Synthetic profiles of polypeptides of human oocytes and normal and abnormal preimplantation embryos

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There is considerable variation in the rate of development *in vitro* of individual preimplantation human embryos. The relationship between the rate of development and patterns of polypeptide synthesis in individual embryos was examined using SDS-PAGE and autoradiography. After incubation in [³⁵S]methionine, 19 polypeptide bands were identified that change between fertilization and the morula stage. Although changes in two of the bands occurred in embryos that were developing normally and in ageing oocytes, and are thus independent of fertilization, the changes identified in the remaining 17 bands occurred only after fertilization. In embryos that were developing abnormally, as assessed by delayed cleavage, cleavage arrest or extensive fragmentation, the alteration in polypeptide synthetic profiles increased with increasing abnormality.

Introduction

Human embryos derived by *in vitro* fertilization (IVF) differ both in the rate at which they develop *in vitro* and in morphology (Plachot *et al.*, 1988; Bolton *et al.*, 1989). It has been estimated that between 16% (Saito *et al.*, 1994) and 46% (Plachot *et al.*, 1989) of embryos show some degree of extracellular fragmentation, and that approximately 30–37% of embryos undergo delayed cleavage (Hardy *et al.*, 1989; Plachot *et al.*, 1989). The rate of blastocyst formation *in vitro*, which varies depending on the culture system (co-culture, sequential media), the time of evaluation and whether the studies involved cavitating, expanding or fully expanded blastocysts, is about 50% (Muggleton-Harris *et al.*, 1995; Wiemer *et al.*, 1995; Ben-Chetrit *et al.*, 1996; Quinn and Margelit, 1996; Turner and Lenton, 1996; Desai *et al.*, 1997; Alves da Motta *et al.*, 1998; Gardner and Lane, 1998; Gardner *et al.*, 1998; Jones *et al.*, 1998a,b; Martin *et al.*, 1998; Shoukir *et al.*, 1998a).

Several reasons have been suggested for the apparently low survival rate of human embryos developing *in vitro*. These include chromosomal abnormalities (Munné *et al.*, 1994, 1995; Almeida and Bolton, 1996); multinucleation of blastomeres (Levy *et al.*, 1998); deficiencies in the culture medium (Artley and Braude, 1993); exposure to increased concentrations of oxygen free radicals during culture *in vitro* (Johnson and Nasr-Esfahani, 1994); paternal (Sathananthan, 1994; Menezo *et al.*, 1995; Shoukir *et al.*, 1998b), cytoplasmic (Braude *et al.*, 1990; Van Blerkom *et al.*, 1995) and genetic factors inherent to each embryo (Warner *et al.*, 1998); and epigenetic causes (Van Blerkom, 1998). Furthermore, major activation of the embryonic genome, which occurs between the four- and eight-cell stages in humans (Braude *et al.*, 1988),

coincides with the highest incidence of developmental arrest. This is also the case in other mammalian embryos developing *in vitro* (mouse: Flach *et al.*, 1982; pig: Davis, 1985; sheep: Crosby *et al.*, 1988; cow: Frei *et al.*, 1989; goat: Sakkas *et al.*, 1989; hamster: Seshagiri *et al.*, 1990; reviewed by Telford *et al.*, 1990; Watson *et al.*, 1993), indicating that developmental arrest of embryos may be associated with failure to activate the embryonic genome (Braude *et al.*, 1988, 1990). It is likely that embryos with different developmental potential express different patterns of protein synthesis, either inherently or as a result of environmental insult. This possibility was investigated in only one study in which the protein synthetic profiles of preimplantation human embryos that had undergone developmental arrest between the pronucleate and eight-cell stages were examined (Artley *et al.*, 1992). Activation of the embryonic genome had occurred in only 50% of the embryos (Artley *et al.*, 1992). To date, there have been no reports of analysis of embryos that either cleave slowly or show extensive fragmentation.

In the present study on human embryos, protein synthetic patterns were determined in preimplantation embryos that were developing normally (Braude *et al.*, 1990), ageing unfertilized oocytes, and embryos showing slow cleavage, developmental arrest, extensive fragmentation or a combination of these. The aim was to investigate the relationship between normal and abnormal development and specific patterns of polypeptide synthesis.

Materials and Methods

Source of oocytes and embryos

Human oocytes and embryos were donated by patients undergoing therapeutic IVF, with their informed consent. This work was approved by King's College Hospital Ethics

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Committee, and licensed by the Human Fertilisation and Embryology Authority (HFEA), license number R0061. Ovarian stimulation, oocyte collection and IVF were carried out as described by Bolton *et al.* (1989) and Waterstone and Parsons (1992), with the exception that the culture medium (Earle's balanced salt solution; EBSS) was supplemented with 0.001% (v/v) synthetic serum replacement (SSR; Imperial Laboratories) and 1% (w/v) human serum albumin.

Oocytes were examined for the presence of pronuclei at 18–20 h after insemination. Only those embryos that developed two pronuclei were included in the study. Embryos resulting from zygotes with one or three pronuclei were excluded. Unfertilized oocytes used for this study came from cohorts in which none of the oocytes developed pronuclei after insemination with suboptimal spermatozoa to ensure analysis of an unselected population of oocytes (WHO Guidelines; Capmany and Bolton, 1993). (Intracytoplasmic sperm injection (ICSI) was unavailable at the time of the study, and patients with male factor infertility were offered at least one treatment cycle; if all of the oocytes failed to fertilize, patients were counselled towards other options.)

Scoring

Embryos were considered to be developing normally if they had developed two pronuclei at 20 h after insemination, and cleaved to the four-, eight-, and 16-cell stages by 45, 69 and 93 h after insemination, respectively (Braude *et al.*, 1990). Embryos were scored for morphology according to the degree of extracellular fragmentation (Fig. 1). Embryos with regular spherical blastomeres or < 10% fragmentation were considered to have good morphology; embryos with irregular blastomeres or > 10% fragmentation were classified as having poor morphology (modified from Bolton *et al.*, 1989). Cleavage was considered to be delayed if two pronuclei had developed at 20 h after insemination and cleavage had occurred, but embryos had failed to cleave to the four-, eight- and 16-cell stages by 45, 69 and 93 h after insemination, respectively. Embryos were considered arrested if they failed to cleave for at least 24 h.

Labelling with [^{35}S]methionine

Oocytes and embryos at 21, 45, 69 or 93 h after insemination were incubated for 3 h in EBSS containing 0.75 mCi [^{35}S]methionine ml^{-1} (specific activity > 1000 Ci mmol^{-1} ; Amersham International, Amersham). After incubation, they were rinsed four times in 1 ml drops of PBS (Sigma, Poole) and the zona pellucida was removed using acid Tyrode's solution (pH 2.5). After a further rinse in PBS, zona-free oocytes and embryos were stored individually in 2–3 μl PBS at -20°C for up to 2 weeks before electrophoresis. A small number of embryos that cleaved during the 3 h incubation in [^{35}S]methionine were excluded from the study.

Polyacrylamide gel electrophoresis

Individual oocytes and embryos were analysed using one-dimensional SDS-PAGE. Immediately before SDS-PAGE,

Fig. 1. Diagrammatic representation of grading system for human embryos using a four-cell embryo. (a,b) Embryos with regular spherical blastomeres or < 10% fragmentation were considered to have good morphology; (c,d) embryos with irregular blastomeres or > 10% fragmentation were considered to have poor morphology (modified from Bolton *et al.*, 1989).

10 μl sample buffer containing 20 mmol dithiothreitol (DTT) l^{-1} was added to each sample (Laemmli, 1970). After loading one oocyte or one embryo per lane, mini-gels (8.5% (v/v) polyacrylamide) were subjected to electrophoresis at 200 V and fixed for 40 min in a mixture of methanol, acetic acid and water (10:2:7 by volume) containing 5% (v/v) fixative enhancer (Bio-Rad, Hemel Hempstead). The gels were stained immediately with Silver Stain Plus (Bio-Rad). After photography, the gels were vacuum dried and exposed to Fuji RX X-ray film for 3–6 weeks at -70°C .

Analysis of data

The autoradiograms were assessed visually and a semi-quantitative estimate of the intensity of individual polypeptide bands was made in relation to the overall intensity of the gel. The analysis was blind and was always performed by the same observer to avoid any possible biasing and differences in the criteria used for scoring the bands. Since 8.5% gels do not give good resolution below 26 kDa and over 90 kDa, only those bands between 30 and 90 kDa were analysed for the study.

In all but two of the bands analysed, a five grade scale of relative intensity was used: absent, very weak, weak, medium and strong. When discrete bands could not be identified clearly, bands were classified as non-analysable (NA), and the data were not included in the analysis.

Statistical analysis

Statistical analysis was performed using Fisher's exact test. The level of significance was $P = 0.05$.

Table 1. Number, cleavage rate and morphology of human embryos that yielded bands after SDS-PAGE and silver staining

Bands after autoradiography	Embryo morphology	Cleavage rate		
		Normal	Slow	Arrested
Analysable (<i>n</i> = 125)	Good	14	35	26
	Poor	7	22	21
Not analysable (<i>n</i> = 16)	Good	1	1	5*
	Poor	–	2	7†

*Four embryos had arrested at the one-cell stage.

†All embryos had two or more cells.

Results

A total of 141 embryos from 60 women (mean of three embryos per patient, range 1–6) and 59 oocytes from 15 women (mean of four oocytes per patient, range 2–5) were processed by SDS-PAGE, all of which yielded analysable bands after silver staining. The only differences identified in oocytes and embryos were among a group of polypeptides of 69 kDa; these were described by Capmany and Bolton (1993) and were not associated with chronological age, rate of development or morphology (data not shown). The morphology and developmental rate of embryos that yielded analysable bands after silver staining are summarized (Table 1).

Seven oocytes and 16 embryos failed to incorporate [³⁵S]methionine. Of the seven oocytes, one was labelled at 69 h after insemination, and two each at 21, 45 and 93 h after insemination. Of the 16 embryos that failed to incorporate [³⁵S]methionine, in 15 development was either slow or arrested, and nine showed poor morphology (Table 1). When only the morphology of the embryos was taken into account, there was a statistically significant difference between the percentage of embryos with good or poor morphology that failed to incorporate [³⁵S]methionine (*P* = 0.03). In addition, when only cleavage-stage arrested embryos were considered, there was a statistically significant difference between the percentage of embryos with good or poor morphology that failed to incorporate [³⁵S]methionine (*P* = 0.05).

SDS-PAGE revealed 19 bands that showed differences between oocytes and embryos with chronological age or stage of development. There were a number of differences in the polypeptide synthetic profiles of embryos that had good morphology and were developing normally compared with ageing oocytes, or embryos with good or poor morphology that were developing slowly or had arrested. These differences were not always consistent within a subgroup of oocytes or embryos. However, certain patterns of polypeptide synthesis were more common in one subgroup than another. These changes are summarized below.

Polypeptide synthesis and normal development

The protein synthetic patterns of 25 embryos with good morphology that were developing normally were analysed (pronucleate: *n* = 11; four-cell: *n* = 5; eight-cell: *n* = 4; 16-cell: *n* = 3; morulae: *n* = 2). The results obtained are summarized

(Table 2). There were changes in 19 bands as development progressed (Fig. 2).

Band 3 resolved differently during fertilization and in normally developing embryos at different stages. In unfertilized oocytes at 21 h after insemination, two bands of low to medium intensity were observed (Fig. 2, lane A). In pronucleate stage embryos of the same chronological age, the band resolved as a single very intense band, consistent with increased synthesis of either a single band or of the same two bands observed in unfertilized oocytes (21 h after insemination; Fig. 2, lane B). At the four-cell stage, synthesis decreased and the band resolved as two bands of equal intensity (45 h after insemination; Fig. 2, lane C), and by the 16-cell stage (morula) only the upper band of the duplex could be detected (93 h after insemination; Fig. 2, lanes D,E).

Polypeptide synthesis in ageing oocytes

Comparison of the polypeptide synthetic profile of human oocytes that had aged *in vitro* (45, 69 and 93 h after insemination) with that of oocytes at 21 h after insemination revealed changes in two polypeptide bands (bands 3 and 11). A similar pattern of synthesis of band 11 was observed in both ageing oocytes and normal embryos of the same chronological age, whereas there was a slight difference in the pattern of synthesis of band 3 between ageing oocytes and normal embryos (Fig. 3).

Polypeptide synthesis and slow development

The protein synthetic patterns of 35 embryos with good morphology that were developing slowly were compared with those of embryos with good morphology that were developing normally. Differences were observed in the pattern of synthesis of 11 polypeptide bands. These differences were classified as follows: (i) changes that proceeded with time (time-dependent; band 10); (ii) changes that depended on the number of cleavage divisions (cleavage-dependent; bands 1, 5, 11 and 18); and (iii) changes that did not appear to be associated with either time or cleavage (bands 2, 3, 9, 12, 15 and 17) (Fig. 4).

Polypeptide synthesis and cleavage arrest

The protein synthetic patterns of 29 pronucleate stage embryos that failed to cleave were analysed at 45 h (*n* = 16)

Table 2. Semi-quantitative analysis of changes in the pattern of synthesis of 19 bands during the development of normal preimplantation human embryos

Band	Molecular mass (kDa)	No pronuclei (21 h after insemination)	Two pronuclei (21 h after insemination)	Four-cell (45 h after insemination)	Eight-cell (69 h after insemination)	Morula (93 h after insemination)
1	87	–	–	–	+	+
2	78	++	–	+	++	++
3*	71	One strong band	One strong band	Two bands	Transition	Upper band
4	67	+	+	++	++	++
5	64	++	++	++	+++	+++
6	61	++	++	+	+	+
7	58	++	++	+	++	+++
8	55	–	+	++	++	++
9	54	+++	+++	++	–	+
10	53	–	+	+	++	++
11	52	–	–	+	+	+
12	49	++	++	+	++	+++
13	47	++	++	+	–	–
14	45	++	++	++	+++	+++
15	44	+++	+++	++	++	++
16	41	++	+++	++	++	++
17	39	++	+	+	++	++
18	37	++	+	+	++	++
19	35	+++	+++	++	++	++

–, not detectable; +, weak; ++, medium; +++, strong.

*The pattern of synthesis of band 3 changed during development from one very strong band (no and two pronuclei stages), to two bands of equivalent intensity (four-cell stage), to weaker synthesis of the lower band (eight-cell stage), to synthesis of the upper band only (16-cell stage).

and 69 h ($n = 13$) after insemination and compared with those of normally developing embryos with good morphology (Fig. 5).

Some of the changes identified in polypeptides synthesized by embryos with good morphology that had arrested during cleavage are shown (Fig. 5). There were similar changes with time in the pattern of synthesis of five polypeptide bands (1, 3, 9, 10 and 18) in both normal and cleavage-stage arrested embryos. The changes in the patterns of synthesis of bands 2, 5, 8, 12 and 15 did not appear to be associated wholly with either cleavage or chronological age.

Polypeptide synthesis and embryo morphology

The protein synthetic profiles of 125 cleavage stage embryos were analysed, of which 75 had good morphology and 50 poor morphology (Table 1). The differences in the patterns of polypeptide synthesis in equivalent stage embryos with good and poor morphology are shown (Fig. 6).

Changes in the synthesis of bands 5, 12 and 17 were identified in embryos with poor morphology that were cleaving at the normal rate. In embryos with poor morphology that were developing slowly, differences were identified in the pattern of synthesis of five polypeptide bands (2, 3, 9, 11 and 17). However, there were fewer common trends among embryos with poor morphology as the degree of developmental anomaly increased, such that hardly any trends could be identified in arrested embryos with poor morphology. The most marked changes were in the patterns of bands 8, 9, 11 and 15.

Discussion

Use of SDS-PAGE and autoradiography identified 19 polypeptide bands that change between fertilization and the morula stage in human oocytes and embryos. Most of the changes occur between the pronucleate and four-cell stages, when there appears to be an overall decrease in polypeptide synthesis, and between the four- and eight-cell stages, coinciding with major activation of the embryonic genome (Braude *et al.*, 1989).

In the preimplantation mouse embryo, changes in the patterns of protein synthesis before activation of the embryonic genome appear to be regulated in one of three ways (Howlett and Bolton, 1985): although most of the changes are dependent on fertilization, others are either accelerated by fertilization or are independent of this process. In the present study, comparison of protein synthetic profiles of normally developing human embryos and ageing oocytes indicated that similar regulation operates in human embryos. Thus, 17 of the 19 polypeptide bands described in this study showed changes that were dependent on fertilization, since they did not occur in ageing oocytes. Of the remaining two bands, one showed changes that were independent of fertilization (band 11) and one (band 3) showed changes that were triggered independently of fertilization, but were only completed in developing embryos.

The changes in polypeptide synthesis at fertilization were described in a study in which the patterns of synthesis of unfertilized oocytes at 21 h after insemination were compared with those of tripronuclear embryos of the same

Fig. 2. Autoradiograph of SDS-PAGE separated polypeptides synthesized by an ageing human oocyte (A) and by human embryos with good morphology that were developing normally (B–E) during 3 h incubation in [35 S]methionine. A, unfertilized oocyte at 21 h after insemination; B, pronucleate stage embryo at 21 h after insemination; C, four-cell embryo at 45 h after insemination; D, eight-cell embryo at 69 h after insemination; E, morula at 93 h after insemination. Approximate molecular masses (kDa) are shown on the left. Numbers on the right indicate bands that changed between fertilization and the morula stage.

age (Dionne *et al.*, 1989). Changes were identified in seven polypeptide bands; two of these (26 and 28 kDa) had molecular masses that were too small for consistent analysis in the mini-gel system used in the present study, and three bands (54, 36.5 and 35.3 kDa) were not identified in the present study. The remaining two bands (42.5 and 41.2 kDa) may correspond to bands 15 and 16 in the present study, although the patterns of synthesis differ markedly between the reports. The differences between the two studies may be the result of the longer incubation in [35 S]methionine (5 h) in the study of Dionne *et al.* (1989), or the analysis of abnormal embryos, since no trippronucleate embryos were included in the present study.

There are four reports of the analysis of the polypeptide synthetic profile of ageing unfertilized human oocytes up to 100 h after insemination (Gifford *et al.*, 1987; Braude *et al.*, 1988; Schultz *et al.*, 1988; Artley *et al.*, 1992). Only Gifford *et al.* (1987) identified any differences between fresh (44–46 h after hCG) and aged unfertilized oocytes (82–86 h after hCG; 42–46 h after insemination), although these differences were not described in detail. In the present study, age-related changes in the pattern of synthesis of two polypeptide bands were identified.

In mice, incorporation of [35 S]methionine into ageing oocytes cultured for 48 h decreased by 92% compared with

Fig. 3. Autoradiograph of SDS-PAGE separated polypeptides synthesized by individual ageing unfertilized human oocytes processed at 21–69 h after insemination. Arrowheads show bands 3 and 11. Molecular mass markers (kDa) are shown on the left.

fresh oocytes (Petzoldt *et al.*, 1980). This decrease was due to lack of transcription from the embryonic genome, which commences in developing embryos of the same chronological age (Flach *et al.*, 1982). Although major activation of the human embryonic genome occurs between the four- and eight-cell stages (69 and 93 h after insemination; Braude *et al.*, 1988), no difference in overall incorporation of [35 S]methionine was apparent from the semi-quantitative analysis of oocytes aged in culture up to 93 h after insemination, indicating that human maternal mRNA is stable until at least 93 h after insemination (136 h after hCG).

Braude *et al.* (1988) and Artley *et al.* (1992) described the polypeptide synthetic patterns of human unfertilized oocytes, and normal and arrested preimplantation embryos. However, to date, there have been no reports describing the polypeptide synthetic patterns of human embryos that are developing slowly or that have poor morphology. In the studies of Braude *et al.* (1988) and Artley *et al.* (1992), changes in the pattern of synthesis of eight polypeptide bands during preimplantation development were described, seven of which appear to correspond to bands identified in the present study. A comparison of the changes in these bands in the present study and in the studies of Braude *et al.* (1988) and Artley *et al.* (1992) is shown (Table 3). It is likely that minor discrepancies among the studies reflect qualitative differences in the embryos. Firstly, the morphology of the embryos is only described in the present study. Secondly, the criteria for normal embryos are more stringent in the present study compared with the earlier studies; no embryos at intermediate cleavage stages (three-, five-, six-, or seven-cell stages) were included in the normal population, and embryos were processed at specific times each day to restrict the heterogeneity in each group to a minimum. In contrast, in the

Fig. 4. Autoradiograph of SDS-PAGE separated polypeptides synthesized by individual human embryos that were developing normally (lanes 1 and 2) or developing slowly (lanes 3–6). Arrows show some of the bands that had different patterns in normally developing embryos and embryos that were cleaving slowly. 2C, two-cell embryo; 3C, three-cell embryo; 4C, four-cell embryo; 6C, six-cell embryo; 8C, eight-cell embryo. Molecular mass markers (kDa) are shown on the left.

study by Artley *et al.* (1992), the control population of embryos, which consisted of 14 non-arrested embryos, included two groups of embryos of the same chronological age but with a different number of cells: one three-, one four- and one five-cell embryo of the same chronological age, and an eight-cell embryo, a morula and a blastocyst of the same age. Alternatively, the discrepancies among the studies may be the result of the different electrophoretic systems used, which can have different resolution capacities.

This is the first report of the polypeptide synthetic patterns of human embryos that develop more slowly than normal. Comparison of the polypeptides synthesized by normal and by slowly developing embryos indicates that some changes in synthesis occur according to the chronological age of the embryo, irrespective of cleavage, and are presumably regulated by mechanisms associated with the period after a triggering event, such as the LH surge (or hCG injection) or fertilization. Other changes appear to be dependent upon a certain number of cell cycles and are presumably regulated by mechanisms associated with cytokinesis. A third group of changes appear to be intermediate, in that they proceed with time, but at a reduced rate in the absence of a certain number of cleavage divisions. Although this may be the result of asynchrony among blastomeres, it is possible that synthesis of these polypeptides is regulated by mechanisms associated with both cytokinesis and time. The results indicate that changes in a number of bands that are time- or cleavage-dependent in slowly developing embryos depend on both mechanisms in arrested embryos. However, since the pattern of polypeptide

synthesis becomes more altered as development progresses in abnormal embryos, it is possible that this only reflects a gross alteration in the pattern of polypeptide synthesis.

The polypeptide synthetic profiles of arrested embryos and embryos with poor morphology display a greater number of differences from normally developing embryos than embryos that are developing slowly. This finding indicates that as developmental abnormality increases, so does the variation in the pattern of polypeptide synthesis. This contention is confirmed by the observation that a significantly larger number of embryos with poor morphology failed to incorporate [35 S]methionine than those with good morphology.

A number of the polypeptide bands (for example 9, 10, 15 and 18) that showed differential expression in abnormal embryos also showed increased synthesis compared with normal embryos. Although upregulation of protein synthesis in embryos that are not developing normally might seem contradictory, it may be the result of the down-regulation of modulators of protein synthesis, such as promoters or enhancers.

Use of the transcriptional inhibitor α -amanitin showed that from the four- to the eight-cell stages, synthesis of bands B, D, E and G (Artley *et al.*, 1992) was regulated at the transcriptional level of the embryonic genome. When normal embryos had cleaved to at least the eight-cell stage, 50% of arrested embryos (25 of 48 cleavage stage embryos and two of six pronucleate stage embryos) had undergone gene activation (Artley *et al.*, 1992). Bands B, D, E and G from the study of Artley *et al.* (1992) appear to correspond to bands 15,

Table 3. Comparison of the pattern of synthesis of the seven polypeptide bands described in the present study and in the studies of Braude *et al.* (1988) and Artley *et al.* (1992)

Band (approximate molecular mass in kDa):			Change in pattern of synthesis during development
(1) Braude <i>et al.</i> , 1988	(2) Artley <i>et al.</i> , 1992	(3) Present study	
A (36 kDa)	A (37 kDa)	19 (35 kDa)	(1) ↓, four-cell; d, eight-cell
		(2) d, two-cell	
		(3) ↓, four-cell	
B (40 kDa)	B (41 kDa)	15 (44 kDa)	(1) ↓, after eight-cell
		(2) ↓, after eight-cell	
		(3) ↓, four-cell	
C (45 kDa)	D (46 kDa)	12 (49 kDa)	(1) a, eight-cell
		(2) a, four-cell	
		(3) ↓, four-cell; ↑, eight-cell	
D (50 kDa)	—	9 (54 kDa)	(1) ↓, four-cell
		(2) Not identified	
		(3) ↓, four-cell	
E (75 kDa)	G (74 kDa)	2 (78 kDa)	(1) a, eight-cell
		(2) a, four-cell	
		(3) a, four-cell	
—	C (44 kDa)	14 (45 kDa)	(1) Not identified
		(2) ↑, two-cell onwards	
		(3) ↓, four-cell; ↑, eight-cell	
—	E (51 kDa)	7 (58 kDa)	(1) Not identified
		(2) a, four-cell	
		(3) ↓, four-cell; ↑, eight-cell	

a = synthesis first detected; d = synthesis undetectable; ↑ = increase in intensity; ↓ = decrease in intensity.

Fig. 5. Autoradiograph of SDS-PAGE separated polypeptides synthesized by individual human embryos that were developing normally (lanes 1 and 3) or were arrested (lanes 2 and 4). Arrows show three of the bands that had different patterns in normally developing and arrested embryos. 4C, four-cell embryo; 8C, eight-cell embryo; 16C, 16-cell embryo. Molecular mass markers (kDa) are shown on the left.

12, 7 and 2 from the present study. If this is correct, the results of the present study indicate that, contrary to the findings of Artley *et al.* (1992), all 47 embryos arrested at the two- to eight-cell stages that were labelled at 69 or 93 h after insemination had activated transcription; normally developing embryos would have reached the eight-cell or morula

Fig. 6. Autoradiograph of SDS-PAGE separated polypeptides synthesized by individual human embryos at different cleavage stages, showing some of the differences (arrows) identified between embryos of the same chronological age and cleavage stage, but with good morphology (GM; lanes 1 and 3) or poor morphology (PM; lanes 2 and 4). 2C, two-cell embryo; 8C, eight-cell embryo. Molecular mass markers (kDa) are shown on the left.

stages, respectively. However, 46% (6/13) of embryos that had arrested at the one-cell stage and were cultured until 69 h after insemination failed to show signs of activation of the embryonic genome. The reason for these discrepancies is not clear. One possibility is that the bands described in the present study do not correspond to those identified by Artley

et al. (1992), although this is unlikely. From the present results, it appears that provided at least one cleavage division has taken place, activation of the embryonic genome proceeds according to an inherent temporal programme, irrespective of subsequent cleavage or the extent of fragmentation of the embryo. Alternatively, failure to activate the embryonic genome could be related to failure to undergo the first round of DNA replication. The use of an inhibitor of DNA synthesis has demonstrated that major activation of the embryonic genome in the mouse requires the first, but not the second, round of DNA replication (Bolton *et al.*, 1984; Howlett, 1986). Unfortunately, in the present study, it was not possible to determine whether embryos that had failed to undergo cytokinesis had undergone DNA replication.

Whether the differences in polypeptide synthesis observed in the present study are the result or the cause of the developmental anomalies analysed remains unclear. It is of interest that there is some variation in the polypeptide patterns of normal embryos. Although this may be explained by asynchrony among blastomeres, it is possible that development is already compromised in some embryos that appear to be normal. In spite of these individual differences, the variation among polypeptide synthetic profiles of embryos that appear to be developing normally is relatively small, which allows identification of trends within different groups of oocytes and embryos. Future studies should involve direct analysis of the RNA present in human embryos at different stages of development with the aim of identifying genes that show differential expression throughout early development.

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