

Short Communications

Human blastocysts from aggregated mono-nucleated cells of two or more non-viable zygote-derived embryos

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Abstract

This study examined the developmental capacity of aggregates of surviving mono-nucleated cells isolated from several non-viable human embryos on day 3 or day 4 after fertilization. The results clearly demonstrate that some blastomeres from non-viable embryos do indeed maintain their developmental potential and regulatory capacity to the extent of being able to contribute to a normally organized blastocyst, with as many as 90% diploid cells. Although the chimaeric nature of such blastocysts excludes them from use in therapeutic IVF, they are of particular relevance to the discussion of embryonic and trophoctodermal stem cell line production.

Keywords: blastocyst, chimaera, developmental capacity, embryo, inner cell mass, stem cell

Clinical evidence accumulated over two decades of human IVF and embryo transfer (Steptoe and Edwards, 1978) shows that between 25 and 50% of the total cytoplasmic volume of an early embryo must be represented in normal cells as a condition for full embryo viability. However, a substantial proportion of embryos produced in the course of routine IVF show abnormal cleavage and lose cells and cytoplasmic volume through cell fragmentation, degeneration, mitotic arrest, and multinucleation (**Figure 1a, b**). Extensive cytogenetic studies have demonstrated a high frequency of chromosomal anomalies in such embryos (Munné and Cohen, 1998). As a consequence, roughly 60% of human IVF embryos do not meet basic viability criteria and in most clinics they are simply discarded at the end of treatment cycles.

Although non-viable in the sense that they are largely incapable of giving rise to a normally organized blastocyst, let alone a clinical pregnancy (Alikani *et al.*, 2000), such embryos often do contain one or more surviving, apparently normal blastomeres.

Experimental chimaerism induced by cell aggregation has been used in several other mammalian species to study the developmental potential and regulatory capacity of whole embryos or isolated blastomeres (reviewed by Tarkowski, 1998). So far, however, this highly flexible approach seems to have been completely overlooked in the study of human embryos.

The present work is part of a wider investigation of the implications of abnormal cleavage and degenerative changes in early human embryos, and followed two key observations: 1) a proportion of individual cells from highly fragmented embryos undergo division and cavitation when cultured in isolation (unpublished), and 2) complete removal of fragments

from some fragmented embryos leads to reorganization of the intact blastomeres and facilitates compaction (Alikani, 2001).

The present study investigates the developmental capacity of aggregates of surviving mono-nucleated cells isolated from several non-viable embryos on day 3 or day 4 after fertilization. On day 3 and day 4, human embryos normally complete their third and fourth cleavage divisions respectively. Discarded embryos used in these experiments were obtained from consenting patients under a protocol approved by the Internal Review Board of Saint Barnabas Medical Centre in 1995 and 1999 and re-approved in 2002. This protocol allows in-depth study of abnormal gametes and embryos.

In 23 experiments, appropriate numbers of mononucleated quarter and eighth blastomeres, or eighth and 16th blastomeres were inserted into emptied host zona pellucidae (**Figure 1a–c**) and placed in culture until day 5 or 6 (for details of micromanipulation and culture, see Willadsen *et al.*, 1982; Gardner *et al.*, 1998; Alikani *et al.*, 2000). In total, 247 usable cells from 107 non-viable (fresh or frozen–thawed) embryos were combined into 36 aggregates, ranging from about 50% to about 150% the normal cell volume. Of these, seven, ranging in aggregate cell number from five to nine cells, arrested completely; 17 others showed some development, but did not blastulate or did so in a disorganized way. The remaining 12 (33%), ranging in aggregate cell number from 6 to 12 cells, formed normally organized blastocyst-like structures with distinct inner cell masses. In most instances, some aggregate cells did not participate in compaction and were completely excluded during blastulation; therefore the blastocysts were on the whole smaller than ordinary blastocysts.

Fluorescence in-situ hybridization (FISH) was carried out using probes for chromosomes 13, 16, 18, 21, 22, X, and Y, on

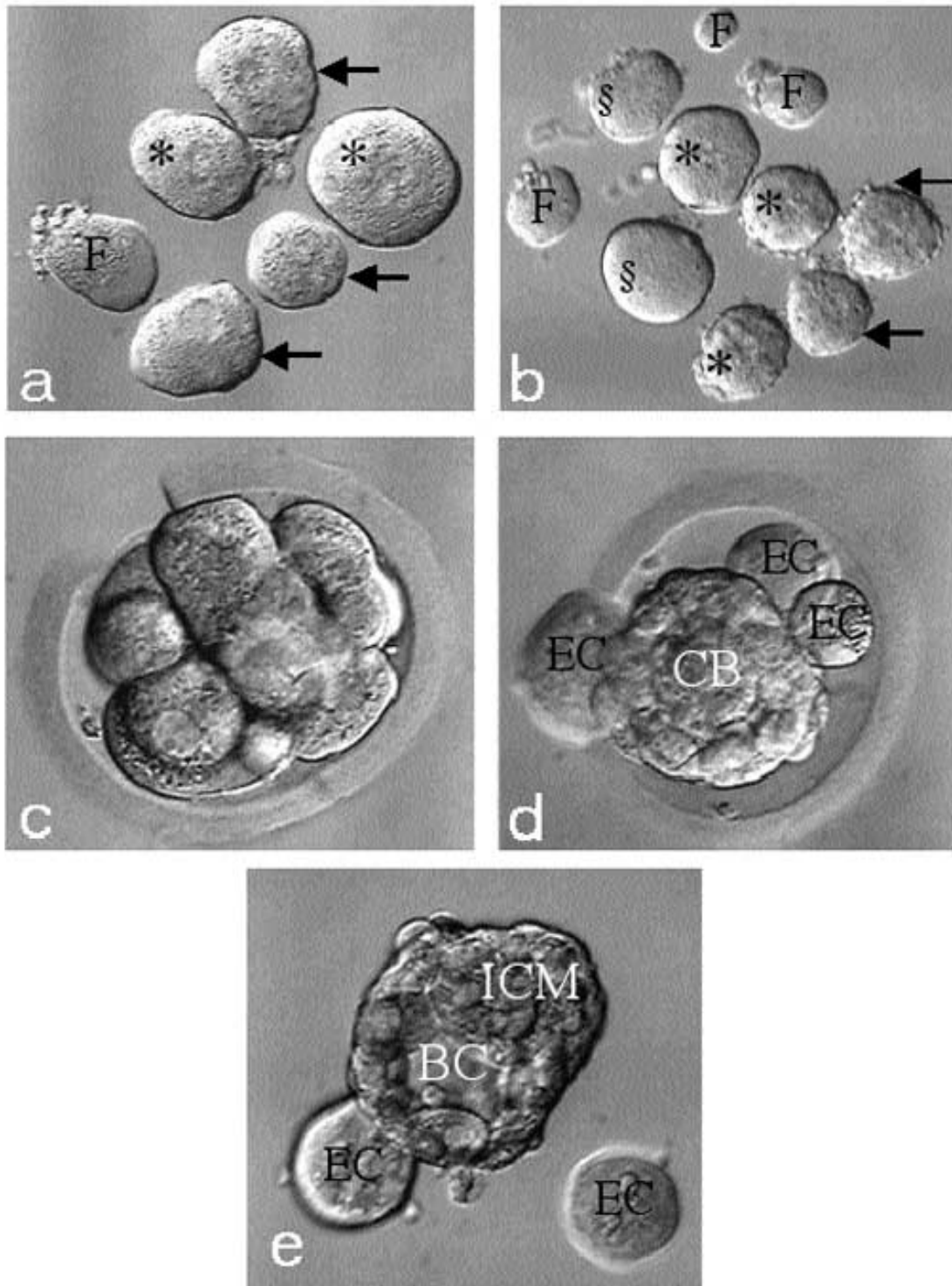


Figure 1. Development of aggregate of mononucleated cells from two non-viable human embryos. (a) Dissociated cells of a non-viable day 3 human embryo, comprising three mononucleated blastomeres (arrows), two multinucleated blastomeres (*), and one large fragment (F). (b) Dissociated cells of the second non-viable day 3 human embryo, comprising two mononucleated blastomeres (arrows), three multinucleated blastomeres (*), two cells with no nuclei visible (§), and three small cytoplasmic fragments (F). Cells marked with arrows in (a) and (b) were used on day 3 to construct a five-cell aggregate (not shown). (c) The aggregate following reconstruction on day 4. Three of the original five blastomeres have divided. Also, by this time, the two cells marked by § in (b) had both divided, each producing a pair of mononucleated cells. The latter have been added to the aggregate; one multinucleated blastomere has been expelled (not shown). (d) Contracted chimaeric blastocyst (CB) on day 5; three cells have been excluded (EC). (e) The chimaeric blastocyst with a well-defined inner cell mass (ICM) on day 6; the blastocyst has hatched and is contracted. Two of the three excluded cells (EC) are visible. (a and b approximately x240; c, d, and e approximately x380).

Table 1. Profiles of seven chimaeric aggregates and fluorescence in-situ hybridization results for the blastocysts they produced.

Experiment	No. non-viable	Aggregate cell number	No. excluded cells at blastulation ^a	Blastocyst cell number ^b	% diploid cells in blastocyst	% abnormal cells in blastocyst	Types of abnormal cells in blastocyst ^c	Sex chimaerism
8a	4	9	2	31	90	10	Poly	Yes
8b	-	6	0	56	89	11	Poly	Yes
13a	4	9	2	39	54	46	Poly/Chao	Yes
21a	8	11	5	31	58	42	Chao	No
22b	4	8	0	38	89	11	Chao	No
25a	7	12	6	27	0	100	Poly/Chao	No
25b	-	7	3	41	52	48	Poly/Chao/Aneu	Yes

^aExcluded cells are those cells not participating in compaction and blastulation.

^bAll blastocysts were analysed on day 5 of development, with the exception of those from experiments 13a and 25a, which were analysed on day 6 of development.

^cPoly = polyploid; Chao = chaotic; Aneu = aneuploid.

all cells of seven blastocysts (Sandalinas *et al.*, 2001). These results are given in **Table 1**. In all, 52–90% of the cells in six blastocysts, containing 31–56 cells, were diploid.

The outcome of these experiments demonstrates that some of the surviving blastomeres in non-viable human embryos do indeed maintain their development potential and regulatory capacity to the extent of being able to contribute to a normally organized blastocyst (**Figure 1e**). Furthermore, a proportion of these blastomeres and their descendent cells are chromosomally normal (**Table 1**).

Clearly, neither the presence of inner (stem) cells nor the confirmation of diploidy in some qualifies these blastocysts as normal, since they are chimaeric. Therefore, in the context of therapeutic IVF, they are unusable. However, this work may be of particular relevance to the discussion of embryonic and trophoctodermal stem cell line production, not least because it points to a source of stem cells that does not require the creation of new embryos or the destruction of existing viable embryos. The possibility of inner cell mass chimaerism in such blastocysts should not be of great concern in the present context.

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