Mosaic and chaotic embryos were excluded from the successfully tested group. The statistical difference in the portion of fragmented and condensed nuclei was reliable only between 4- and 5-cell stages (0.01 > P_{(x2)} > 0.001). Nevertheless, the percentage of successfully tested 3-, 4- and 5-cell embryos was similar because of the exclusion of mosaic embryos.

Tendencies noted by us suggest that the minimal blastomere fragmentation (up to 20% of perivitelline space) does not preclude the normal nuclear status allowing successful ploidy testing. The presence of condensed chromatin is a critical factor for interphase cytogenetic investigations in single early blastomeres.

**Discussion**

Various mitotic rate and morphology patterns can be observed among human preimplantation embryos obtained and cultured at identical conditions and even derived from the same patients using the same protocol for ovarian stimulation. This diversity originates during gametogenesis and cleavage. Probably some disturbances of nucleo-cytoplasmic interactions leading to cell and nuclear abnormalities are involved (Evsikov and Verlinsky, 1998). There are three possible explanations of the link between cell fragmentation and apoptosis in cleaving embryos. First, if apoptotic changes of chromatin at this stage are provoked by blastomere fragmentation, the fragmentation must occur earlier than chromatin condensation; second, if the chromatin damage is the reason for blastomere death, the cell fragmentation must be the later process; finally, these processes might be unrelated. In the literature there are data supporting all of these schemes to be probable in mammalian pre-embryos (Warner et al., 1998; Antczak et al., 1999). Our results are closer to the first mechanism and do not exclude the last one. The evidence for activity of genes responsible for PCD and early steps of apoptosis in cleaving embryos supports the same idea (Jurisicova et al., 1996). DNA breaking is proved by positive terminal transferase-mediated DNA end labelling (TUNEL) reaction in blastomere nuclei with chromatin appearance similar to those observed by us. Taking into account these data and the pictures of apoptotic nuclei previous-

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**Table 1. Proportion of abnormal nuclei in class A and B embryos**

<table>
<thead>
<tr>
<th>Class</th>
<th>No. of embryos</th>
<th>No. of tested cells</th>
<th>Fragmented nuclei</th>
<th>Nuclei with condensed chromatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>75</td>
<td>19 (25%)</td>
<td>14 (19%)</td>
</tr>
<tr>
<td>B</td>
<td>22</td>
<td>81</td>
<td></td>
<td>14 (17%)</td>
</tr>
</tbody>
</table>

**Table 2. Proportion of successfully tested 3-, 4-, and 5-cell stage embryos**

<table>
<thead>
<tr>
<th>Cleavage stage</th>
<th>No. of embryos</th>
<th>% of fragmented nuclei</th>
<th>% of condensed nuclei</th>
<th>Embryos with determined ploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 cells</td>
<td>14</td>
<td>17%</td>
<td>19%</td>
<td>11 (79%)</td>
</tr>
<tr>
<td>4 cells</td>
<td>20</td>
<td>15%</td>
<td>10%</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>5 cells</td>
<td>8</td>
<td>23%</td>
<td>30%</td>
<td>6 (75%)</td>
</tr>
</tbody>
</table>
ly described in various tissues as well as in mammalian preimplantation embryos (El-Shershaby and Hinchcliffe, 1974; Mohr and Trounson, 1982; Kerr et al., 1987), we can accept that the chromatin anomalies registered in our study were related to apoptosis.

It appears that some checkpoints of the cell cycle are eliminated in mammalian preimplantation embryos, allowing accumulation of faults of chromosome distribution. The checkpoints are introduced at some point during cleavage, leading to normal cell cycle control at the blasocyst stage (Hartwell and Weinert, 1989; Eviskov and Verlinsky, 1998). It is supposed that at the morula- blasocyst transition a negative selection starts against aneuploid cells by PCD in order to prevent their involvement in the embryonic inner cell mass. Some critical level of aneuploidy results in self-destruction of the embryo. The percentage of aneuploid and mosaic embryos is substantial at the cleavage stage – from 20 to more than 50% according to different authors (Plachot et al., 1989; Coonen et al., 1994; Handside, 1996). It is noted that blastomere morphology corresponds to the aneuploidy at this stage, but no relation between the cleavage rate and correctness of chromosome complement has been demonstrated (excluding arrested embryos) (Pellestor et al., 1994; Magli et al., 2000). Therefore, we consider it premature at this stage to discuss our percentage of aneuploid and mosaic embryos. In our work apoptotic changes were observed in blastomeres without surrounding fragments, i.e. in some of the analysed 3–5-cell embryos the injury appears first in the nucleus and it is not necessarily accompanied with poor cell morphology. Nearly one third of blastomeres tested by us had nuclei damaged to some extent. The fact that the embryonic cell number was 3 to 5 was probably significant for our results. We must have in view that the presence of less than 5 blastomeres at the third day post in vitro insemination is considered a cleavage anomaly and is related to a negative prognosis for embryo survival (Betteridge, 1995). The authors who perform preimplantation ploidy investigations report FISH failures caused by biopsy of multinucleated or enucleated blastomeres, but they do not comment on them in the context of apoptotic changes of the chromatin. In order to decrease the risk of biopsy of abnormal blastomere, at the reproductive centres applying preimplantation genetic diagnosis (PGD) it is accepted that more suitable for their aim are the embryos with at least 7 cells at the third day post in vitro insemination – such mitotic rate is a criterion for good developmental potential and allows biopsy of two blastomeres per embryo (Munne et al., 1993; Vandervorst et al., 1998; De Vos and Van Steirteghem, 2001). It is possible that at the 7–8-cell stage the chromatin damage is more closely related to cytoplasmic fragmentation, making the affected cells easier to identify.

**References**


