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Programmed cell death and human embryo fragmentation

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The quality of embryos produced by in-vitro fertilization (IVF) is variable. Many embryos contain unequal sized blastomeres and multiple cellular fragments. Embryos with excessive fragmentation have limited developmental potential both *in vitro* and *in vivo*. Histologically, some blastomeres of fragmented embryos resemble cells undergoing apoptosis as a result of programmed cell death (PCD). The objective of the present study was to determine if the morphological features of apoptosis are observed in fragmented human preimplantation embryos, supporting the possible involvement of PCD in early human embryo arrest and demise. Using combined nuclear and terminal transferase-mediated DNA end labelling (TUNEL) on arrested, fragmented human embryos, we were able to detect extensive condensation and degradation of chromatin, compatible with apoptosis. Electron microscopy confirmed the typical morphological features of apoptosis. No such abnormalities were observed in spare embryos with regular sized blastomeres without fragmentation. The high incidence of condensed chromatin, TUNEL detection of degraded DNA, cell corpses and apoptotic bodies in fragmented human embryos strongly suggest that PCD is triggered in human embryos at a stage prior to blastocyst formation. At such early stages, occurrence of apoptosis seemed to be detrimental, leading to preimplantation embryo death.

Key words: apoptosis/fragmentation/preimplantation embryo/programmed cell death

Introduction

The quality of embryos produced by in-vitro fertilization (IVF) is variable; <50% of embryos cleave regularly to give equal-sized blastomeres without fragmentation. The remaining embryos often contain variable-sized blastomeres with multiple cellular fragments enclosed within the zonae pellucidae. Subsequent in-vitro development of these fragmented embryos is impaired, often leading to cleavage arrest and embryo degeneration. Upon transfer, fragmented embryos have limited developmental potential and rarely result in pregnancy (Plachot and Mandelbaum, 1990; Erenus *et al.*, 1991). Interestingly, cytogenetic observations of spare human embryos confirmed the presence of a spectrum of nuclear anomalies, e.g. multinucleated or anucleated blastomeres, floculent and fragmented nuclei (Hardy *et al.*, 1989, 1993; Winston *et al.*, 1991). A wide range of chromosomal abnormalities including premature chromosome condensation and a high degree of aneuploidy and polyploidy were reported in spare human embryos (Papadopoulos *et al.*, 1989; Zenzes and Casper, 1992). Compared to embryos with good morphology, a higher incidence of these cytogenetic abnormalities was found in embryos with fragmentation (Michaeli *et al.*, 1990; Pellestor *et al.*, 1994; Munné and Cohen, 1994).

Our knowledge of embryo fragmentation following IVF in different mammalian species is very limited. The morphological appearance of fragments in early embryos and previous reports describing nuclear abnormalities (Hardy *et al.*, 1993) led us to hypothesize that fragmentation in these embryos is the consequence of programmed cell death (PCD) with typical features of apoptosis.

PCD refers to physiological cell death, which is a normal part of development. True PCD can be precisely predicted in time and space in developing organisms (Cohen, 1994). Because this cell death is truly genetically programmed it requires activation of specific genes involved in the execution of cell death. Often, but not always, this leads to the presence of a common series of morphological changes termed apoptosis (Kerr *et al.*, 1972). Cells dying via apoptosis have very distinct cellular morphology, easily recognizable from ischaemic cell death followed by necrosis. Typical characteristics of apoptosis, previously described in various tissues, include nuclear chromatin condensation with subsequent DNA degradation into oligonucleosomal fragments (demonstrable by the appearance of DNA 'laddering' on gel electrophoresis) and abnormal nuclear shape. Cytoplasmic changes include cellular shrinkage as a result of extensive budding, and the appearance of multiple cellular fragments which are directly proportional to the size of the dying cell. Cellular fragments contain cytoplasm, intact cytoplasmic organelles, and occasionally, pieces of condensed chromatin. Because the cellular membrane remains intact, the fragments often exclude vital dyes.

The objective of the present study was to determine

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whether the morphological features of apoptosis are observed in fragmented human preimplantation embryos, supporting the possible involvement of PCD in early human embryo arrest and demise.

Materials and methods

In-vitro fertilization and embryo culture

Spare human preimplantation embryos were obtained from the IVF programme, Division of Reproductive Sciences, Department of Obstetrics and Gynaecology, at the University of Toronto. Patients who chose not to freeze their spare embryos for future transfers were asked to donate them for research and informed consent was obtained. This research was approved by the human ethics committee of the Toronto Hospital.

Ovarian stimulation was carried out using a gonadotrophin-releasing hormone (GnRH) agonist (Lupron; Abbott Pharmaceuticals, Montreal, Canada) in a long protocol, and human menopausal gonadotrophin (HMG, Pergonal; Serono Canada, Oakville, Ontario or Humegon; Organon Canada, Scarborough, Ontario, Canada). Both IVF and embryo transfer were performed using standard techniques as previously described (Segal and Casper, 1992). Briefly, ovum retrieval was carried out 36 h following injection of 10 000 IU human chorionic gonadotrophin (Profasi; Serono). After retrieval, oocytes were cultured for 3 h in human tubal fluid medium (HTF) supplemented with 10% human serum albumin at 37°C an atmosphere of 5% CO_2, O_2 and 90% N_2. Fertilization resulted from insemination with 50 000–100 000 washed spermatozoa per dish containing one to three ova. Between 18 and 22 h after insemination, oocytes were examined for the presence of two pronuclei and 43–45 h after insemination up to three of the most rapidly and evenly dividing embryos were transferred (at the 2- to 6-cell stage) to the patient's uterus.

Spare embryos of variable quality that appeared to arise from normally fertilized oocytes with 2 pronuclei were used for subsequent analysis. Embryos were cultured in HAM's F10 medium (Gibco BRL, Burlington, Canada) supplemented with 10% human serum at 37°C in 5% CO_2 and 95% air. Assessment of embryo quality and developmental stage was recorded daily until embryos showed cleavage arrest with no further progress compared to the previous 24 h.

Electron microscopy

For scanning electron microscopy (SEM) and transmission electron microscopy (TEM), embryos were washed in phosphate-buffered saline (PBS) and fixed for 1 h in 2.5% glutaraldehyde diluted in Sorrensen's phosphate buffer, post-fixed in 1% osmium tetraoxide, and dehydrated in serial dilutions of alcohol followed by propylene oxide. The final dehydration was done in hexamethyldisilizane (Polysciences, Warrington, PA, USA). Samples were coated with gold particles and analysed using a Hitachi 2500 scanning electron microscope.

Specimens for TEM were fixed similarly to those for SEM, dehydrated through an alcohol series, embedded in Spurr and serially sectioned. Semi-thin sections (1 μm) were cut and stained with 1% Toluidine Blue, while ultrathin sections (70 nm) were stained with alcoholic uranyl acetate followed by Reynold's lead citrate, and were examined by a Hitachi 7000 electron microscope.

Combined nuclear and fragmented DNA labelling

To analyse the status of chromatin in arrested, fragmented embryos, we used a combined technique for simultaneous nuclear and terminal transferase-mediated DNA end labelling (TUNEL). Briefly, embryos were stained with 4,6-diamidino-2-phenylindole (DAPI; Sigma, St. Louis, MO, USA) at a concentration of 0.02 mg/ml for 1 h and washed in medium for 10 min. The zonae pellucidae were removed using acid Tyrode's, and embryos were immediately fixed for 10 min at room temperature on microscope slides in 4% paraformaldehyde diluted in PBS. After air drying, slides were stored at −20°C until further use.

TUNEL was performed according to White *et al.* (1994) with a few modifications. After thawing, slides were washed in PBS. Specimens were preincubated in One-Phor-All Buffer (Pharmacia, Quebec, Canada) consisting of 10 mM Tris-acetate, 10 mM magnesium acetate and 50 mM potassium acetate supplemented with 0.1% Triton X. Afterwards, the embryos were overlayed with reaction cocktail, which contained One-Phor-All Buffer with 0.1% Triton X, 0.25 IU/μl terminal transferase (Pharmacia, Quebec, Canada), 6 μM dATP and 3 μM Bio-dUTP (Sigma). Reactions were carried out in a humidified chamber for 1 h at 37°C after washing the slides twice for 10 min in PBS, and incorporated biotinylated nucleotides were detected with streptavidin–Texas Red conjugate (Calbiochem, San Diego, CA, USA) diluted 1:150 in PBS plus 0.1% Triton X. To decrease non-specific binding, conjugate incubation was performed at 4°C for 30 min. After three washes in cold PBS, slides were examined and photographed using a Leica fluorescent microscope with appropriate filters.

Results

Our combined approach allowed us to distinguish between chromatin status and DNA fragmentation associated with apoptosis as opposed to necrosis. When live healthy cells are exposed to DAPI, staining is restricted to chromatin. When staining is observed in the cytoplasm, this suggests a lack of integrity of the cellular membrane and is a sign of necrosis. The status of chromatin condensation can be assessed by the intensity of DAPI labelling and the shape of the chromatin. Normal, uncondensed chromatin has pale uniform DAPI staining and the nucleus is oval. Condensed chromatin stains brightly with DAPI, and is often irregular in shape. These features were identified as apoptosis positive. In comparison with DAPI staining, TUNEL labelling is based on an enzyme reaction and reflects the integrity of the DNA. Negative labelling is equivalent with background, while positive labelling is very bright (Figure 1). Necrotic cells can be distinguished from apoptotic ones by the distribution of TUNEL labelling, which is cytoplasmic and uniform in necrotic blastomeres and punctuate in apoptotic cells.

Nuclear analysis of arrested embryos

A total of 229 human embryos, arrested at different stages of development ranging from the 2-cell stage to uncompacted morulae, were studied using DAPI and TUNEL analysis. Out of these, 203 showed various degrees of fragmentation. Within this population we observed several different categories of staining: (i) no nuclear or DNA abnormalities (DAPI and TUNEL negative, 13%); (ii) nuclear staining only, with condensed chromatin and intact DNA (DAPI positive, TUNEL negative, 30%); (iii) nuclear staining only, with condensed chromatin and DNA fragmentation (DAPI and TUNEL positive, 21%); (iv) normal uncondensed chromatin and DNA fragmentation (DAPI negative, TUNEL positive, 1.5%); (v) diffuse cytoplasmic staining corresponding to necrotic cells

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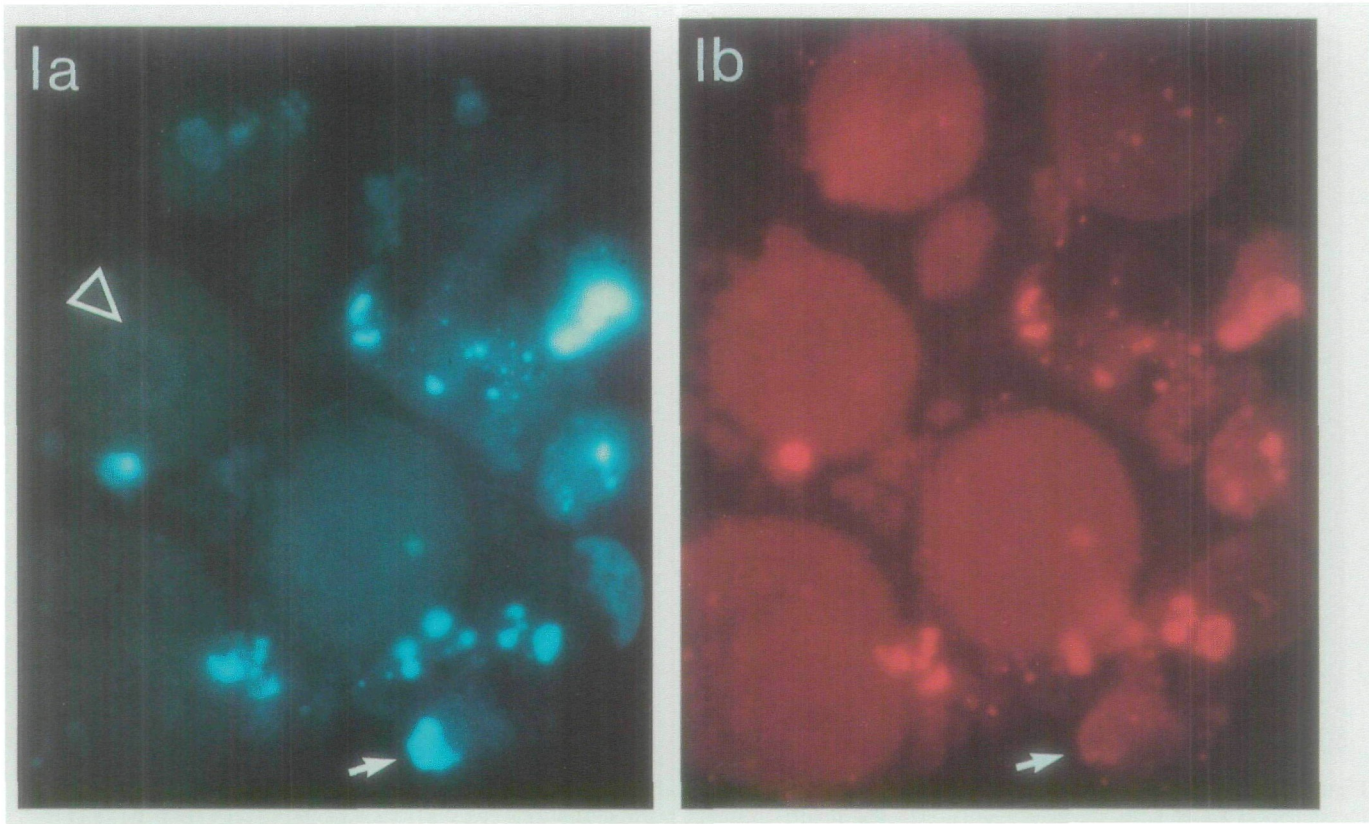

Figure 1. Combined nuclear 4,6-diamidino-2-phenylindole (DAPI)/terminal transferase-mediated DNA end labelling (TUNEL) analysis (original magnification ×400). (**a**) Fragmented human embryo arrested with four normal appearing blastomeres and several cellular fragments. As assessed by DAPI staining chromatin within a few cellular fragments is heavily condensed and misshapen. In contrast, the nucleus in normal blastomere is lightly stained (arrowhead). (**b**) DNA fragmentation in condensed chromatin is extensive as observed by the intense TUNEL signal. However, as indicated by the arrow, some condensed chromatin did not display fragmented DNA. No labelling can be seen within blastomeres with normal nucleii.

Table I. Nuclear status in arrested and fragmented human embryos

Embryo morphology	Nuclear morphology[a]	DAPI[b]	TUNEL[b]	No. embryos
Normal	Normal	–	–	26
Fragmented	Apoptotic	+	–	61
Fragmented	Apoptotic	+	+	42
Fragmented	Apoptotic and necrotic	+	–	42
Fragmented	Apoptotic and necrotic	+	+	8
Fragmented	Normal	–	–	24
Fragmented	Normal	–	+	3
Fragmented	Necrotic	+	+	11
Fragmented	Necrotic	–	–	12

DAPI = 4,6-diamidino-2-phenylindole; TUNEL = transferase-mediated DNA end labelling.
[a]Condensed chromatin was scored as apoptotic while diffuse staining extending into the cytoplasm was scored as necrotic.
[b]DAPI and TUNEL staining was scored (+) if signal was strong and (–) if staining was weak. See Figure 1 for example.

(DAPI positive with or without TUNEL signal, 5.4 and 5.9% respectively); (vi) fragmented embryos displaying evidence of both apoptosis and necrosis in at least two, but more often in more blastomeres of the same embryo (24.6%, see Table I).

An additional 26 apparently normal embryos, with no visible cellular fragmentation, had normal looking nuclei as judged by the DAPI staining pattern and were negative with respect to TUNEL. In three of these embryos, we observed multinucleated blastomeres. In contrast, 153 of 203 fragmented embryos (75.4%) displayed the hallmarks of apoptosis with or without some normal nuclei.

Ultrastructural analysis of fragmented embryos

Evidence of apoptosis was confirmed by light, transmission, and scanning electron microscopy (Figures 2 and 3); ~17 fragmented embryos which arrested with variable numbers of cells were serially sectioned. In 15 embryos, we observed several undegraded cell corpses with dense cytoplasm, multiple cellular fragments which contained normal appearing cytoplasmic organelles, and dense masses resembling condensed chromatin. Despite condensed cytoplasm in these cell corpses, intact mitochondria and several other organelles were visible. Corpses were not phagocytosed and were always found in the intercellular space within the zona pellucida (Figure 3a)

Some of the cellular fragments showed secondary necrotic changes with disrupted cellular membranes and swelled cytoplasmic organelles (Figure 3a). These necrotic changes were also evident in some non-apoptotic blastomeres (disrupted cell membrane, swelled organelles and dilated lysosomes). These observations were consistent with the diffuse DAPI/TUNEL signal observed in some embryos.

While embryos varied with the amount of fragmentation, each embryo also contained a few apparently normal cells

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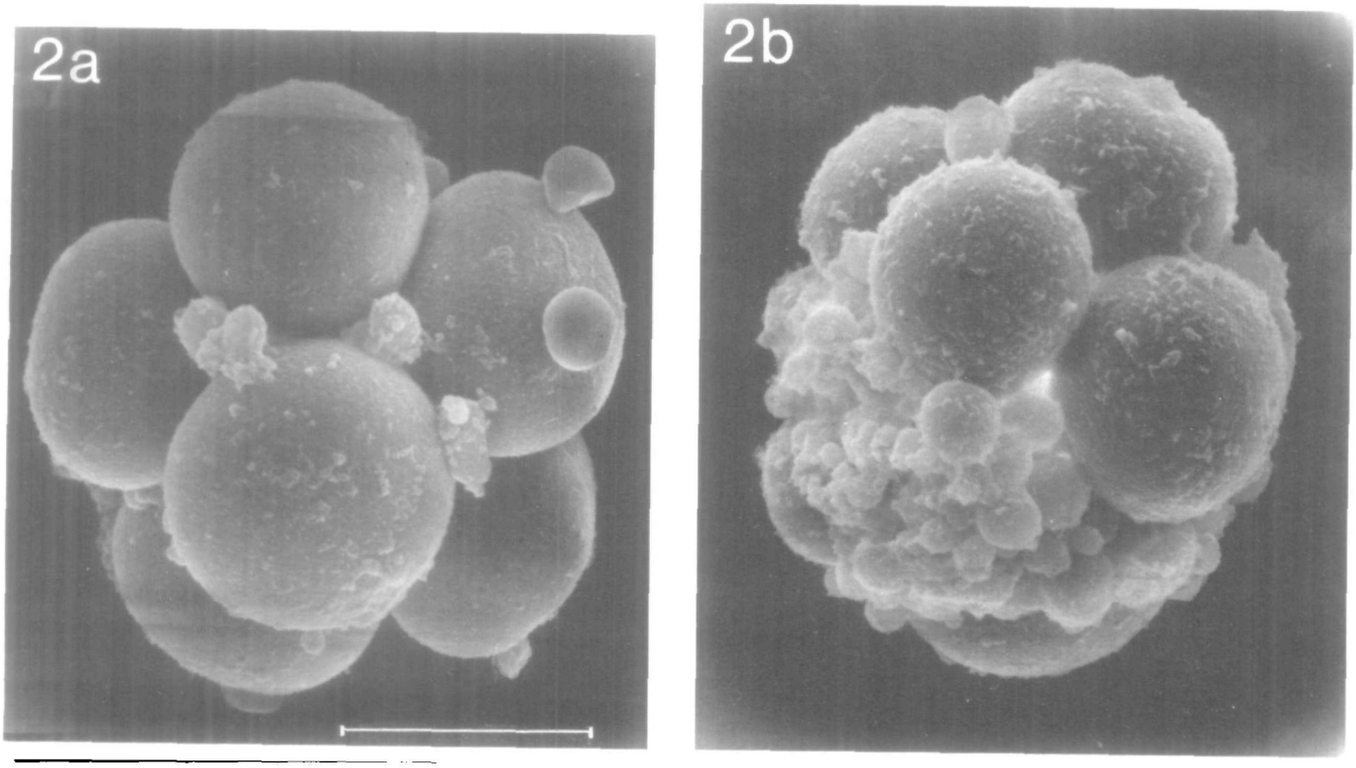

Figure 2. Scanning electron micrographs of human embryos at day 3 after insemination (original magnification ×1200). (**a**) 8-cell stage human embryo with blastomeres of regular size and almost no fragmentation. The smaller cells with smooth surfaces are polar bodies Bar = 25 μm. (**b**) Human embryo of the same age and at the same developmental stage with a few normal blastomeres and excessive cellular fragmentation. Fragments are comparably smaller in size than normal blastomeres and fill ~25% of the total volume of embryo within the zona pellucida

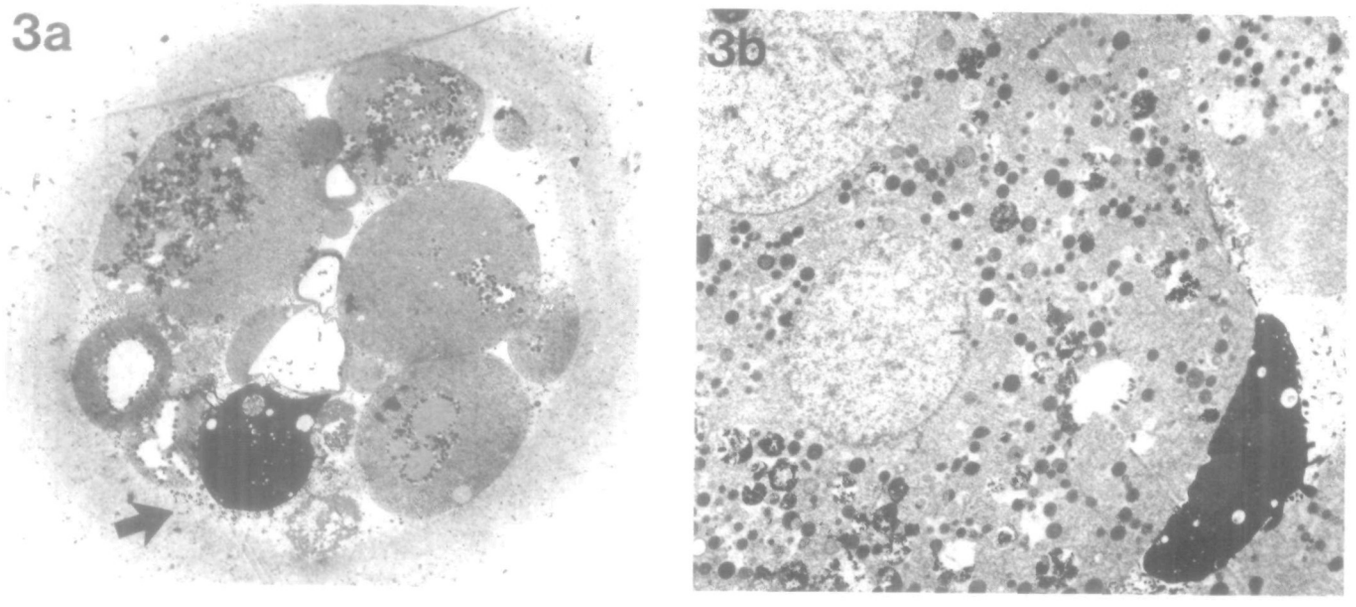

Figure 3. Transmission electron micrograph sections of fragmented human embryos. (**a**) Fragmented human embryo that arrested in development with ~six normal blastomeres and excessive cellular fragmentation at day 4 after insemination. Arrow indicates remnants of apoptotic blastomere with several apoptotic bodies, some of which are undergoing secondary necrosis. The remaining part of the cell–cell corpse has heavily condensed cytoplasm (original magnification ×1000). (**b**) Unphagocytosed cell corpse found in compacting 14-cell embryo with low amount of fragmentation. The corpse had several intact mitochondria present in condensed cytoplasm Note also slight budding and pinching of the parts of cytoplasm Most of the cells within this embryo appeared normal. However, a multinucleated blastomere similar to those found in several embryos, can be seen (original magnification ×5000).

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with regular, round nuclei and no extensive chromatin condensation. In addition, some blastomeres with fragmentation, also contained two or three nuclei of normal size and shape (Figure 3b).

Discussion

Programmed cell death through apoptosis is characterized by a number of well defined morphological features including chromatin condensation and cellular shrinkage. Another of these features, DNA oligonucleosomal fragmentation, can be visualized by the appearance of a DNA ladder pattern on agarose gel electrophoresis. The small number of cells found in human preimplantation embryos makes gel electrophoresis impractical. However, an in-situ technique utilizing TUNEL, has been developed to demonstrate lack of DNA integrity (Gavrieli *et al.*, 1991). This technique has allowed investigators to trace the events which follow the triggering of apoptosis, and has revealed a rapid progression from blebbing to phagocytosis, occurring over a period of a few hours. In some cells, secondary necrosis rather than phagocytosis is the culmination of apoptosis (Kerr *et al.*, 1987; Welsh, 1993).

Our experiments provide clear evidence of apoptosis in human preimplantation embryos, using the combined techniques of DAPI/TUNEL, TEM, SEM and stereomicroscopic observations. The present study concentrated on early cleavage stage embryos which arrested and failed to develop to the blastocyst stage *in vitro*. The distinguishing feature of these embryos was excessive blastomere fragmentation, which was easily detectable under a dissection microscope. Embryos were processed 24 h after the last cleavage and had reached variable stages of development or arrest. Therefore, embryos with asynchronously dying blastomeres were sampled at different times with respect to the first apoptotic process that produced the original fragments. This may account for the different categories of embryos observed in our population (Table I). We hypothesize that embryos with a few fragments but no condensed DAPI/TUNEL signals may represent an earlier stage of apoptosis than those with fragments and both condensed DAPI/TUNEL signals. Embryos showing evidence of necrotic changes may be more advanced still, although we cannot rule out the possibility that some cells/embryos die through arrest-mediated necrosis rather than apoptosis. We do not know whether the preponderance of DAPI signal over co-incident DAPI/TUNEL represents an experimental limitation or an effective means of distinguishing cells at different stages in the apoptotic pathway.

Blastomeres probably have no phagocytic capability since we found cell corpses in the intercellular space, but none which were engulfed by other cells. Alternatively, blastomere corpses may not promote phagocytosis, possibly through an inability to express cell surface molecules (apogens), responsible for recognition of apoptotic cells (Ellis *et al.*, 1991; Rotello *et al.*, 1994). Thus, within developing preimplantation embryos, cell corpses and fragments which are not phagocytosed effectively, may undergo secondary necrosis, which in turn may trigger arrest and subsequent necrosis of surrounding blastomeres. We did not find phagocytosed cell corpses in the cytoplasm of surrounding blastomeres. However our population of studied embryos represented those that fail to reach the blastocyst stage. Fragmented embryos that managed to reach the blastocyst stage, may have the ability to deal with cell corpses more effectively than their arrested counterparts. Trophoblast and trophoectoderm, the first differentiated cell type that arise in the embryo during blastocyst formation, was reported to possess highly effective phagocytic activity (Drake and Rodger, 1987).

These observations support the hypothesis that PCD and the resultant apoptosis is responsible for a significant proportion of fragmented human embryos, and reinforces the original morphologic description of Hardy *et al.* (1989, 1993). However, extremely fragmented embryos were excluded from their study, which may be one reason that the morphological indicators of apoptosis (chromatin condensation and fragmentation) were observed in a much smaller number of embryos. Interestingly, a higher number of anucleated cells were found in embryos of poor morphology (Hardy *et al.*, 1993). These cells/fragments could have been mistaken for large apoptotic bodies that do not contain pieces of condensed chromatin.

Several articles have described the morphologic appearance of dying cells in mammalian embryos at the blastocyst stage. Analysis of ultrathin sections of blastocysts of various mammalian species revealed the presence of cells with clumped, condensed chromatin, swelling of the endoplasmic reticulum, multiple cellular fragments and several cellular corpses (El-Shershaby and Hinchliffe, 1974; Mohr and Trounson, 1982). Occasionally, parts of cell corpses were found undigested in the cytoplasm of surrounding cells. The presence of cell death was observed in blastocysts obtained *in vivo*, as well as in embryos cultured *in vitro* following IVF. The morphological description, as well as the published photographs, show typical signs of apoptosis. It has been proposed that the blastocyst is the first stage during mammalian embryogenesis where one can observe PCD (Parchment, 1993). Furthermore, apoptosis at this stage was coupled with elimination of redundant inner cell mass (ICM) cells with trophectodermal potential. Our results indicate that PCD occurs at even earlier stages of embryo development, with detrimental effects leading to embryo demise in some cases.

In summary, our results demonstrate the morphological appearance of apoptosis in fragmented human embryos. These findings strongly suggest that PCD is triggered in human embryos at a stage prior to blastocyst formation. In view of the fact that cellular fragmentation is one of the consequences of apoptosis in somatic cells and that fragmented embryos display many morphological hallmarks of apoptosis (chromatin condensation, DNA fragmentation and the presence of cell corpses), our observations suggest that embryo fragmentation is a result of activated PCD in some blastomeres. However, this hypothesis cannot be tested directly at the present time because we do not know the triggers of cell death in early embryos. Further efforts in our laboratory are now aimed at identifying genes whose activation or suppression results in the initiation of PCD, and the triggers for such gene expression in human embryos. We believe this research will prove valuable

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in the development of strategies to prevent, or reduce, embryo fragmentation and death.

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References

Cohen, J.J. (1994) Apoptosis: physiologic cell death. *J. Lab. Clin. Med.*, **124**, 761–765.

Drake, B.L. and Rodger, J.C. (1987) Phagocytic properties of cultured murine trophoblast. *Placenta*, **8**, 129–139.

Ellis, R.E., Jacobson, D.M. and Horvitz, H.R. (1991) Genes required for the engulfment of cell corpses during programmed cell death in *C. elegans*. *Genetics*, **129**, 79–94.

El-Shershaby, A.M. and 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.

Erenus, M., Zouves, C., Rajamahendran, P. *et al.* (1991) The effect of embryo quality on subsequent pregnancy rates after *in vitro* fertilization. *Fertil. Steril.*, **56**, 707–710.

Gavrieli, Y., Sherman, Y. and Ben-Sasson, A.B. (1991) Identification of programmed cell death *in situ* via specific labelling of nuclear DNA fragmentation. *J. Cell Biol.*, **119**, 493–501.

Hardy, K., Handyside, A.H. and Winston, R.M. (1989) The human blastocyst: cell number, death and allocation during late preimplantation development in vitro. *Development*, **107**, 597–604.

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

Kerr, J.F.R., Wyllie, A.H. and Currie, A.R. (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer*, **26**, 239–257.

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

Michaeli, G., Feigin, M., Ghetler, Y. *et al.* (1990) Chromosomal analysis of unfertilized oocytes and morphologically abnormal preimplantation embryos from an *in vitro* fertilization program. *J. In Vitro Fertil. Embryo Transfer*, **7**, 341–346.

Mohr, L.R. and Trounson, A.O. (1982) Comparative ultrastructure of hatched human, mouse and bovine blastocysts. *J. Reprod. Fertil.*, **66**, 499–504.

Munné, S. and Cohen, J. (1994) Monospermic polyploidy and atypical embryo morphology. *Hum. Reprod.*, **9**, 506–510.

Papadopoulos, G., Templeton, A.A., Fisk, N. and Randall, J. (1989) The frequency of chromosomal anomalies in human preimplantation embryo after in-vitro fertilization. *Hum. Reprod.*, **4**, 91–98.

Parchment, R.E. (1993) The implications of a unified theory of PCD, polyamines, oxyradicals and histogenesis in the embryo. *Int. J. Dev. Biol.*, **37**, 75–83.

Plachot, M. and Mandelbaum, J. (1990) Oocyte maturation, fertilization and embryonic growth *in vitro*. *Br. Med. Bull.*, **46**, 675–694.

Pellestor, F., Girardet, A., Andreo, B. *et al.* (1994) Relationship between morphology and chromosomal constitution in human preimplantation embryos. *Mol. Reprod. Develop.*, **39**, 141–146.

Rotello, R.J., Fernandez, P.A. and Yuan, J. (1994) Anti-apogens and anti-engulfens: monoclonal antibodies reveal specific antigens on apoptotic and engulfment cells during chicken embryonic development. *Development*, **120**, 1421–1431.

Segal, S. and Casper, R.F. (1992) Progesterone supplementation increases luteal phase endometrial thickness and estradiol levels in in-vitro fertilization. *Hum. Reprod.*, **7**, 1210–1213.

Welsh, A.O. (1993) Uterine cell death during implantation and early placentation. *Microsc. Res. Tech.*, **25**, 223–245.

White, K., Grether, M.E., Abrams, J.M. *et al.* (1994) Genetic control of PCD in *Drosophila*. *Science*, **264**, 677–683.

Winston, N.J., Braude, P.R., Pickering, S.J. *et al.* (1991) Incidence of abnormal morphology and nucleocytoplasmic ratios in 2-, 3- and 5-day human pre-embryos. *Hum. Reprod.*, **6**, 17–24.

Zenzes, M.T. and Casper, R.F. (1992) Cytogenetics of human oocytes, zygotes, and embryos after *in vitro* fertilization. *Hum. Genet.*, **88**, 367–375.

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