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Mitochondrial morphology during preimplantational human embryogenesis

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The structure, distribution, and function of mitochondria during human oogenesis and early development is reported. Oogonia show a sparse and even distribution of mitochondria, which are oval or elongated. Except around nuclei, growing oocytes from small antral follicles have more dense rounded or oval mitochondria, associated with the rough endoplastic reticulum. Mitochondria in fully grown, germinal vesicle (GV) oocytes present an inert appearance, with a dense matrix and a few arch-like or transverse cristae. At this stage mitochondria are usually absent from the cortical part of the cytoplasm. Mitochondria in metaphase I and II oocytes, including fertilized oocytes, present a similar structure, but they are numerous and evenly spread in the ooplasm, associating closely with vesicles or aggregates of tubular smooth endoplasmic reticulum. The most substantial change in distribution occurs at the pronuclear stage, when there is a central conglomeration of mitochondria around the pronuclei in both monospermic and dispermic embryos, which persists up to syngamy. In structure and distribution, mitochondria in blastomeres of 2–16-cell embryos remain virtually unchanged and resemble those of mature oocytes, though perinuclear aggregation can be evident. Mitochondria are usually excluded from meiotic and mitotic spindles but locate peripherally, apparently providing energy for centrosomal, cytoskeletal, and chromosomal activity during cell division. Morphogenetic changes in mitochondrial structure occur in the 8-cell cleaving embryo, the morula and the blastocyst (apparently accompanying the onset of nuclear and mitochondrial transcription), when they become progressively less electron dense and often develop clear areas in their matrices. Elongating mitochondria with inner mitochondrial membranes arranged into transverse cristae appear in expanding blastocysts, in the trophoblast, embryoblast, and endodermal cells. These mitochondria seem to play a role in blastocyst differentiation, expansion, and hatching, with their morphological changes reflecting increased cellular activity.

Key words: embryo/human/mitochondria/mtDNA/oocyte/ultrastructure

Introduction

Mitochondria, the chief energy transducers of cells, have come to prominence in the study of human embryo development and in-vitro embryo culture (Bavister, 1995; Jansen and

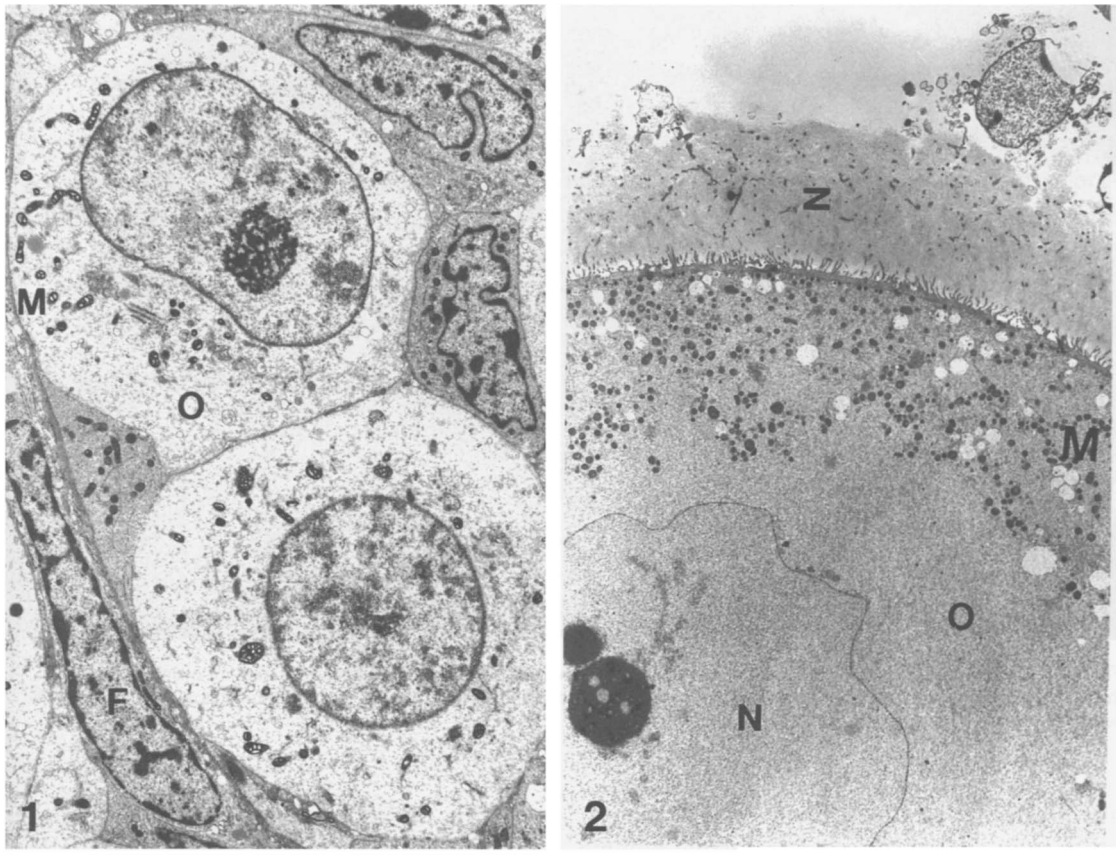


Figure 1. Oogonia from fetal ovary. Mitochondria (M) are sparse and variable in form. Note reticulated nucleolus. F = follicle cell; O = oogonia. Original magnification ×5250 (reproduced from Sathananthan *et al.*, 2000, with permission).

Figure 2. Growing primary oocyte in an early antral follicle from adult ovary. The mitochondria (M) are mostly located in peripheral ooplasm (O). N = nucleus; Z = zona. Original magnification ×2275.

De Boer, 1998). The embryo during this time changes its metabolic requirements *in vitro*, corresponding perhaps with its in-vivo transport from the oviduct (or Fallopian tube) to the uterine environment. *In vitro*, the human embryo requires a pyruvate or lactate-rich culture medium during early cleavage and then seems to prefer a glucose-rich substrate medium later in preimplantation development (Bavister, 1995). This can now be achieved *in vitro* by sequential culture of human embryos in so-called stage-specific culture media (Jones *et al.*, 1998). Such a change of substrate requirements could reflect an inherent change in mitochondrial structure and function, since these are the organelles of cellular respiration and ATP synthesis that ultimately utilize these substrates. Furthermore mitochondria exhibit morphological changes that seem to coincide with this metabolic transition.

Mitochondria are the most prominent organelles in maturing oocytes and embryos, identifiable by transmission electron microscopy (TEM). The other readily distinguishable organelles present are cortical granules, smooth or rough endoplasmic reticulum (SER or RER), the Golgi apparatus, and lysosomes (see Sathananthan *et al.*, 1986, 1993). Mitochondria are apportioned to various blasto-

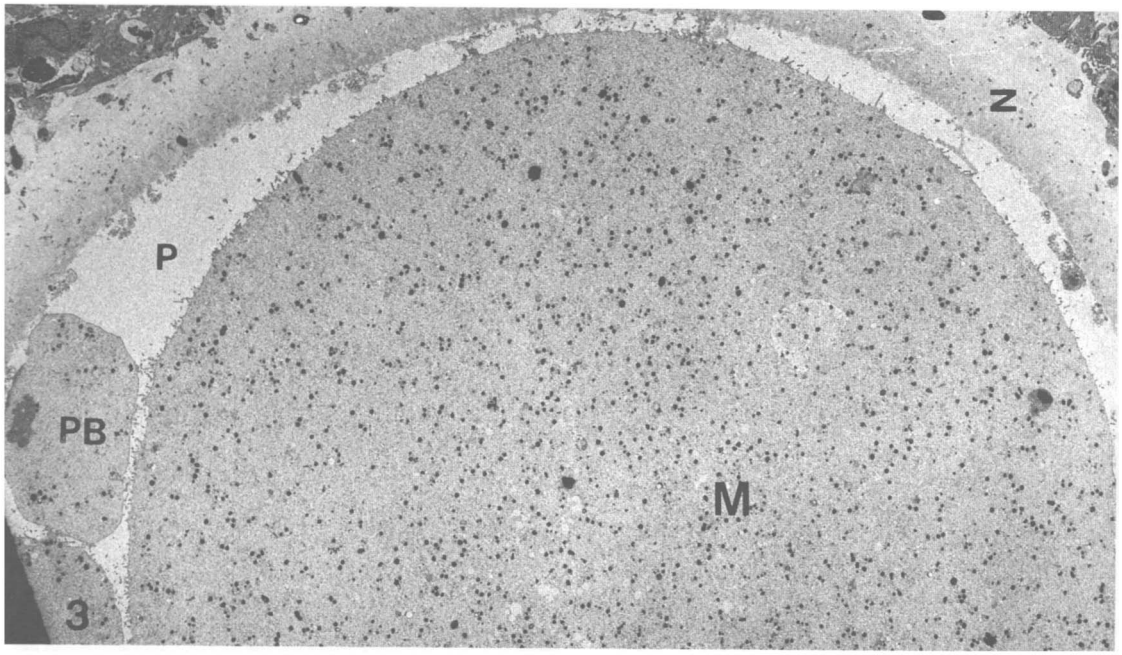


Figure 3. Mature fertilized oocyte, 3 h after insemination. Mitochondria (M) are very numerous and are evenly distributed throughout the ooplasm. Note that the polar body (PB) also contains mitochondria. P = perivitelline space; Z = zona pellucida. Original magnification ×1820.

meres during cleavage up to the blastocyst stage, with no increase in cytoplasmic volume during repeated mitoses (cell divisions) of embryonic cells.

The TEM studies presented here deal principally with the intracellular distribution and microstructure of mitochondria from the mature oocyte to the stage at which the blastocyst hatches out of the zona pellucida, in preparation for implantation into the endometrium. Some observations on primordial germ cells as well as growing and maturing oocytes are also made, and are relevant to culture of ovarian tissue and oocytes *in vitro*. There have been several previous studies of mitochondrial morphology in human oocytes or embryos, some of which have focused on oocyte or embryo culture (see Dvorak and Tesarik, 1985; Makabe *et al.,* 1989; Van Blerkom, 1989; Wartenberg, 1989; Sathananthan *et al.*, 1990, 1993; and also see Motta, 2000). Studies of mammalian embryos have shown that mitochondria do undergo morphogenetic changes in response to increased metabolism, oxygen consumption, carbon dioxide production, or substrate utilization during early development (for review, see Van Blerkom and Motta, 1979).

Here we report changes in mitochondrial structure and distribution during oogenesis, oocyte maturation, and preimplantation development, and attempt to relate these changes to known biochemical and genomic transitions.

Mitochondria were examined by routine TEM in serial sections of human oocytes and embryos collected in an IVF laboratory. Fetal and adult ovaries were also examined for mitochondria in oogonia and in oocytes of primordial and small antral follicles. All material was routinely fixed in glutaraldehyde and osmium tetroxide, and serial sections were stained with alcoholic uranyl acetate and Reynold's lead citrate (Sathananthan, 1993; Sathananthan *et al.*, 1993).

Mitochondrial distribution

In general, oogonia in fetal ovaries and the oocytes of primordial follicles in adult ovaries show mitochondria in a perinuclear distribution, whereas oocytes from small antral follicles show a peripheral distribution (Figures 1 and 2). Mitochondria of healthy, large, germinal vesicle oocytes (obtained from tertiary follicles) show a perinuclear distribution, being excluded from the cortical cytoplasm. In mature (metaphase II) oocytes and in oocytes soon after fertilization, mitochondria are usually evenly distributed (Figure 3), but then conspicuously conglomerate around the pronuclei as pronuclear formation occurs (Figure 4). Mitochondria also tend to locate around some nuclei in early blastomeres and in cells of morulae and blastocysts. However, they are

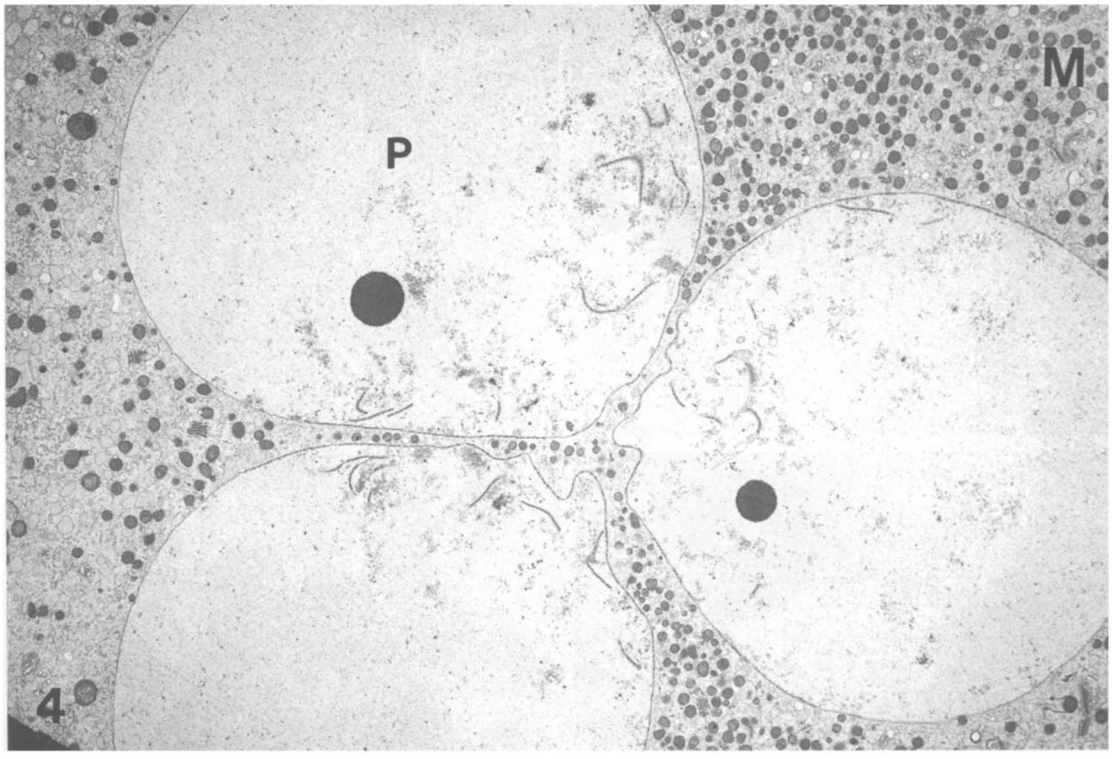


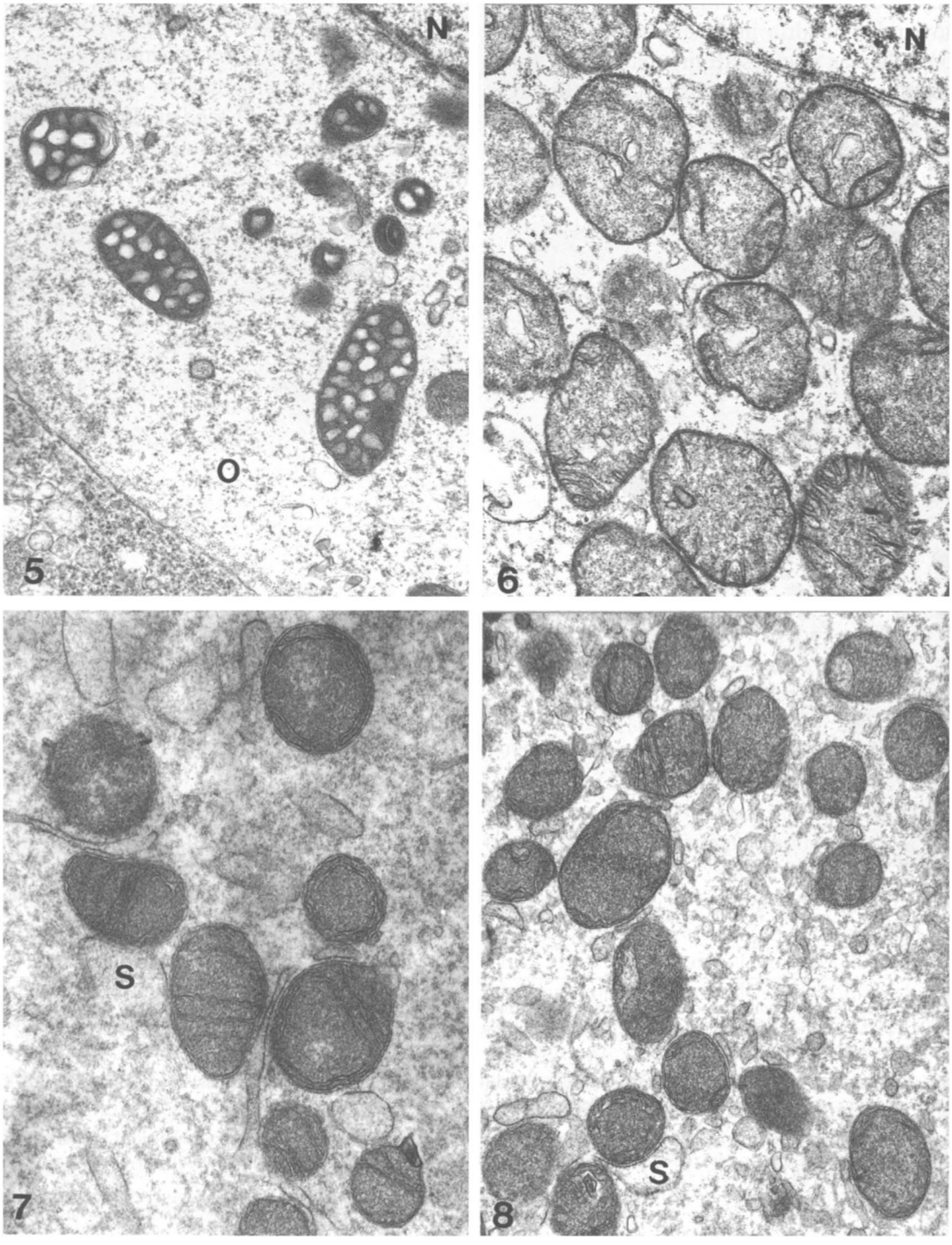
Figure 4. Pronuclear stage zygote with three pronuclei. The mitochondria (M) conglomerate in the central ooplasm around the pronuclei (P). This occurs in both normal (two pronuclei) and three pronuclear zygotes. Original magnification ×3500.

Figure 5. Oogonium showing mitochondria in detail. Mitochondria are oval to elongated, show a dense matrix, and the inner mitochondrial membranes form tubular cristae, resembling those of steroid-secreting cells. O = ooplasm; N = nucleus. Original magnification ×35 000.

Figure 6. Primordial follicular oocyte showing mitochondria in detail. Mitochondria are spherical and inner mitochondrial membranes form irregular cristae, among a less dense matrix. N = nucleus. Original magnification ×35 000.

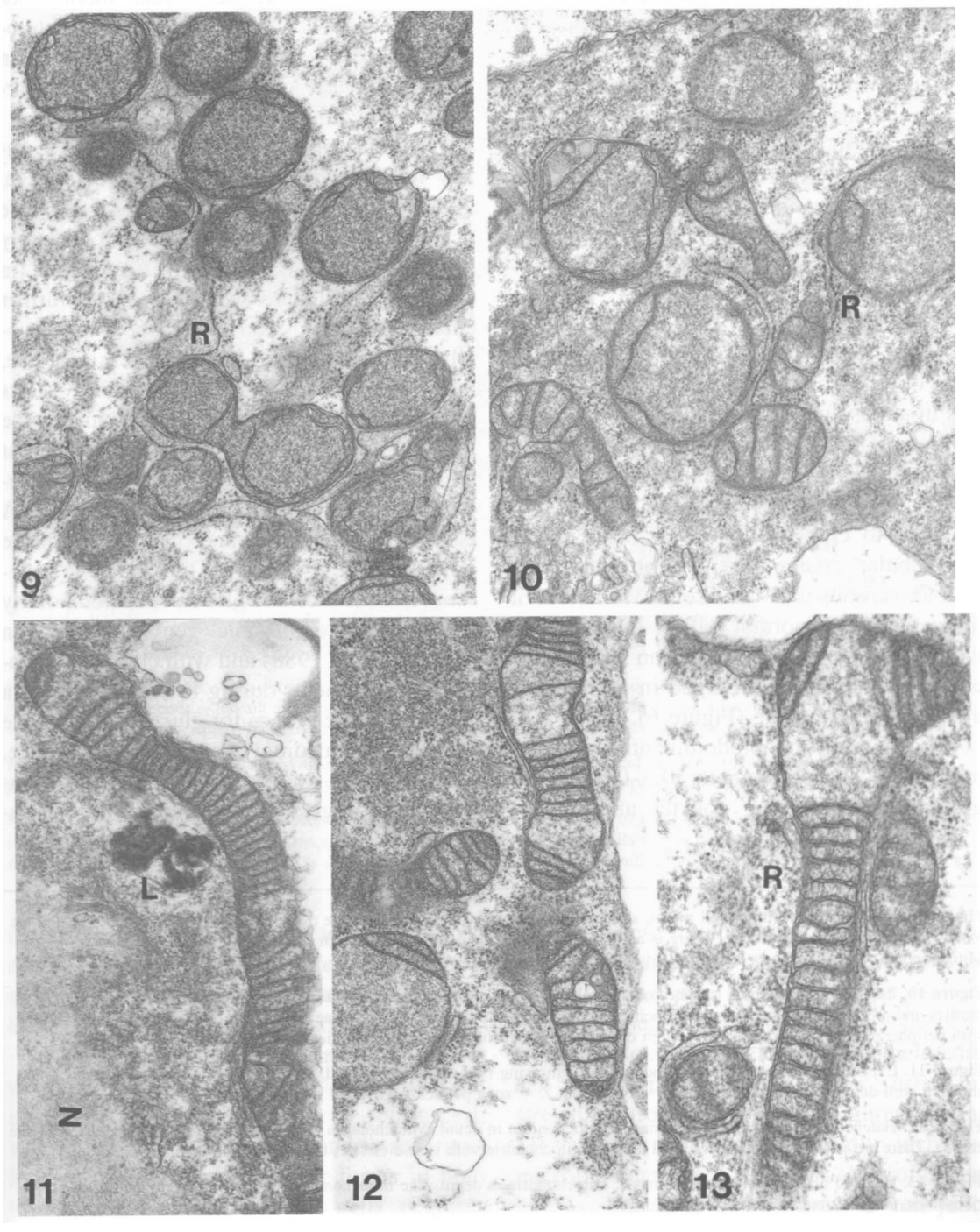
Figure 7. Mature metaphase II secondary oocyte showing mitochondria in detail. The mitochondria are rounded to oval with a dense matrix and arch-like or transverse cristae. Note the association with smooth endoplasmic reticulum (S). Original magnification ×43 750.

Figure 8. Two-cell embryo, showing mitochondria in detail. Mitochondria are unchanged from those of mature oocytes. S = smooth endoplasmic reticulum. Original magnification ×35 000.



usually excluded from meiotic and mitotic spindles, aggregating instead at the periphery.

Mitochondria are usually associated with elements of the SER or RER (Figures 7–10), depending on the stage of development. SER manifests as isolated vesicles or aggregates of tubules. Mitochondria are found on the periphery of the latter in maturing oocytes.



Mitochondria are also associated with decondensing sperm heads in association with vesicular SER (Figure 15) and then during construction of the male and female pronuclear envelopes. Sperm mitochondria are easily distinguishable from oocyte mitochondria by their shape (oval to elongate) and cristal structure and their association with sperm centrioles, axonemes and male pronuclei (Figures 14, 16). Sperm mitochondria have clearly been involved in sperm motility, which ceases at fertilization; they might then be involved in the release of the sperm centrosome from the sperm neck to form a sperm aster.

Mitochondrial structure

TEM observations of oocytes during oogenesis and in a closely knit series of preimplantation human embryos show that mitochondria undergo some significant changes in structure during this time. Mitochondria of fetal oogonia are oval to elongated, have a dense matrix and tubular cristae that resemble those of steroid secreting cells (Figure 5). Mitochondria in oocytes of primordial follicles are rounded, with a less dense matrix and an inner mitochondrial membrane arranged in the form of a few irregular cristae (Figure 6).

Mitochondria are predominantly spherical to oval in shape (diameter 0.3–0.5 μm) in growing and maturing oocytes and in very early cleavage stage blastomeres (Figures 7 and 8). They present an inert-looking appearance, with a dense matrix and a few arch-like cristae located peripherally or transversely. Clear blebs beneath the surface membranes are sometimes seen. Occasionally giant mitochondria (diameter 1–2 μm) can be seen in mature oocytes. Mitochondria that are apparently duplicating, fusing, or double are rare.

This mitochondrial microstructure is virtually unchanged until about the 8-cell stage, after which a subtle but progressive decrease occurs in matrix density until about the early blastocyst stage (Figure 9), after which (in expanding and hatching blastocysts) further changes in fine structure occur more dramatically. In the 8–16-cell stages and in morulae, mitochondria become comparatively less dense, but the cristal structure remains the same as in early embryos. Developing clear areas in the mitochondrial matrix might coincide with the location of mitochondrial DNA (see Fawcett, 1981) and possibly indicate initiation of transcription. This change in structure after the 8-cell stage coincides temporally with known embryonic genome activation (Braude *et al.*, 1988) and with changing metabolic requirements during IVF culture, with a developing preference for glucose as substrate in the culture medium over pyruvate or lactate. Mitochondrial studies should help in the concocting of progressive culture media to optim-

Figure 9. Early blastocyst (inner cell mass), showing mitochondria in detail. Note the decreasing density of the matrix and the mostly peripheral arch-like cristae. There is an association of mitochondria with rough endoplasmic reticulum (R). Mitochondria of morulae show a similar structure. Original magnification ×35 000.

Figure 10. Expanding blastocysts (trophectoderm cell), showing mitochondria in detail. Some mitochondria have smaller profiles and are beginning to elongate as their inner membranes form transverse cristae; others are larger and spherical, still with peripheral, arch-like cristae. R = rough endoplasmic reticulum. Original magnification ×35 000.

Figure 11. Expanding blastocyst (trophectoderm cell), showing mitochondria in detail. Highly elongated mitochondrion showing well-defined transverse cristae. L = lysosome; Z = zona pellucida. Original magnification ×35 000.

Figure 12. Hatched blastocyst (inner cell mass), mitochondria in detail. Mitochondria have elongated and developed transverse cristae. There are still some larger, rounded to oval mitochondria with sparse cristae. Original magnification ×35 000.

Figure 13. Hatched blastocyst (inner cell mass), mitochondria in detail. The mitochondrion is apparently elongating. R = ribosomes. Original magnification ×52 500.

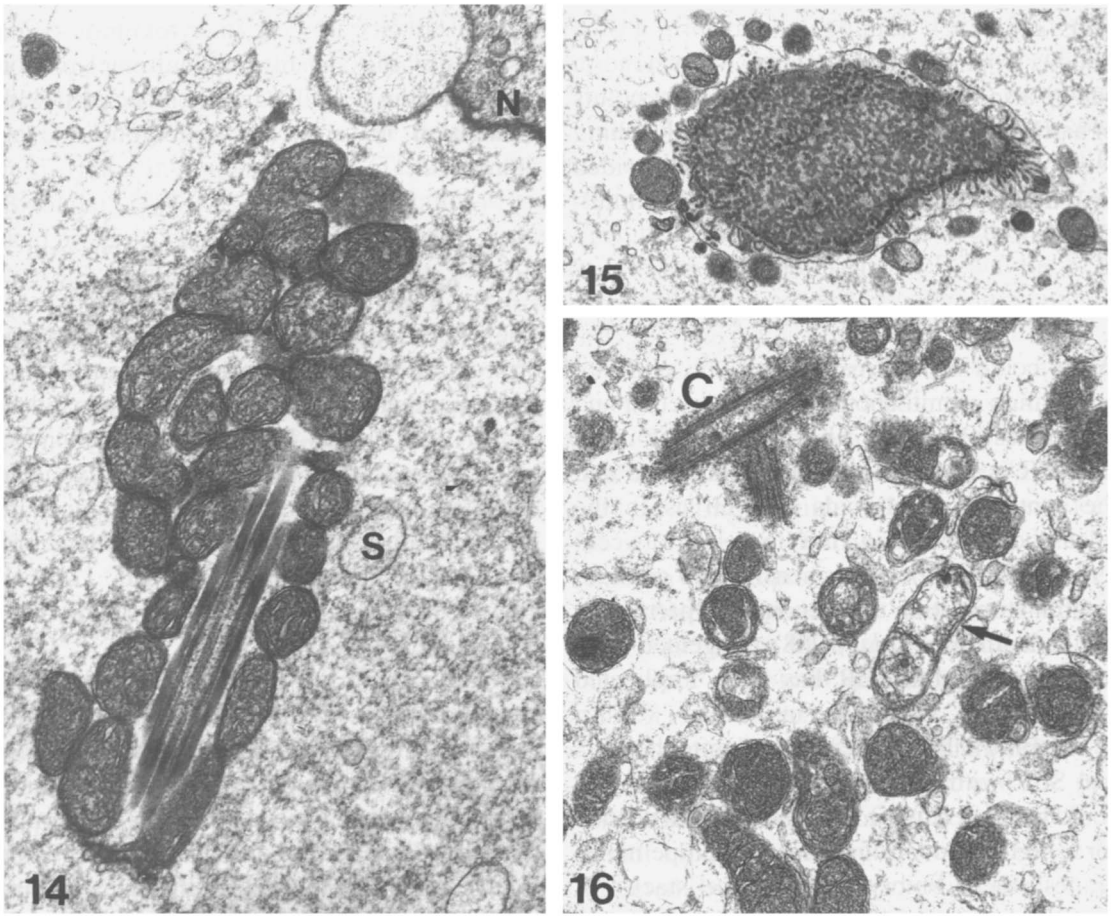


Figure 14. Sperm midpiece mitochondria within the ooplasm, 3 h after insemination. The mitochondria are easily distinguished from oocyte mitochondria, having abundant inner mitochondrial membranes (cristae) with a lucent matrix, while still associated with the axoneme. N = sperm nuclear envelope; S = smooth endoplasmic reticulum. Original magnification ×35 700.

Figure 15. Oocyte mitochondria at syngamy closely associated with a sperm head that has failed to decondense (silent polyspermy). Oocyte mitochondria usually associate with normal decondensing sperm heads. Original magnification ×17 500.

Figure 16. Sperm mitochondria in a pronuclear-stage zygote. The morphology of most of the oocyte mitochondria is little changed (compared with Figure 7). One sperm mitochondrion (arrow) has lost its matrix density and cristae and is probably degenerating. The sperm centriole (C) is duplicating at this stage. Original magnification ×35 000.

ize early embryo development to the blastocyst stage *in vitro*: the substrate and oxygen requirements of preimplantation embryos have been discussed previously (Bavister, 1995.)

The most dramatic changes in mitochondrial morphology occur during the differentiation, expansion, and hatching of the blastocyst, when rounded to oval mitochondria are transformed into elongated tubular forms in both trophoblast and embryoblast cells (Figures 10–13). These mitochondria have inner membranes that increase in extent to form well-defined transverse cristae, a clear sign of increased metabolic activity. During blastocyst expansion extremely elongated mitochondria can be aligned parallel to the surface in stretching trophoblast cells (Figures 10 and 11), which also show markedly increased microfilament (actin) activity. Microfilament bundles attach to desmosomes at cell junc-

tions, effectively joining cells to form a continuous trophoblast epithelium. Mitochondria can be also found in trophoblast cells at points of hatching. Blastocyst expansion and hatching require energy, which is presumably provided by oxidative phosphorylation within the mitochondria.

Mitochondrial functional correlates

What could be the reason for the constant association of mitochondria with vesicular and aggregates of tubular SER? In somatic cells, the SER is involved in lipid metabolism and the synthesis of steroids as well as in the release of calcium in muscle cells (Fawcett, 1981). SER in oocytes is sensitive to gonadotrophin stimulation and becomes hypertrophic (Sathananthan *et al.*, 1988). Together with mitochondria, the SER has been implicated with calcium signalling in oocytes (Sousa *et al.*, 1997). The association of mitochondria and SER with pronuclei of fertilized oocytes could be related to the lipid metabolism needed for a high turnover of nuclear envelope membranes; these embryos also have stacks of annulate lamellae, which closely resemble nuclear membranes in form. The mitochondrial conglomeration persists to syngamy, when the first cleavage spindle is formed.

Mitochondria are normally located in the periphery of spindles and may be important in cell division, presumably providing ATP for microtubular activity including chromosomal movements and for cytokinesis, which is mediated by microfilament activity. If so, hypoxia and oxidative stress could affect ATP production in oocytes and embryos, and inhibit microtubular activity during meiosis and mitosis and microfilament activity during cytokinesis, and also affect the expansion and hatching of the blastocyst (Tarín, 1996;Van Blerkom *et al.*, 1998). Mitochondrial dysfunction could thus result in aberrant chromosomal segregation, resulting in aneuploidy of the mature oocyte. Defects in cytokinesis could also cause mosaicism and multinucleolation among cleavage cells, and be associated with retardation or arrest in embryonic development.

The fate of sperm mitochondria and other organellae components

The whole sperm midpiece and tail are incorporated into the human oocyte at fertilization (Sathananthan and Chen, 1986; Sathananthan *et al.*, 1986, 1990, 1993). The mitochondria are still associated with the sperm axoneme 3 h after insemination (Figure 14) and are gradually dissociated from the axoneme by the time the pronuclei are formed, 12–16 h after insemination (Sathananthan, 1996, 1997; Sathananthan *et al*, 1996).

Another important contribution of the spermatozoon to the fertilized oocyte is the plasma membrane, which is almost totally incorporated into the oolemma in zipper-like fashion during gamete fusion, resulting in the formation of a mosaic zygote plasma membrane (Sathananthan and Chen, 1986; Sathananthan *et al.*, 1986, 1990, 1993). Another incorporated component is the expanding sperm nuclear envelope, which is partly inserted into the developing male pronucleus, together with maternal SER. At fertilization there is, in summary, a complicated interaction of cellular membranes, including mitochondrial membranes, about which very little is known.

Sperm mitochondria introduced into the oocyte cytoplasm at fertilization become progressively translucent (Figure 16). While they are traceable to about the 8-cell stage, their fate is unknown, although they are generally believed to degenerate (Ankel-Simons and Cummins, 1996). However, before degeneration, they might still have an important role. One of the last functions of sperm mitochondria (sperm motility having ceased) seems to be to effect release of the sperm centrosome,

which contains the functional centriole, from a 'black box' beneath the basal plate, to organize the sperm aster. This could be associated with the calcium transient detected at fertilization in human oocytes (Tesarik, 1997; Battaglia, 1998), as there is a calcium-activated protein, centrin, associated with the sperm centriole (Sutovsky *et al.*, 1996). In humans, the spermatozoon contributes the functional centrosome that activates the embryo to cleave by repeated mitoses (Sathananthan *et al.*, 1991, 1996; Simerly *et al.*, 1995). The mechanics of cell division of the human embryo have so far been partly unravelled (Schatten, 1994; Sathananthan *et al.*, 1996; Sathananthan, 1997).

Mitochondrial movements and the cytoskeleton

The dramatic changes in mitochondrial distribution in the oocyte after fertilization and during early cleavage in the embryo are dependent upon cytoskeletal reorganization. One of the most important cell organelles involved in activation of the oocyte at fertilization, the centrosome, organizes the cytoskeletal system of the oocyte and, later, of the embryonic cells, during both interphase and mitosis. The dominant centrosome contains the sperm centriole (introduced at fertilization), reshapes the cytoskeletal architecture of the oocyte, is involved in the formation of the sperm aster, moves the male and female pronuclei towards each other, and ultimately organizes the first mitotic spindle after syngamy, when male and female chromosomes come together on a metaphase plate (Sathananthan *et al.*, 1991, 1996; Schatten, 1994). During this process, it attracts maternal γ-tubulin to form a zygote centrosome. All these events are mediated by cytoskeletal mictrotubules composed of α-tubulin, which reorganize the cellular organelles of the fertilized oocyte, including mitochondria, the SER, and the Golgi. Their spatial relationship is important to preserve the structural integrity of the ooplasm. This contributes partly to the microtrabecular lattice within the cell, first conceptualized by Keith Porter (see Fawcett, 1981). This lattice is attached to the cell surface membrane and to membranous cell organelles such as mitochondria, the RER, and the SER, and its meshes are traversed by microtubules and fine microfilaments composed of actin. It is well known that microtubules are involved in the movement of chromosomes during mitosis and in directing secretory vesicles elaborated by Golgi membranes within the cell by acting as guide-rails during the process of cell secretion. Since, at this time, the early embryo has a preference for pyruvate as a metabolic substrate to glucose, oxidative phosphorylation by mitochondria rather than anaerobic glycolysis is presumed to provide the ATP for such movements.

Mitochondria, Golgi, and microtubules are abundant in fertilized oocytes, and the peripronuclear gathering of mitochondria is no doubt brought about by microtubular activity directed by centrosomes. Microtubules are very sensitive to heat and to ageing. Their disruption would result in aberrant redistribution of organelles within the zygote, severely compromising embryonic cell structure and development. This is clearly evident in oocytes and embryos ageing *in vitro* (Sathananthan and Trounson, 1989; Sathananthan *et al.*, 1993).

Mitochondria in degenerating oocytes and blastomeres

The mitochondria of ageing oocytes and embryonic cells tend to clump together, become increased in electron-density, and are associated with swollen, translucent vesicles of SER. The latter form vacuoles and mitochondria align on their periphery. During degeneration, mitochondria can acquire inner dense granules, resembling lysosomes, or can

show a disorganized matrix structure. They may become attenuated and curl around vesicular SER. These mitochondria tend to aggregate together in the ooplasm, forming clouds in degenerating oocytes and conglomerating around nuclei in blastomeres (Sathananthan and Trounson, 1989).

Conclusions

Mitochondria seem to undergo profound morphogenetic changes during oogenesis and embryogenesis, reflecting changing metabolic requirements during early development. Mitochondria of preovulatory oocytes and early cleavage stage embryos present an 'inert' appearance when compared to those of morulae and blastocysts, which appear as more active mitochondria in structure. They seem to be most active during blastocyst differentiation expansion, and hatching. Dramatic changes in distribution of mitochondria are also observed, particularly during pronuclear formation after fertilization, when a centralized conglomeration of mitochondria appears around pronuclei. It appears that mitochondrial structure and function substantially change during early development, and this should be considered when sequential media are made for culture of human embryos to blastocysts.

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References

Ankel-Simons, F. and Cummins, J.M. (1996) Misconceptions about mitochondria and mammalian fertilization: implications for theories on human evolution. *Proc. Natl Acad. Sci. USA*, **93**, 13859–13863.

Battaglia, D.E. (1998) Questions about oocyte activation: answers from ICSI? In Filicori, M. and Flamigni, C. (eds), *Treatment of Infertility: The New Frontiers.* Communications Media for Education, New Jersey, USA, pp. 249–256.

Bavister, B.D. (1995) Culture of preimplantation embryos: facts and artefacts. *Hum. Reprod. Update*, **1**, 91–148.

Braude, P., Bolton, V. and Moore, S. (1988) Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature*, **332**, 459–461.

Dvorak, M. and Tesarik, J. (1985) Differentiation of mitochondria in the human preimplantation embryo grown *in vitro. Scr. Med. (Brno)*, **58**, 161–170.

Fawcett, D.W. (1981) *The Cell.* Saunders, Philadelphia, USA.

Jansen, R.P.S. and de Boer, K. (1998) The bottleneck: mitochondrial imperatives in oogenesis and ovarian follicular fate. *Mol. Cell. Endocrinol.*, **145**, 81–88.

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

Makabe, S., Nottola, S.A. and Motta, P.M. (1989) Life history of the human germ cell: Ultrastructural aspects. In van Blerkom, J. and Motta, P.M. (eds). *Ultrastructure of Human Gametogenesis and Early Embryogenesis.* Kluwer Academic, Boston, USA, pp.33–60.

Motta, P.M., Nottola, S.A., Makabe, S. and Heyn, R. (2000) Mitochondrial morphology in human fetal and adult female germ cells. *Hum. Reprod.*, **15** (Suppl. 2), 129–147.

Sathananthan, A.H. (1993) Ultrastructure in fertilization and embryo development. In Trounson, A. and Gardner, D.K. (eds), *Handbook of In Vitro Fertilization.* CRC Press, Boca Raton, FL, USA, pp 237–261.

Sathananthan, A.H. (ed.) (1996) *Visual Atlas of Human Sperm Structure and Function for Assisted Reproductive Technology.* National University Hospital and Serono, Singapore, 279 pp.

Sathananthan, A.H. (1997) Mitosis in the human embryo: The vital role of the sperm centrosome (centriole) – review. *Histol. Histopathol.*, **12**, 827–856.

Sathananthan, A.H. and Chen, C. (1986) Sperm–oocyte membrane fusion in the human during monospermic fertilisation. *Gamete Res.*, **15**, 177–186.

Sathananthan, A.H. and Trounson, A. (1989) Effects of culture and cryopreservation on human oocyte and embryo ultrastructure and function. In Van Blerkom, J. and Motta, P.M. (eds), *Ultrastructure of Human Gametogenesis and Early Embryogenesis.* Kluwer Academic, Boston, USA, pp 181–200.

Sathananthan, A. H., Trounson, A.O. and Wood C. (1986) *Atlas of Fine Structure of Human Sperm Penetration, Eggs and Embryos Cultured in vitro.* Praeger Scientific, Philadelphia, USA, 279 pp.

Sathananthan, A.H., Ng, S.C., Ratnam, S.S., *et al.* (1988) Are we overstimulating in IVF? *Singapore J. Obstet. Gynaecol.*, **19**, 83–88.

Sathananthan, A.H., Bongso, A., Ng, S.C. *et al.* (1990) Ultrastructure of preimplantation human embryos co-cultured with human ampullary cells. *Hum. Reprod.*, **5**, 309–318.

Sathananthan, A.H., Kola, I., Osborne, J. *et al.* (1991) Centrioles in the beginning of human development. *Proc. Natl Acad. Sci. USA*, **88**, 4806–4810.

Sathananthan, A.H., Ng, S.C., Bongso, A. *et al.* (1993) *Visual Atlas of Early Human Development for Assisted Reproductive Technology.* National University Hospital and Serono, Singapore, 209 pp.

Sathananthan, A.H., Ratnam, S.S., Ng, S.C. *et al.* (1996) The sperm centriole: its inheritance, replication and perpetuation in early human embryos. *Hum. Reprod.*, **11**, 345–356.

Sathananthan, A.H., Selvaraj, K. and Trounson, A. (2000) Fine structure of human oogonia in the foetal ovary. *Mol. Cell. Endocrinol.*, **161**, 3–8.

Schatten, G. (1994) The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. *Dev. Biol.*, **165**, 299–335.

Simerly, C., Wu, G.J., Zoran, S. *et al.* (1995) The paternal inheritance of the centrosome, the cell's microtubule-organizing center in humans, and the implications for infertility. *Nature Med.*, **1**, 47–52.

Sousa, M., Barros, A., Silva., J. *et al.* (1997) Developmental changes in calcium content of ultrastructurally distinct subcellular compartments of preimplantation human embryos. *Mol. Hum. Reprod.*, **3**, 83–90.

Sutovsky, P., Hewitson, L., Simerly, C. *et al.* (1996) Molecular medical approach for alleviating infertility and understanding assisted reproduction technology. *Proc. Assoc. Am. Phys.*, **108**, 432–443.

Tarín, J.J. (1996) Potential effects of age-associated oxidative stress on mammalian oocytes/embryos. *Mol. Hum. Reprod.*, **2**, 717–724.

Tesarik., J. (1997) Confocal laser scanning microscopy in the study of calcium signalling during human oocyte activation at fertilization. In Motta, P.M. (ed). *Microscopy of Reproduction and Development : A Dynamic Approach.* Antonio Delfino Editore, Rome and Milan. pp. 205–211

Van Blerkom, J. (1989) Developmental failure in human reproduction associated with preovulatory oogenesis and preimplantation embryogenesis. In Van Blerkom, J. and Motta, P.M. (eds), *Ultrastructure of Human Gametogenesis and Early Embryogenesis.* Kluwer Academic, Boston, USA, pp. 125–180.

Van Blerkom, J. and Motta, P.M. (1979) *The Cellular Basis of Mammalian Reproduction.* Urban and Schwarzenberg, Baltimore and Munich.

Van Blerkom, J., Sinclair, J. and Davis, P. (1998) Mitochondrial transfer between oocytes: potential applications of mitochondrial donation and the issue of heteroplasmy. *Hum. Reprod.*, **13**, 2857–2868.

Wartenberg, M. (1989) Ultrastructure of foetal ovary including oogenesis. In Van Blerkom, J. and Motta, P.M. (eds), *Ultrastructure of Human Gametogenesis and Early Embryogenesis.* Kluwer Academic, Boston, USA, pp. 61–84.