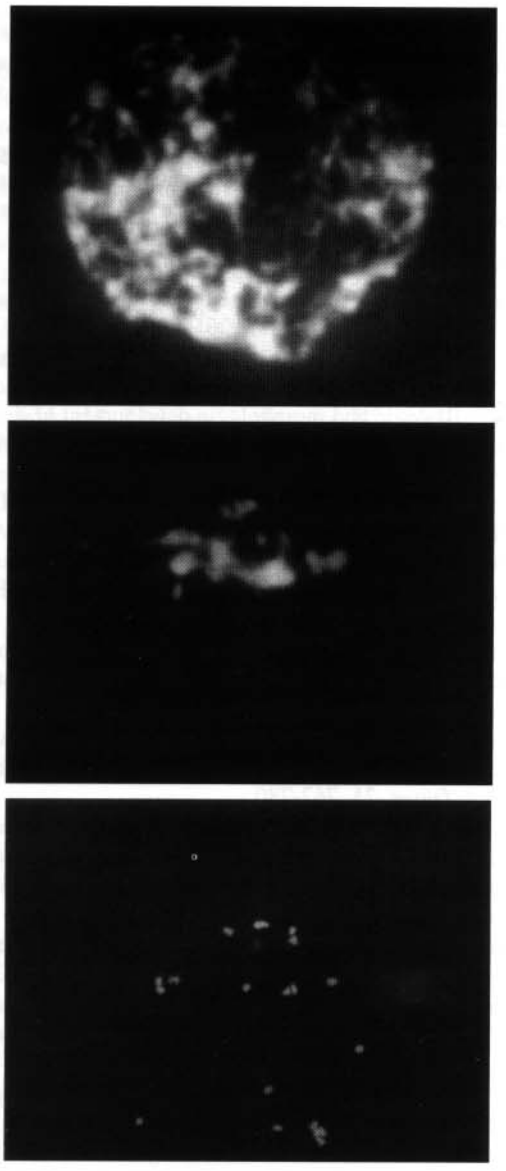

Fig. 3. Subsequent stages of chromatin condensation in blastomere nuclei
A/ an early stage of chromatin condensation;
B/ advanced chromatin condensation;
C/ dense chromatin bodies.

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 ($0.01 > P_{(\chi 2)} > 0.001$). 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 preembryos (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 these observed by us. Taking into account these data and the pictures of apoptotic nuclei previous-

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

Class	No. of embryos	No. of tested cells	Fragmented nuclei	Nuclei with condensed chromatin
A 20	75	19 (25%)	14 (19%)	
B 22	81	15 (19%)	14 (17%)	

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

Cleavage stage	No. of embryos	% of fragmented nuclei	% of condensed nuclei	Embryos with determined ploidy
3 cells	14	17%	19%	11 (79%)
4 cells	20	15%	10%	15 (75%)
5 cells	8	23%	30%	6 (75%)

ly described in various tissues as well as in mammalian preimplantation embryos (El-Shershaby and Hinchliffe, 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 blastocyst stage (Hartwell and Weinert, 1989; Evsikov and Verlinsky, 1998). It is supposed that at the morula-blastocyst 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; Handyside, 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

Alikani, M., Calderon, G., Tomkin, G., Garrisi, J., Kokot, M., Cohen, J. (2000) Cleavage anomalies in early human embryos and survival after prolonged culture in vitro. *Hum. Reprod.* **14,** 2634-2643.

Antczak, M., Van Blerkom, J. (1999) Temporal and spatial aspects of fragmentation in early human embryos: possible effects on developmental competence and association with the differential elimination of regulatory proteins from polarized domains. *Hum. Reprod.* **14,** 429-447.

Betteridge, K. J. (1995) Phylogeny, ontogeny and embryo transfer. *Theriogenology* **44,** 1061-1098.

Buster, J. E., Busillo, M., Rodi, I. A., Cohen, S. W., Hamilton, M., Simon, J. A., Thorneycroft, I. H., Marshall, J. R. (1985) Biologic and morphologic development of donated human ova recovered by nonsurgical uterine lavage. *Am. J. Obstet. Gynecol.* **153,** 211-217.

Coonen, E., J. C. Harper, F. C. S. Ramaekers, J. D. A. Delhanty, A. H. N. Hopman, J. P. M. Geraedts, A. H. Handyside (1994) Presence of chromosomal mosaicism in abnormal preimplantation embryos detected by FISH. *Hum. Genet.* **94,** 609-615.

Delimitreva, S., Zhivkova, R., Vatev, I. (2001) Supernumerary human preembryos provide potential for preimplantation genetic diagnosis. *Folia Biol. (Praha)* **47,** 88-91.

De Vos, A., Van Steirteghem, A. (2001) Aspects of biopsy procedure prior to preimplantation genetic diagnosis. *Prenat. Diagn.* **21,** 767-780.

El-Shershaby, A. M., Hinchliffe, J. R. (1974) Cell redundancy in the zona-intact preimplantation mouse blastocyst: a light and electron microscope study of dead cells and their fate. *J. Emb. Exp. Morphol.* **31,** 643-654.

Evsikov, S., Verlinsky, Y. (1998) Mosaicism in the inner cell mass of human blastocysts. *Hum. Reprod.* **13,** 3151-3155.

Giorgetti, C., Terrou, P., Auquier, P. (1995) Embryo score to predict implantation after in vitro fertilization: based on 957 single embryo transfers. *Hum. Reprod.* **10,** 1427-2431.

Handyside, A. H. (1996) Mosaicism in the human preimplantation embryo. *Reprod. Nutr. Dev.* **36,** 643-649.

Hardy, K., Winston, R. M. L., Handyside, A. H. (1993) Binucleate blastomeres in preimplantation human embryos in vitro: failure of cytokinesis during early development. *J. Reprod. Fertil.* **98,** 549-558.

Hartwell, L. H., Weinert, T. A. (1989) Checkpoints: controls that ensure the order of cell cycle events. *Science* **246,** 629-634.

Hoover, L., Baker, A., Check, J. H., Lurie, D., O'Shaughenessy, A. (1995) Evaluation of a new embryo-grading system to predict pregnancy rates following in vitro fertilization. *Gynecol. Obstet. Invest.* **40,** 151-157.

Jurisicova, A., Varmuza, S., Casper, R, F. (1996) Programmed cell death and embryo fragmentation. *Mol. Hum. Reprod.* **2,** 93-98.

Kerr, J. F. R., Searle, J., Harmon B. V., Bishop, C. J. (1987) Apoptosis. In: *Perspectives on Mammalian Cell Death,* ed. Potten, C. S., pp. 93-128, Oxford University Press, Oxford.

Magli, M. C., Jones, G. M., Gras, L., Gianarolli, L., Korman, I., Trounson, A. O. (2000) Chromosome mosaicism in day 3 aneuploid embryos that develop to morphologically normal blastocyst in vitro. *Hum. Reprod.* **15,** 1781-1786.

Mohr, L. R., Trounson, A. O. (1982) Comparative ultrastructure of hatched human, mouse and bovine blastocysts. *J. Reprod. Fertil.* **66,** 506-509.

Munne, S., Weier, H.-U.G., Stein, J., Grifo, J., Cohen, J. (1993) A fast and efficient method for simultaneous X and Y in situ hybridization of human blastomeres. *J. Assist. Reprod. Genet.* **10,** 82-90.

Munne, S., Alikani, M., Cohen, J. (1994) Monospermic polyploidy and atypical embryo morphology. *Hum. Reprod.* **9,** 506-510.

Pellestor, F., Girardet, A., Andreo, B., Arnal, F., Humeau, C. (1994) Relationship between morphology and chromosomal constitution in human preimplantation embryo. *Molec. Reprod. Dev.* **39,** 141-146.

Plachot, M., Mandelbaum, J., Junca, A. M., de Grouchy, J., Salat-Baroux, J., Cohen, J. (1989) Cytogenetic analysis and developmental capacity of normal and abnormal embryos after IVF. *Hum. Reprod.* **4,** (suppl.), 99-103.

Vandervorst, M., Liebaers, I., Sermon, K., Staessen, C., De Vos, A., Van de Velde H., Van Assche, E., Joris, H., Van Steirteghem, A., Devroey, P. (1998) Successful preimplantation genetic diagnosis is related to the number of available cumulus-oocyte complexes. *Hum. Reprod.* **13,** 3169-3176.

Vatev, I. (1988) Human in vitro fertilization and embryo transfer program at the Medical Academy, Sofia, Bulgaria. *J. In Vitro Fert. Embryo Transfer* **5,** 48-49.

Vatev, I. Ts., Tarlatzis, B. C., Dukaki, C., Bontis, J., Lagos, S., Mantalenakis, S. (1993) Human assisted fertilization by micromanipulation. *Hum. Reprod.* **8,** 121.

Vatev, I., Karagyozov I., Istatkov, M., Srebreva, M. (1998) Successful application of the fallopian sperm perfusion method and intracytoplasmic sperm injection (ICSI) technique for treatment of human infertility. *Balkan J. Med. Genet.* **3,** 115-119.

Warner, C. M., Cao, W., Exley, G. E., McElhinny, A. S., Alikani, M., Cohen, J., Scott, R. T., Brenner, C. A. (1998) Genetic regulation of egg and embryo survival. *Hum. Reprod.* **13,** 178-196.