FISH analysis for chromosomes 13, 16, 18, 21, 22, X and Y in all blastomeres of IVF pre-embryos from 144 randomly selected donated human oocytes and impact on pre-embryo morphology

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BACKGROUND: The data are compiled from two multicentre, prospectively randomized studies on the effect of follicular fluid meiosis-activating sterol (FF-MAS) on human oocytes. The donated oocytes were exposed either to test doses of FF-MAS or to control solutions. The data from the control groups are presented with chromosomal status of the embryos correlated to embryo morphology. METHODS: The study includes 144 randomly selected donated human oocytes. The nucleus from each blastomere was fixed separately and fluorescence in-situ hybridization (FISH) using seven probes (13, 16, 18, 21, 22, X and Y) was performed. RESULTS: Analysis of 103 pre-embryos containing 479 blastomeres resulted in 424 blastomeres with clear FISH signals. Of these blastomeres, 55% were normal diploid and 45% were abnormal. At a pre-embryonic level, 53% were classified as normal containing >50% normal blastomeres while 31% of the pre-embryos were classified as uniformly normal. Abnormality rate was significantly increased in the pre-embryos with unevenly sized blastomeres and with increasing degree of fragmentation at 68 h after fertilization. Applying criteria for good embryo quality significantly increased the rate of chromosomally normal pre-embryos from 53 to 75%. CONCLUSIONS: The data demonstrate the high degree of genetic heterogeneity in a randomly selected pool of donated pre-embryos from an IVF programme. Further, we found that uniformity of blastomere size, degree of fragmentation and cleavage kinetics reflect the cytogenetic status of the pre-embryo and are therefore important in the selection of pre-embryos.

Key words: aneuploidy/chromosomal constitution/embryo quality/embryo selection/whole embryo analysis

Introduction

One of the key factors for a successful outcome in an assisted reproductive programme is the selection of pre-embryos with the highest developmental potential. For many years pre-embryos have been selected based on parameters considered important quality indicators, such as fragmentation, cell number and cell size (Cummins et al., 1986; Claman et al., 1987; Puissant et al., 1987; Staessen et al., 1992; Steer et al., 1992; Shulman et al., 1993; Giorgetti et al., 1995; Ziebe et al., 1997; Hardarson et al., 2001; Van Royen et al., 2001). Further, it is known that age of the woman is one important factor affecting the quality of the pre-embryos (Munné et al., 1995; Battaglia et al., 1996; Lim and Tsakok, 1997; Ziebe et al., 2001). However, we have very little knowledge on the variation of intrinsic chromosomal anomalies. The development and implementation of techniques for preimplantation genetic diagnostic programmes have made it possible to assess the chromosomal constitution without destroying the pre-embryo (Handyside et al., 1990; Pellestor, 1995). Most studies published have been based on either single or dual cell biopsy in an attempt to predict the chromosomal status of the rest of the pre-embryo, or on all blastomeres from suboptimal surplus pre-embryos that would otherwise have been discarded (Munné et al., 1994, 1995, 1997; Kuo et al., 1998). It is still uncertain to what extent these findings are representative of the whole pre-embryo, and in particular for good quality pre-embryos normally selected for transfer. However, some studies based on whole embryo analysis suggest that in surplus good quality embryos the chromosomal complement in the pre-embryo is quite often compromised (Harper et al., 1995; Kligman et al., 1996; Delhanty et al., 1997).

Limited information is available relating the morphological appearance of the pre-embryo to the chromosomal status of the...
pre-embryo. It has been suggested that pronuclear morphology could be indicative of pre-embryo quality (Manor et al., 1999; Tesarik et al., 1999) and that pre-embryos containing uneven sized blastomeres have an increased aneuploidy rate (Hardarson et al., 2001). Further, there is evidence that growth retardation in addition to accelerated cleavage could be an indication of chromosomal abnormalities (Magli, 1998; Bialaska et al., 2002). Other studies have demonstrated increased chromosomal abnormality rates with increased degree of fragmentation/poor embryo morphology (Munne et al., 1994; Pellestor et al., 1994; Bialaska et al., 2002)

The aim of this study was to assess the chromosomal constitution for seven chromosomes in all blastomeres from pre-embryos originating from randomly selected human oocytes donated by IVF patients, and to correlate the morphological appearance of the pre-embryos to the cytogenetic findings.

Materials and methods

Trial design

The data are part of two phase 1, multicentre, prospectively randomized, double blind, controlled in-vitro studies (Bergh et al., unpublished; Loft et al., 2003). An independent Data Monitoring Board (Quintiles AB, Denmark) was responsible for monitoring the trial and kept the code of randomization, which was first broken after completion of the study. These safety and efficacy studies were conducted from March 2000 to June 2001 at the Fertility Clinics, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; Sahlgrenska University Hospital, Gothenburg, Sweden; and Carlanderska Hospital, Gothenburg, Sweden. The data from the control groups are hereby presented where chromosomal status of the embryos is correlated to embryo morphology.

To minimize the inter-clinical variation, the same batches of culture medium, utensils and donor sperm were used at all three clinics. In addition to the primary morphological scoring at each site, a consolidated reassessment was performed by three embryologists (one from each centre) based on high-resolution computer-based image recording of the oocytes and pre-embryos. Evaluation of the cytogenetic results was performed as a consolidated evaluation by three embryologists (one from each centre) together with a geneticist from Quest Diagnostics Inc. (USA)

Patients

The inclusion criteria were indication for IVF or ICSI treatment, female age between 25 and 37 years (both inclusive) and regular menstrual cycles. Further, a minimum of six (Denmark) to 10 (Sweden) aspirated oocytes should be available to the couple after donation.

Exclusion criteria were any known medical condition or genetic disorders that could preclude IVF/ICSI treatment or interfere with the interpretation of the results of the study, and the use of any investigational drug within 30 days prior to oocyte retrieval.

Treatment and donation

The studies included 143 women, who donated 144 oocytes. Patients used long protocols with GnRH (Synarel®, Pharmacia, Denmark; Suprefact®, Aventis Pharma, Denmark) for down-regulation for ≥14 days or short protocols using GnRH antagonists (Orgalutran®, Organon, Denmark; Cetrotide®, Serono, Denmark). Patients were stimulated with recombinant FSH (Puragon®, Organon; Gonal-F®, Serono) with an average daily dose between 100 to 300 IU. hCG (10 000 IE Profasi®, Serono; Pregnyl®, Organon) was administered when ≥3 follicles of 17 mm were registered by ultrasound.

Oocyte aspiration was performed according to the normal procedure of each clinic. Depending on the number of oocytes the patients wanted to donate, the selection procedures were as follows: the third aspirated oocyte from the first ovary, the third aspirated oocyte from the second ovary, an oocyte randomly chosen among the remaining oocytes. The time interval from aspiration to randomization by treatment group was <90 min.

All oocytes were inseminated using donor sperm from one of three sperm donors of proven fertility and normal karyotype (Cryos International Sperm Bank, Aarhus, Denmark), except for two oocytes that were inseminated using the husband’s semen sample.

Cell culture and embryo evaluation

In total, 144 cumulus-enclosed oocytes were allocated to the control groups.

The oocytes were cultured in 4-well dishes (Nunc, Denmark) in 0.5 ml standard IVF medium (IVF-20; Vitrolife, Sweden). After 4 h of culture, insemination was performed by addition of 150 000 sperm cells or by ICSI. Pre-embryo evaluation was performed at 26, 44, 50 and 68 h (± 1 h) after insemination. At these time-points the pre-embryos were scored for: cell number; degree of fragmentation (no fragmentation, 0–10, 11–20, 21–50 or >50% fragmentation); localization of fragments (local or dispersed); equally or unequally sized blastomeres, defined as ≥25% difference in size; and cytoplasmic appearance (homogeneous or dark/granulated/vacuolated).

High-resolution digital images were stored at each time point using the FertiGrab system (Image House Medical A/S, Denmark) for later re-assessment of the pre-embryo score.

Fixation

At 70 h (± 2 h) after insemination, all blastomeres from all cleaved pre-embryos were fixed individually by dissolving the zona pellucida in pronase (5 mg/ml) (Sigma, USA) followed by incubation in Ca²⁺/Mg²⁺-free medium (EB-10; Vitrolife, Sweden) until segregation of the individual blastomeres. Nucleus/nuclei from each blastomere were then fixed on a silanized slide (Cat. no. S1308; Oncor, USA) in an HCl/Tween-20 solution (0.01 M/0.1%) (Coonen et al., 1994). After fixation the slides were left to dry and then dehydrated in phosphate-buffered saline followed by 70–90–99% ethanol series and the slides were packed in containers with silica gel and stored at −20°C until shipment for analysis.

Cytogenetic analysis and evaluation

Fluorescence in-situ hybridization (FISH) analysis for chromosomes 13, 16, 18, 21, 22, X and Y was performed at an independent laboratory (Quest Diagnostics Inc.).

The FISH analysis was performed by sequential hybridization using MultiVysion PB and CEP X/CEP Y (alpha satellite) (Vysis, USA). Locus-specific probes were used for chromosomes 13, 21, 22 and centromeric probes for chromosomes 16, 18, X and Y.

The cytogenetic evaluation included documentation of the number of nuclei in each blastomere and classification of each blastomere as normal (diploid constitution), abnormal (multinucleated and/or fragmented nuclei/nuclei and/or an abnormal chromosomal constitution or no nuclei seen, neither during fixation or after FISH labelling) or not classifiable for the tested chromosomes (nucleus was found, but FISH signals were missing completely or very loose chromatin or covered by cytoplasmic residue). Based on the result in the blastomeres, a classification as overall normal, overall abnormal or not classifiable was applied to each pre-embryo (definition 1). A
genetically ‘overall normal’ pre-embryo was defined and classified as a pre-embryo with >50% of its analysed cells having nuclei with a normal constitution concerning these probed chromosomes. Each embryo was also classified as either uniformly normal, uniformly abnormal, mosaic ploidy, mosaic aneuploidy, mosaic chaotic or not classifiable (definition 2). Mosaic pre-embryos were defined as follows. (i) Ploidy: a pre-embryo with normal diploid constitution in combination with a multiplied cell line, e.g. 2n/3n or 2n/4n. However, 2n/4n embryos where the tetraploid blastomere had the chromosomes separated in 2 diploid nuclear compartments were considered normal. (ii) Aneuploidy: a pre-embryo with single chromosomes added or deleted e.g. 2n/2n + 13. (iii) Chaotic pattern: a pre-embryo with more than 2 cell lines with nuclei showing randomly different chromosome complements.

Statistics
To analyse the dependency of overall normal pre-embryos and uniformly normal pre-embryos with regards to the different morphology parameters, graphic models were used. This approach was chosen because graphic models are able to take correlations between the explanatory variables in a statistical model into account (Edwards, 1995). In this way a potential bias caused by a complex correlation structure between the morphology parameters would be minimized.

Besides using the morphology parameters as explanatory variables, age, trial and centre were also included in the model. This analysis was conducted at both 44 and 68 h after insemination. Additional tests were done using Fisher’s exact test.

To measure the level of agreement between embryologists (local observations versus reassessment observation, as well as between the three embryologists at reassessment), Kappa statistics were used to calculate concordance rates on cleavage stage, fragmentation, and morphology at 68 h after insemination.

All statistical tests were two-sided with a significance level of 0.05. The data were analysed using SAS version 6.12 (SAS Institute Inc., USA) run on a UNIX platform.

Ethical approval
Independent Scientific and Ethics Committees in Denmark and Sweden approved the study. Written informed consent was obtained from both partners and the trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP).

Results
A total of 144 oocytes donated by 143 women were included in this study. This resulted in 103 (72%) pre-embryos containing 479 blastomeres that were analysed by FISH 70 h after insemination. A total of 424 (89%) blastomeres gave conclusive results with clear FISH signals (Table I). Of these, 235 (55%) were normal diploid and 189 (45%) were abnormal.

Evaluating the results at a pre-embryonic level, a total of 53% were classified overall normal containing >50% normal blastomeres while 47% of the pre-embryos were abnormal in the majority of the cells. Thirty-one per cent of the pre-embryos were normal in all blastomeres while 69% contained at least one abnormal blastomere. Of the abnormal pre-embryos, 9% were uniformly abnormal, 11% were mosaic aneuploid, 6% had mosaic ploidy and 39% were mosaic chaotic (Table II). The chromosomal abnormality rate was significantly increased with increasing age of the woman donating the oocytes (P = 0.0078).

Cytogenetic findings and pre-embryo morphology
For each pre-embryo the cytogenetic analysis at day 3 (70 h after insemination) was evaluated in relation to pre-embryo morphology 44 and 68 h after insemination.

Blastomere size
Cytogenetic analysis 70 h after insemination showed a significantly increased rate of chromosomal abnormality for pre-embryos containing unevenly sized blastomeres, both for pre-embryo scoring performed at 44 h (P = 0.0002) and at 68 h (P < 0.0001).

Fragmentation
No significant impact of the presence of fragmentation on the chromosomal constitution was found for pre-embryos when scored at 44 h after insemination. However, when scored at 68 h after insemination the degree of fragmentation was significantly associated with an increase in the rate of abnormal pre-embryos (P = 0.0285) (Table III).

Cleavage rate
The cleavage rate (defined as the number of pre-embryos scored as containing >4 blastomeres at 44 h) was not related to the cytogenetic outcome at 70 h after insemination. The number of pre-embryos scored as containing <6 cells at 68 h after insemination showed a non-significant trend (P = 0.0709) towards an increase in chromosomal abnormality rate (Table III).

Localization of fragments
In the pre-embryos displaying fragments there was no impact on whether the fragments were classified as local or dispersed.

### Table I. Inclusion data

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of women</td>
<td>143</td>
</tr>
<tr>
<td>No. of donated oocytes</td>
<td>144 (100)</td>
</tr>
<tr>
<td>No. of cleaved pre-embryos</td>
<td>111 (77)</td>
</tr>
<tr>
<td>No. of pre-embryos with FISH result</td>
<td>103 (72)</td>
</tr>
<tr>
<td>No. of blastomeres from the cleaved pre-embryos</td>
<td>479 (100)</td>
</tr>
<tr>
<td>Blastomeres with FISH results</td>
<td>424 (89)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

FISH = fluorescent in-situ hybridization.

### Table II. Chromosomal classifications

<table>
<thead>
<tr>
<th>Classification</th>
<th>Cytogenetic evaluation</th>
<th>Classified as overall normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniformly normal</td>
<td>32 (31)</td>
<td>32</td>
</tr>
<tr>
<td>Uniformly abnormal</td>
<td>9 (9)</td>
<td>0</td>
</tr>
<tr>
<td>Mosaic aneuploid</td>
<td>11 (11)</td>
<td>9</td>
</tr>
<tr>
<td>Mosaic ploidy</td>
<td>6 (6)</td>
<td>1</td>
</tr>
<tr>
<td>Mosaic chaotic</td>
<td>40 (39)</td>
<td>13</td>
</tr>
<tr>
<td>Other</td>
<td>5 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>103 (100)</td>
<td>55</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.
upon the rate of chromosomally abnormal pre-embryos at any of the time-points.

**Cytoplasmic appearance**

Evaluation of the appearance of the cytoplasm in blastomeres was not related to the rate of chromosomally abnormal pre-embryos at any time-point.

**Early cleavage**

Early cleavage was defined as cleaved pre-embryos 26 h after insemination. There was no correlation between time of first cleavage and chromosomal abnormality rate.

**Good quality pre-embryos**

Analysing the subset of transferable pre-embryos consisting of pre-embryos with \( \geq 6 \) blastomeres and \( < 20\% \) fragmentation at 68 h post insemination resulted in a significant increase in the rate of chromosomally overall normal pre-embryos, from 53\% (55/103 normal pre-embryos) to 67\% (32/48 normal pre-embryos) \((P = 0.012)\).

Adding an extra selection criteria of equally sized blastomeres to the group of transferable pre-embryos increased the rate of chromosomally overall normal pre-embryos to 75\% (21/28 normal) which was significantly increased compared with the total group \((P < 0.007)\) (Figure 1).

The rate of chromosomally normal blastomeres was significantly increased with pre-embryo selection ranging from 55\% in the total group to 65\% in the transferable group \((P < 0.0001)\) and to 68\% in the selected group \((P < 0.0001)\).

The rate of uniformly normal pre-embryos increased nonsignificantly from 31\% in the total group to 43\% in the selected group \((P = 0.011)\).

**Kappa analyses**

The kappa values were calculated in order to express the degree of variation between the embryologists performing independent assessment of cleavage and morphology at 68 h after insemination. Overall the concordance between the three embryologists at reassessment observations was high. The highest level of agreement was found for cleavage rate (Kappa value 0.89–0.93). The kappa value for classification of fragmentation ranged from 0.82 to 0.92.

**Discussion**

The data presented here demonstrate that selected morphological features were indicative of the chromosomal status of the pre-embryo. Applying strict morphological criteria when selecting pre-embryos for transfer considerably increased the chance of selecting a chromosomally overall normal embryo from 53 to 75\%.

Selection of pre-embryos based on the morphological appearance of the pre-embryo increased the percentage of chromosomally normal blastomeres in the embryo from 55 to 68\%. The number of blastomeres in the pre-embryo that must be chromosomally normal in order to produce a viable pregnancy needs further clarification.

In the present study, an overall normal pre-embryo was defined as a pre-embryo with \( \geq 50\% \) of its cells having a normal diploid constitution for the chromosomes tested. While 50\% is an arbitrary threshold, this level is supported by a study of Bialanska et al. (2002), which suggested that the developmental potential of the pre-embryo may be impaired if the majority of its cells are abnormal.

We found that 31\% of the pre-embryos were uniformly normal, having a normal diploid constitution in all blastomeres. This is in accordance with the finding by Iwarsson et al. (1999), who found 27.9\% uniformly normal pre-embryos on frozen-thawed good morphology pre-embryos when testing for six chromosomes, and by Bialanska et al. (2002), who found 29.6\% uniformly normal pre-embryos in surplus embryos when analysing for three to five chromosomes.

The present study, based on whole pre-embryo analysis, from prospectively randomly selected donated oocytes from patients in an assisted reproductive programme, showed that 45\% of all blastomeres are chromosomally abnormal. When assessing the effect of aneuploidy screening as reported in a number of studies (Magli et al., 1998; Gianaroli et al., 1999; Munné et al., 1999; Kahraman et al., 2000), it is important to...
consider that these studies based the evaluation of the total pre-embryo upon the results from only one or two biopsied blastomeres. Our results indicate that close to 50% of all blastomeres carry an abnormal chromosomal constitution, and suggest that the risk of misdiagnosis could be considerable. However, the chance of correct diagnosis increases significantly when applying pre-embryo selection based on morphology and development prior to biopsy, as shown in this study as well as in other studies (for review see Pellestor, 1995).

Despite our finding that morphological evaluation does appear to assist the avoidance of cytogenetically abnormal embryos, a significant proportion of the good quality embryos harbour chromosome abnormalities that are likely to be lethal. It is in the detection of these embryos that preimplantation genetic diagnosis provides an advantage.

Our data demonstrate a significant increase in chromosomal abnormalities with increasing female age. While this is not a novel finding, it is important to realize that this increase was found despite the limited female age range in the inclusion criteria of 25–37 years.

The importance of morphological evaluation of the pre-embryos was illustrated by the findings that pre-embryo selection based on homogeneity of blastomere size, degree of fragmentation and cleavage stage significantly increased the ratio of chromosomally normal pre-embryos from 53 to 75%. Previous studies (Cummins et al., 1986; Puissant et al., 1987; Staessen et al., 1992; Shulman et al., 1993; Giorgetti et al., 1995; Ziebe et al., 1997) have demonstrated that pre-embryo morphology is the key tool when selecting pre-embryos with the highest implantation potential. A number of morphology parameters including impaired cleavage rates, high degree of fragmentation, differences in blastomere size and the presence of multinucleated blastomeres have previously been shown to influence negatively the implantation potential of the pre-embryo. The present study indicates that this may in part be caused by chromosomal errors in these pre-embryos.

We found that the presence of unevenly sized blastomeres was associated with a significant increase in chromosomal abnormality rate. This is in line with the finding from previous studies (Hardarson et al., 2001; Magli et al., 2001). This may explain why transferred pre-embryos with unevenly sized blastomeres, or uneven cell numbers often having different size, have a significantly lowered implantation and pregnancy potential (Giorgetti et al., 1995; Ziebe et al., 1997; Hardarson 2001).

Increasing amounts of fragmentation in the pre-embryos at 68 h after fertilization was significantly correlated with increased chromosomal abnormality rates. This finding is in accordance with previous publications (Bongso et al., 1991; Munné et al., 1995; Magli et al., 2001). Assuming that an increased chromosomal abnormality rate is associated with a decreased implantation and pregnancy potential, this could explain the lowered implantation and pregnancy rates after transfer of fragmented pre-embryos as found in several studies (Staessen et al., 1992; Giorgetti et al., 1995; Ziebe et al., 1997; Ebner et al., 2001). Ebner et al. (2001) found an increased malformation rate after transfer of highly fragmented pre-embryos and the authors concluded that this might be due to a higher percentage of chromosomal disorders.

Previous studies (Giorgetti et al., 1995; Ziebe et al., 1997) have demonstrated that increasing amounts of fragments assessed at 44 h were associated with compromised implantation and pregnancy rates. We found no correlation between degree of fragmentation in the pre-embryos scored at 44 h after fertilization and the chromosomal abnormality rate. One reason for this discrepancy could be that fragmentation may be indicative of other quality-compromising errors in the pre-embryo, and it is possible that the effect of the chromosomal complement was not yet manifest.

It has been suggested that pre-embryos should cleave with an optimal cleavage rate (Cummins et al., 1986; Staessen et al., 1992; Giorgetti et al., 1995; Claman et al., 1987; Ziebe et al., 1997; Magli et al., 2001) with too fast or too slow cleavage rates indicating a compromised developmental potential. In this study, pre-embryos with a slow cleavage rate resulting in <6 cells at 68 h after fertilization had a tendency (P = 0.011) towards an increased chromosomal abnormality rate.

The chromosomes selected for analysis in this study are most often associated with trisomies with potential of delivery, i.e. chromosome X, Y, 13, 18 and 21 and with trisomies frequently found in spontaneous abortions, i.e. chromosome 16 and 22 (Munné et al., 1999). The low rate of multiple chromosome abnormalities found in spontaneous abortions (Reddy, 1997) suggests that severe errors, such as chaotic pre-embryos, are incompatible with sustained development.

The blastomeres in this study were analysed for seven chromosomes. Studies using comparative genomic hybridization (CGH) analysing for all chromosomes suggest that 25% of the pre-embryos are uniformly normal in all blastomeres, and that 50–58% of the pre-embryos have ≥50% of their blastomeres normally diploid. However, these studies were performed on surplus pre-embryos that were either selected for low fragmentation and normal development (Wells and Delhanty, 2000) or frozen pre-embryos cultured further after thawing (Voullaure et al., 2000). Therefore the true abnormality rate in an unselected pool of pre-embryos from assisted reproduction remains unknown. However, there is a striking correlation between our findings and the abnormality rates reported in these studies.

Previous studies applying FISH analysis to oocytes have shown that the majority of oocytes aspirated in IVF cycles are chromosomally normal (Plachot, 2001; Honda et al., 2002; Pellestor et al., 2002). This is in agreement with the chromosomal abnormalities derived from meiotic errors found in this study (9%). This suggests that the majority of errors occur at the postzygotic stage, either in oocytes that are already compromised but in which the effect is not yet seen in the chromosomes, or in oocytes whose in-vitro environment is insufficient, causing errors in cell cycle regulation.

In conclusion, we found a large genetic heterogeneity in a randomly selected pool of pre-embryos from couples participating in an assisted reproductive programme. Further, this study demonstrates that a number of morphological parameters such as the homogeneity of blastomere size, degree of fragmentation and presumably cleavage rate to a large extent
reflect the cytogenetic status of the pre-embryo and thus are important in the selection of pre-embryos with the highest implantation potential. There is still an urgent need to clarify how 'normal' a pre-embryo needs to be in order to be able to implant and give rise to a healthy baby. We do not know to what extent chromosomal abnormalities compromise the developmental potential of the pre-embryo and whether any corrective mechanism exists within the pre-embryo that may compensate for various degrees of chromosomal errors. Further, the chromosomal errors demonstrated in this and other studies may not be the causal reason for impaired pre-embryo quality but could rather be a result of an incompetent or immature cytoplasm affecting a number of parameters in the pre-embryo—including normal segregation of chromosomes.

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References


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