

Culture media and laboratory methods

Over the study period, there were no changes in the laboratory procedures and the culture media utilized. Sequential media system (G1 and G2 media from Scandinavian Science AB Products, Gothenburg, Sweden) was used for embryonic culture. Embryos were cultured in G1 medium on days 1 and 2. They were transferred to G2 medium following AH on the morning of embryo transfer. Culture medium was refreshed every day. Cleavage stage embryos were graded as follows: grade 1 embryo: no fragmentation with equal-sized homogeneous blastomeres; grade 2 embryo: <20% fragmentation with equal-sized homogeneous blastomeres; grade 3 embryo: 20–50% fragmentation with unequal-sized blastomeres; grade 4 embryo: >50% fragmentation with unequal-sized blastomeres.

Assisted hatching method

Mechanical assisted hatching (partial zona dissection)

Three-dimensional partial zona dissection was performed as previously described (Cieslak *et al*., 1999). Briefly, the embryo was held in position by gentle suction from the holding pipette and the microneedle was passed through the zona pellucida at the largest perivitelline space and advanced tangentially. The embryo was then released from the holding pipette and held by the microneedle. The microneedle was brought to the bottom of the holding pipette and the embryo was gently rubbed against until a cut was made. Once again the embryo was rotated until the slit was visible at the 12 o'clock position. The embryo was firmly held by the holding pipette and the zona pellucida was cut in a similar manner creating a cross-shaped slit. The embryos were then returned to the culture medium and stored until transfer.

Acid Tyrode assisted hatching

This was performed as previously described (Cohen *et al*., 1990). Embryos were stabilized with a holding pipette held at 9 o'clock position and a 10 μm pipette containing acid Tyrode (ZD-10 Ref 1006; Vitrolife Fertility Systems, Gothenburg, Sweden) solution was oriented at the 3 o'clock position next to an area of empty perivitelline space. A 30 μm diameter defect in the zona was then created by using a mouth-controlled delivery system to blow the acid Tyrode over the external surface of the zona. Embryos were then rinsed several times to wash the excess acid Tyrode and returned to the standard culture media until transfer.

Laser hatching

A 1480 nm diode laser in a computer-controlled non-contact mode was used for laser hatching (IVF Workstation and Zona Laser Treatment System, Hamilton Thorne Instruments, Beverley, MA, USA). The IVF Workstation that uses a compact diode laser is attached to an Olympus IX-70 inverted microscope below the objective turret. The Laser Assisted Hatching software is designed for easy positioning, focus and measurement of embryos and simple alignment of the laser. The laser has three preset energy intensities of low (35 mW), medium (45 mW) and high (55 mW) that can be delivered in a single 25 ms pulse with a single click of the mouse controller. Low power is used for perforating very thin (<10 μm) zona or to minimize exposure, medium power for drilling the zona of most embryos (10–15 μm), and high power for perforating thick (>15 μm) or hard zona pellucida.

Assisted hatching by pronase thinning of the zona pellucida

This was performed as previously described (Fong *et al*., 1998). A more dilute solution of pronase was used (10 IU/ml pronase was diluted 10× by the G2 medium). The embryos were transferred to the pronase solution (Protease, Sigma P-8811; Sigma Aldrich Co Ltd, Irvine, UK) in G2 medium under oil for ~60 s for initial stretching and softening of the zona pellucida. The aim was to thin the zona pellucida without complete removal. The embryos were then quickly examined on the heated stage of an inverted microscope to observe if the zona had expanded in size, was faint and if the perivitelline space had increased in size. If these criteria were not met, the embryos were further incubated with pronase for an additional 30–60 s. The embryos were than transferred to fresh G2 medium and gently washed twice and placed into the incubator until transfer.

Statistics

Results were analysed using one-way analysis of variance and χ^2 and Fisher's exact tests when applicable. $P < 0.05$ was accepted as significant.

Results

Indications for AH in the four groups are shown in Table I. The majority of the patients had more than one indication for AH. Mean female age, number of previous failed treatment cycles, basal FSH levels, thickness of the zona pellucida, number of oocytes retrieved, two-pronuclear fertilization rate, cleavage rate, the incidence of grade 1 and 2 embryos, and the number of embryos transferred, were similar between the four groups (Table II). However, there were significant differences ($P < 0.05$) between the control group and the other groups regarding patient's age, the number of previous IVF/ICSI cycles, mean basal FSH levels, number of oocytes retrieved, and mean zona pellucida thickness. Implantation rate per transferred embryo, clinical pregnancy rate per embryo transfer, multiple pregnancy rate and abortion rates were similar in all groups including the controls.

Table I. Indications for assisted hatching in the study population

	Partial zona dissection	Acid tyrode	Diode laser	Pronase zona thinning
Previous implantation failure	218/239 (91.2)	164/191 (85.8)	184/219 (84.0)	132/145 (91.0)
Thick zona	47/239 (19.6)	39/191 (20.4)	58/219 (26.4)	40/145 (27.5)
Elevated basal FSH level	22/239 (9.2)	14/191 (7.3)	18/219 (8.2)	13/145 (8.9)
Female aged >35 years	110/239 (46.0)	94/191 (49.2)	108/219 (49.3)	60/145 (41.4)
Poor embryo quality	64/239 (26.7)	78/191 (40.8)	79/219 (36.0)	40/145 (27.6)

Table II. Patient characteristics and cycle outcome with different methods of assisted hatching

	Group 1 Partial zona dissection	Group 2 Acid tyrode	Group 3 Diode laser	Group 4 Pronase zona thinning	Group 5 Controls
No. of embryo transfer cycles	239	191	219	145	188
Mean ± SD age (years)[a]	34.4 ± 3.3	34.6 ± 3.2	35.0 ± 3.3	34.5 ± 3.1	29.8 ± 3.0
Mean ± SD no. of previous assisted reproduction cycles[a]	1.9 ± 0.6	1.7 ± 0.5	2.1 ± 0.8	2.1 ± 0.7	0
Mean ± SD basal FSH (mIU/ml)[a]	6.9 ± 1.1	7.1 ± 1.2	7.4 ± 1.3	7.1 ± 1.3	3.9 ± 1.0
No. of oocytes retrieved	2295	1872	2081	1407	2313
(mean ± SD)[a]	(9.6 ± 2.2)	(9.8 ± 2.2)	(9.5 ± 2.1)	(9.7 ± 2.2)	(12.3 ± 3.0)
Oocytes injected	1791	1445	1624	1126	1783
2-Pronuclear fertilization rate (%)	70	69	70	70	69.9
Degeneration rate (%)	3.6	4.0	3.3	3.9	3.1
Cleavage rate (%)	98	98.4	98	98.2	98
No. (%) G1 + G2 embryos	702 (57)	574 (58.3)	653 (58.5)	455 (59.0)	795 (65)
Zona thickness (μm)[a]	14.4 ± 2.0	14.9 ± 2.0	14.8 ± 2.0	14.3 ± 2.1	12.1 ± 1.9
No. of embryos transferred	932	763	876	537	652
(mean ± SD)	(3.8 ± 1.1)	(3.9 ± 1.0)	(4.0 ± 0.9)	(3.7 ± 1.1)	(3.4 ± 0.7)
Implantation/embryo (%)	174/932 (18.6)	133/763 (17.4)	166/876 (18.9)	103/537 (19.1)	142/652 (21.6)
Clinical pregnancy/embryo transfer (%)	118/239 (49.3)	88/191 (46.0)	106/219 (48.4)	68/145 (46.8)	91/188 (48.4)
Multiple pregnancy rate (%)	50.8	45.4	50.0	48.5	45.1
Singletons/twins/triplets	46/60/12	48/35/5	53/46/7	35/31/2	50/31/10
Abortion rate (%)	18.6	20.4	19.8	17.6	16.4

[a]The control group is significantly different from the other groups (analysis of variance with Tukey post-hoc test, $P < 0.05$).

The duration of procedure for partial zona dissection and acid Tyrode zona drilling was ~40 s, whereas AH by diode laser took ~20 s per embryo. The pronase zona thinning procedure lasted ~60 s for the whole batch of available embryos. The embryologists rated laser hatching as technically the easiest procedure.

Discussion

The results of this study show that different methods of AH yield similar outcomes. Implantation and pregnancy rates in the AH groups were similar to a control group with more favourable (younger, lower basal FSH level, increased oocyte yield and thinner zona pellucida of the embryos) characteristics. As the study is not randomized, several biases may have been introduced that may cast doubt on the conclusions. However, there were no changes in the clinical and laboratory protocols during the study period. The same stimulation protocols with pure or recombinant gonadotrophins were used. There was no change in the culture media utilized. Laboratory and clinical personnel were the same. Similar technique and catheters were used for transferring embryos. There was no difference between all four treatment groups regarding patient characteristics such as mean female age, mean number of previous unsuccessful treatment cycles and mean basal FSH levels. Embryo characteristics were also similar such as fertilization, cleavage and degeneration rates, the incidence of grade 1 and 2 embryos, zona thickness and the number of embryos transferred. Therefore, taking into consideration all of the above, we believe it is unlikely that significant bias was introduced.

There is no universal agreement regarding the efficacy of AH for improving the outcome of assisted reproduction. Prospective randomized studies have yielded conflicting results. Variable patient selection criteria and different methodologies employed make interpretation of data more difficult. No randomized trials have been conducted comparing mechanical, chemical, or laser hatching. According to the results of our study, it is unlikely that the method of AH has any major impact on clinical outcome.

Prior to implantation, the blastocyst has to hatch from the zona pellucida. It is believed that creating an artificial hole in the zona pellucida facilitates in-vivo hatching of the blastocyst and may improve the efficacy of implantation. This so-called AH process was reported to benefit certain subgroups of poor prognostis patients or patients with poor embryonic morphology. Initial studies by Cohen *et al*. with partial zona dissection revealed improved implantation rates in patients with elevated FSH levels or embryos with thicker zona pellucida and/or poor morphology (Cohen *et al*., 1992). However, implantation rates were not significantly increased in patients with normal basal FSH levels where AH was performed on all embryos.

In a selected patient group consisting of women >40 years, elevated basal FSH levels and a prior history of multiple IVF failures, Schoolcraft showed a significantly higher implantation rate in the AH group (64%) compared with controls (19%) (Schoolcraft *et al*., 1994). The same group, in a more recent study, showed increased implantation and pregnancy rates by AH in patients aged >40 years (Schoolcraft *et al*., 1995). However, AH yielded no benefit in an unselected good prognosis patient population as demonstrated in one study (Hurst *et al*., 1998), in which the pregnancy rate for the hatching group was 23% compared with 43% for the control group.

Randomized studies, however, have yielded variable results (Hellebaut *et al*., 1996; Chao *et al*., 1997; Hurst *et al*., 1998; Lanzendorf *et al*., 1998; Magli *et al*., 1998; Nakayama *et al*., 1999; Isik *et al*., 2000). Meta-analysis of results is extremely difficult due to different patient selection criteria and different methodologies employed for AH. The American Society of Reproductive Medicine concluded: 'assisted hatching may be clinically useful and individual assisted reproduction programmes should evaluate their own patient populations in order to determine which subgroups may benefit from the procedure. The routine or universal performance of assisted hatching in the treatment of all IVF patients appears, at this point, to be unwarranted' (American Society of Reproductive Medicine, 2000).

Assisted hatching may be of benefit for patients having embryos with thick zona pellucida. Thick zona pellucida and/or decreased variation of the zona pellucida may be associated with advanced female age and poor embryo scores have been shown (Gabrielsen *et al*., 2000). Videocinematographic evaluation of zona pellucida thickness variation (ZPTV) correlated strongly with pregnancy. Mean ZPTV was 28.6 and 17.9 for conception versus nonconception cycles.

It is questionable whether different methods of AH yield similar outcomes. Randomized studies are lacking that compare AH methods with regard to embryo implantation rate or subsequent blastocyst formation and in-vitro hatching. Furthermore, potential complications of different AH methods, such as embryo degeneration and the occurrence of monozygotic twinning, need to be carefully evaluated and compared.

Assisted hatching was initially performed using mechanical means. Mechanical hatching by partial zona dissection has been evaluated thoroughly in several studies, some of which were randomized. The limitation of this technique is the difficulty of creating a hole of consistent size.

A second technique that allows a larger and more consistent hole size is the use of acid Tyrode solution for drilling of the zona pellucida. However, variability and exposure to acid with its possible embryotoxicity remain as potential problems.

Enzymatic methods to dissolve or thin the zona pellucida have been described recently. Pronase solution can be used to circumferentially thin the zona (Fong *et al*., 1997). This method has been used on blastocysts (Fong *et al*., 1998). The authors reported a 53% pregnancy and 33% implantation rate for zona-free blastocyst transfer in women with more than two previous failed IVF attempts. The safety of the method has been recently shown in a study by the same group where ultrastructure of the embryos subjected to pronase solution was examined (Fong *et al*., 2001).

The latest armamentarium added to the list of AH options is the laser. The 1.48 μm infrared diode laser appears to be the most suitable for use in the laboratory. This system allows non-contact, microscope objective-delivered accessibility of laser light to the target with minimal absorption by the culture dish and the medium (Schoper *et al*., 1999). Laser hatching has been proposed as more reproducible, controlled, and technically easier compared with mechanical and chemical means. Antinori *et al*. reported that zona thinning using the Er:YAG laser was associated with a significant increase in the implantation and pregnancy rates in a population of women with repeated implantation failures and also among those undergoing treatment with IVF for the first time (Antinori *et al*., 1996). A recent prospective randomized study found no difference in implantation and pregnancy rates in a population aged <37 years and with no previous implantation failure (Baruffi *et al*., 2000).

Different methods of AH have not been compared in prospective studies. Although the current study is retrospective, consecutive patients with similar characteristics underwent AH with four different methods in a 2 year time period. The method employed was selected according to the availability of that particular method at that particular time. The results of this study indicate that there is no difference between different methods of AH when employed in a poor prognostic patient population. The aim of the study was not to question the efficacy of AH but rather to study whether there are differences regarding the modalities used to affect the procedure. The results of the study indicated that different modalities do not impact on the outcome. This is valuable information as available methods are associated with different potential harmful effects, varying time utilization for the embryology laboratory, additional training period when the modality is switched for another, and different costs. It appears that reasonable pregnancy rates can be obtained with AH in what can be defined as a poor prognostis patient population. The high incidence of multiple pregnancies recorded in this study suggests caution regarding embryo transfer policy. Regarding the efficacy of AH, the current study was not designed to test this endpoint. Only a large scale randomized study should answer the value of AH in poor prognostis patients or in an unselected patient population.

References

Antinori, S., Selman, H., Caffa, B., Panci, C., Dani, G. and Versaci, C. (1996) Zona opening of human embryos using a non-contact UV laser for assisted hatching in patients with poor prognosis of pregnancy. *Hum. Reprod.*, **11**, 2488–2492.

Baruffi, R., Mauri, A., Petersen, C., Ferreira, R., Coelho, J. and Franco, J. (2000) Zona thinning with noncontact diode laser in patients aged <37 years with no previous failure of implantation: a prospective randomized study. *J. Assist. Reprod. Genet.*, **17**, 557–560.

Chao, K., Chen, S., Chen, H., Wu, M., Yang, Y. and Ho, H. (1997) Assisted hatching increases the implantation and pregnancy rate of in vitro fertilization–embryo transfer but not that of IVF-tubal embryo transfer in patients with repeated IVF failures. *Fertil. Steril.*, **67**, 904–908.

Cieslak, J., Ivakhnenko, V., Wolf, G., Shelag, S. and Verlinsky, Y. (1999) Three-dimensional partial zona dissection for preimplantation genetic diagnosis and assisted hatching. *Fertil. Steril.*, **71**, 308–13.

Cohen, J., Inge, K. and Suzmann, M. (1989) Video-cinematography of fresh and cryopreserved embryos: a retrospective analysis of embryonic morphology and implantation. *Fertil. Steril.*, **51**, 820–827.

Cohen, J., Elsner, C., Kort, H., Malter, H., Massey, J., Mayer, M.P. and Weimer, K. (1990) Impairment of the htaching process following IVF in the human and improvement of implantation by assisted hatching using micromanipulation. *Hum. Reprod.*, **5**, 7–13.

Cohen, J., Alikani, M., Trowbridge, J. and Rosenwaks, Z. (1992) Implantation enhancement by selective assisted hatching using zona drilling of human embryos with poor prognosis. *Hum. Reprod.*, **7**, 685–688.

Edirisinghe, W., Ahnonkitpanit, V., Promviengchal, S., Suwajanakorn, S., Pruksananonda, K., Chinpilas, V. and Virutamasen, P. (1999) A study failing to determine significant benefits from assisted hatching: patients selected for advanced age, zonal thickness of embryos and previous failed attempts. *J. Assist. Reprod. Genet.*, **16**, 294–301.

Fong, C., Bongso, A., Ng, S. *et al.* (1997) Ongoing normal pregnancy after transfer of zona free blastocysts: implications for embryo transfer. *Hum. Reprod.*, **12**, 557–560.

Fong, C., Bongso, A., Ng, S., Anan Kumar, J., Trounson, A. and Ratnam, S. (1998) Blastocyst transfer after enzymatic treatment of the zona pellucida: improving in-vitro fertilization and understanding implantation. *Hum. Reprod.*, **13**, 2926–2932.

Fong, C., Bongso, A., Sathananthan, H., Ho, J. and Ng, S. (2001) Ultrastructural observations of enzymatically treated human blastocysts: zona free blastocyst transfer and rescue of blastocysts with hatching difficulties. *Hum. Reprod.*, **16**, 540–546.

Gabrielsen, A., Bhatnager, P., Petersen, K. and Lindenberg, S. (2000) Influence of zona pellucida thickness of human embryos on clinical pregnancy outcome following in vitro fertiliuzation treatment. *J. Assist. Reprod. Genet.*, **17**, 323–328.

Graham, M., Hoeger, K. and Phipps, W. (2000) Initial IVF–ET experience with assisted hatching performed 3 days after retrieval followed by day 5 embryo transfer. *Fertil. Steril.*, **74**, 668–671.

Hellebaut, S., De Sutter, P., Dozortsev, D., Onghena, A., Qian, C. and Dhont, M. (1996) Does assisted hatching improve implantation rates after in vitro fertilization or intracytoplasmic sperm injection in all patients? A prospective randomized study. *J. Assist. Reprod. Genet.*, **13**, 19–22.

Hurst, B., Tucker, M., Awoniyi, A. and Schlaff, W. (1998) Assisted hatching does not enhance IVF success in good prognosis patients. *J. Assist. Reprod. Genet.*, **15**, 62–64.

Isik, A., Vicdan, K., Kaba, A. and Dagli, G. (2000) Comparison of zona manipulated and zona intact blastocyst transfers: a prospective randomized trial. *J. Assist. Reprod. Genet.*, **17**, 135–139.

Lanzendorf, S., Nehchiri, F., Mayer, J., Oehninger, S. and Muasher, S. (1998) A prospective randomized double blind study for the evaluation of assisted hatching in patients with advanced maternal age. *Hum. Reprod.*, **13**, 409–413.

Magli, M., Gianoroli, L., Ferraretti, A., Fortini, D., Aicardi, G. and Montanaro, N. (1998) Rescue of implantation potential in embryos with poor prognosis by assisted zona hatching. *Hum. Reprod.*, **13**, 1331–1335.

Mandelbaum, J. (1996) The effects of assisted hatching on the hatching process and implantation. *Hum. Reprod.*, **11**, 43–50.

Nakayama, T., Fujiwara, H., Yamada, S., Tastumi, K., Honda, T. and Fuji, S. (1999) Clinical application of a new assisted hatching method using a piezomicromanipulator for morphologically low quality embryos in poor prognosis infertile patients. *Fertil. Steril.*, **71**, 1014–1018.

Schoolcraft, W., Schlenker, T., Gee, M., Jones, G.S. and Jones, H.W.Jr (1994) Assisted hatching in the treatment of poor prognosis in vitro fertilization candidates. *Fertil. Steril.*, **62**, 551–554.

Schoolcraft, W., Schlenker, T., Jones, G. and Jones, W. (1995) In vitro fertilization in women age 40 and over: the impact of assisted hatching. *J. Assist. Reprod. Genet.*, **12**, 581–583.

Schoper, B., Ludwig, M., Edenfeld, J., Al-Hasani, S. and Diedrich, K. (1999) Possible applications of lasers in assisted reproductive technologies. *Hum. Reprod.*, **14** (Suppl. 1), 186–193.

Tucker, M., Luecke, N., Wilker, S. and Wright, G. (1993) Chemical removal of the outside of the zona pellucida on day 3 human embryos has no impact on implantation rate. *J. Assist. Reprod. Genet.*, **10**, 187–191.

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