Prospective hatching of embryos developed from oocytes exhibiting difficult oolemma penetration during ICSI

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BACKGROUND: The hormonal milieu during ovarian stimulation is known to affect oolemma behaviour as well as zona pellucida thickness and structure. This led us to investigate whether a special subgroup of patients with oocytes where penetration of the oolemma is difficult during ICSI may benefit from assisted hatching. METHODS: A total of 77 couples (mean age: 32.9 ± 4.6 years; range: 22–38) had oocytes that could hardly be penetrated by the ICSI pipette. Nineteen patients underwent two ICSI cycles, giving a total number of 96 cycles, which were randomly split into either the study group (n = 52) or the non-hatching group (n = 44). Hatching was done using a non-contact 1.48 mm wavelength diode laser. Implantation and pregnancy rates were recorded. RESULTS: The pregnancy rate was 36.6% (19/52) in the study group and 13.6% (6/44) in the non-hatching group (P < 0.05). In addition, a higher number (P < 0.05) of embryos implanted in the study group (23/106; 21.7%) than in the non-hatching group (9/92; 9.8%). CONCLUSIONS: Once oolema penetration during ICSI has proven difficult, prospective hatching of embryos considered for transfer may increase their implantation behaviour.

Key words: assisted reproductive techniques/ICSI/oolema penetration/ovarian stimulation

Introduction

ICSI (Palermo et al., 1992) has progressively replaced previous micromanipulation techniques because high rates of survival and fertilization can be achieved.

ICSI is a rather invasive method, as both the zona pellucida and the oolemma have to be penetrated. Once the zona pellucida is breached, the oolemma may display various reactions to the penetrating glass pipette (Nagy et al., 1995; Palermo et al., 1996; Dumoulin et al., 2001). Three distinct types of oolemma response can be observed. Most of the injected oocytes show normal breakage of the membrane, as assessed by formation of an invagination rupturing at the approximate centre of the oocyte. A second type of response, sudden breakage of the oolemma without creation of a funnel may be observed. This phenomenon is correlated with decreased rates of survival and fertilization (Nagy et al., 1995; Palermo et al., 1996; Dumoulin et al., 2001; Ebner et al., 2001). The third type of membrane response is called ‘difficult breakage’ and is characterized by delayed penetration. Frequently, several penetration attempts are necessary prior to successful passage of the oolemma.

Palermo et al. (Palermo et al., 1996) support the hypothesis that the hormonal environment during ovarian stimulation may affect oolemma behaviour (Amsterdam and Aharoni, 1994). In addition, thickness and three-dimensional structure of the zona pellucida were also found to be affected by the hormonal environment, namely estradiol (Bertrand et al., 1996; De Mola et al., 1997; Green, 1997). Consequently, membranes with a difficult breakage pattern may serve as a marker for an impaired zona pellucida.

Hence, the ability to hatch and implant may be adversely influenced, although neither fertilization rate nor embryo quality was affected in such oocytes (Nagy et al., 1995; Palermo et al., 1996). This led us to investigate if a special subgroup of patients with oocytes difficult to inject may benefit from prospective hatching using a non-contact 1.48 mm wavelength diode laser (Rink et al., 1994).

Materials and methods

Since assisted hatching using a non-contact 1.48 mm wavelength diode laser has become fully accepted in routine IVF laboratory work, approval of our internal Institutional Review Board was not obtained.

Over a period of 16 months, a total of 77 couples (mean age: 32.9 ± 4.6 years; range: 22–38) presented with oocytes that could hardly be penetrated by the ICSI pipette. Nineteen patients had two ICSI attempts, giving a total of 96 cycles, which were randomly allocated to either the study group (n = 52) or the non-hatching group (n = 44). Patients failing to achieve pregnancy the first time were allocated to the other cohort on the second occasion (n = 19). No patients suffering from endometriosis or polycystic ovarian syndrome were included in the present study, in order to minimize a possible negative influence of oocyte quality (Pellicer et al., 1995; Aboulghar et al., 1997).

A long protocol was used to perform controlled ovarian stimulation. © European Society of Human Reproduction and Embryology 1317
In detail, pituitary desensitization was achieved by application of a gonadotrophin-releasing hormone agonist (Suprecur®; Hoechst, Frankfurt, Germany) and stimulation of the ovaries was started with an individually adjusted dose of HMG (Menogon®; Ferring, Kiel, Germany). Ovulation was induced by administering 5000–10 000 IU of HCG (Pregnyl®; Organon, Oss, The Netherlands) prior to ultrasound guided oocyte collection.

Micromanipulation was performed on an inverted microscope (×200 magnification, Olympus, Vienna, Austria) with Hoffman Modulation Contrast (Modulation Optics Inc., Greenvale, NY, USA) using hydraulic micromanipulators (Luigs and Neumann, Ratingen, Germany).

The technique of ICSI has been described in detail elsewhere (Palermo et al., 1995). Briefly, sperm were immobilized mechanically by damaging their tails with an injection pipette (Eppendorf, Hamburg, Germany) at a magnification of ×400. For injection, the oocyte was held in place with a holding pipette (Eppendorf) at 9 o’clock. The first polar body was located on the 6 o’clock position. As soon as the equatorial plane of the oocyte was focused, the ICSI pipette was pressed against the zona pellucida, creating a characteristic funnel at 3 o’clock.

According to our study design, all oocytes involved could hardly be penetrated. This breakage pattern was characterized by extreme elasticity of the oolemma, which impeded injection and required several attempts to penetrate the membrane. In order to ensure an adequate survival rate, we introduced a slightly adapted injection process combining a pressing phase (to the opposite region of the membrane) to minimize the influence of bad quality glass tools. All ICSIs were performed by the same embryologist, so that interindividual differences in technique could be excluded.

Fertilization and survival of the injected oocytes were assessed 18–20 h after injection. The presence of two pronuclei (2PN) as well as two polar bodies characterized normal fertilization (2PN). Early embryo development as assessed by the number of blastomeres and the percentage of fragmentation was evaluated 42–44 h after injection.

Most embryo transfers were performed on day 3 (48 in the study group and 39 in the non-hatching group). For organizational reasons, transfer had to be done on day 2 in 7.7 and 11.4% respectively.

All embryos considered for transfer were hatched using a non-contact 1.48 mm wavelength diode laser (Fertilase®; MTM, Montreux, Switzerland), which ensured precise and localized lysis of the zona pellucida. A series of tangential shots (Mantoudis et al., 2001), each with an irradiation time of 12 ms (1.2 mJ), was applied as soon as the equatorial plane was focused.

Fisher’s exact test, χ²-test, and Student’s t-test were applied to the present data. All tests were two-tailed with a significance of 95% (P < 0.05).

Results

A total of 753 MII oocytes showed oolemmas that could hardly be penetrated. Two distinct pronuclei were found in 540 oocytes (71.7%). Approximately 10% (72/753) of all injected oocytes showed signs of degeneration after ICSI. A mean number of 2.1 ± 0.7 embryos was transferred (n = 198). Twenty-five clinical pregnancies occurred (26.0%). The overall implantation rate was 16.2% (32/198).

Twelve women who did not become pregnant with the non-hatching protocol were referred to the hatching group. This resulted in five clinical pregnancies. On the other hand, none of seven patients who failed to achieve pregnancy, though all embryos were hatched had successful treatment without hatching (P = 0.047; χ²-test).

Table I clearly indicates that study and control group were comparable (P > 0.05) with regard to controlled ovarian hyperstimulation (COH) characteristics (e.g. medication, dose, basal FSH, estradiol on the day of ovulation induction).

In addition, number and quality (number of blastomeres, percentage of fragmentation) of transferred embryos were similar (Table II). Assisted hatching was found to influence significantly the rates of pregnancy and implantation (P < 0.05).

In this study, there was no statistical difference in the outcome of day 2 (n = 9) and day 3 transfers (n = 87); therefore results were pooled without affecting statistical evaluation.

Discussion

It is generally recognized that COH using a long protocol may affect oocyte quality. Maturation of abnormal oocytes which
otherwise would have become atretic may occur (Serhal et al., 1997). Abnormal oocyte morphology has been associated with the hormonal environment to which the gametes are exposed (De Sutter et al., 1996). This could be related to a progesterone and estradiol deficiency that is closely connected with maturation of nucleus and cytoplasm (Thibault, 1977).

Palermo et al. described a correlation between different breakage patterns of the oolemma and peripheral estradiol concentrations (Palermo et al., 1996). In detail, they were able to show that oocytes with sudden breakage of the membrane were found in patients with low concentrations of estradiol at the time of ovulation induction compared with the other two oolemma response patterns ($P = 0.01$).

Unlike this previous study, we could not include data from oocytes with sudden breakage of the oolemma, since the number of women who showed such gametes during the present investigation period ($n = 10$) was too low for statistical analysis. In addition, oocytes with normal breakage were excluded from this study, since no data on assisted hatching of this type of oocytes are available. However, retrospective analysis of a selected control group with 'normal breakage', by taking the immediate antecedent or subsequent cycle to an abnormal membrane pattern (Palermo et al., 1996), confirmed the finding that there is no significant difference in estradiol concentration ($P > 0.05$) between oocytes with difficult and normal membrane response (data not shown).

However, the mean estradiol concentration of the patients in our study (1690 ± 970 pg/ml) corresponded to that previously published for patients with a difficult breakage pattern of the oolemma (Palermo et al., 1996).

Since the extreme elasticity of the oolemma impeded injection in such cases, several attempts to penetrate the membrane were required. This additional manipulation may cause an increased rate of oocyte damage (Ebner et al., 2001). This is consistent with the findings of Xia, who reported decreased rates of oocyte survival after ICSI in patients with estradiol concentrations $>10,000$ pmol/ml (Xia, 1997).

It should be kept in mind that the ICSI technique and pipette may have contributed to the difficult penetration process (Nagy et al., 1995; Joris et al., 1998; Dumoulin et al., 2001). However, repeated change of the injection pipette ensured that this influence was kept to a minimum. In addition, the fact that extremely elastic oolemmas were diagnosed in 19 patients in two consecutive cycles rather suggests a suboptimal hormonal environment.

An identical response of the oolemma to at least three ICSI pipettes of different lots supports this finding, which is in contrast with the results of Palermo et al., who stated that the number of patients displaying a homogeneous membrane pattern is limited and that membrane behaviour is rather an individual characteristic (Palermo et al., 1996). Differences in stimulation as well as in laboratory set up may have contributed to this divergence.

An oolemma exposed to an unfavourable hormonal environment during oocyte maturation may show changes in its lipoprotein structure. Consequently, the contribution of the membrane to the formation of the zona pellucida, though yet undetermined (Van Blerkom and Motta, 1989), may be inhibited. Therefore, both oolemma and zona pellucida may be impaired by a possible steroid imbalance.

Some data provide evidence that patients with elevated day 3 FSH concentrations (De Mola et al., 1997), advanced female age (Bertrand et al., 1996; Gabrielsen et al., 2000) or increased HMG dose (Bertrand et al., 1996) may present affected zonae. However, no low-response patients (e.g. high basal FSH and HMG dose, advanced age) were included in our study (Table I).

In addition, estradiol shows a negative influence on the zona pellucida by increasing its thickness (Bertrand et al., 1996; De Mola et al., 1997). According to Bertrand et al., zona thickness is a characteristic of oocytes existing before fertilization (Bertrand et al., 1996). It has been suggested that there is an optimal zona thickness beyond which fertilization is difficult (Bertrand et al., 1996). Since thickness of the zona did not exceed 20 μm in the majority of the oocytes evaluated, a possible damage of the zona pellucida can rather be attributed to changes in its acellular protein-carbohydrate composition (‘prefertilization zona hardening’) than to increased thickness (Tucker et al., 1993).

Consequently, the main functions of the zona pellucida may be impaired. Firstly, its role in zona–permeatozoon receptivity may be limited. However, by performing ICSI a possible failure of fertilization with conventional IVF can be avoided. Secondly, the blastocyst’s ability to hatch from the zona pellucida prior to implantation may be affected. Artificial generation of a hole in the zona pellucida is known to overcome this problem and enhances the hatching ability of the embryo (Cohen et al., 1992; Chao et al., 1997; Lanzendorf et al., 1998; Magli et al., 1998). This benefit has not been reported for unselected groups of patients (Hellebaut et al., 1996; Tucker et al., 1996).

The results of the present investigation support the hypothesis that specific subgroups of patients may benefit from assisted hatching. A difficult breakage pattern of the inner membrane may serve as marker for a dysfunction of the zona pellucida. Prospective hatching of such embryos was found significantly to increase rates of implantation and clinical pregnancy.

There is evidence from animal (Amsterdam and Aharoni, 1994; Kimura and Yanagimachi, 1995) and human studies (Nagy et al., 1995; Palermo et al., 1996) that there is a link between hormone profile and membrane behaviour. Further studies, including hormonal assays of follicular fluid, should be performed in order to bring out the actual correlation between hormones and oocyte morphology.

References


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