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Sperm-Oocyte Membrane Fusion in the Human During Monospermic Fertilization

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Sperm-oocyte membrane fusion has been observed during monospermic fertilization of a human oocyte in vitro.

Women were stimulated with both clomiphene citrate and human menopausal gonadotropin and were given human chorionic gonadotropin before a LH-surge. Twelve oocytes, collected at laparoscopy from six women who became pregnant by IVF, were allowed to mature for 7–14 hours in vitro and inseminated with preincubated sperm, fixed between 1–3 hours after insemination, and examined by transmission electron microscopy.

Membrane fusion had occurred in one ovum 2 hours after insemination, and the oocyte had resumed maturation and was at anaphase II of meiosis. Cortical granules had been exocytosed, and some of their contents were visible at the surface close to the oolemma all around the oocyte. The sperm that fused with this oocyte was acrosome-reacted and had been partly incorporated into the ooplasm, while the anterior two-thirds of its head was phagocytosed by a tongue of cortical ooplasm. Membrane fusion had occurred between the oolemma and the plasma membrane overlying the postacrosomal segment of the sperm head, posterior to the equatorial vestige. Sperm chromatin had not decondensed, and serial sections revealed a midpiece attached to the basal plate and a tail located deeper in the ooplasm, all devoid of plasma membrane. Supplementary sperm penetrating the inner zona, approaching the perivitelline space, had undergone the acrosome reaction but had a persistent vestige of the equatorial segment of the acrosome with intact plasma membrane. Evidence of sperm chromatin decondensation was seen in other oocytes, 3 hours after insemination, which were at telophase II of meiosis. Eight oocytes penetrated by sperm were monospermic, while four were unfertilized.

The general pattern of sperm fusion and incorporation appears to conform to that seen in most other mammals. The study also reveals that sperm have to complete the acrosome reaction before fusing with the egg.

Key words: Sperm-egg fusion, sperm incorporation, acrosome reaction, human IVF, electron microscopy

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INTRODUCTION

Sperm-oocyte membrane fusion has not yet been demonstrated in the human during monospermic fertilization. This process, however, has been observed during polyspermic oocyte interaction in zona-free [Soupart and Strong, 1975] and zona-punctured [Sathananthan et al, 1986] human oocytes, respectively. Many studies on sperm-egg fusion have been reported in mammals, particularly laboratory rodents and rabbits [see Gwatkin, 1977; Edwards, 1980; Bedford et al, 1979; Soupart, 1980; Yanagimachi, 1981], but there has been some controversy on the regions of sperm membrane involved in fusion.

Earlier reports [Lopata et al, 1980; Sathananthan, 1984] dealt with the events of human fertilization soon after sperm penetration, 3 hours after insemination in vitro. The present study attempts to demonstrate the early events of gamete interaction and sperm incorporation using eggs and sperm of couples who had success with in vitro fertilization (IVF). A complimentary study traced the sequence of sperm penetration through the egg vestments of these human oocytes, 1–3 hours after insemination [Chen and Sathananthan, 1986].

MATERIALS AND METHODS

The clinical, IVF, and electron microscopic procedures used in this study have already been reported [Chen and Sathananthan, 1986]. Briefly, oocytes were obtained from women with tubal or unexplained infertility who became pregnant by IVF. The sperm used was from their partners who had normal semen parameters. The women were stimulated with clomiphene followed by human menopausal gonadotropin and were given human chorionic gonadotropin (hCG). Follicular development was monitored by ultrasound and estradiol estimations. Oocytes were recovered at laparoscopy 32–34 hours after hCG injection and matured in Whittingham's T_6 culture medium for 7–14 hours before insemination. Semen was washed, centrifuged, layered in the same culture medium, and used within 1 hour of collection. About 20,000 sperm were incubated with each oocyte in a tube containing 1 ml of culture medium.

Twelve oocytes collected from six women were used for this study. All six women became pregnant and gave birth to normal babies after transfer of four embryos each. These embryos (2–8-cell stages) were developed in culture from 24 fertilized ova obtained in the same treatment cycles that provided oocytes for this investigation. The oocytes were examined at various intervals by transmission electron microscopy, 1–3 hours after insemination. They were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3), postfixed in 1% aqueous osmium tetroxide, processed, and embedded in Araldite. Each oocyte was serially sectioned from pole to pole with glass and diamond knives. Alternate series of thick (1 μm) and thin sections ($\sim$70 nm) were cut, stained with toluidine blue or alcoholic uranyl acetate/Reynolds lead citrate, respectively. Thin sections were examined with a Philips 301 electron microscope.

RESULTS

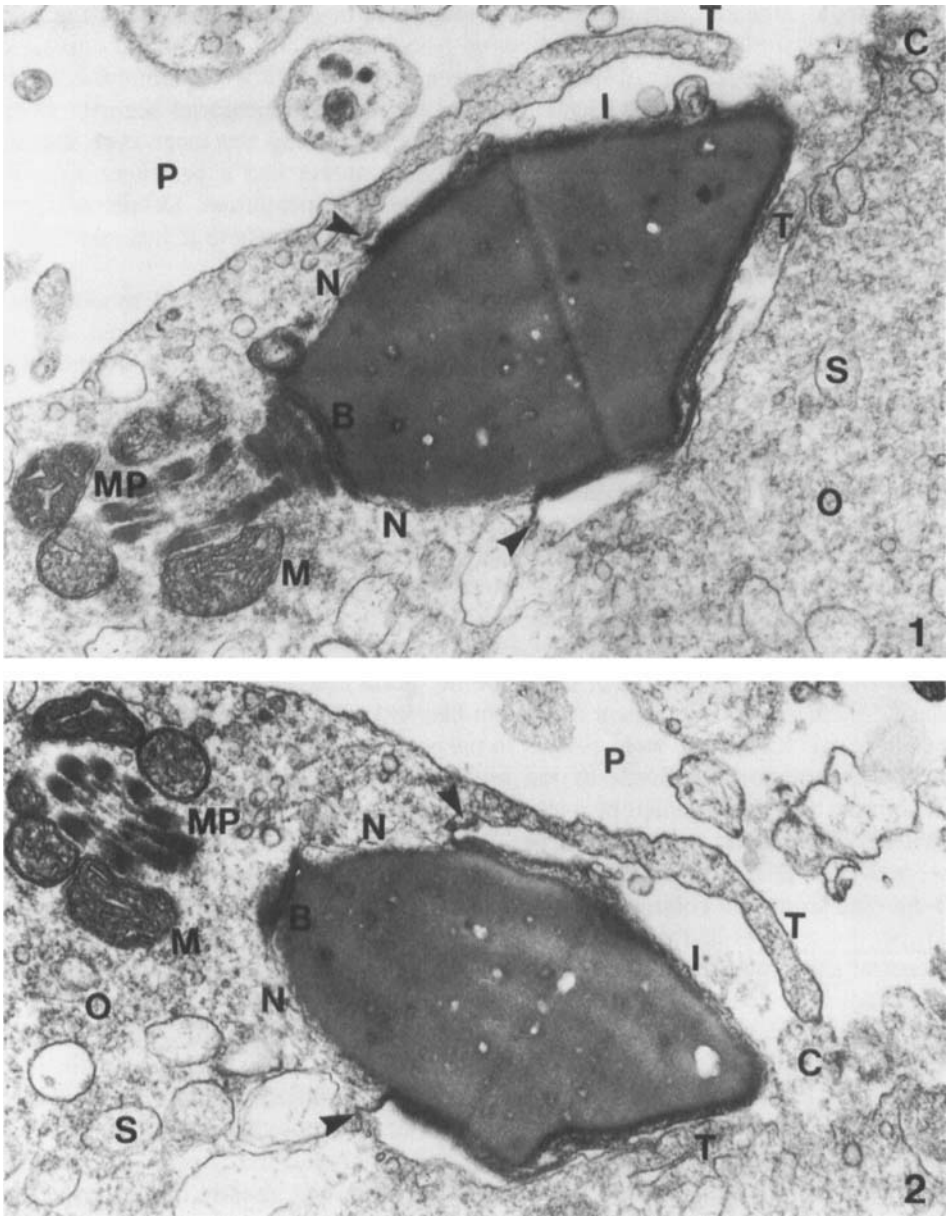
When examined after insemination, most oocytes had one to five layers of cumulus cells showing variable amounts of lipid globules, and the corona cells had

retracted their processes from the oolemma. Variable numbers of sperm were bound to the zona pellucida, and this was also evident in oocytes obtained from the same women. The acrosome reaction had begun 1 hour after insemination, and unreacted, partially reacted, and reacted sperm were seen penetrating between cumulus cells and entering the zona. Partially reacted sperm had an intact equatorial segment of the acrosome. Only acrosome-reacted sperm were penetrating the inner zona and approaching the perivitelline space. Most of these sperm had a persistent spur-like vestige of the equatorial segment with intact plasma membrane. Details of sperm penetration through the cumulus and zona are reported elsewhere [Chen and Sathananthan, 1986].

All oocytes had matured in culture, but only 8 ova showed monospermic fertilization, 2–3 hours after insemination. Four oocytes were not fertilized. Sperm-oocyte membrane fusion was seen in one of two oocytes 2 hours after insemination and sperm nuclear decondensation in the ooplasm was evident in seven ova 2–3 hours after insemination. All ova penetrated by sperm were undergoing the cortical reaction and had initiated second polar body extrusion. Serial sections of an oocyte showing sperm fusion (Figs. 1–5) revealed that the sperm had already been incorporated posterior to the acrosome region, and the anterior two-thirds of its head was in the process of being phagocytosed by a flap or tongue of cortical ooplasm. The sperm head was held more or less tangential to the egg surface, while its midpiece and tail were oriented obliquely with respect to the surface and were located deeper in the ooplasm. Membrane fusion had evidently occurred between the oolemma and plasma membrane of the postacrosomal region of the sperm head extending from the equatorial vestige (Figs. 1, 2). Sperm chromatin had still not decondensed, and the nuclear envelope and basal plate were evident in the posterior third of the sperm head.

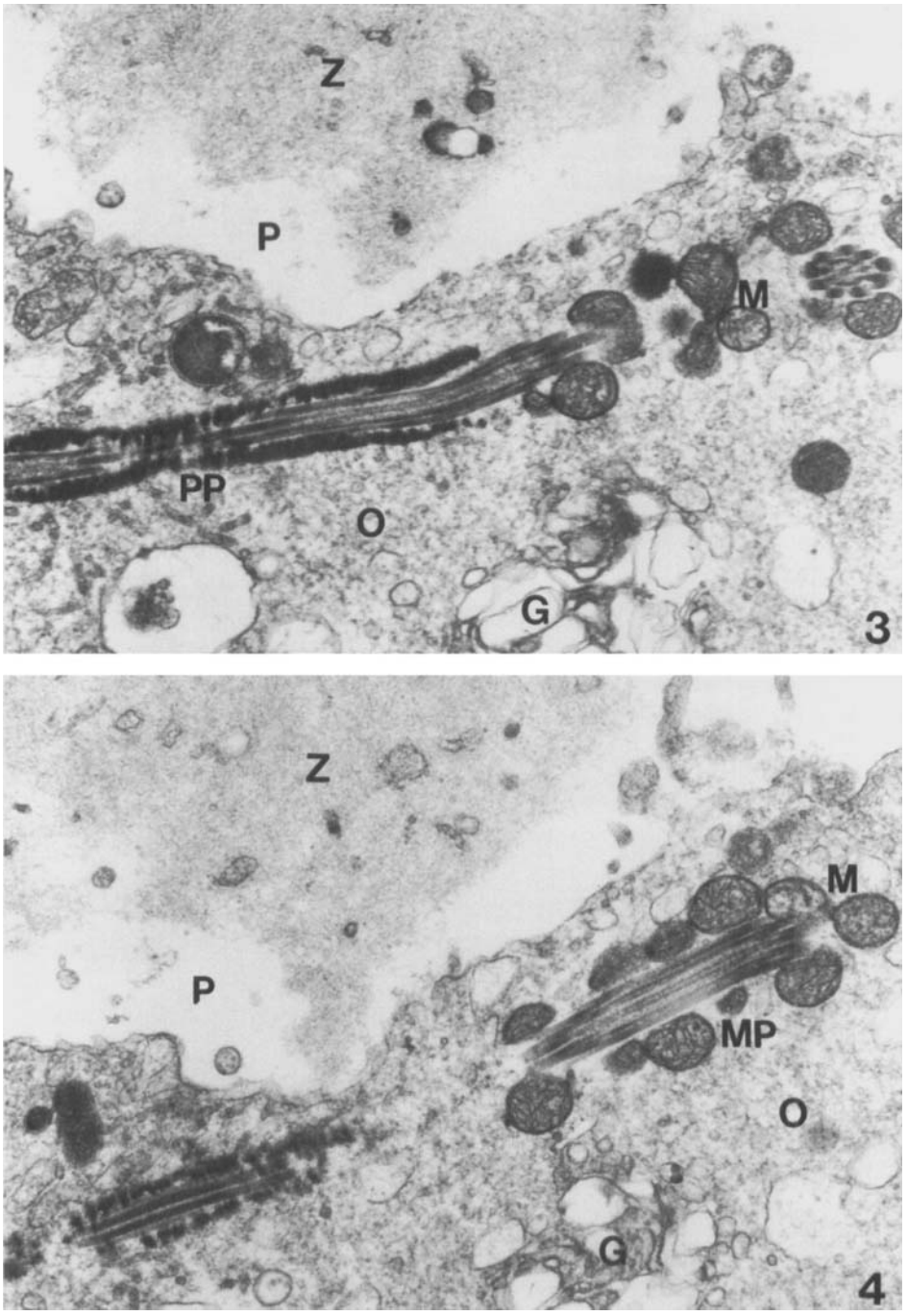
The anterior two-thirds of the sperm head had an exposed inner acrosome membrane and was engulfed by a tongue-like process (Fig. 2) extended from the egg-surface. This process was much larger than surface microvilli. The midpiece and tail were evident posterior to the basal plate but were devoid of plasma membrane (Figs. 3–5). The segmental columns were attached to the basal plate in the neck region (Fig. 1), but the sections had not passed through the centrioles. The midpiece consisted of a central axoneme surrounded by dense fibres and mitochondria. The principal piece of the tail was sectioned longitudinally (Fig. 3) or obliquely at various points (Fig. 5) and showed the axoneme surrounded by dense fibres and ribs of the fibrous sheath. Unidentified filamentous structures, more uniform and smaller than microvilli, were found near the principal piece (Fig. 5). A prominent fertilization cone was not evident at this stage of sperm incorporation. Products of exocytosed cortical granules were visible close to the oolemma (Figs. 3, 5) and all around the oocyte. In the ovum showing sperm fusion, second polar body formation had already begun, and chromosomes were at anaphase II of maturation (Fig. 6).

Various stages of sperm chromatin decondensation were seen in serial sections of penetrated ova. Fully or partially expanded sperm chromatin associated with either centrioles or their microtubules were detected in the ooplasm. Remnants of disintegrating intrinsic nuclear envelope and smooth endoplasmic reticulum forming a new nuclear envelope were evident in the periphery of a fully decondensed sperm nucleus (Fig. 7), which retained its overall shape and was attached to a centriole (Fig. 8). Sections of sperm tails with intact plasma membrane were occasionally seen in the ooplasm.

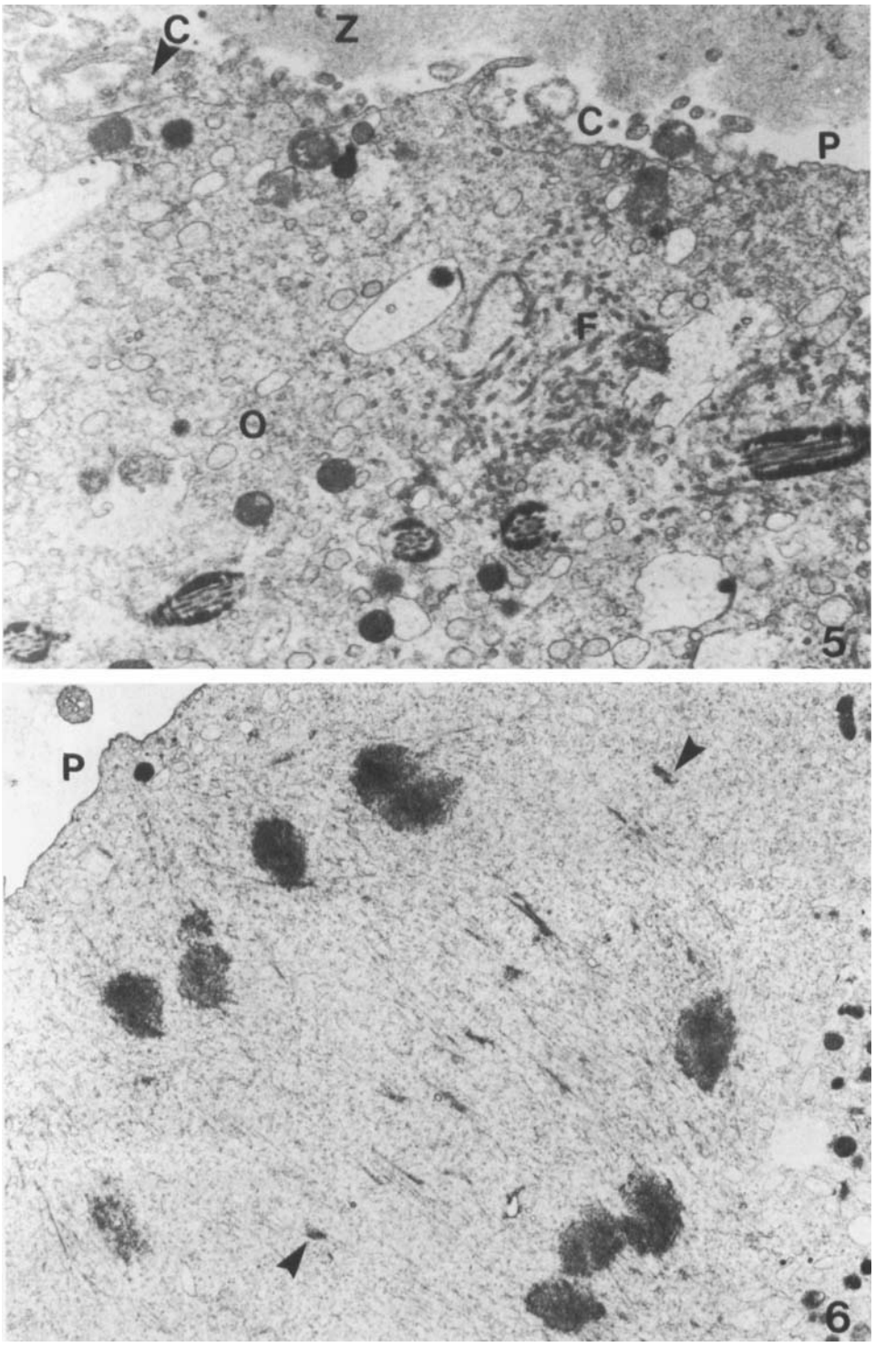


Figs. 1–5. Electron micrographs of serial sections of a human ovum showing sperm fusion and incorporation (2 hours postinsemination).

Figs. 1, 2. Sperm head sectioned at two levels depicting membrane fusion and phagocytosis by the egg. The anterior two-thirds of the acrosome-reacted sperm head has been engulfed by a tongue of cortical ooplasm (T). The oolemma has fused with the postacrosomal segment of the sperm plasma membrane posterior to the equatorial vestige (arrowheads). The basal plate (B) and midpiece (MP) have already been incorporated into the ooplasm (O). Sperm chromatin has still not decondensed. ×34,400. C, contents of cortical granules; I, inner acrosome membrane; M, sperm mitochondria; N, sperm nuclear envelope; P, perivitelline space; S, smooth endoplasmic reticulum.



Figs. 3, 4. Incorporated sperm midpiece (MP) and principal piece of the tail (PP) seen at two levels close to the egg surface. Note absence of sperm plasma membrane. ×24,300. G, Golgi complex; M, sperm mitochondria; O, ooplasm; P, perivitelline space; Z, zona pellucida.


C
Z
C
P
F
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5
P
6

DISCUSSION

The general pattern of gamete fusion, sperm incorporation, and nuclear decondensation in the human conforms to that seen in other eutherian mammals [see Yanagimachi, 1981; Bedford, 1982, 1983]. The possible fusogenic zone of the human sperm during initial gamete membrane interaction seems to be the plasma membrane extending from the equatorial vestige over the postacrosomal region. This is conjectured from observations that most supplementary sperm penetrating the inner zona and perivitelline space have a persistent equatorial vestige or spur [Chen and Sathananthan, 1986] and that the anterior limit of fusion of the oolemma corresponds to the location of this vestige. Further evidence supporting this view comes from a study of numerous sperm fusions with a mature oocyte, mechanically denuded of its zona [Sathananthan et al, 1986]. Although there are differences in the manner in which sperm approach the egg-surface during polyspermic penetration of zona-free eggs, there is clearly evidence that the process of membrane fusion is initiated in the postacrosomal region of the sperm head extending from this equatorial vestige. In mammals, there are some differences of opinion about the fusogenic zone of the sperm head [see review by Yanagimachi, 1981], where it is believed that the plasma membrane of the postacrosomal region or of the equatorial segment or both are capable of fusing with the oolemma. It must, however, be noted that the persistent equatorial vestige is not always obvious in acrosome-reacted human sperm, especially after long hours of insemination [Sathananthan et al, 1982]. This vestige, also seen by Soupart and Strong [1974], is small and stumpy and not easily detectable. Once initial sperm fusion has occurred between the midsegment of the sperm plasma membrane and oolemma, the anterior region of the sperm head, covered by exposed inner acrosome membrane, is incorporated by the egg cortex by a process akin to phagocytosis, which has also been observed in other mammals [see Yanagimachi, 1978; Bedford, 1982, 1983]. This clearly shows that the inner acrosome membrane is not capable of fusing with the oolemma. Sperm first make contact with the oolemma using this membrane as they break through the zona [Chen and Sathananthan, 1986]. Further, Sathananthan et al [1986] have shown that unreacted sperm are incapable of fusing with zona-punctured oocytes and that they remain passive in the perivitelline space making contact with microvilli or the oolemma. From the foregoing observations, there is now little doubt that only acrosome-reacted sperm are capable of fusing with egg, as reported in other mammals [Yanagimachi, 1981].

An obvious fertilization cone seen 3 hours after insemination [Lopata et al, 1980; Sathananthan, 1984] was not evident at this stage of fusion (2 hours postinsemination) and was not always seen during polyspermic incorporation [Sathananthan et al, 1986]. The absence of plasma membrane in the midpiece and tail indicates that

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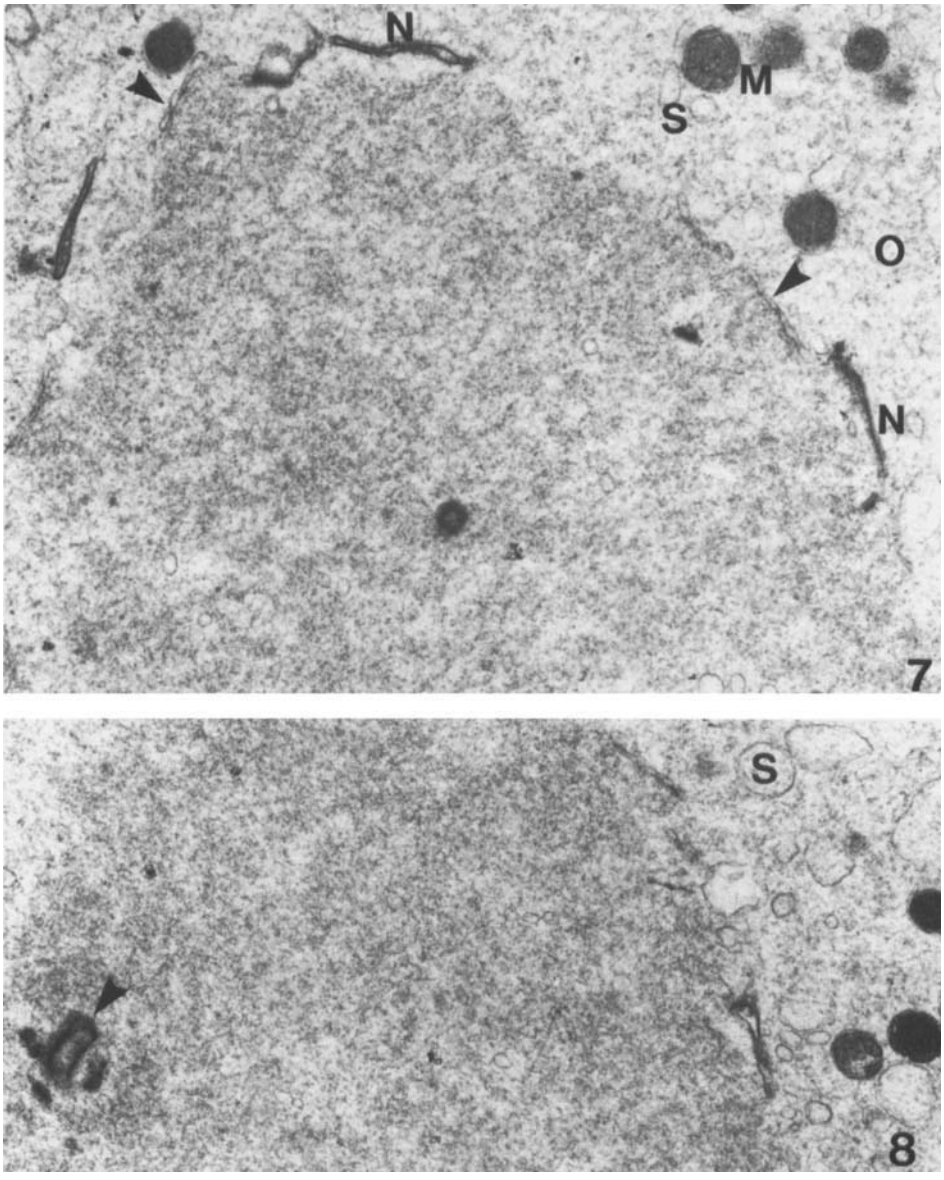
Fig. 5. Principal piece of the sperm tail cut obliquely at various points, located deeper in the ooplasm (O). Unidentified filaments (F) of uniform thickness are associated with the tail. Note released products of cortical granules (C) at the surface of the ovum. ×17,640. P, perivitelline space; Z, zona pellucida.

Fig. 6. A section of the same ovum (Figs. 1–5) showing beginning of second polar body formation (anaphase of second maturation). Chromosomes have separated and a dense interbody (arrowheads) is being elaborated at the equator of a wide spindle. There are no asters or centrioles. The first polar body was seen in the perivitelline space (P) close to this spindle. ×10,710.



Figs. 7, 8. Parts of a decondensing sperm nucleus in the ooplasm of a fertilized ovum (3 hours postinsemination). Its chromatin has fully expanded, and remnants of the intrinsic sperm nuclear envelope (N) and endoplasmic reticulum of oocyte origin (arrowheads) are seen at the periphery. The sperm nucleus (Fig. 8) from a serial section of the same ovum is associated with a centriole (arrowhead). The ovum was at telophase of the second meiosis. ×24,300. M, egg mitochondria; O, ooplasm; S, smooth endoplasmic reticulum.

membrane fusion had already occurred in these regions, as they were incorporated first. The progressive incorporation of the tail by membrane fusion in zipper-like fashion [see Yanagimachi, 1978] was clearly seen during polyspermic fertilization [Sathananthan et al, 1986]. Intermixing of considerable lengths of sperm plasma membrane with the oolemma could also be expected during monospermic fertilization, as there was no trace of this membrane even though these structures were superficially located in the egg. Phagocytosis of the tail might also occur because flagellar segments with intact plasma membrane were occasionally encountered in the ooplasm. Occasional phagocytosis of the tail was also observed during polyspermic penetration [Sathananthan et al, 1986]. Both intermixing of membranes and phagocytosis have been indicated for other mammals [Yanagimachi, 1978; Bedford, 1982].

All penetrated ova had evidently matured in culture and were activated. During sperm decondensation, the intrinsic sperm nuclear envelope was either lacking or had disorganized, while remnants of centrioles and flagellar microtubules were still associated with the decondensing sperm nucleus. It is believed that mature oocytes have a sperm-nucleus-decondensing factor, absent in immature eggs [see Yanagimachi, 1978, 1981]. Eventually all components of the tail are gradually incorporated into the ooplasm. These early events are vividly portrayed in the study of polyspermic egg penetration [Sathananthan et al, 1986]. The fate of the intrinsic sperm nuclear envelope and other cytoplasmic organelles, however, is not clear, although they are said to be "incorporated" into the ooplasm. The involvement of sperm mitochondrial DNA and flagellar microtubules in future embryonic development is a possibility [Yanagimachi, 1981]. Identification of cortical granule contents at the egg surface and completion of meiotic maturation confirms that the oocytes were activated [see Sathananthan, 1984].

REFERENCES

Bedford JM (1982): Fertilization. In Austin CR, Shorty RV (eds): "Gametes and Fertilization: Reproduction in Mammals." Cambridge: Cambridge University Press, Vol. 1, pp 128–163.

Bedford JM (1983): Form and function of eutherian spermatozoa in relation to the nature of egg vestments. In Beier HM, Lindner HR (eds): "Fertilization of the Human Egg In Vitro." Berlin: Springer-Verlag, pp 133–146.

Bedford JM, Moore HDM, Franklin LE (1979): Significance of the equatorial segment of the acrosome of the spermatozoon in eutherian mammals. Exp Cell Res 119:119–126.

Chen C, Sathananthan AH (1986): Early penetration of human sperm through the vestments of human eggs in vitro. Arch Androl (in press).

Edwards RG (1980): "Conception in the Human Female." London: Academic Press.

Gwatkin RBL (1977): "Fertilization Mechanisms in Man and Mammals." New York: Plenum Press.

Lopata A, Sathananthan AH, McBain JC, Johnston WIH, Spiers AL (1980): The ultrastructure of the preovulatory human egg fertilized in vitro. Fertil Steril 33:12–20.

Sathananthan AH (1984): Ultrastructural morphology of fertilization and early cleavage in the human. In Trounson A, Wood C (eds): "In Vitro Fertilization and Embryo Transfer." Edinburgh: Churchill Livingstone, Chapter 9, pp 131–158.

Sathananthan AH, Trounson AO, Wood C, Leeton JF (1982): Ultrastructural observations on the penetration of human sperm into the zona pellucida of the human egg in vitro. J Androl 3:356–364.

Sathananthan AH, Ng SC, Edirisinghe R, Ratnam SS, Wong PC (1986): Sperm-oocyte interaction in the human during polyspermic fertilization in vitro. Proc Fourth World Conf of In vitro Fertilization, Melbourne, Australia. Gamete Res (in press).

Soupart P (1980): Fertilization. In Hafez ESE (ed): "Human Reproduction: Conception and Contraception." New York: Harper & Row, Chapter 20, pp 453–470.

Soupart P, Strong PA (1974): Ultrastructural observations on human oocytes fertilized in vitro. Fertil Steril 25:11–44.

Soupart P, Strong PA (1975): Ultrastructural observations on polyspermic penetration of zona pellucida-free human oocytes inseminated in vitro. Fertil Steril 36:523–537.

Yanagimachi R (1978): Sperm-egg association in mammals. Curr Top Dev Biol 12:83–105.

Yanagimachi R (1981): Mechanisms of fertilization in mammals. In Mastroianni L Jr, Biggers JD (eds): "Fertilization and Embryonic Development In Vitro." New York: Plenum Press, Chapter 5, pp 81–182.