Human Reproduction, Vol. 15, (Suppl. 4), pp. 49–58, 2000

The blastocyst

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Preimplantation development culminates in the formation of the blastocyst. Until the 16-cell stage, the blastomeres of the morula are quite distinct and can easily be identified. The process of compaction, which involves the formation of a variety of junctions between the individual blastomeres starts ~4 days after fertilization (the day on which insemination occurs is counted as day 0). This leads to blastomere adhesion, and the boundaries between the blastomeres become difficult to identify (Figure 6.1). Gap junctions provide channels for the passage between cells of small molecules such as sugars, amino acids and nucleotides. The cells of the compacted embryo become highly polarized during compaction (Figure 6.2 and 6.3). On day 5 after fertilization, the blastocyst starts to form (Figure 6.4). This involves the formation of the blastocoel, a fluid-filled cavity. Figures 6.5–6.30 represent the different stages and aspects of blastocyst development.

The blastocyst consists of two cell types. The inner cell mass gives rise to the embryo proper and certain extraembryonic membranes. The differentiated cells of the trophectoderm form a fluid-transporting epithelium responsible for the accumulation of fluid in the blastocoel and blastocyst expansion. The trophectoderm gives rise to extraembryonic tissues only. The last event of preimplantation development prior to implantation is hatching. This is the escape of the embryo from the zona pellucida, which occurs between days 6 and 7.

In human IVF, the embryos are routinely transferred to the uterus on day 2 or 3 of development, when they have between 4 and 8 cells. As the embryonic genome is fully activated after the 8-cell stage, it may be beneficial to delay embryo transfer until after the transition from maternal to embryonic genome, making it possible to identify embryos with poor developmental potential. Blastocyst transfer, therefore, facilitates the selection of the best embryos with high implantation potential, thereby reducing the need to transfer several embryos. Better synchronization between embryo development and the surrounding environment may be achieved, reducing the risk of embryos expulsion after transfer.

The first human blastocysts were obtained in standard medium in 1971 (Steptoe *et al.*, 1971); the same medium was also able to support blastocyst hatching (Edwards and Surani, 1978). Although ~50% of embryos grew to blastocysts in routine media, the implantation rate was greatly improved with the introduction of embryo co-culture on feeder cells. The most widely-used feeder cell system, in terms of the number of patients who entered a co-culture protocol, is a monolayer of monkey kidney epithelial cells (Vero cells). However, the technique is time-consuming, and there is a risk of viral or bacterial infection of the cells if they are not prepared to a high standard 'for clinical use'. Recently, co-cultures have been successfully replaced by the use of sequential media.

Gardner *et al.* (1998) have developed a sequential culture system involving two media, G1 and G2 the compositions of which are derived from those of tubal and uterine fluids. In these media, 46.5% of normally fertilized zygotes develop to the blastocyst stage, most on day 5. In patients with a moderate to good response to gonadotrophin stimulation, the implantation rate of the blastocysts (50.5% fetal heart beat) was greater than that of cleavage stage embryos transferred on day 3 (30.1%).

The use of a glucose/phosphate-free simple culture medium (P1) for up to 72 h of culture and a complex, glucose-containing medium (blastocyst medium), makes it possible to obtain 40–54% expanded blastocysts with high implantation potential after 120 h of culture (Alves da Motta *et al.*, 1998; Behr *et al.*, 1999).

Blastocysts are classified according to their morphology: (i) early blastocysts have a small cavity occupying <50% of the volume of the embryo; (ii) expanding blastocysts have a large blastocoel cavity with well-defined trophectoderm and inner cell mass; (iii) expanded blastocysts have a fully expanded blastocoel that results in an increased diameter (215–280 μm) and an extremely thin zona pellucida.

Significantly higher pregnancy rates are obtained with the transfer of fully expanded blastocysts compared with early blastocysts, indicating that, as for cleavage stage embryos, the implantation rate is correlated with the rate of development. Compacted morulae on day 5 have a low implantation potential.

A significant positive correlation has been observed between the number of blastocysts formed and the number of oocytes, pronuclear zygotes and 8-cell embryos formed (Jones *et al.*, 1998b). Therefore, it is important to determine the efficacy of extended culture in sequential media for patients with a low response to gonadotrophins.

The predictive value of embryo morphology on day 3 for subsequent blastocyst formation is limited. Indeed, only 51% of the embryos that developed to the blastocyst stage had been preselected for transfer on day 3 (Rijnders and Jansen, 1998).

A paternal influence on embryo development has been suggested. Indeed, the fertilising spermatozoon does contribute in many ways to post-fertilization events. Poor semen quality

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may be due to biochemical abnormalities having a negative effect on chromatin and/or DNA. In IVF, sperm counts of $<10\times10^6$/ml are associated with poor quality embryos, fewer blastocysts and a decreased pregnancy rate. The development of intracytoplasmic sperm injection (ICSI) makes further studies of the role of paternal factors in early human embryogenesis possible. Shoukir *et al.* (1998) showed that the rate of embryo development to the blastocyst stage was lower after ICSI (26.8%) than after IVF (47.3%). The low rate of blastocyst development may be due to several factors, e.g. poor sperm characteristics or technical problems associated with the ICSI procedure itself. Such impairment in embryo development was, however, not observed by Gardner *et al.* (1998).

Thus, the production of viable blastocysts in a simple, commercially-available culture system is a major goal in the advancement of clinical IVF.

References

Alves da Motta, E.L., Alegretti, J.R., Baracat, E.C. *et al.* (1998) High implantation and pregnancy rates with transfer of human blastocysts developed in preimplantation stage one and blastocyst media. *Fertil. Steril.*, **70**, 659–663.

Behr, B., Pool, T.B., Milki, A.A. *et al.* (1999) Preliminary clinical experience with human blastocyst development *in vitro* without co-culture. *Hum. Reprod.*, **14**, 454–457.

Edwards, R.G. and Surani, M.A.H. (1978) *Uppsala J. Med. Sci.*, **22**, 39–50.

Formigli, L., Roccio, G., Belotti, G. *et al.* (1990) Non-surgical flushing of the uterus for pre-embryo recovery: possible clinical applications. *Hum. Reprod.*, **5**, 329–335.

Gardner, D.K., Schoolcraft, W.B., Wagley, L. *et al.* (1998) A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. *Hum. Reprod.*, **13**, 3334–3340.

Hartshorne, G.M. and Edwards, R.G. (1996) Early embryo development. In Adashi, E.Y., Rock, J.A. and Rosenwaks, Z. (eds) *Endocrinology, Surgery and Technology.* pp. 435–450. Raven Press, N.Y.

Jones, G.M., Trounson, A.O., Gardner, D.K. *et al.* (1998a) Evolution of a culture protocol for successful blastocyst development and pregnancy. *Hum. Reprod.*, **13**, 169–177.

Jones, G.M., Trounson, A.O., Lolatgis, N. *et al.* (1998b) Factors affecting the success of human blastocyst development and pregnancy transfer following in-vitro fertilization and embryo transfer. *Fertil. Steril.*, **70**, 1022–1029.

Rijnders, P.M. and Jansen, C.A.M. (1998) The predictive value of day 3 embryo morphology regarding blastocyst formation, pregnancy and implantation rate after day 5 transfer following in-vitro fertilization or intracytoplasmic sperm injection. *Hum. Reprod.*, **13**, 2869–2873.

Shoukir, Y., Chardonnens, D., Campana, A. *et al.* (1998) Blastocyst development from supernumerary embryos after intracytoplasmic sperm injection: a paternal influence? *Hum. Reprod.*, **13**, 1632–1637.

Steptoe, P.C., Edwards, R.G. and Purdy, J.M. (1971) *Nature*, **229**, 132–133.

Figure Legends

Figure 6.1
Compacted morula on day 4 with granulated (g) and agranulated (a) areas and a few small fragments (f) (courtesy of M.Pieters, Rotterdam, The Netherlands).

Figure 6.2
Compacted morula on day 4 beginning to cavitate (courtesy of A.Veiga, Barcelona, Spain).

Figure 6.3
Beginning of cavitation (arrows) in a day 4 embryo, demonstrating an advanced development (courtesy of M.Pieters, Rotterdam, The Netherlands).

Figure 6.4
A cavity has started to form, surrounded by the cells of the morula (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 6.5
Morula on day 5 after oocyte retrieval. These delayed embryos were demonstrated to have a low implantation rate (courtesy of M.Scholtes, Düsseldorf, Germany).

Figure 6.6
Transition stage between morula and early blastocyst after 5 days of culture. These delayed embryos have been shown to have an impaired implantation rate (courtesy of M.Scholtes, Düsseldorf, Germany).

Figure 6.7
Blastocyst on day 5 after oocyte retrieval before expansion occurs. Note the inner cell mass, which is a positive sign of embryo development (courtesy of M.Scholtes, Düsseldorf, Germany).

Figure 6.8
(**6.8.1**) Day 5 blastocyst with a small blastocoel and a non-delineated inner cell mass; some cells are not compacted yet. (**6.8.2**) The same blastocyst on day 6: expansion has occurred, but the inner cell mass shows a highly fragmented appearance and no compaction (courtesy of K.Koziot, Warsaw, Poland).

Figure 6.9
This early blastocyst containing <50 cells displays fragmentation (f) in or next to the inner cell mass (cm) giving a slightly abnormal aspect (courtesy of M.Pieters, Rotterdam, The Netherlands).

Figure 6.10
Expanding day 5 blastocyst showing a large compacted inner cell mass (cm) (courtesy of M.Pieters, Rotterdam, The Netherlands).

Figure 6.11
Expanding human blastocyst (day 5 post-insemination) cultured in G2 medium supplemented with human serum albumin. Note the prominent inner cell mass (top left) and the flattening of the trophectoderm cells as the central cavity expands. Original magnification $\times300$ (courtesy of G.Jones, Melbourne, Australia).

Figure 6.12
Expanding blastocyst in the afternoon of day 5. The inner cell mass is clearly visible and the zona pellucida has become thinner (courtesy of G.Cassuto, Paris, France).

Figure 6.13
Blastocyst on day 5 after oocyte retrieval, shortly before hatching. The zona pellucida is extremely thin and the blastocoelic cavity is very large (courtesy of M.Scholtes, Düsseldorf, Germany).

Figure 6.14
Different stages of embryonic development after 5 days of embryo culture. Three blastocysts showing varying amounts of inner cell mass and different size according to the stage of expansion and beginning of hatching (courtesy of M.Scholtes, Düsseldorf, Germany).

Figure 6.15
Fully expanded human blastocyst (day 6 post-insemination) cultured in G2 medium supplemented with human serum albumin. Note the prominent inner cell mass (bottom left), flattened trophectoderm cells and thinned zona pellucida. Original magnification $\times300$ (courtesy of G.Jones, Melbourne, Australia).

Figure 6.16
The blastocoelic cavity has enlarged, the trophectoderm has become thinner and the inner cell mass is evident at the bottom of the figure. Expansion of the blastocoel has caused the zona pellucida to stretch and become thinner (courtesy of G.Hartshorne, Warwick, UK. Permission as in 6.4).

Figure 6.17
Hatching human blastocyst on day 6 post-insemination, cultured in G2 medium supplemented with human serum albumin. Note the prominent inner cell mass (lower pole) and the hatching trophectoderm (left) (courtesy of G.Jones, Melbourne, Australia).

Figure 6.18
The inner cell mass is still inside the zona (left) and half the trophectoderm has already hatched. Original magnification $\times300$ (courtesy of G.Jones, Melbourne, Australia).

Figure 6.19
Hatching human blastocyst on day 7 post-insemination. The inner cell mass, hatching from the zona, shows non-compacted, degenerated cells (courtesy of K.Koziot, Warsaw, Poland).

Figure 6.20
Hatching blastocyst showing inner cell mass and trophectoderm on both sides. A rupture of the embryo at this point may produce monozygotic twins after implantation (courtesy of M.Plachot, Paris, France).

Figure 6.21
Blastocyst hatching through a large hole in the zona pellucida. A large dark cell in the inner cell mass is visible through the trophectoderm (courtesy of G.Hartshorne, Warwick, UK. Permission as in 6.4).

Figure 6.22
Blastocyst hatching in the afternoon of day 6 (courtesy of G.Cassuto, Paris, France).

Figure 6.23
Differentially labelled human blastocyst viewed by epifluorescence microscopy. Outer trophectoderm nuclei labelled in orange and inner cell mass nuclei labelled in green (courtesy of K.Hardy and A.Handyside, London, UK).

Figure 6.24
Normal appearance of a day 6 hatched blastocyst showing a clearly differentiated and compact inner cell mass (c) next to an empty zona pellucida (zp) (courtesy of M.Pieters, Rotterdam, The Netherlands).

Figure 6.25
The zona pellucida has been shed, the blastocyst has expanded further and individual cells of trophectoderm are distinguishable (courtesy of G.Hartshorne, Warwick, UK. Permission as in 6.4).

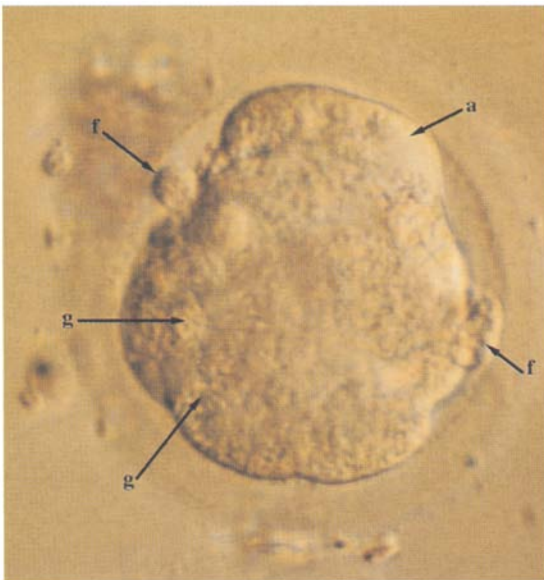
Figure 6.26
Hatched human blastocyst (courtesy of day 6 post-insemination) cultured in G2 medium supplemented with human serum albumin. Note the abundant cell numbers and the absence of a zona pellucida. Original magnification ×300 (courtesy of G.Jones, Melbourne, Australia. Published with permission from Jones, G.M., Trounson, A.O., Gardner, D.K. *et al. Hum. Reprod.* (1998a) **13**, 169–177).

Figure 6.27
The zona pellucida after hatching on day 7. Note the presence of some cells and/or debris in the empty zona (courtesy of G.Cassuto, Paris, France).

Figure 6.28
Artificially hatched human blastocysts prior to embryo transfer. Expanded blastocysts (courtesy of day 6 post-insemination) cultured in G2 medium supplemented with human serum albumin were briefly exposed to a 0.2% pronase solution to remove the zona pellucida enzymatically. Original magnification ×150 (courtesy of G.Jones, Melbourne, Australia. Permission as in 6.26).

Figure 6.29
This blastocyst was recovered by non-surgical flushing of the uterine cavity of a donor woman, 5 days after she was artificially inseminated with spermatozoa from the recipient's husband; the recovered blastocyst was immediately transferred to the recipient who delivered a healthy female child at term (courtesy of L.Formigli, Milano, Italy. Published with permission from Formigli, L., Roccio, C., Belotti, G. *et al. Hum. Reprod.* (1990) **5**, 329–335).

Figure 6.30
Scanning electron micrograph of a hatching blastocyst. In the frame, the magnification of the surface of the hatching embryo (courtesy of B.Dale, Naples, Italy).



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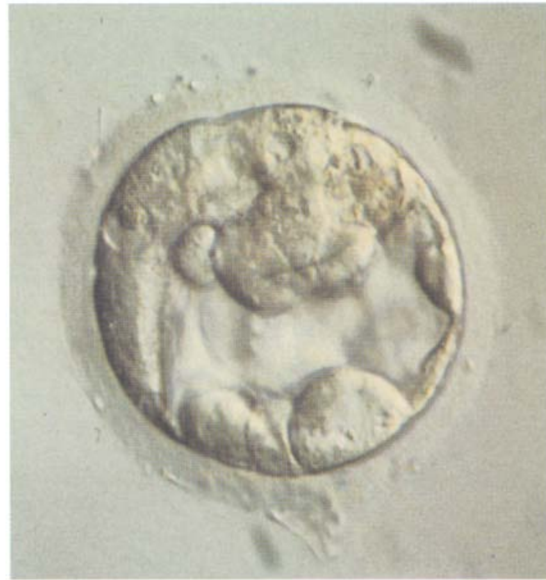

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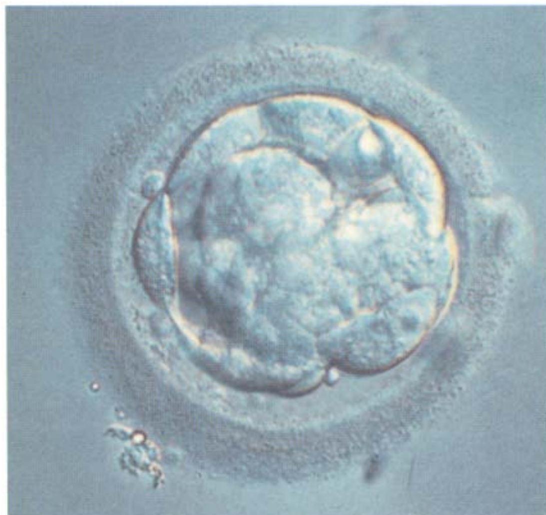

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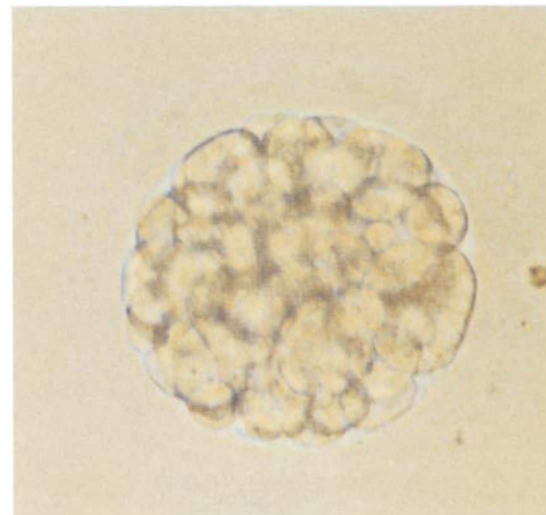

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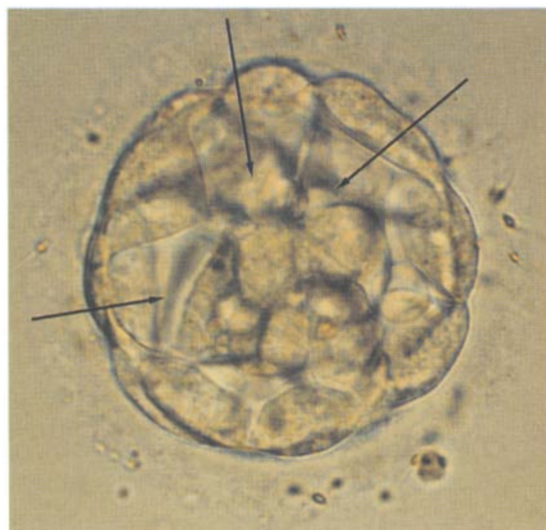

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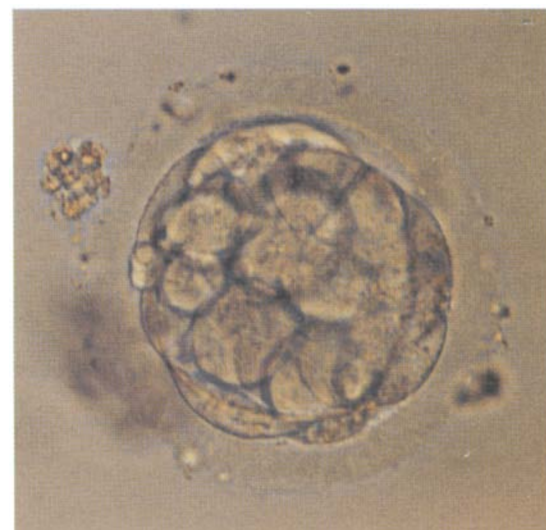

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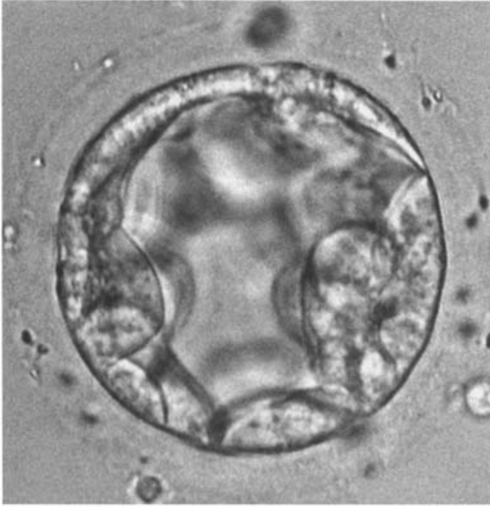

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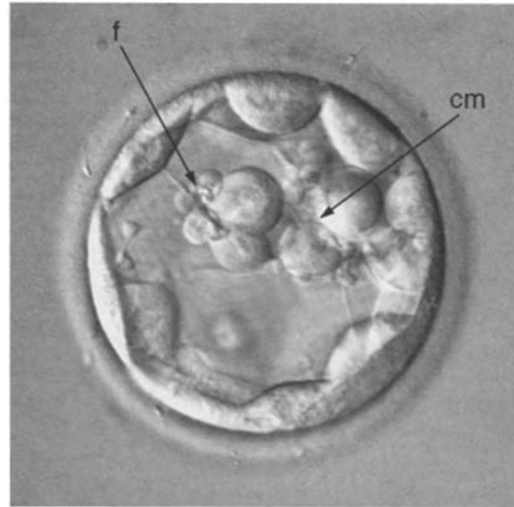

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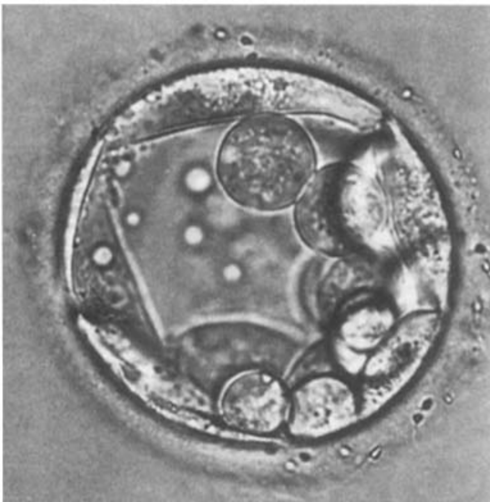

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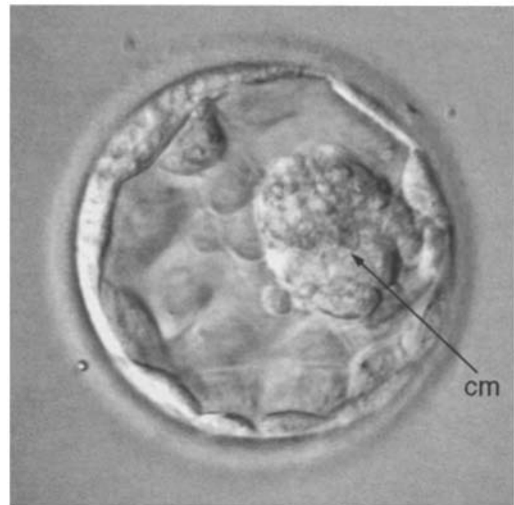

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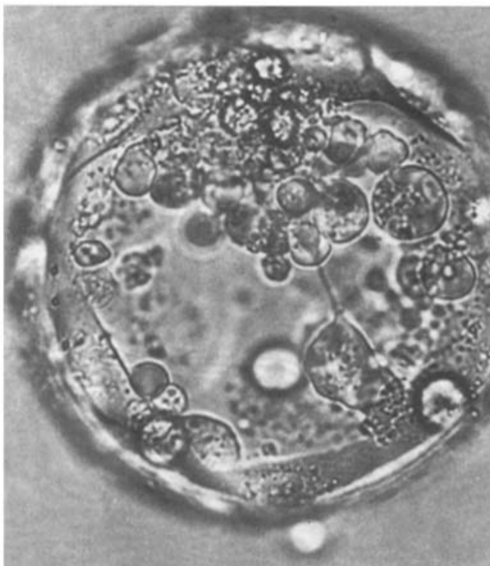

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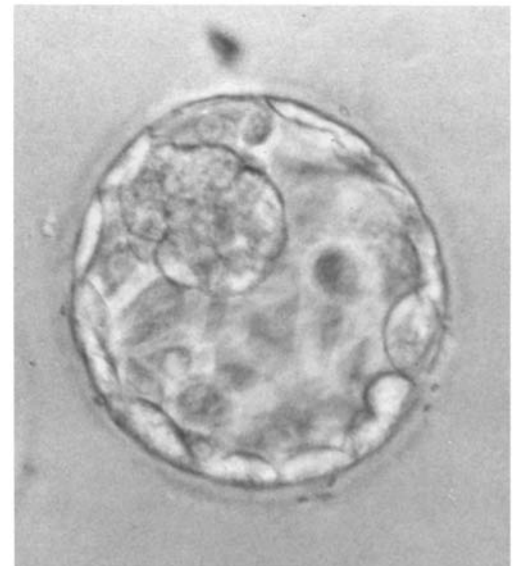

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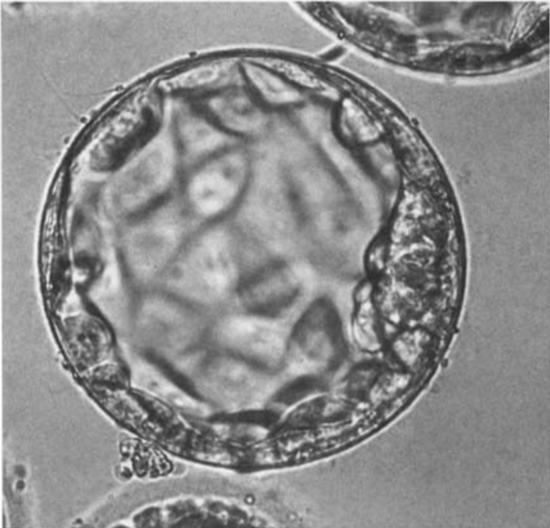

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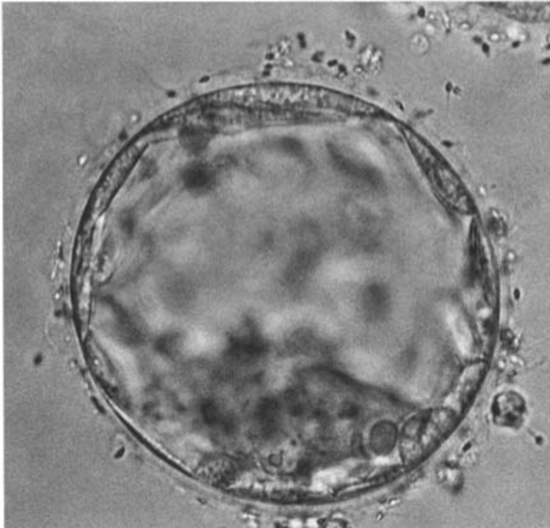

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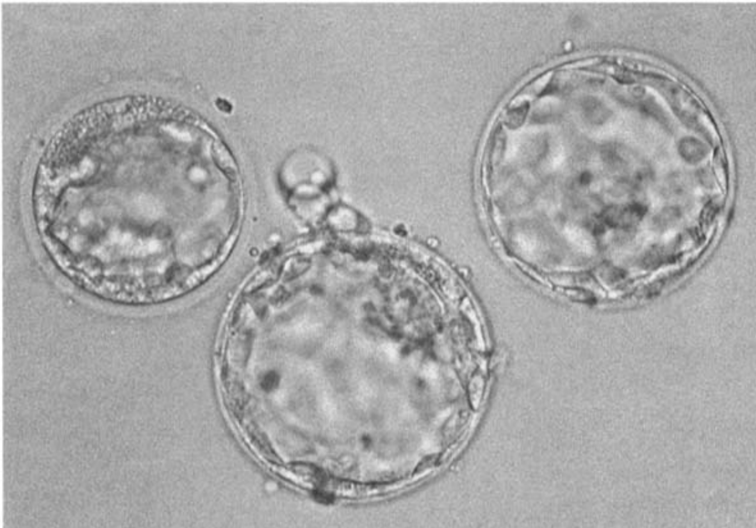

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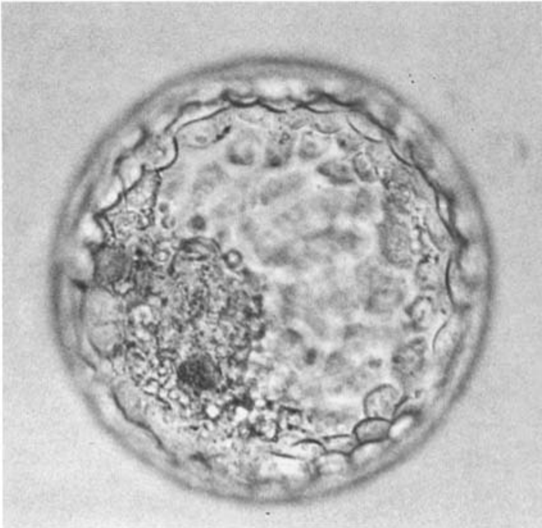

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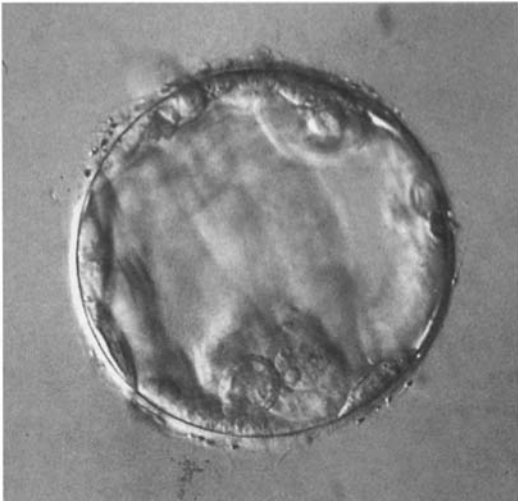

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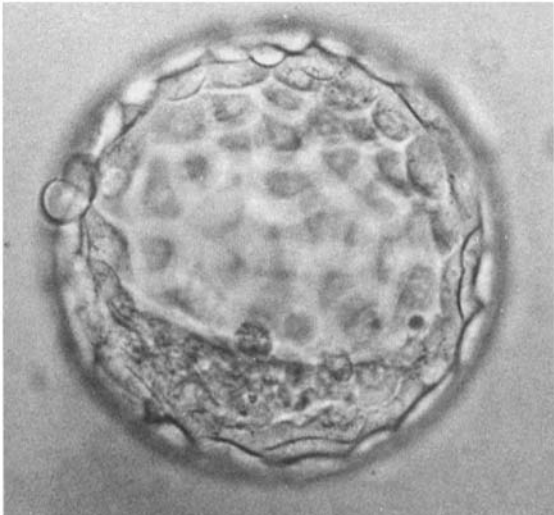

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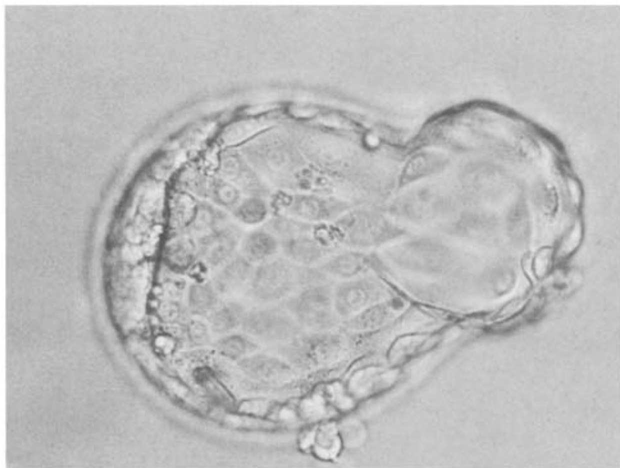

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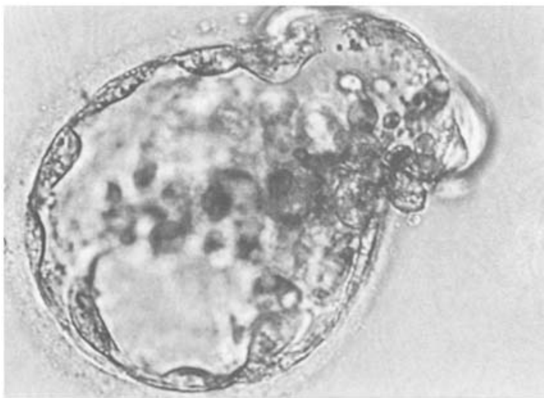

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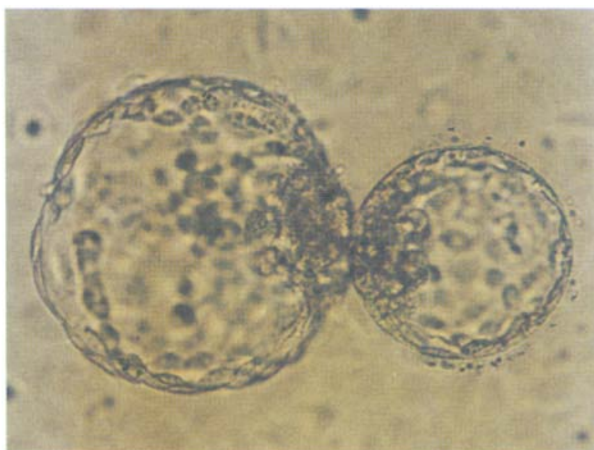

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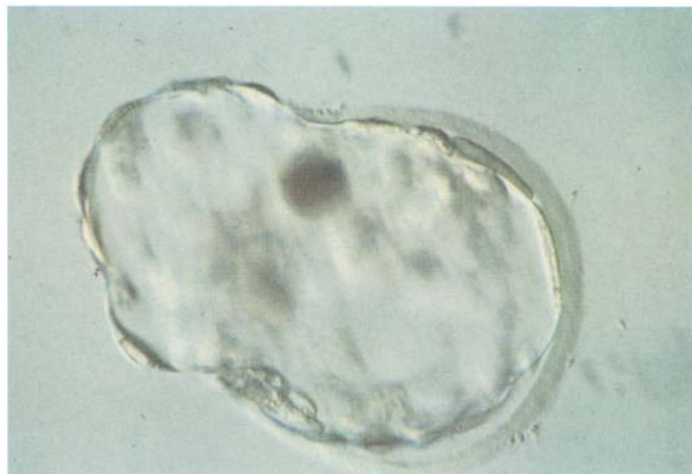

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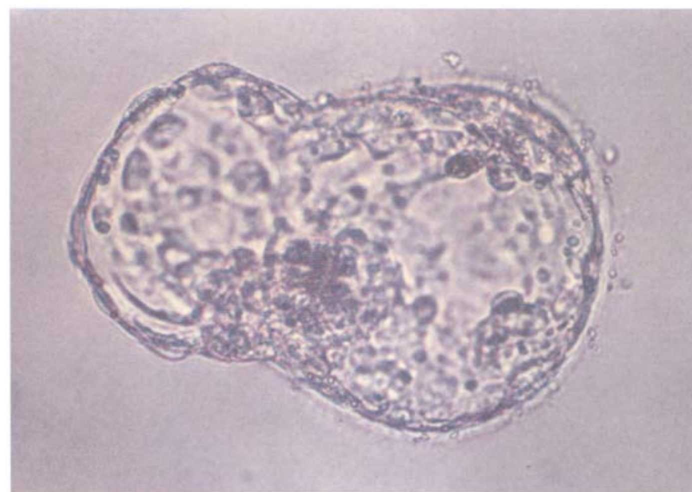

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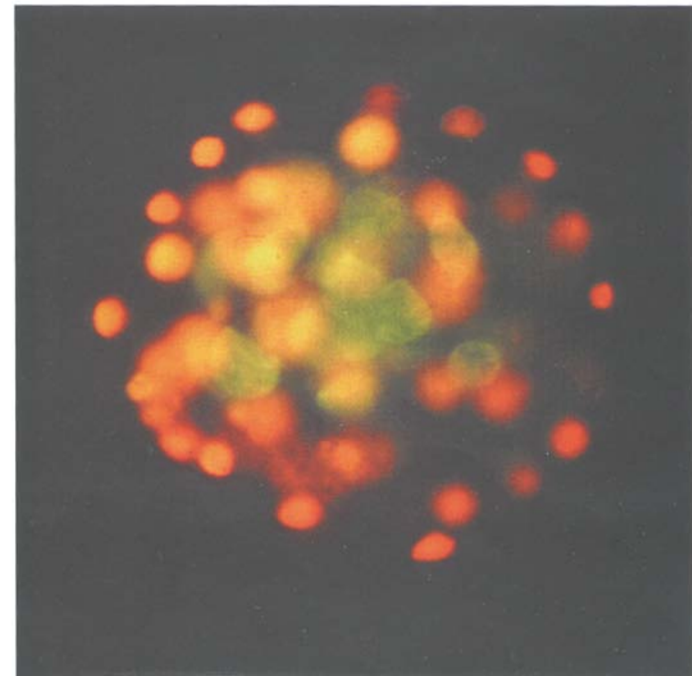

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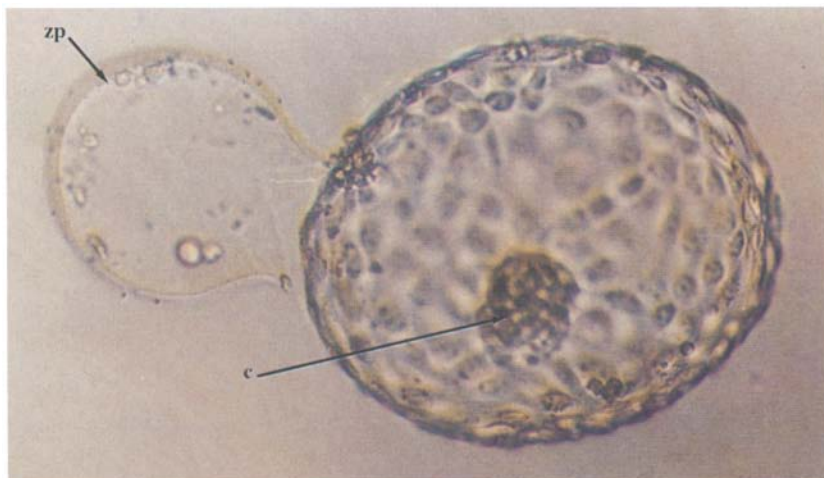

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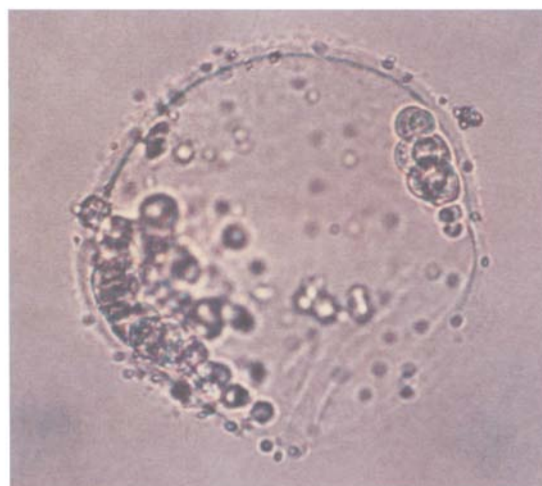

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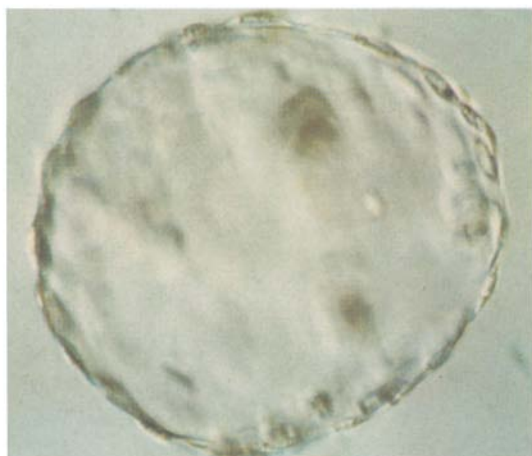

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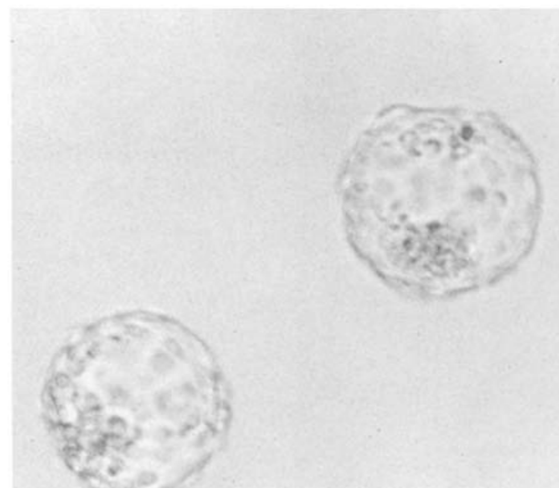

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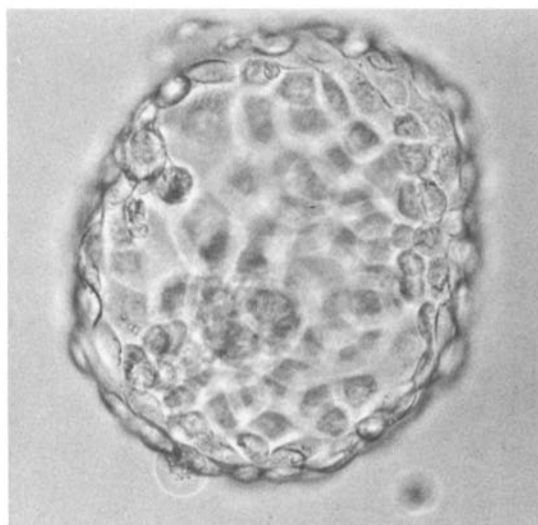

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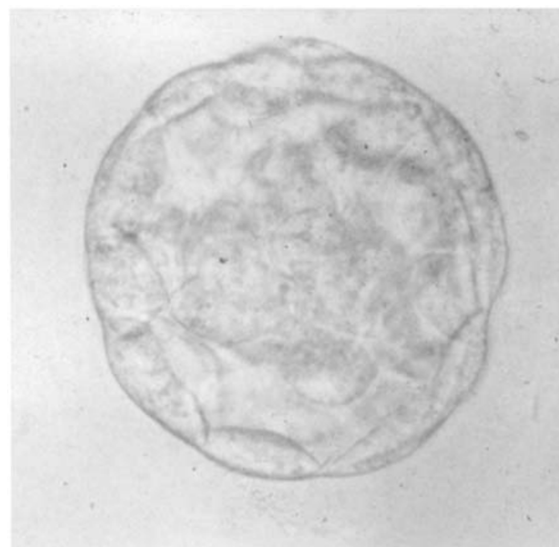

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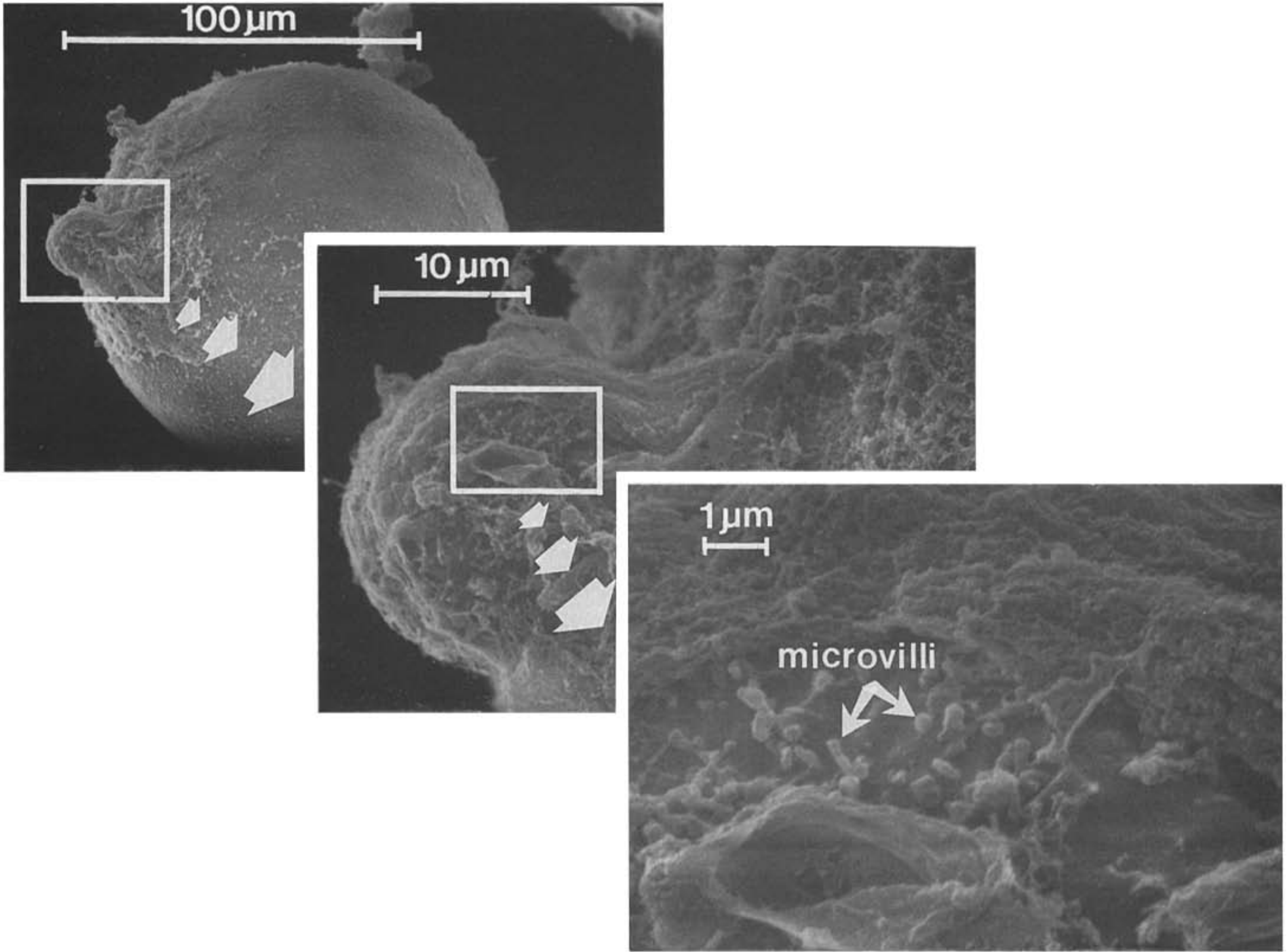

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