

50% fragmented cells, granulated cytoplasm and/or arrested cell division. More than 60% of their nuclei were fragmented or had an apoptotic appearance – from relatively dispersed joined parts to entirely separated small fragments. Some blastomeres with normal shape and size had two to four not very small nuclei with typical interphase chromatin. The total number of fluorescent signals per multinucleated blastomere varied. It is an important fundamental question whether this fragmentation is a sign of beginning apoptosis, whether the abnormal chromosomal status causes eventual apoptosis or whether this abnormal chromosomal number is a consequence of fragmentation due to other reasons (Warner et al., 1998; Antczak and Van Blerkom, 1999; Iwarsson et al., 1999; Pampfer and Donnay, 1999). It is recommended to perform biopsy of mononucleated blastomeres only (Munne et al., 1994).

Although we analysed a rather small group of embryos, our data for FISH – success and chromosomal mosaicism – are close to these in other studies. The mosaicism is the major methodology limit of preimplantation ploidy diagnosis because it introduces an inherent possibility of errors (Coonen et al., 1994; Handyside, 1996; Verlinsky et al., 1997; Evsikov and Verlinsky, 1998). Misdiagnosis or doubt diagnosis due to mosaicism could be reduced by analysing two blastomeres biopsied from an eight-cell embryo – this is the approach in the centers which report on live-born healthy children after IVF-PGD procedures (Delhanty et al., 1993; Handyside, 1996; Verlinsky et al., 1996).

References

- 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.
- Benadiva C., Kligman, I., Munne, S. (1996) Aneuploidy 16 in human embryos increases significantly with maternal age. *Fertil. Steril.* **66**, 248-255.
- Coonen, E., Harper, J. C., Ramaekers, F. C. R., Delhanty, J. D. A., Hopman, A. H. N., Geraedts, J. P. M., Handyside, A. H. (1994) Presence of chromosomal mosaicism in abnormal preimplantation embryos detected by FISH. *Hum. Genet.* **94**, 609-615.
- Delhanty, J. D., Griffin, D. K., Handyside, A. H., Harper, J., Atkinson, G. H. G., Pieters, M. H. E. C., Winston, R. M. L. (1993) Detection of aneuploidy and chromosomal mosaicism in human embryos during preimplantation sex determination by fluorescent in situ hybridisation (FISH). *Hum. Mol. Genet.* **2**, 1183-1185.
- Delimitreva, S., Zhivkova, R., Janakiev, P., Vatev, I. (1998) Pilot models of preimplantation genetic diagnosis by PCR and FISH in human gametes and cleaving embryos. *Comptes rendus de l'Academie bulgare des Sciences* **51**, 107-110.
- Evsikov, S., Verlinsky, Y. (1998) Mosaicism in the inner cell mass of human blastocysts. *Hum. Reprod.* **13**, 3151-3155.
- Handyside, A. H., Pattison, J. K., Penketh, R. J. A., Delhanty, J. D. A., Winston, R. M. L., Tuddenham, E. G. D. (1989) Biopsy of human preimplantation embryos and sexing by DNA amplification. *Lancet* **1**, 347-349.
- Handyside, A. H., Lesko, J. G., Tarin, J. J., Winston, R. M. L., Hughes, M. R. (1992) Birth of a normal girl after in vitro fertilization and preimplantation diagnosis testing for cystic fibrosis. *N. Engl. J. Med.* **327**, 905-909.
- Handyside, A. H. (1996) Mosaicism in the human preimplantation embryo. *Reprod. Nutr. Dev.* **36**, 643-649.
- Iwarsson, E., Lundqvist, M., Inzunza, J., Ahrlund-Richter, L., Sjoblom, P., Lundqvist, O., Simberg, N., Nordenskjold, M., Blennow, E. (1999) A high degree of aneuploidy in frozen-thawed human preimplantation embryos. *Hum. Genet.* **104**, 376-382.
- Munne, S., Weier, H.-U. G., Stein, J., Grifo, J., Cohen, J. (1993) Reproductive genetics. A fast and efficient method for simultaneous X and Y in situ hybridisation 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.
- Munne, S., Weier, H.-U. G. (1996) Simultaneous enumeration of chromosomes 13, 18, 21, X and Y in interphase cells for preimplantation genetic diagnosis of aneuploidy. *Cytogen. Cell Genet.* **75**, 263-270.
- Pampfer, S., Donnay, I. (1999) Apoptosis at the time of embryo implantation in mouse and rat. *Cell Death Differ.* **6**, 533-545.
- Pellicer, A., Rubio, C., Vidal, F., Minguez, Y., Gimenez, C., Egozcue, J., Remohi, J., Simon, C. (1999) In vitro fertilization plus preimplantation genetic diagnosis in patients with recurrent miscarriage: an analysis of chromosome abnormalities in human preimplantation embryos. *Fertil. Steril.* **71**, 1033-1039.
- Staessen, C., Van Steirteghem, A. C. (1997) The chromosomal constitution of embryos developing from abnormally fertilized oocytes after intracytoplasmic sperm injection and conventional in-vitro fertilization. *Hum. Reprod.* **12**, 321-327.
- Vandervorst, M., Liebars, 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 Transf.* **5**, 48-49.
- Vatev, I., Tarlatzis, B., Delimitreva, S. (1997) Possible use of intracytoplasmic sperm injection in some cases with male and female immunological infertility. *J. Assist. Reprod. Genet.* **14**, S122.
- Vatev, I., Fitchev, P., Tabakova, P., Dimitrov, M., Yanakieva, T., Yordanov, V., Vakrilov, G. (1990) The in vitro fertilization and embryo transfer program at the First Woman's Hospital "T. Kirkova", Sofia, Bulgaria. *J. Assist. Reprod. Genet.* **7**, 119-122.
- Vatev, I., Karagyozov, I., Istatkov, M., Srebrev, M. (1998) Successful application of the fallopian sperm perfusion method and intracytoplasmic sperm injection (ICSI) technique for the treatment of human infertility. *Balkan J. Mol. Genet.* **3**, 115-119.
- Vatev, I., Tarlatzis, B. C., Dukaki, C., Bontis, J., Lagos, S., Mantalenakis, S. (1993) Human assisted fertilization by micromanipulation. *Hum. Reprod.* **8**, 121-124.

Verlinsky, Y., Rechitsky, S., Cieslak, J., Strom, C., Lifchez, A. (1996) Birth of a healthy girl after preimplantation gender determination using a combination of polymerase chain reaction and fluorescent in situ hybridization analysis. *Fertil. Steril.* **65**, 358-360.

Verlinsky, Y., Munne, S., Simpson, J. L., Kuliev A., Ray, P., Sermon, K., Martin, R., Strom, C., Van Seirteghem, A.,

Veiga, A., Drury, K., Williams, S., Ginsberg, N., Wilton, L. (1997) Current status of preimplantation diagnosis. *J. Assist. Reprod. Genet.* **14**, 72-75.

Warner, C. M., Cao, W., Exley, G. E., McElhinny, A. S., Alicani, M., Cohen, J., Scott, R. T., Brenner, C. A. (1998) Genetic regulation of egg embryo survival. *Hum. Reprod.* **13**, Suppl. 3, 178-190.