

The Status of the Chromatin of Human Preimplantation Embryos with Good Morphology

(chromatin / cell fragmentation / FISH / IVF / preimplantation embryo)

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Abstract. The data about the relation and succession of blastomere fragmentation, cleavage rate and chromatin anomalies in preimplantation mammalian embryos are empirical and controversial at present. In this work we studied the proportion of nuclear fragmentation and condensation in 3–5-cell stage human embryos with no or minimal blastomere fragmentation (morphological class A and B, respectively) and the possibilities to perform FISH chromosomal analyses with them. We observed different stages of chromatin damage in blastomere nuclei corresponding to the steps of nuclear apoptotic changes well known in many cell types. The ploidy analysis of chromosomes 1, 5, 19 and X was determined as successful in embryos which had at least 2 out of 3, 3 out of 4 or 3 out of 5 normal nuclei with an equal number of FISH signals. There was no difference in the percentage of abnormal nuclei among the A- and B-class 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 analysis of single early blastomeres.

A substantial part of mammalian embryos are lost before their implantation into the uterus. This applies to both *in vivo* and *in vitro* fertilization (Buster et al., 1985; Munne et al., 1994; Giorgetti et al., 1995). The preimplantation embryo destruction can be provoked by cell cycle errors leading to genomic anomalies, by the *in vitro* fertilization (IVF) procedure *per se*, cultivation conditions and probably some unknown factors. It is accepted that genetic mechanisms of programmed cell death (PCD) with subsequent apoptosis are involved (Hardy et al., 1993; Hoover et al., 1995; Jurisicova et al., 1996). In this way, the rate of aneuploidy, cell and nuclear fragmentation and blastomere number are the main criteria for assessment of *in vitro* derived human

preimplantation embryos prior to intrauterine transfer. The data about relations and succession of these phenomena are empirical and controversial at present – according to some investigations the blastomere fragmentation in cleaving embryos illustrates the apoptotic process (Jurisicova et al., 1996; Warner et al., 1998); other studies support the opposite thesis that apoptosis in early preembryos can be a consequence rather than a cause of fragmentation (Antczak et al., 1999); some authors suggest that cell morphology and chromosomal disorders are related, but on the other hand there is no evidence that genome and chromosomal errors impair preimplantation development (Coonen et al., 1994; Alikani et al., 2000).

In our work we studied the proportion of nuclear condensation and fragmentation in 3–5-cell stage human embryos with no or minimal blastomere fragmentation and the possibilities to perform fluorescent *in situ* hybridization (FISH) chromosomal analyses with them.

Material and Methods

The IVF, intracytoplasmic cell injection (ICSI) and embryo transfer procedures were carried out according to the protocols applied in the Human IVF Unit "Technobioassistance", based at the Department of Biology, Medical Faculty of Sofia (Vatev, 1988; Vatev et al., 1993; Vatev et al., 1998). The investigated embryos were donated for research purposes in the cases when more than the required number of embryos with good morphology and adequate mitotic rate were available on the day of embryo transfer (there is no legislation concerning embryo donation and cryopreservation in Bulgaria). The investigation was approved by the Ethical Committee of the Preclinical University Centre – Medical University of Sofia, and the patients gave informed consent for the supernumerary embryos to be used for scientific research.

We used 42 preembryos at the stage of 3–5 cells with no or minimal blastomere fragmentation. All of them originated from normally fertilized two-pronucleated zygotes and the cell division was registered less than 24 hours before fixation (to exclude "arrested" embryos). The embryo grading system with 4 morphological

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Abbreviations: FISH – fluorescent *in situ* hybridization, FITC – fluorescein isothiocyanate, ICSI – intracytoplasmic cell injection, IVF – *in vitro* fertilization, PCD – programmed cell death.

classes from A to D, accepted in many IVF centers, was applied (Hoover et al., 1995; Vandervorst et al., 1998). Twenty of the tested embryos were subdivided in class A (with no blastomere fragmentation) and 22 of them in class B (the volume of cell fragments occupied up to 20% of the perivitelline space).

Zonae pellucidae were removed by Tyrode solution, pH 2.5, adjusted by HCl. The embryos were incubated for 20 min at room temperature in hypotonic solution (1% sodium citrate with 5% bovine serum albumin (BSA) to prevent adhesion to the dish), dropped in minimal volume onto slides immediately cleaned by acetone and fixed by methanol/acetic acid (3 : 1). Fixation was controlled under a phase-contrast microscope. The fixative was dropped until the complete disappearance of the cytoplasm. FISH was carried out according to Munne et al. (Munne et al., 1993; Delimitreva et al., 2001). Directly FITC (fluorescein isothiocyanate)-labelled probes (Boehringer Mannheim GmbH, Mannheim, Germany) specific for centromeric regions of chromosomes 1, 5, 19 were denatured simultaneously with nuclear DNA and hybridized for 5 h. The nuclei were counterstained with propidium iodide (1 mg/ml) diluted in glycerol/PBS (9 : 1). Antifade reagent DABCO (3% diazabicyclo[2.2.2]octane – Sigma, St. Louis, MO) was supplied and fluorescence of DNA was observed by Zeiss Aksioscop 20 (Zeiss, Oberkochen, Germany). After washing in PBS, pH 8, with 1% Nonidet-P40 (Sigma) and dehydration in ethanol (70, 85 and 100%), the same hybridization procedure for detection of the X chromosome was performed using FITC-labelled centromeric probes.

Results

A hundred and fifty-six blastomeres were tested. The nuclei of 94 of them with typical interphase chromatin with homogeneous fluorescence or mitotic chromosomes were classified as normal. Successful FISH reaction was registered in all normal nuclei (Fig. 1). Fragmented or flocculent nuclei were observed in 34 blastomeres. In five of them there were 2–3 separate

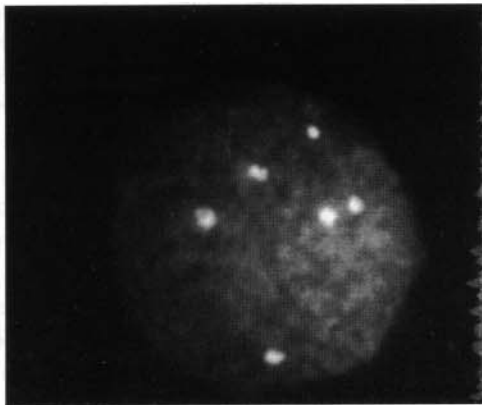


Fig. 1. FISH reaction for chromosomes 1, 5 and 19 (6 spots) in an interphase blastomere nucleus

fragments in a blastomere, up to 8 flocculi were joined like clover leaves in the others. When the chromatin appeared relatively homogeneous, some FISH signals were visible in the fragments, but their number was chaotic and was not usable as a result (Fig. 2). Twenty-eight blastomeres had unfragmented nuclei at different stages of condensation. No FISH signals were seen in the nuclei or nuclear fragments at any stage of chromatin condensation.

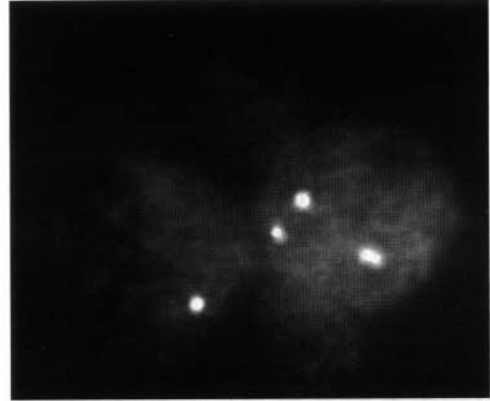


Fig. 2. Abnormal number of FISH signals for chromosomes 1, 5, and 19

According to our observations, the probable chronology of chromatin condensation in the blastomere of an early cleaving human embryo is the following: the fluorescence becomes uneven, the chromatin forms at first a fine network, later the structure becomes rougher, relatively large bright and dark regions appear in the nuclei (Fig. 3a); the contrasting parts increase their size, but links between them are still visible (Fig. 3b), the nuclear volume decreases significantly at this stage; in the next stage the chromatin disperses into small oval dense pieces (Fig. 3c). In fact, these pictures correspond to the steps of nuclear apoptotic changes well known in many cell types (Mohr and Trounson, 1982; Kerr et al., 1987; Jurisicova et al., 1996).

Although our group of embryos was small, some tendencies were noticed. We did not observe a statistically reliable difference in the percentage of abnormal nuclei among the embryo classes A and B ($P_{(\chi^2)} > 0.05$). The portion of fragmented and condensed nuclei for A-class embryos was 25% and 19%, respectively, and for B-class 19% and 17% (Table 1).

The proportion of successfully tested 3-, 4- and 5-cell embryos was described in Table 2. The ploidy analysis of tested chromosomes was determined as successful in 32 embryos, which had at least 2 out of 3, 3 out of 4 or 3 out of 5 normal nuclei with an equal number of FISH signals. Two of them had an abnormal number of some of the identically labelled 1, 5 and 19 chromosomes. Mosaic distribution of the X chromosome was found in four embryos and in one embryo, chromosomes 1, 5 and 19 were distributed chaotically.