

The embryo

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This section covers the period from first cleavage of the fertilized oocyte (~24 h post-insemination) up to, but not including, blastocyst formation at ~5–6 days post-insemination.

Key events

Several key events underlie development at this time: (i) cleavage of the fertilized oocyte through about five mitotic cell divisions, not associated with any increase in size; (ii) conversion from control by maternally inherited genetic transcripts to de-novo transcripts from the embryonic genome; (iii) the differential expression of imprinted genes, depending upon whether they were inherited from the maternal or paternal gamete; (iv) the initial steps in differentiation, including the differential orientations and molecular polarity adopted by blastomeres, their subsequent divisions resulting in their increasingly restricted developmental potential and their developmental fate; and (v) the expression of key molecules mediating contact and communication between blastomeres.

Normal development

The first cleavage occurs meridionally in human and mammalian embryos, resulting in two blastomeres which contain approximately similar allocations of the animal and vegetal poles of the oocyte (Figure 4.1). The second cleavage division occurs with slight asynchrony between the first-cleaving and the second-cleaving blastomeres. This is associated with some rotation, and results in a characteristic 'pyramidal' orientation of blastomeres at the 4-cell stage, as shown in Figures 4.2 and 4.5.

The major switch between transcripts of maternal and embryonic genes occurs between the 4- and 8-cell stages (Figures 4.2 and 4.3), although certain genes may be expressed earlier (Ao *et al.*, 1994), and several sex-determining genes are active from pronuclear stages (Edwards and Beard, 1997). Some genes are expressed differently depending upon whether they were inherited from the maternal or paternal genome. The exact time of this imprinting is unclear, but some imprinted genes (e.g. *SNRPN*), are already being differentially expressed by the time of generalized embryonic genome activation (Huntriss *et al.*, 1998).

Around the 8-cell stage, increased surface contact results in

previously discrete blastomeres becoming indistinguishable. This is known as compaction (Figure 4.4) and is a prerequisite for formation of the blastocoelic cavity since it allows the formation of an interior compartment bounded by impermeable tight junctions. From this point, the interior of the embryo is conditioned by the surrounding blastomeres and lacks continuity with the embryo exterior. The fourth and fifth cleavage divisions occur, usually during or after compaction, but individual cleavages are difficult to follow from this point.

Abnormal development

Very few human embryos have the characteristic 'normal' appearance discussed above. Many have gross or discrete anomalies clearly evident on light microscopy, which may have variable significance for the embryo's subsequent potential, e.g. fragmentation or abnormal cellular orientation. Other anomalies may only become evident with additional observation or experimentation. For example, a single interphase nucleus per cell is normal, but at other times of the cell cycle, nuclear material cannot be observed by light microscopy. Also, genetic anomalies may exist unnoticed in a normal appearing embryo, which may result in developmental arrest sooner or later.

Some examples of known anomalies are presented in the figures. There may be many different manifestations of these problems, and it is important to be aware of their underlying causes. Moreover, different anomalies may coexist in the same embryo. Yet frequently we see that embryos which appear highly unpromising have implanted to produce a normal healthy baby. Our understanding of embryo development is simplistic and the tests which can be done in an infertility clinic are minimal, so the term 'abnormal embryo' must be applied only with extreme caution.

Zona anomalies

The zona pellucida is formed early in follicle growth, principally by the oocyte. It is normally a regular sphere comprising three unique proteins, ZP1, ZP2 and ZP3. Unusual forms occur sporadically, and sometimes regularly in particular patients, including various shapes, thicknesses and colours. These may reflect some abnormality of oocyte function during follicle growth, and can affect sperm penetration. If such eggs are normally fertilized (by IVF or ICSI) the zona anomaly rarely affects embryo development, unless its morphology is so severe as to interfere with blastomere orientations or hatching (Figures 4.6 and 4.7).

Nuclear anomalies

Any chromosomal anomalies already present in the zygote are amplified by the successive rounds of DNA replication occurring

during cleavage. It is well known that many gametes are aneuploid and that chromosomal anomalies are frequent in early embryos (Kuo *et al.*, 1998). In addition, mitosis can become disordered during these rapid cell divisions, producing a range from occasional errors, compatible with embryo survival, through complex mosaic forms, to completely chaotic embryos, incompatible with further development. Mitosis must be accompanied by controlled cytoplasmic division for normal cleavage to continue, however, uncoupling is possible since both multinucleated cells (Figures 4.8–4.10) and anucleate fragments (Figures 4.11–4.18) occur frequently in human embryos. In human IVF treatment, we rarely have information on the genetic content of an embryo, but multinucleation or nuclear fragmentation may be associated with a genetic anomaly and low developmental potential. Such embryos are normally avoided if there is a choice even though live births have been recorded.

Cytoplasmic anomalies

The cytoplasm of cleaving embryos is normally pale, and clear or finely granular in appearance. However, if viewed on time lapse video, the activity of cytoplasmic organelles is spectacular. Cytoplasmic anomalies occur occasionally and include unusual colours or granular texture, and inclusions such as vacuoles or dense bodies. Their origins and importance vary, and their appearance is subject to differing interpretation by different individuals.

Cleavage anomalies

Precise bisection of the zygote or blastomere into two daughter cells relies upon the position of the spindle and the activity of the cytoskeletal elements involved in cytokinesis. Slight deviations from normal are probably unimportant, but major imbalances may suggest an underlying problem with these fundamental mechanisms. Regularity of cleavage should be considered carefully, as, for example, it is expected that two cells in a normal, 'regular' 5-cell embryo would be smaller than the others (see Figure 4.19), and a 3-cell embryo with identical sized blastomeres may be cause for concern.

Fragmentation

Fragmentation of blastomeres is arguably not an anomaly since it occurs in very many cleaving embryos. Several different forms arise (Antczak and Van Blerkom, 1999). Some apparent fragments are transient and never separate from the parent cell, while others may separate, lyse or move within the embryo. Distinguishing between small cells and large fragments may be difficult (Figure 4.11). Fragmentation may affect a small proportion of the embryo (Figure 4.12) or all of its cells, and its onset may be early or late. The distribution of fragments is also variable. It may be limited to a small area around the cleavage furrow of several cells (Figure 4.13), or may completely destroy one or more cells (Figure 4.14). The fragments themselves may be small or large. Its aetiology is likely to be multifactorial, and certain patients always have embryos with a high proportion of fragments. The impact of fragmentation upon the subsequent development of embryos may depend upon whether critical functions such as blastomere polarity and communication are compromised (Antczak and Van Blerkom, 1999). It is also possible that the cells which

fragment may never have had the potential to participate in a viable embryo and their fragmentation facilitates their exclusion from the rest of the embryo. Whether fragment removal by micromanipulation may confer benefits for subsequent development is controversial.

Rate of development

It has been recognized for many years that the slowest progressing embryos may have a lower developmental potential than those progressing normally. Moreover, the earliest cleaving zygotes may have the highest chances of producing a successful pregnancy (Edwards *et al.*, 1984), even if they present other anomalies such as fragmentation or irregularity (Sakkas *et al.*, 1998). Assessment of the rate of development requires observation more than once daily. Embryos which do not cleave further within 24 h are normally considered to have arrested. Developmental arrest can occur at any stage in an apparently normal embryo and frequently occurs in embryos which accumulate visible anomalies. In the absence of any problems with culture conditions, it reflects an underlying cause incompatible with further development, such as a gross nuclear anomaly (Figure 4.17), failure of embryonic genome activation, or cytoplasmic deterioration. Compaction (Figure 4.4, 4.20 and 4.21) relies upon genes which must be expressed by the embryonic genome and are not amongst the mRNA complement inherited from the oocyte. Failure of compaction might therefore be an indication of failure of embryonic genome activation, and may occur in individual blastomeres or the entire embryo (Figure 4.11).

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Figure Legends

Figure 4.1

Normal 2-cell embryo 30 h post-insemination (courtesy of M.Plachot, Paris, France).

Figure 4.2

Normal 4-cell embryo 2 days post-insemination. Note crosswise orientation of blastomeres (courtesy of K.Koziot, Warsaw, Poland).

Figure 4.3

Normal non-compacting 8-cell embryo 3 days post-insemination (courtesy of K.Koziot, Warsaw, Poland).

Figure 4.4

Normal morula. The embryo has compacted, the individual cell boundaries have become indistinguishable, and a few fragments have been excluded into the perivitelline space (courtesy of G.Hartshorne, Warwick, UK. Published with permission from Hartshorne, G.M. and Edwards, R.G. In *Endocrinology*. Raven Press N.Y. 435–450).

Figure 4.5

Six 4-cell embryos 42 h after ICSI. Note orientation of blastomeres in characteristic crosswise fashion (courtesy of E.Kontogianni, Athens, Greece).

Figure 4.6

(4.6.1) Normal 4-cell embryo and immature oocyte at the germinal vesicle stage joined together by abnormally developed zona pellucida at 42 h post-insemination; original magnification $\times 1700$. (4.6.2) Line drawing of the above. Possibly two zonae fused during the early oocyte development (courtesy of H.Balakier, Toronto, Canada).

Figure 4.7

Normal 4-cell embryo with a large, thickened elongated zona pellucida, 40 h post-insemination (courtesy of M.C.Magli, Bologna, Italy).

Figure 4.8

(4.8.1) Two-cell embryo containing a mononucleated and a binucleated blastomere 41 h post-insemination (courtesy of H.Balakier, Toronto, Canada. Published with permission from Balakier H. and Cadesky, K. *Hum. Reprod.* (1997) **12**, 800–804). (4.8.2) Line drawing of the above. Although such embryos may carry chromosomal abnormalities and have decreased developmental capability, they may implant and can result in a live birth (2/19 transfers with exclusively multinucleated embryos).

Figure 4.9

(4.9.1) Two-cell embryo with heavily multinucleated blastomeres (~6–7 nuclei per cell) 41 h post-insemination (courtesy of H.Balakier, Toronto, Canada. Published with permission from Balakier, H. and Cadesky, K. *Hum. Reprod.* (1997) **12**, 800–804). (4.9.2) Line drawing of the above. Note the different sizes of the nuclei, some considerably smaller than normal, suggesting that the nuclei are fragmenting. Most such abnormal embryos arrest in early cleavage, however, occasionally they may form trophoblastic vesicles or abortive blastocysts which theoretically could give rise to blighted ova when they implant.

Figure 4.10

Sections (5 μm) of multinucleated embryos. (4.10.1) 2-cell and (4.10.2) 4-cell (courtesy of M.Plachot, Paris, France).

Figure 4.11

Ten-cell embryo with unequal sized blastomeres 68 h after ICSI. Compaction has not begun (courtesy of J.Mandelbaum, Paris, France).

Figure 4.12

Three-cell embryo with 15–20% cytoplasmic fragments 40 h after ICSI (courtesy of J.Mandelbaum, Paris, France).

Figure 4.13

Four-cell embryo 40 h after ICSI. Two blastomeres have a visible nucleus, two blastomeres are small in size, and there is <10% cytoplasmic fragmentation (courtesy of J.Mandelbaum, Paris, France).

Figure 4.14

Eight-cell embryo with one blastomere fragmenting 64 h post-insemination (courtesy of L.Gianaroli, Bologna, Italy).

Figure 4.15

Section (5 μm) of a fragmenting 2-cell embryo. Many organelle-free small fragments are evident (courtesy of M.Plachot, Paris, France).

Figure 4.16

Fragmented human embryo labelled for chromatin and actin. A high rate of fragmentation is evident in this 4-cell embryo observed using confocal microscopy. Numerous fragments are visible due to fluorescein isothiocyanate (FITC) labelling of the actin cortex. Chromatin is completely fragmented in two blastomeres whereas a mitosis is visible in one blastomere (courtesy of J.F.Guerin and R.Levy, Lyon, France).

Figure 4.17

Morphologically normal arrested embryo showing high levels of chromatin anomalies. Complete disorganization of chromatin in a 6-cell arrested embryo observed by confocal microscopy. Chromatin is totally fragmented in two blastomeres and two other blastomeres are binucleate. Note that in one binucleate blastomere, chromatin condensation is evocative of apoptosis (courtesy of J.F.Guerin and R.Levy, Lyon, France. Published with permission from Levy, R., Benchaib, M., Cordanier, H. *et al. Mol. Hum. Reprod.* (1998) **4**, 775–783).

Figure 4.18

Scanning electron micrographs of a fragmenting 4-cell embryo. The anterior (a) and posterior (b) surfaces of a morphologically normal human 4-cell embryo with ~20% of the cellular mass in fragmentation. (c,d and e) are magnifications of the areas outlined in (a). Note that there are no regional differences in form or density of the microvilli. (f) The surface structure of a cytoplasmic fragment. (a and b) scale bar = 30 μm ; (c–f) scale bar = 1 μm ; (courtesy of B.Dale and E.Tosti, Napoli, Italy. Published with permission from Dale, B. *et al.* (1991) *Mol. Reprod. Dev.*, **29**(1) 22–28 (J.Wiley and Co.).

Figure 4.19

Normal 5-cell embryo 40 h after insemination. Note the relative sizes of the blastomeres, suggesting cleavage from a normal regular 4-cell embryo (courtesy of M.C.Magli, Bologna, Italy).

Figure 4.20

Scanning electron microscopy of an 8-cell human embryo. Anterior (4.20.1) and posterior (4.20.2) aspects of a morphologically normal 8-cell human embryo. (4.20.3) Magnification of a contact area of two front compacting blastomeres in (4.20.1). Note the lack of microvilli. (4.20.1 and 4.20.2) Scale bar = 30 μm ; (c) scale bar = 4 μm (courtesy of B.Dale and E.Tosti, Napoli, Italy. Published with permission from Dale, B. *et al.* (1995) *Zygote*, **3**, 31–36 (Cambridge University Press).

Figure 4.21

Intercellular communication in the early human embryo. Half light and half fluorescence photographs of human embryos after injection of Lucifer Yellow (LY) into single blastomeres. The zona pellucida was removed manually with steel needles. (a) 4-cell stage 15 min post injection, (b) LY injected into a single blastomere of a 10 cell embryo. (c) Early compacted morula 20 min after microinjection of LY into a cell. (d) Late blastocyst 10 min after injection of LY into a trophectoderm cell. The blur to the right is the LY-containing micropipette. icm = inner cell mass; te = trophectoderm cells (courtesy of B.Dale and E.Tosti, Napoli, Italy. Permission as in 4.20).

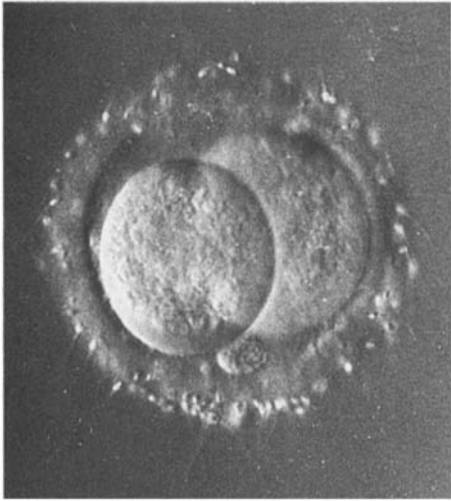


Figure 4.1

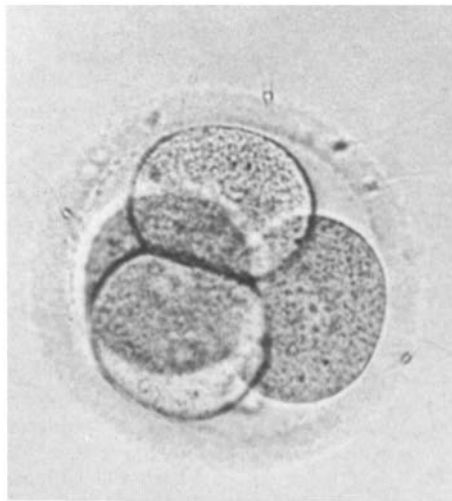


Figure 4.2



Figure 4.3



Figure 4.4

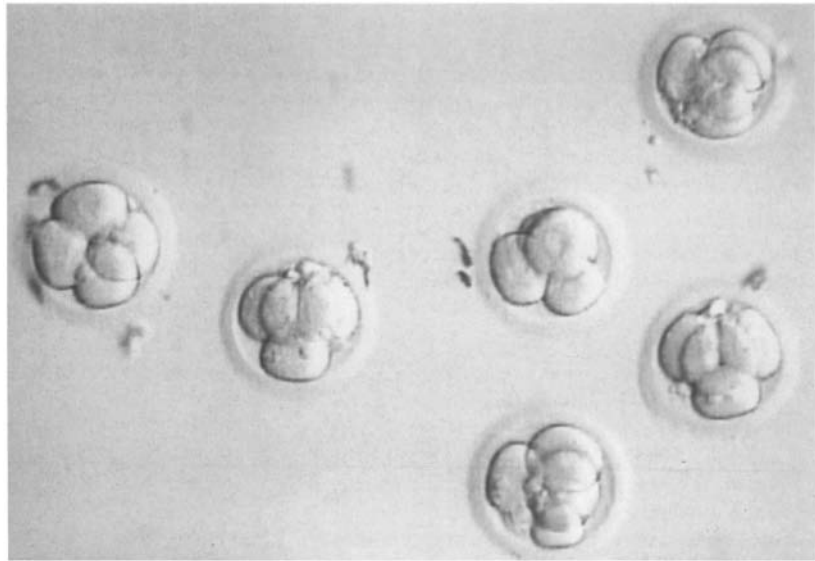


Figure 4.5



Figure 4.6.1

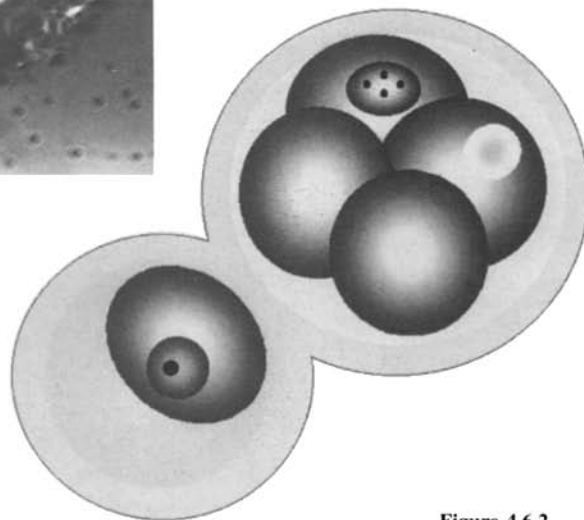


Figure 4.6.2



Figure 4.7



Figure 4.8.1

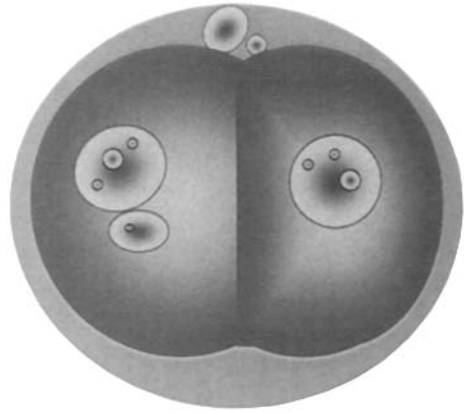


Figure 4.8.2

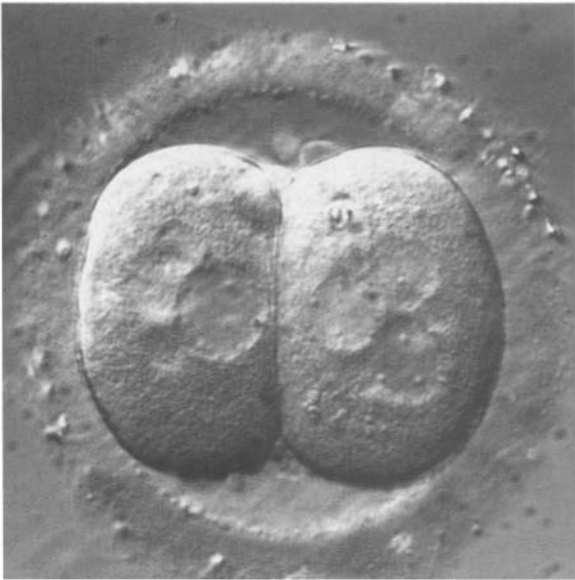


Figure 4.9.1

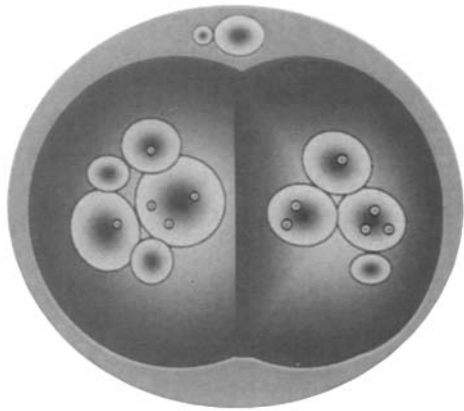


Figure 4.9.2

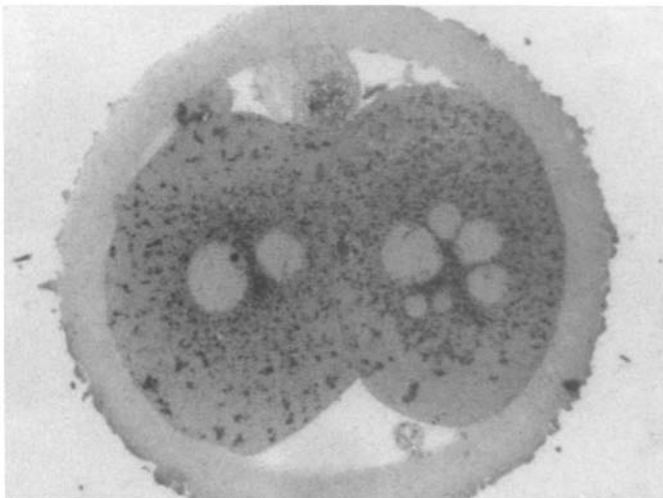


Figure 4.10.1

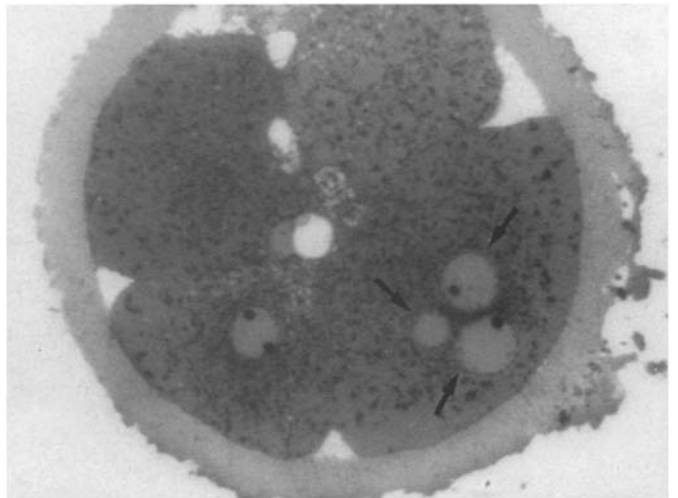


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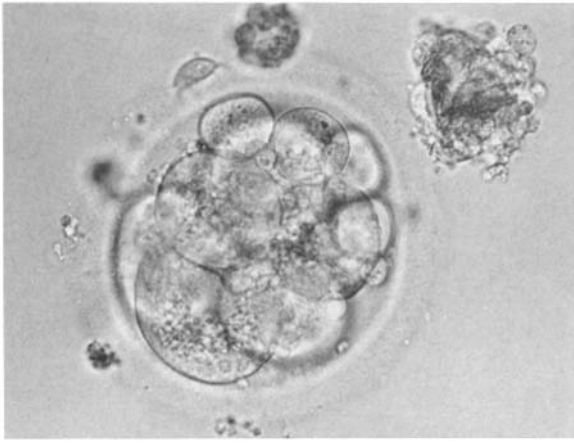


Figure 4.11



Figure 4.12

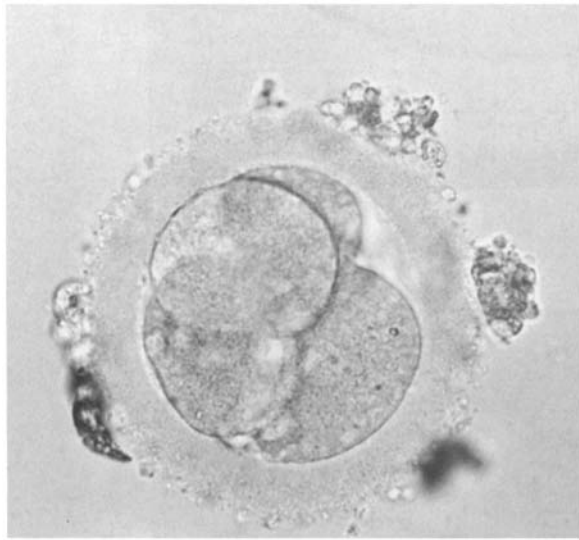


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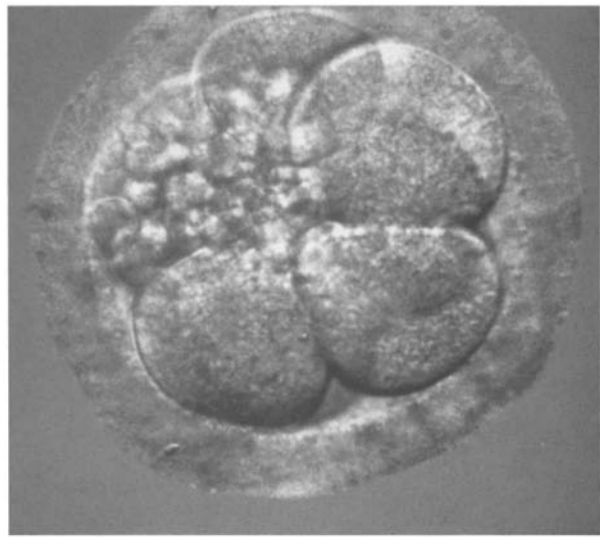


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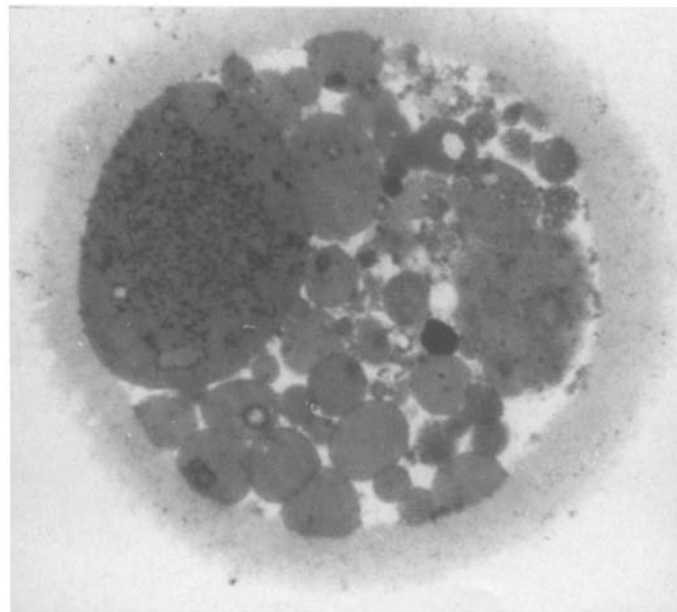


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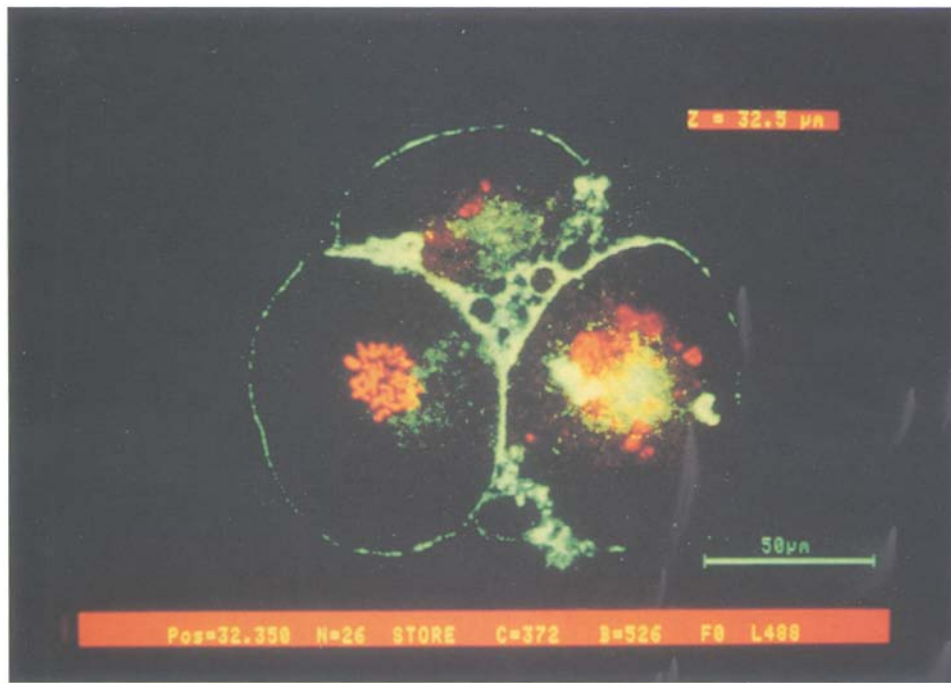


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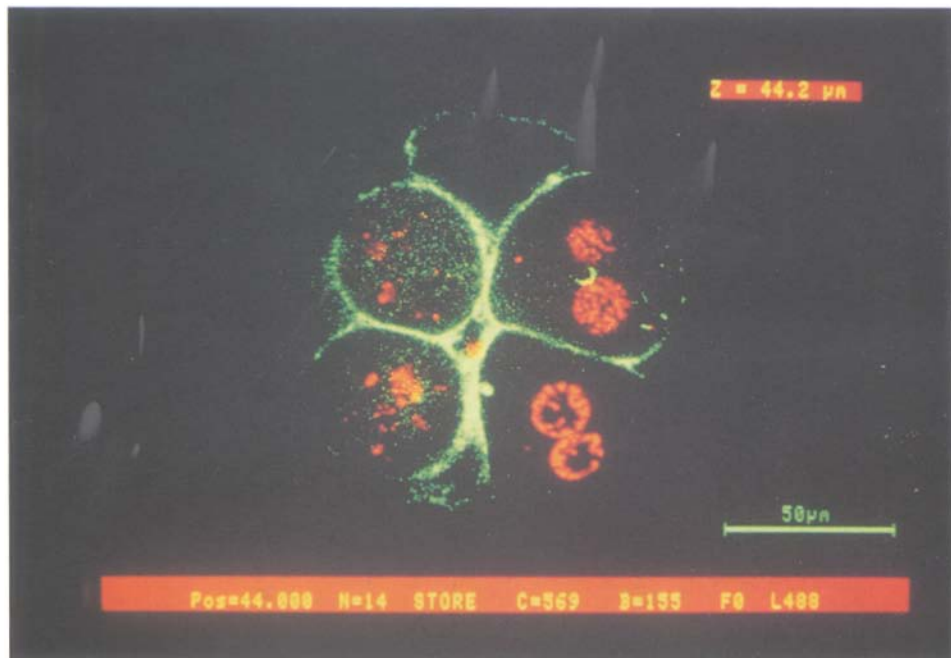


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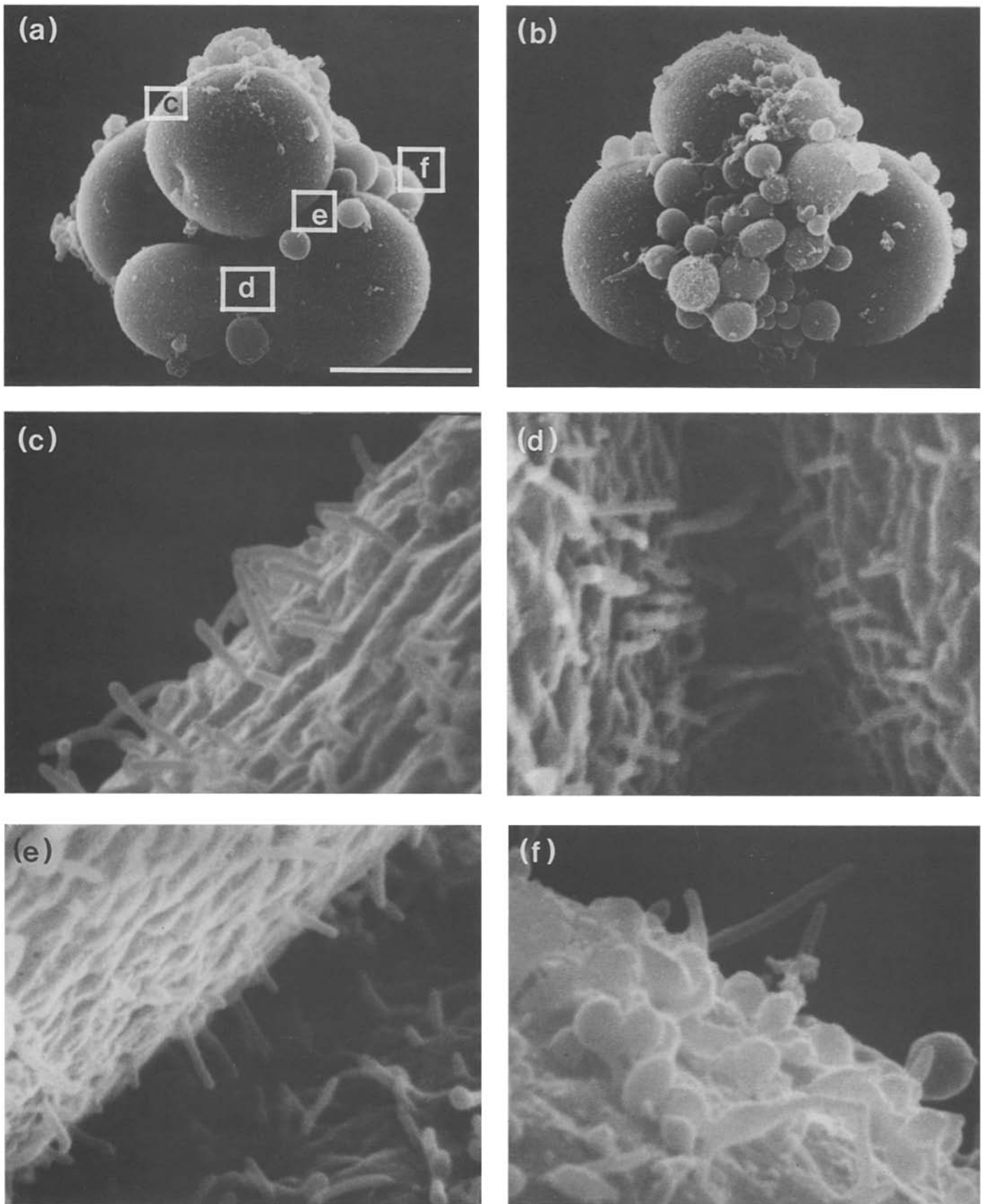


Figure 4.18



Figure 4.19

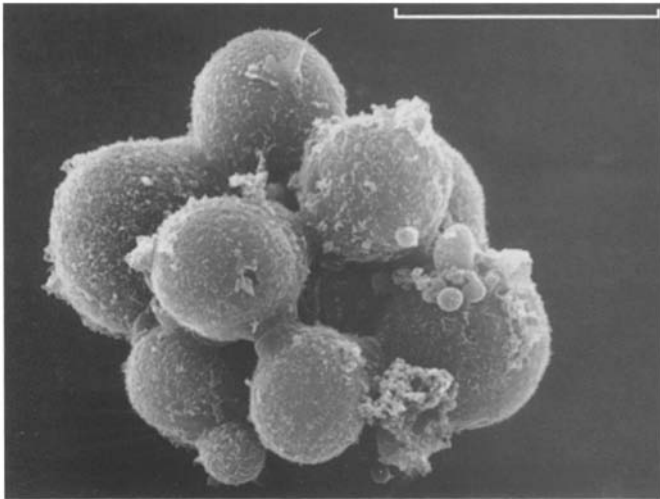


Figure 4.20.1

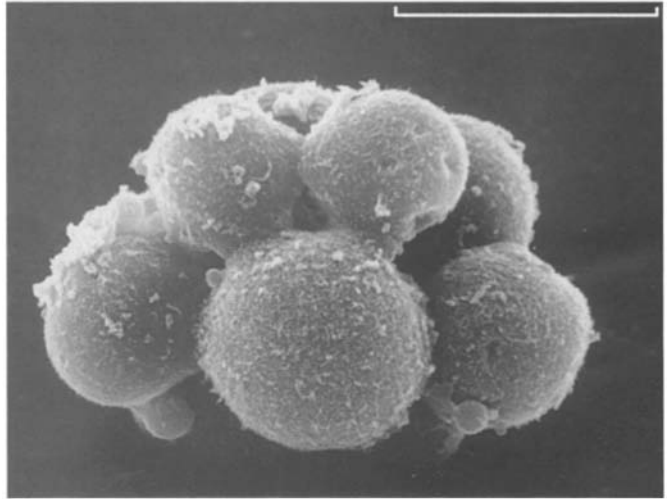


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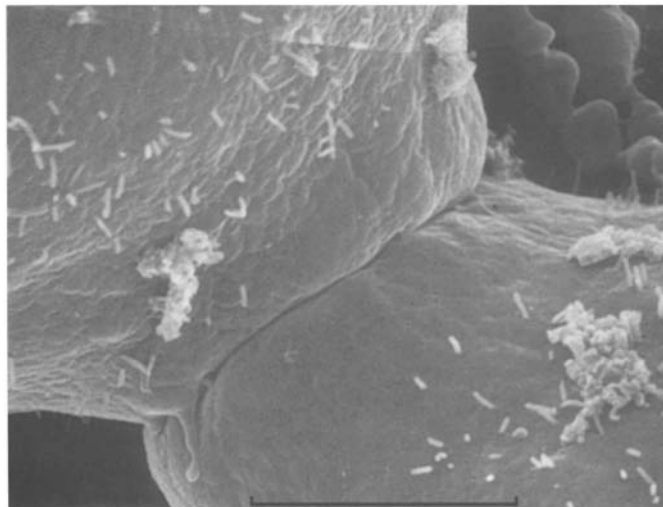


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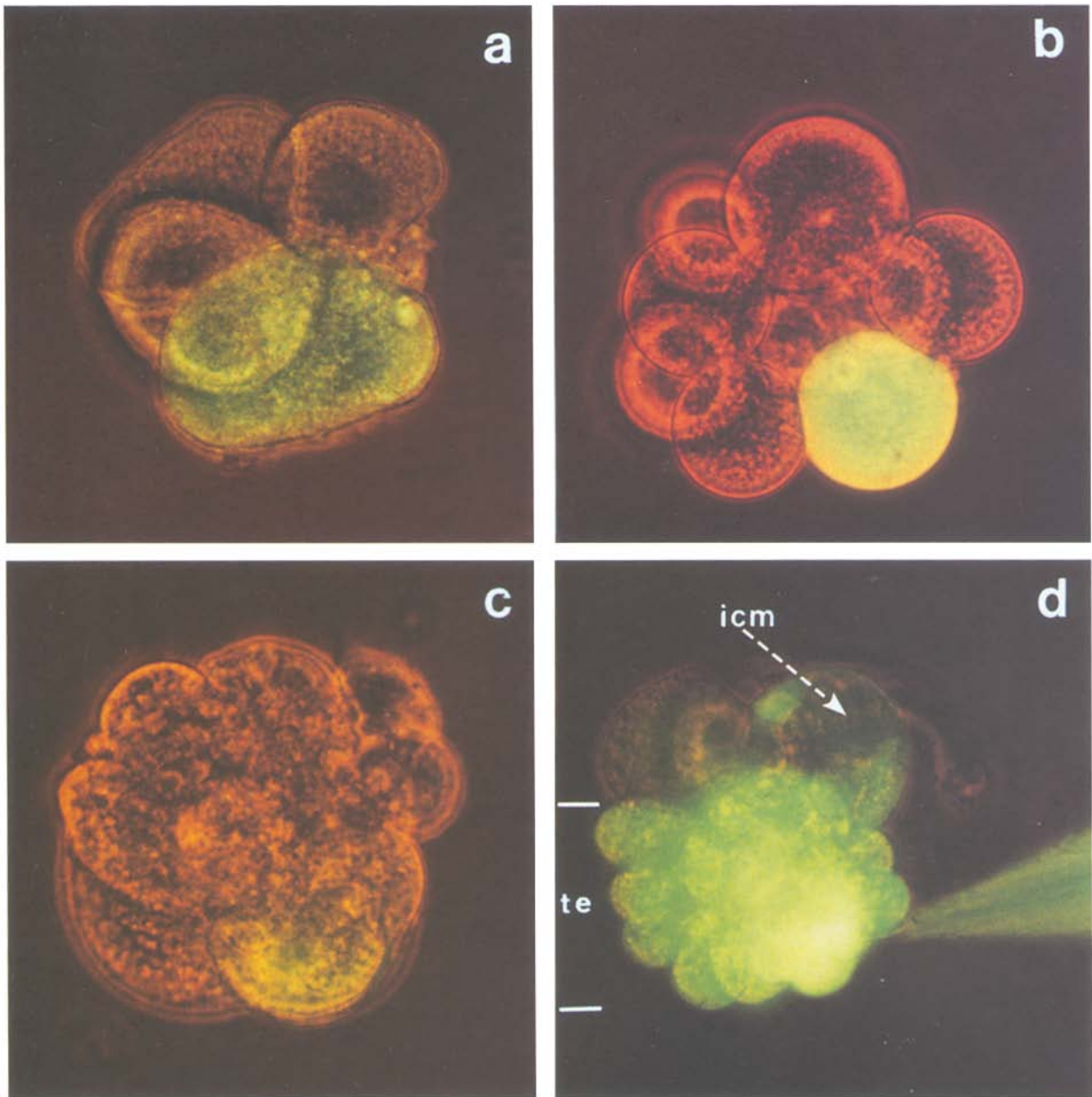


Figure 4.21