Embryos with high implantation potential after intracytoplasmic sperm injection can be recognized by a simple, non-invasive examination of pronuclear morphology


Laboratoire d'Eylau, 55 Rue Saint-Didier, 75116 Paris, France

Abstract

Embryos are conventionally selected for transfer based on the evaluation of the cleavage speed and extent of blastomere fragmentation. Here we examined whether the predictive value of these criteria, as indicators of the chance of embryo implantation, can be further potentiated by adding previously described criteria reflecting the regularity of pronuclear development. In a group of embryos selected for transfer in 380 fresh embryo transfer cycles according to the conventional criteria, the transfer of only those embryos that developed from zygotes judged normal at the pronuclear stage (pattern 0) gave significantly higher pregnancy (44.8%) and implantation (30.2%) rates compared with the pregnancy (22.1%; \( P < 0.05 \)) and implantation rates (11.2%; \( P < 0.001 \)) for the transfers of only those embryos that developed from zygotes judged abnormal (non-pattern 0). The transfer of only one pattern 0 embryo was sufficient for the optimal chance of pregnancy (no differences in pregnancy rates after transfer of one, two or three pattern 0 embryos), whereas the transfer of two pattern 0 embryos mostly resulted in a twin pregnancy. The inclusion of the criteria based on pronuclear morphology can thus lead to the application of a single embryo transfer policy and optimize the selection of embryos for transfer and cryopreservation. 

Key words: implantation rate/multiple pregnancy rate/implantation rate/pronuclear morphology/single-embryo transfer

Introduction

Several studies have shown a relationship between various aspects of zygote morphology on the one hand, and the viability of the future embryo on the other (Wright et al., 1990; Balakier et al., 1993; Van Blaricom et al., 1995; Payne et al., 1997; Scott and Smith, 1998; Garello et al., 1999; Goud et al., 1999). However, the evaluation criteria used in some of these studies were invasive in nature, while others required the use of non-standard laboratory equipment or the application of laborious and time-consuming protocols. In a previous study (Tesarik and Greco, 1999) we described relatively simple criteria of pronuclear morphology evaluation that can be assessed during a single, non-invasive observation on human zygotes, performed between 12 and 20 h after in-vitro insemination or intracytoplasmic sperm injection (ICSI). With the use of these criteria, the probability of developmental arrest at the early cleavage stages and the risk of the development of embryos with multinucleated blastomeres or overall poor morphology can be predicted (Tesarik and Greco, 1999). However, the relationship between the implantation potential of the embryos developing from zygotes showing different patterns of pronuclear morphology was addressed only marginally in that study. In particular, it remained to be determined whether abnormal pronuclear morphology can predict poor implantation potential of embryos with normal cleavage speed and morphological appearance at cleavage stages. The examination of this relationship is important to assess the value of pronuclear morphology as an additional criterion to be used for embryo selection for transfer.

If a subpopulation of embryos with a high implantation potential can be recognized by a combined evaluation of pronuclear and cleavage-stage morphology, the application of these criteria in the decision about the number and grade of embryos to be transferred will not only improve pregnancy rates but will also reduce the risk of multiple pregnancies by avoiding the transfer of several high-grade embryos at the same time.

To address these issues, we compare here the pregnancy and implantation rates in fresh embryo transfer cycles in which embryos that show good morphology at the cleavage stages but that had developed from zygotes with different patterns of pronuclear morphology were used. We also show how the relationship between the number of embryos transferred and the incidence of multiple pregnancies is dependent on the pronuclear morphology of the zygotes from which these embryos developed.

Materials and methods

This study involved 380 ICSI treatment cycles with ejaculated spermatozoa. Ovarian stimulation, ICSI, and embryo culture and transfer were performed using the standard protocols (Tesarik and Greco, 1999). Zygotes at the pronuclear stage were inspected once, 14–17 h after ICSI. The morphological parameters evaluated in this study included the number of nucleolar precursor bodies (NPB) and their distribution in each pronucleus (polarized versus non-polarized) as previously described (Tesarik and Greco, 1999). These criteria were simplified in this study by including the originally described patterns 1–5 (abnormal) into a single group of non-pattern 0 zygotes as opposed to pattern 0 (normal). Briefly, zygotes with the following characteristics were considered as pattern 0: (i) the difference in the number of NPB between both pronuclei did not exceed three; (ii) the distribution of NPB (random versus polarized) was the same in both pronuclei; and (iii) there were at least three NPB in each pronucleus. This assessment required the changing of focus during observation
until the whole volume of both pronuclei was inspected. Before this examination, zygotes were rotated until both pronuclei were clearly visible and there was no optical superimposition of both abutted pronuclei. The number and spatial distribution of NPB in each pronucleus were noted. However, these observations were not taken into account for the selection of embryos for transfer. Instead transferrable embryos were selected, 2 days after ICSI, by using the conventional criteria of cleavage speed and morphology. Only cases in which all embryos transferred were characterized as good morphology ones were included in this study. Good morphology embryos were characterized as embryos with equal-sized blastomeres (with the exception of 3-cell embryos for which the presence of one bigger and two smaller, equal-sized blastomeres was considered a feature of good morphology), <10% of intrazonal space occupied by cell fragments and whose blastomeres all had clear, non-granulated cytoplasm (Tesarik and Greco, 1999). Pregnancy and implantation rates were calculated for cycles resulting in a clinical pregnancy. Only clinical pregnancies with an embryonal sac in an intrauterine position and positive heart activity visible on ultrasound were taken into account.

Means and percentages were compared by using Student’s t-test and χ² test (StatView®; Abacus Concepts, Berkeley, CA, USA) respectively.

Results

Out of the 380 transfer cycles, mixed transfer of embryos developing from pattern 0 and non-pattern 0 pronuclear zygotes was performed in 205 cases. Of the remaining 175 cases, only embryos (n = 189) developing from normal morphology (pattern 0) pronuclear zygotes were transferred in 98 cycles (group A), and only embryos (n = 161) developing from abnormal morphology (non-pattern 0) pronuclear zygotes were transferred in 77 cycles (group B). The mean number of embryos per transfer in group A and group B was thus 1.93 and 2.09 respectively. Cleavage speed and morphology of the cleavage stages at the time of transfer did not differ significantly between groups A and B. However, both the pregnancy rate (P < 0.05) and the implantation rate (P < 0.001) were significantly higher in group A compared with group B (Table I).

In a second part of this study, all the 380 transfer cycles were divided into two partially overlapping groups, the one in which at least one pattern 0 embryo was transferred (Table II), irrespective of the number of co-transferred non-pattern 0 embryos, and the other in which at least one non-pattern 0 embryo was transferred (Table III), irrespective of the number of co-transferred pattern 0 embryos. Accordingly, cases with mixed transfers of both pattern 0 and non-pattern 0 embryos appeared in both groups. However, the pregnancy and implantation rates were related exclusively to the number of pattern 0 embryos transferred in the former group (Table II) and to the number of non-pattern 0 embryos transferred in the latter (Table III). The mean overall number of embryos (including pattern 0 and non-pattern 0 ones) transferred in a single cycle was similar in both groups (1.98 and 2.04 respectively).

In the pattern 0 group, the pregnancy rates were 33.6, 35.7 and 44.4% for transfer cycles with 1, 2 and 3 pattern 0 embryos respectively. These differences were not statistically significant. However, there were significantly more (P < 0.05) singleton pregnancies after the transfer of only one pattern 0 embryo as compared with the transfer of two embryos which mostly resulted in a twin pregnancy (Table II). The twin and triplet pregnancies developing after the transfer of only one pattern 0 embryo must have resulted from the implantation of co-transferred non-pattern 0 embryos in mixed transfer cycles or by monozygotic twinning. Two of the four pregnancies after

<table>
<thead>
<tr>
<th>Group</th>
<th>Transfers</th>
<th>Transferred embryos</th>
<th>Pregnancies</th>
<th>Embryonal sacs</th>
<th>% pregnancy rate</th>
<th>% implantation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>98</td>
<td>189</td>
<td>44</td>
<td>57</td>
<td>44.9 (44/98)a</td>
<td>30.2 (57/189)b</td>
</tr>
<tr>
<td>B</td>
<td>77</td>
<td>161</td>
<td>17</td>
<td>18</td>
<td>22.1 (17/77)a</td>
<td>11.2 (18/161)b</td>
</tr>
</tbody>
</table>

Values with the same superscript were significantly different.

Table II. Relationship between the number of pattern 0 embryos transferred and the development of singleton and multiple pregnancies in 181 fresh embryo transfer cycles

<table>
<thead>
<tr>
<th>Embryos transferred</th>
<th>Transfer cycles</th>
<th>No. (%) of pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Singleton</td>
<td>Twin</td>
</tr>
<tr>
<td>1</td>
<td>24 (24.7)</td>
<td>7 (7.2)</td>
</tr>
<tr>
<td>2</td>
<td>14 (16.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>1 (8.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table III. Relationship between the number of non-pattern 0 embryos transferred and the development of singleton and multiple pregnancies in 199 fresh embryo transfer cycles

<table>
<thead>
<tr>
<th>Embryos transferred</th>
<th>Transfer cycles</th>
<th>No. (%) of pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Singleton</td>
<td>Twin</td>
</tr>
<tr>
<td>1</td>
<td>24 (24.7)</td>
<td>7 (7.2)</td>
</tr>
<tr>
<td>2</td>
<td>14 (16.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>1 (8.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
the transfer of three pattern 0 embryos were triplet (Table II). Interestingly, the same analysis for transfer cycles with non-pattern 0 embryos shows an inverse relationship between the pregnancy rates and the number of embryos transferred (Table III), probably because the number of co-transferred pattern 0 embryos decreased with the increasing number of non-pattern 0 embryos in the transferred cohort.

Discussion
The criteria of pronuclear morphology evaluation used in this study were defined in a previous paper in which a relationship between pronuclear morphology and several parameters reflecting the normality of embryo preimplantation development, namely the occurrence of cleavage arrest, the incidence of blastomere multinucleation and cleavage stage embryo morphology, was demonstrated (Tesarik and Greco, 1999). In addition to introducing a potentially useful criterion for the selection of human embryos for fresh transfer or cryopreservation as early as the pronuclear stage, thus circumventing a number of ethical, administrative and ethical concerns regarding the cryostorage and eventual destruction of embryos at later stages of preimplantation development (Evans and Evans, 1996; Schäfer et al., 1996; Edwards and Beard, 1997), a combination of these new criteria with the conventional cleaving-embryo quality evaluation criteria (Staessen et al., 1993), with the recently formulated strict embryo criteria (Van Royen et al., 1999; Gerris et al., 1999), or with criteria based on oocyte morphology (Serhal et al., 1997; Xia, 1997; Ebner et al., 1999) might enable a more reliable prediction of embryo developmental potential and thus a further reduction of the number of embryos to be transferred at a time as compared with the current practice. This study has as least partly satisfied these expectations.

Though prospective in terms of the definitive characterization of the study design and the pronuclear morphology evaluation criteria before its beginning, this study is based on a retrospective cohort analysis, since it did not apply these criteria for embryo selection for transfer. Instead, embryos were selected according to the conventional criteria reflecting cleavage speed, blastomere regularity and the volume occupied by anuclear cell fragments. According to these conventional criteria, the morphological grade of the embryos selected for transfer did not differ between the group of embryos that developed from pattern 0 zygotes and those developed from non-pattern 0 zygotes, and the pronuclear grading thus represented an additional, possibly independent evaluation criterion. The inclusion of this additional criterion made it possible to recognize a subpopulation of good morphology embryos that had a very high probability of implanting successfully after transfer. In fact, the implantation rate of the embryos developing from pattern 0 zygotes was almost three-fold higher than that of the embryos developing from non-pattern 0 zygotes although the cleavage speed and morphology did not differ between these two groups of embryos.

In other terms, the developmental inferiority reflected by an abnormal pattern of pronuclear development does not always lead to a visible impairment of cleavage speed or excessive embryo fragmentation and may thus go unnoticed during the early cleavage stages. Because important developmental processes in human pronuclei, including the first zygotic round of DNA replication and an early wave of RNA synthesis, are known to be associated with the development of pronuclear structure and ultrastructure (Tesarik and Kopecky, 1989, 1990), it may be speculated that asynchrony or alteration of these processes can be at the origin of molecular abnormalities whose morphological consequences appear later in development, perhaps after the major activation of the embryonic genome between the 4-cell and the 8-cell stage (Tesarik, 1987). It has to be noted, however, that the absence of a difference in the morphological appearance and cleavage speed between these two groups of embryos is due to the fact that both groups consisted of the best embryos available that had been selected for transfer in each treatment cycle. If compared in the whole, unselected group of embryos, those embryos developing from pattern 0 zygotes show better morphology and are less likely to arrest cleavage as compared to embryos developing from non-pattern 0 zygotes (Tesarik and Greco, 1999). This suggests that the adverse consequences of the pronuclear abnormalities may become patent during prolonged embryo culture to the blastocyst stage. This hypothesis, however, remains to be tested. A prospective study, in which embryos are chosen for transfer exclusively on the basis of pronuclear morphology, is needed in order to decide about the prognostic value of pronuclear morphology by itself as compared to the recently described strict embryo criteria, applied to cleavage-stage embryos, with which high implantation rates can also be achieved (Gerris et al., 1999; Van Royen et al., 1999).

It is important to note that the incidence of multiple pregnancies was very high when more than one pattern 0 embryo was replaced in a single transfer cycle, irrespective of the number of non-pattern 0 embryos that accompanied them in mixed transfer cycles. In fact, the incidence of twin pregnancies was almost twice that of singleton pregnancies when two pattern 0 embryos were transferred, and half of the clinical pregnancies developing after the transfer of three pattern 0 embryos were triplet. On the other hand, the overall pregnancy rate did not differ significantly between the transfer cycles in which one, two or three pattern 0 embryos were replaced. These observations have two practical implications. Firstly, it appears sufficient to transfer a single ‘top quality’ embryo (a good morphology cleaving embryo having developed from a pattern 0 zygote) to achieve the optimal chance of pregnancy. In this respect, the value of the pronuclear criteria, used alone or in combination with the previously described criteria for single embryo transfer (Gerris et al., 1999; Van Royen et al., 1999; Vilska et al., 1999), remains to be determined by a prospective study. Secondly, the transfer of two or more such embryos entails a great risk of multiple pregnancy. Hence, if more than one embryo is to be transferred, it appears reasonable to avoid a simultaneous transfer of more than one embryo fulfilling, at the same time, the criteria of good morphology at the pronuclear and at the cleavage stage.

This transfer policy would lead to more high quality embryos being cryopreserved, preferentially accompanied by lower grade embryos in individual straws for later common transfer.
Because the evaluation criteria concerning pronuclear morphology can also be used alone to select appropriate embryo groups for early cryopreservation at the pronuclear stage (Tesarik and Greco, 1999), and because pronuclear zygotes appear to survive the freezing and thawing procedures better as compared with cleavage-stage embryos (Veeck et al., 1993; Al-Hassani et al., 1996; Hoover et al., 1997), this policy may further increase the chance of pregnancy while reducing the risk of multiple implantations.

In conclusion, cleaving embryos developing from zygotes with normal morphology at the pronuclear stage have a better implantation potential than embryos developing from abnormal morphology zygotes, even if the cleavage speed and morphology of the embryos selected for transfer is the same. Even though this study only dealt with ICSI-derived zygotes, the pronuclear criteria used here had been originally defined by analysing a mixed group of embryos of which some originated from ICSI and others from conventional in-vitro fertilization (Tesarik and Greco, 1999). It is thus conceivable that the same selection criteria can be applied in both situations. High implantation rates (>30%) can be achieved by elective transfer of embryos developing from normal morphology zygotes, which may make it possible to transfer only one embryo at a time.

Pronuclear morphology is a valuable additional criterion for elective embryo transfer. It can be used to make the decision of which embryos will be transferred in the fresh state and which will be frozen as early as the pronuclear stage. As an additional criterion to the criteria reflecting cleavage speed and blastomere morphology, the evaluation of pronuclear morphology is useful to determine the most suitable number of embryos for transfer so as to achieve the optimal chance of conception while reducing the risk of multiple pregnancies.

References


Received on November 29, 1999; accepted on February 24, 2000

Pronuclear morphology and implantation rate