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Ultrastructural Characteristics of Human Oocytes Fixed at Follicular Puncture or After Culture

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The material consisted of 73 oocytes obtained at follicle aspiration in ovarian-stimulated women. Oocytes in various stages of maturation were either immediately fixed or cultured before fixation. Observations by transmission electron microscopy disclosed that 20% of the immature oocytes, which appeared normal in the light microscope, had commenced atresia, and one immediately fixed oocyte surrounded by a normal-appearing preovulatory cumulus mass was found to be degenerated. Further, a cumulus mass judged as preovulatory contained an oocyte possessing a germinal vesicle. Light microscopy is thus not always adequate for judging the condition of ova surrounded by cumulus cells. Cytoplasmic changes which were regarded as being related to oocyte maturation were a decrease in the number of vacuoles and multivesicular bodies, an increase in the volume of the endoplasmic reticulum, a clustering of mitochondria, and the appearance of aggregates of tubuli of the smooth endoplasmic reticulum surrounded by mitochondria and mitochondria–vesicle complexes. Certain features were found to cause misinterpretations at examination of oocytes in a light microscope. For instance, polar bodies can be mimicked by corona cells in the perivitelline space and pronuclei in oocytes by large mitochondria–vesicle complexes in the ooplasm. Consequently, not all oocytes in which polar body-like or pronuclei-like structures are observed are necessarily fertilized oocytes.

KEY WORDS: ultrastructure; human oocyte; maturation; oocyte culture.

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INTRODUCTION

Oocytes obtained at follicle puncture are classified clinically as immature, intermediate, or preovulatory (1). By definition, a preovulatory, fertilizable oocyte has extruded its first polar body. However, a polar body cannot easily be observed in the light microscope, as cumulus corona cells surrounding the oocyte obscure the view.

The exact stage of maturation of an oocyte at the time of incubation with spermatozoa is therefore usually not known. Consequently, not all oocytes classified as preovulatory become fertilized. Even though failure of fertilization can be due to various disorders of the sperm cells or the oocyte, misjudging immature or "overmatured" oocytes for mature oocytes would lower the efficacy of in vitro fertilization (IVF).

In an IVF program it is often difficult to judge the exact stage of maturation of an oocyte and its morphology due to the fact that the oocytes are almost always surrounded by corona or cumulus corona cells at collection. Further, difficulties are encountered when judging cytoplasmic features even in naked oocytes because of the low resolution in the light microscope. The aim of the present study, therefore, is to describe the ultrastructure of immediately fixed and cultured oocytes obtained in ovarian-stimulated cycles and to ascertain whether the impression of an oocyte in the light microscope corresponded to that found in the transmission electron microscope.

MATERIALS AND METHODS

The material consisted of 73 oocytes obtained from 58 ovarian-stimulated women admitted to the

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department because of infertility, due mainly to damage of the fallopian tubes. For stimulation, the women were given clomiphene citrate and human chorionic gonadotropin (hCG) or, in a few cases, clomiphene citrate, human menopausal gonadotropin, and hCG. Follicle aspiration was carried out at midcycle, usually day 14, 34 to 36 hr after the hCG injection. The size of the follicles varied, but all follicles that could be seen were aspirated. Some very small follicles were aspirated when the operation was performed as a laparotomy. Follicular fluid volumes were measured and the fluids surveyed for oocytes within 5 min of follicle aspiration.

The clinical classification of collected ova was made by judging the amount, dispersion, and viscosity of cumulus corona cells surrounding the oocytes, as visualized in a stereomicroscope. Immature oocytes were either denuded or surrounded by a corona consisting of a few to several layers of cells, whereas intermediate oocytes were surrounded by a small or medium-large, moderately dispersed and viscous cumulus mass. Oocytes were classified as preovulatory when surrounded by a large, dispersed, and viscous cumulus mass (1).

Some oocytes were fixed immediately. A few immature and intermediate oocytes were taken for culture and kept in the incubator for 5 hr or for 2–3 days before fixation. Almost all the preovulatory oocytes were preincubated for 5 hr, incubated with spermatozoa for 24 hr, and then cultured for 1–2 days or, in one case, 7 days, before fixation (Table I).

Table I. Time from Oocyte Recovery to Fixation

Stage at recovery[a]	0 hr	5 hr	2–3 days
Immature with a GV (N = 10)			
N	6	2	—
A	—	—	—
PA	1[b]	1	—
Immature without a GV (N = 25)			
N	12	2	2[c]
A	5	—	—
PA	4	—	—
Intermediate (N = 7)			
N	3	—	3[c]
A	—	—	—
PA	1	—	—
Preovulatory (N = 31)			
N	5	—	18
A	1	—	7[d,e]
PA	—	—	—
Total N	38	5	30

[a] N, normal; A, atretic; PA, preatretic.
[b] Oocyte clinically classified as preovulatory.
[c] One immature and two intermediate oocytes, each possessing a polar body at fixation.
[d] One oocyte showed several fragments and a well-preserved cytoplasm in each fragment.
[e] Oocyte fixed 8 days after recovery.

Follicular fluid volumes in follicles yielding immature oocytes ranged from 0.1 to 2.0 ml and those yielding intermediate oocytes ranged from 1.0 to 3.5 ml. Follicles from which preovulatory oocytes were obtained varied considerably in content, ranging between 1 and 37 ml (mean, 6 ml).

Preparation of spermatozoa and methods for fertilization and oocyte culture have been described in detail elsewhere (2). In this study some minor changes were made at incubation of the oocytes with spermatozoa and at oocyte culture. Thus, the number of spermatozoa at incubation was 500,000, while at culture, test tubes, without lipid paraffin to cover the medium, were used. The gas in the incubator was 5% O_2, 5% CO_2, and 90% N_2 and the medium used was Earle's medium. Patient serum was added to the medium used for incubation with spermatozoa (10% serum) and culture (15% serum). Spermiograms were analyzed from the sperm samples used for IVF, and only two were classified as seriously pathological.

Estradiol-17β and progesterone in plasma obtained from the women on the days around oocyte collection were analyzed by conventional radioimmunoassay. The day of ovulation was presumed to be the day after the peak in plasma estradiol-17β, coinciding with a rise in plasma progesterone (3). Accordingly, all preovulatory oocytes from women in whom plasma hormone levels had been analyzed (all but five) were collected on the presumed day of ovulation.

Fixation of the oocytes was done by placing them in a solution of 2.5% glutaraldehyde and culture medium without serum (room temperature, pH 7.4). Immediately fixed oocytes were examined in a light microscope at fixation. In oocytes surrounded by several layers of corona cells or by a cumulus mass, the general appearance of the ooplasm and the presence of a polar body were difficult to judge. Cultured oocytes were examined at fixation, shortly after the removal of the remaining corona cells surrounding some oocytes. The general appearance of the cytoplasm and the presence of a polar body at fixation were noted.

Oocytes that were denuded or surrounded by only a few layers of corona cells at fixation could be judged and were regarded as atretic when dis-

playing a dense retracted ooplasm. Normal-appearing oocytes displayed a homogeneous, nonretracted cytoplasm.

Preparation of the oocytes for transmission electron microscopy (TEM) was performed in the same manner in all cases. Thus, after fixation for a few days, the oocytes were washed in phosphate buffer, postfixed in 1% osmium tetroxide in phosphate buffer, dehydrated in increasing concentrations of ethanol, and embedded in Epon 812. Sections were stained with uranyl acetate and lead citrate. Sections, from one to four levels, from each specimen were examined.

RESULTS

Condition of the Oocytes at Fixation

The maturation stages of the oocytes and their condition at fixation are presented in Table I. Oocytes clinically classified as immature were of two kinds, viz., oocytes possessing a germinal vesicle (GV) and oocytes without a GV at serial sectioning. Oocytes without a GV were usually surrounded by several layers of cells at collection. Ten oocytes possessed a GV. Among the cultured preovulatory oocytes, all the normal ones possessed a polar body.

Two kinds of degenerating oocytes were found at TEM. Oocytes were classified as atretic when displaying extensive vacuolization throughout the cytoplasm and a condensed ground substance. Oocytes showing numerous vacuoles in the center of the cytoplasm were classified as preatretic. Apart from numerous vacuoles, these oocytes showed a well-preserved cytoplasm.

General Characteristics of Normal-Appearing Oocytes as Judged by TEM

Immature oocytes were surrounded by corona cells which extended cytoplasmic projections deep into the zona pellucida (Fig. 1). In mature oocytes, these projections had been retracted. In one immature oocyte, a few corona cells were observed, trapped in the perivitelline space (Fig. 2). Numerous slender microvilli, 4–5 μm long, extended from the surface of the cells.

The cytoplasm membrane possessed numerous microvilli, 1–2 μm long, which projected into the narrow perivitelline space. The oocyte cytoplasmic ground substance consisted of a fine flocculent material throughout the ooplasm. Mitochondria were rounded, with a diameter of about 0.5 μm, and contained a few, concentric inner membranes (Fig. 3). Golgi complexes appeared scattered in the cytoplasm. Each Golgi had a size of about 0.5 × 1 μm and consisted of four to six membranes surrounded by small vesicles. Lysosomes, with a diameter of about 2 μm, were observed in many oocytes. Annulate lamellae were seen only occasionally in a few oocytes.

Immature Oocytes Possessing a Germinal Vesicle

The cytoplasm contained membrane-bound cortical granules, with a diameter of 0.3 to 0.5 μm, in varying numbers located close to the plasma membrane and deep within the cytoplasm. The mitochondria were evenly distributed, though clusters were occasionally seen peripherally. Usually, there was a close association between the mitochondria and the smooth endoplasmic reticulum (SER) which appeared as small irregular profiles or sometimes as flattened sacs with a size of about 0.5 to 1 μm. Some vacuoles with a diameter of 1–2 μm were observed in the center of the oocytes. Flattened sacs of SER were often found in their vicinity. Multivesicular bodies were present in the oocytes. These structures, which had a diameter of about 1 μm, contained several doughnut-shaped inclusions and, occasionally, one or two dark bodies (Fig. 4). In two oocytes, a few small aggregates of closely packed SER tubuli were noted.

The germinal vesicle, which was usually spherical and had a diameter of about 20 μm, was located centrally or, in a few oocytes, close to the cell membrane. In the latter oocytes, a cone-formed zone of cytoplasm with relatively few organelles was noted between the nucleus and the oocyte plasma membrane. The nuclear envelope, consisting of a double membrane, showed regular dense spots representing nuclear pores. A large nucleolus was observed in some nuclei, with dark chromatin concentrated around it. Two oocytes cultured for 5 hr showed an irregular outline of their nuclear membranes.

Immature Oocytes Without a Germinal Vesicle

In immature oocytes, which were fixed immediately, the cytoplasmic organelles and their distribution were generally rather similar to those in oocytes in the GV stage. However, in some oocytes,

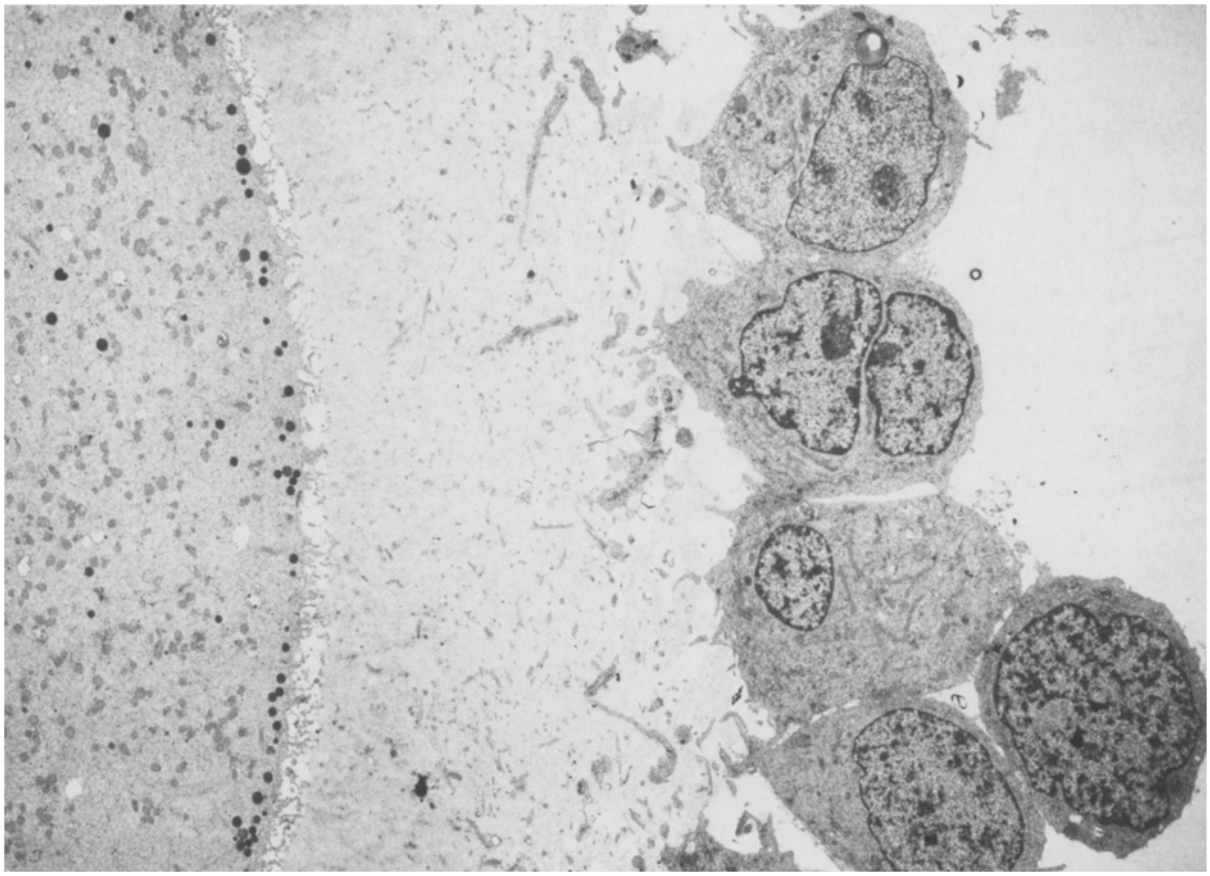

Fig. 1. Immediately fixed immature oocyte. Cytoplasmic projections from the corona radiata cells are extending into the zona pellucida. The perivitelline space is narrow and numerous microvilli are projecting into this space. Cortical granules are located by the cell membrane and deep in the cytoplasm. Mitochondria are scattered in the cytoplasm. A few vacuoles are observed. ×3000; reduced 5% for reproduction.

cortical granules were observed only by the cell membrane, and none deep in the cytoplasm (Fig. 5a). Some vacuoles were observed in most of the oocytes but multivesicular bodies were rarely seen. The close association between SER and mitochondria was even more pronounced than in oocytes possessing a GV. In areas where no SER was seen, mitochondria were sparse, and where profiles of SER were common, mitochondria tended to cluster. Moreover, SER in the form of flattened sacs was more common than in the GV stage (Fig. 6). In two oocytes a few small aggregates of flattened tubuli of SER were observed. One of these oocytes also possessed a few (three or four) small mitochondria–vesicle (MV) complexes.

Intermediate Oocytes

The general appearance and distribution of the cytoplasmic organelles in intermediate oocytes which were fixed immediately resembled in the main those of preovulatory, immediately fixed oocytes. Multivesicular bodies were not seen in any of the oocytes. In one oocyte a chromosome was found close to the cell membrane. It also contained a few small MV complexes.

Preovulatory Oocytes

In cultured preovulatory oocytes, cortical granules were usually located close to the cell membrane in a single layer. Membrane-bound vacuoles were infrequent and multivesicular bodies were lacking. SER often appeared as distended rounded sacs with a diameter of about 1 to 3 μm, as aggregates of tubuli, or as vesicles surrounded by mitochondria (MV complexes).

MV complexes were found in all the cultured oocytes. The complex consists of a single membrane-

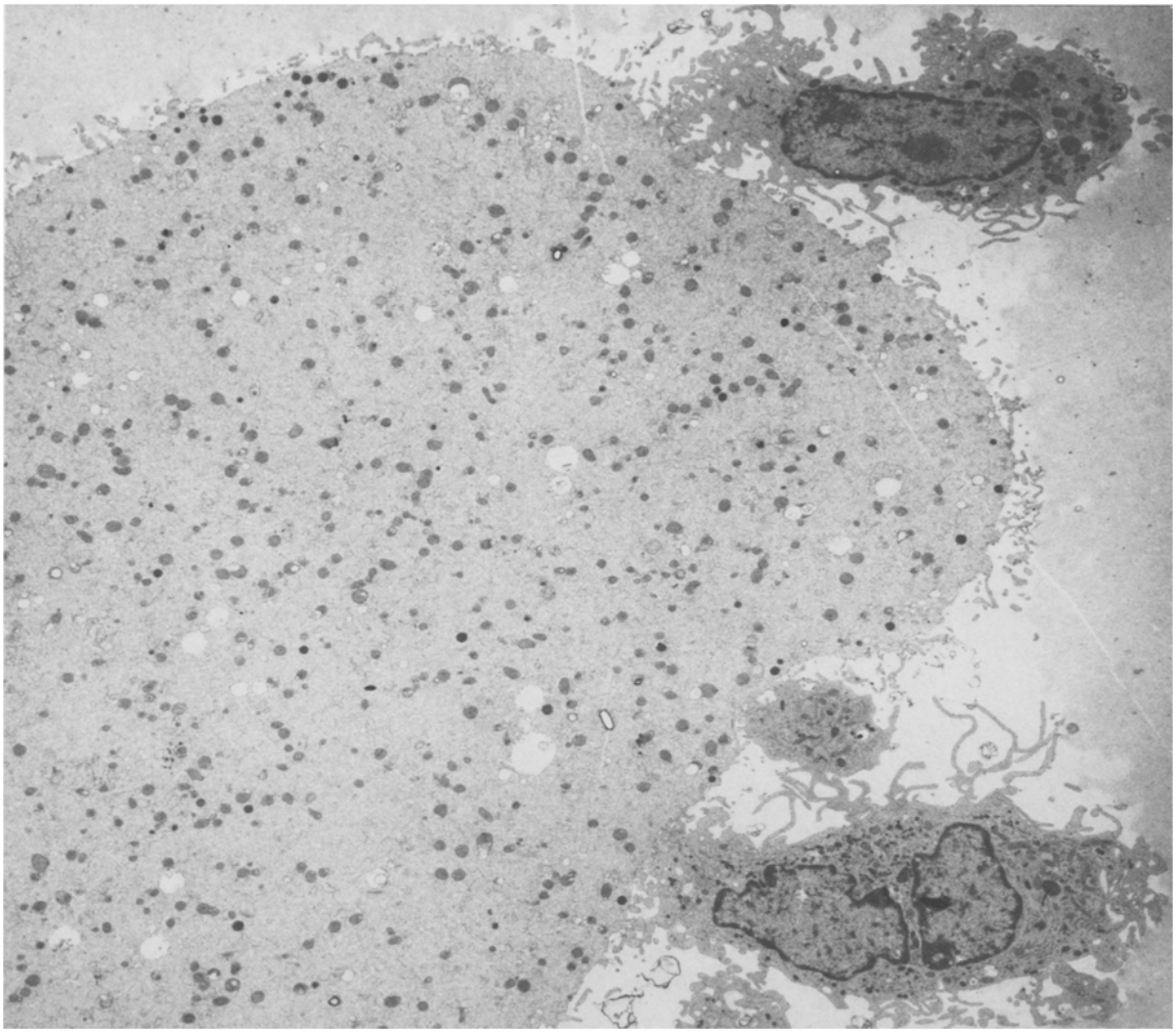

Fig. 2. Immediately fixed immature oocyte. A few corona cells with long and slender microvilli are observed in the perivitelline space in this oocyte. ×2000; reduced 5% for reproduction.

bound vesicle, containing a medium-dense flocculent substance, intimately surrounded by a rim of usually 5 to 20, occasionally more, rounded mitochondria (Fig. 7). No signs of communication between MV complexes were found. The number of complexes varied from a few to about 50 in sections from different oocytes (Fig. 8). Their size varied too, the smallest having a diameter of about 1.5 μm and the largest a diameter of about 5 μm. In two cultured oocytes a few large MV complexes with a diameter of about 10 to 15 μm were observed (Fig. 9).

In most cultured oocytes, more or less prominent aggregates of flattened tubuli of SER, usually surrounded by one to three layers of mitochondria, were observed. The structures were about as large as MV complexes, and even unusually large aggregates with a diameter of 10 to 15 μm were observed. Occasionally, these structures were seen in close association with MV complexes. In some oocytes, both mitochondria–SER aggregates and MV complexes were numerous. In these oocytes, the conventional appearance of SER profiles was rare and almost all the mitochondria were clustered around the two types of structures (Fig. 8).

In preovulatory oocytes which were immediately fixed, aggregates of SER tubuli and MV complexes were few, but otherwise no differences were observed between these oocytes and cultured preovulatory oocytes.

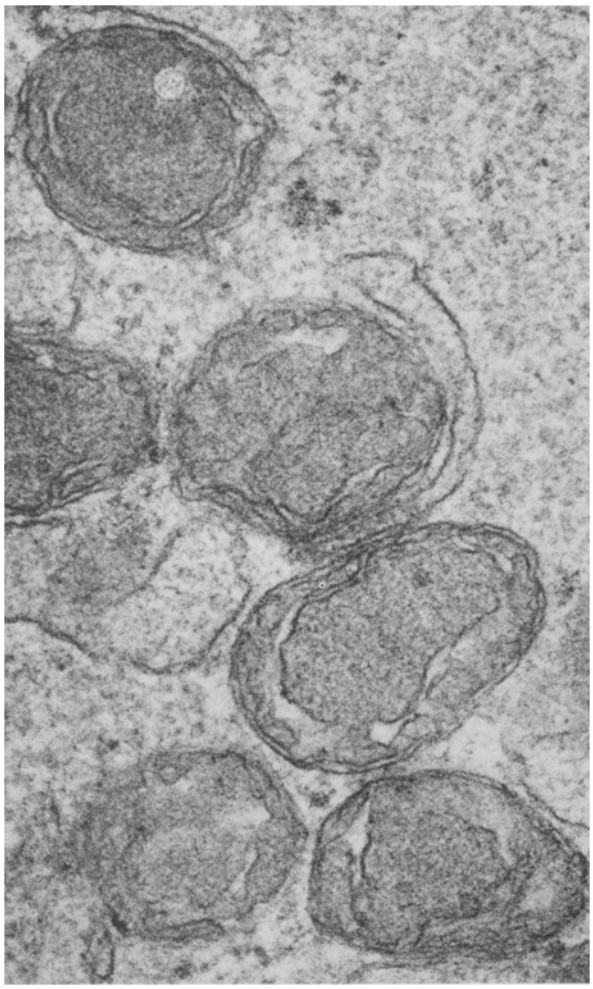

Fig. 3. Rounded mitochondria with a few peripherally located crista. Profiles of SER are seen in close association with some of the mitochondria. ×100,000.

Chromosomes were only occasionally included in the randomly taken sections and then were seen as condensed chromosomes located in the periphery of the cell.

All three intermediate oocytes and one of the two immature oocytes cultured for 2–3 days resembled cultured preovulatory oocytes and showed several aggregates of mitochondria–SER and MV complexes.

Ultrastructure of Degenerating Oocytes

In atretic oocytes, vacuoles were numerous. Further, microvilli were sparse or lacking. Of the immediately fixed preovulatory oocytes, one was atretic. Another immediately fixed oocyte, which was classified as preovulatory at collection, possessed a GV and was found to be preatretic.

The oocyte fixed after 8 days showed numerous vacuoles in the center of the cytoplasm and also one large pronuclear-like structure. Higher magnification revealed that the structure, which had a diameter of 25 μm, consisted of a single membrane-bound vesicle surrounded by mitochondria, that is, a large MV complex. Preatretic oocytes (Fig. 5b) showed many vacuoles but intact microvilli.

DISCUSSION

Oocyte maturation comprises: breakdown of the germinal vesicle membrane, formation of the first meiotic spindle, extrusion of the first polar body, formation of the second meiotic spindle, and arrest of meiosis (4). At this stage the oocyte is ovulated. Ovulation occurs about 36 hr after oocyte maturation commenced (5). The fertilizable life span of the ovulated human oocyte is not known but probably measurable in hours as in other mammalian ova (6). If the oocyte is fertilized, meiosis is resumed, the second polar body is extruded, and the female and male pronuclei are formed.

Various maturation stages of human oocytes, obtained from ovarian wedges or by aspirating ovarian follicles—or, in a few cases, obtained from the fallopian tube—have been examined in the electron microscope (7–13). Maturation of oocytes in vitro has also been studied (14–17). Accordingly, maturation in vivo is characterized by an increase in the number of cytoplasmic organelles. In the resting oocyte of the primordial follicle, organelles are located juxtanuclearly. Golgi complexes, mitochondria, and profiles of the smooth endoplasmic reticulum are not prominent, whereas annulate lamellae are rather common. In maturing oocytes the converse is found and the organelles are distributed throughout the ooplasm. Moreover, microvilli develop on the cell membrane and cortical granules appear. In vitro matured oocytes show essentially the same changes during maturation as do oocytes developed in vivo. However, cultured oocytes lack a well-developed Golgi apparatus but have acquired spheroidal aggregates of tubuli of SER (14). The number of cortical granules increased markedly in preovulatory oocytes incubated for 3 to 6 hr after collection (18).

The cytoplasmic changes which we observed among the oocytes and which could be related to oocyte maturation were a decreased number of vac-

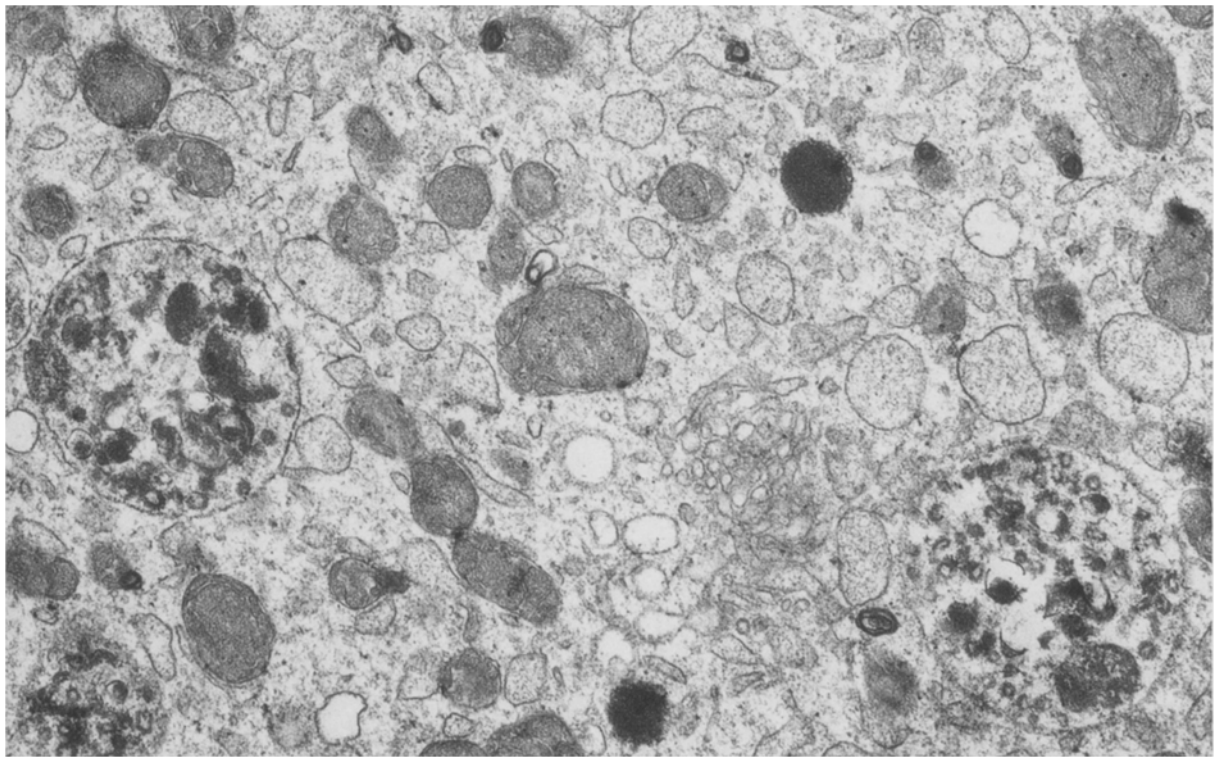

Fig. 4. Immediately fixed oocyte in the GV stage. Several multivesicular bodies are observed in the cytoplasm. The mitochondria are rounded and contain few concentric cristae. The mitochondria are scattered in the cytoplasm but usually associated with SER, which appears as small irregular profiles and as small sacs. A small Golgi complex and a few dark cortical granula are seen. ×26,000; reduced 5% for reproduction.

uoles and multivesicular bodies, an increase in volume of the profiles of the endoplasmic reticulum, a clustering of mitochondria, and the appearance both of aggregates of tubuli of SER surrounded by mitochondria and of MV complexes.

The presence of numerous vacuoles probably indicates imminent atresia. Some vacuoles, however, were observed in immediately fixed preovulatory oocytes as well as in blastomeres of eggs in early cleavage stages (2). We therefore believe that a certain degree of vacuolization need not constitute a pathological sign. The function of these vacuoles, however, is still not known.

Multivesicular bodies were common in oocytes in the GV stage but rare in later maturation stages. Multivesicular bodies belong to the lysosome system and probably have a digestive function (19), maybe at germinal vesicle breakdown. The endoplasmic reticulum is of the smooth variety (SER) and is observed as small irregular profiles early in maturation but as circular profiles in cultured preovulatory oocytes. The SER was closely associated with mitochondria, and in areas where SER was common, mitochondria were also numerous. It might be that synthesis of products within the SER is energy demanding or that mitochondria are provided with products from the SER. The shift in shape from a narrow to a circular profile implies an increase in volume of the reticulum, but it is not known what substance within the reticulum accounts for this increase. Since the reticulum is of the smooth variety, the change could be related to an increase in the production of carbohydrates or lipids, to the production of some steroid hormones, or to calcium metabolism (19).

The distribution of mitochondria changed during maturation. Mitochondria were irregularly scattered in GV oocytes, clustered and closely associated with SER in immature oocytes without a GV, and arranged around spherical cisternae or rounded aggregates of tubuli of the endoplasmic reticulum in cultured preovulatory oocytes. The presence of aggregates of mitochondria–SER has been described earlier (14), but their function is still now known. It seems that the aggregates appear before the formation of MV complexes and that the two

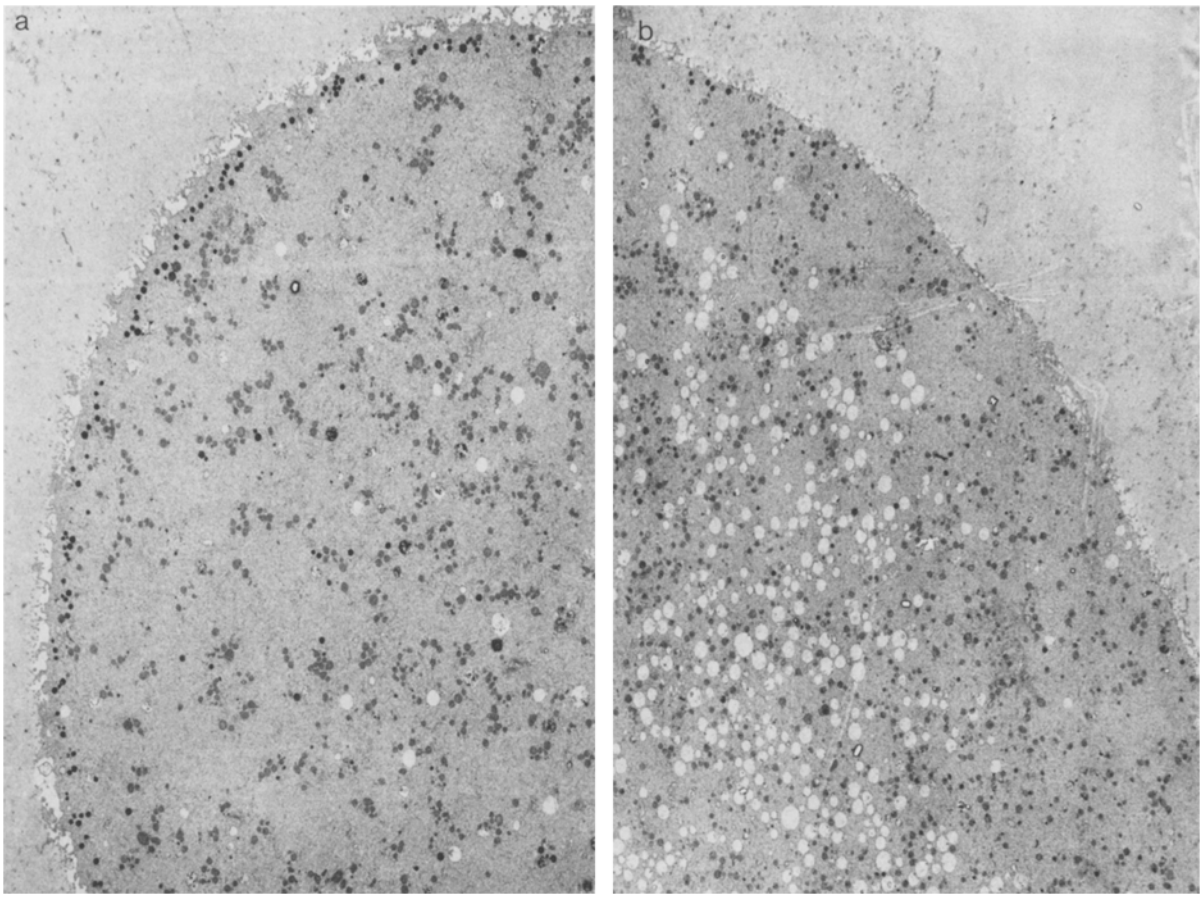

Fig. 5. (a) Immediately fixed immature oocyte without a GV. Cortical granules are located only by the cell membrane. Mitochondria are evenly distributed throughout the ooplasm. A few vacuoles are seen. (b) Immediately fixed immature oocyte with numerous vacuoles. This oocyte was regarded as preatretic. ×2100; reduced 5% for reproduction.

types of structures belong to the same functional system. In cultured oocytes with many aggregates of mitochondria–SER, MV complexes were usually also frequent, and vice versa.

MV complexes have been found in early cleavage stages, but they rapidly diminished in number during cleavage (2). The functional implication of the complexes is as yet unknown, but it can be assumed that the mitochondria cooperate with the vesicles of the endoplasmic reticulum in producing some substance(s) which has a function related to fertilization and/or early cleavage. The complexes could be required at, for instance, the activation of the oocyte at fertilization by releasing calcium ions into the cytoplasm (20). The number of complexes varies among the oocytes, the greatest number and also the largest complexes being observed in some cultured oocytes. Since all oocytes submitted to IVF are incubated for 5 hr before the fertilization attempt, it might be that those which are fertilizable at recovery get "overmatured" after incubation and that an abundance of MV complexes is a sign of "overmaturation."

The only obvious difference between cultured and immediately fixed preovulatory oocytes was that aggregates of tubuli of SER and MV complexes were less numerous in the latter. These observations strengthen our opinion that the appearance of the MV complexes is related to oocyte maturation. A few unusually large MV complexes were observed in some cultured oocytes. These complexes, which were located centrally or peripherally, could mistakenly be interpreted as pronuclei under the light microscope. An MV complex, however, contains no nucleoli which can help in distinguishing it from a pronucleus.

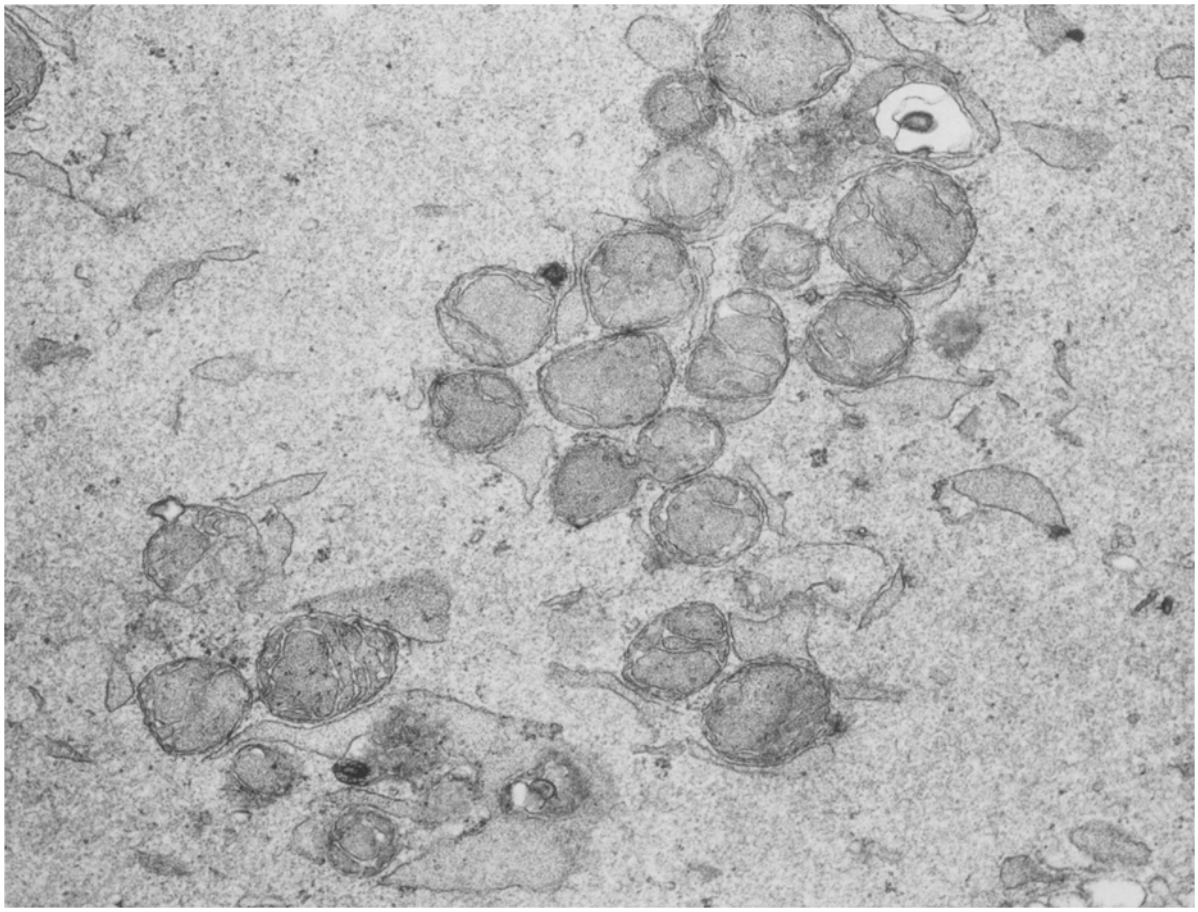

Fig. 6. Immediately fixed immature oocyte without a GV. There is a close association between SER and mitochondria. Where SER is common, mitochondria tend to aggregate. At this stage of maturation, SER appears in the form of flattened sacs. ×45,000; reduced 5% for reproduction.

Cortical granules, which are released at fertilization, induce in the zona a reaction which prevents polyspermia (18). Cortical granules have been found to increase in number during egg maturation and to be concentrated at the cell membrane in mature oocytes (18). In this study, cortical granules were found to be located close to the cell membrane but also deep within the cytoplasm in GV oocytes. In preovulatory oocytes, the density of cortical granules near the cell membrane varied in different sections of an oocyte, but in some there was a rim of cortical granules in a single layer by the cell membrane and hardly any deep in the cytoplasm. However, even some immediately fixed immature oocytes without a GV possessed cortical granules only by the cell membrane and none deep within the cytoplasma, at examination of several randomly taken sections from each of the oocytes. Thus, the location of cortical granules does not seem to be a reliable indicator of the maturation stage of an oocyte. Since polyfertilization sometimes occurs, both in vivo and in vitro, it would seem that the mechanism of cortical granule release rather than the maturity of the oocyte accounts for the failure in the blocking of several spermatozoa from entering the egg cell.

Corona cells were occasionally seen inside the zona pellucida. These cells had numerous long projections differing from the small microvilli on the cells of the corona radiata. Since rat granulosa cells are known to form long projections when exposed to, for instance, prostaglandins (21), it might be that the perivitelline space contains a high concentration of a substance which is capable of affecting the appearance also of human granulosa cells. A secretion of prostaglandin by the oocyte would be appropriate since prostaglandins have a direct effect on human sperm function (22). It should be mentioned also that corona cells trapped in the perivitelline

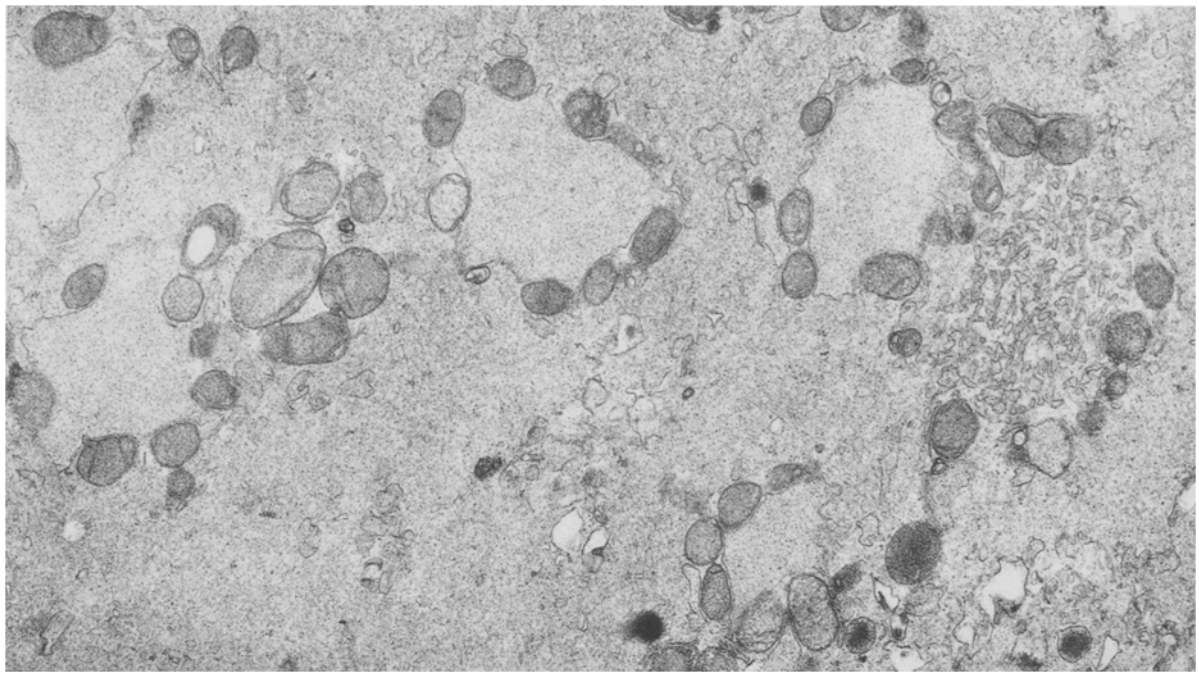

Fig. 7. Cultured preovulatory oocyte. MV complexes and aggregates of SER and mitochondria are commonly observed in cultured oocytes. ×17,000; reduced 5% for reproduction.

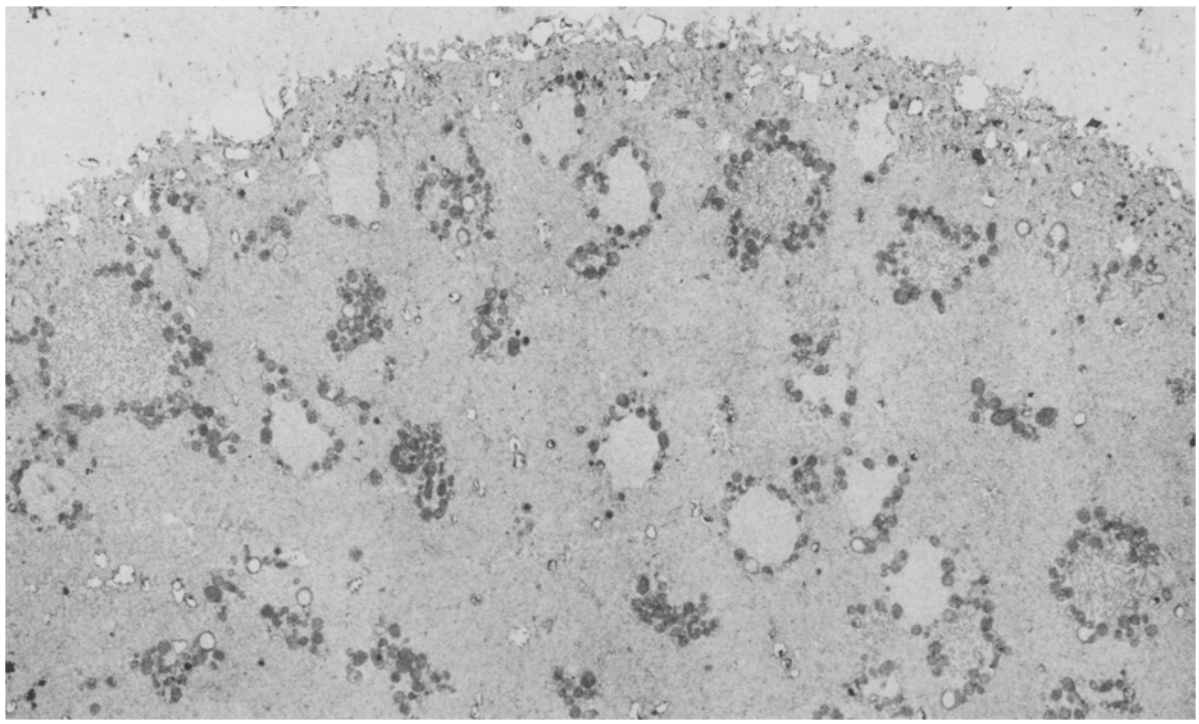

Fig. 8. Same oocyte as in Fig. 7. Numerous MV complexes and aggregates of SER–mitochondria are observed. Almost all the mitochondria are associated with these structures. ×2500; reduced 5% for reproduction.

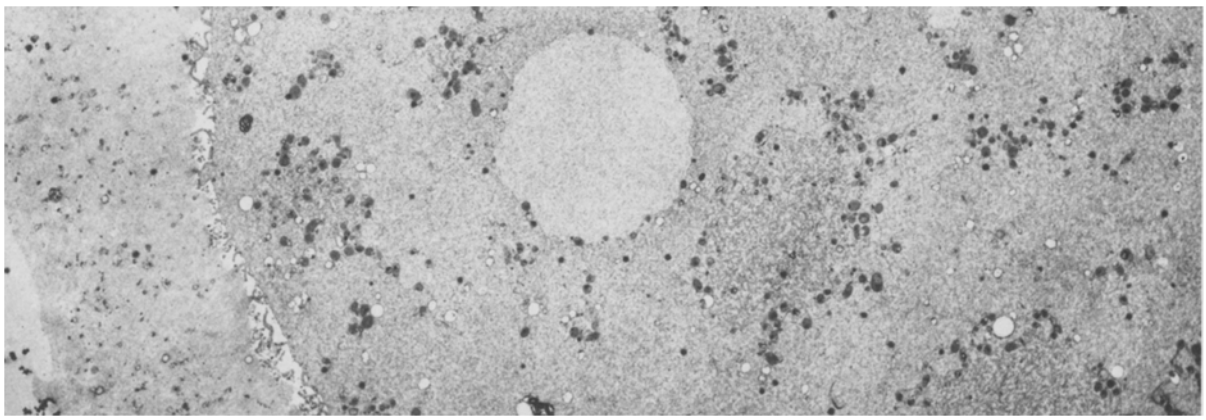

Fig. 9. Cultured preovulatory oocyte. A large MV complex is seen. ×1500; reduced 5% for reproduction.

space can be mistaken under the light microscope for extruded polar bodies (15) or cytoplasmic fragments.

Some oocytes, both immediately fixed and cultured, showed signs of degeneration. Degenerating oocytes were of two kinds, viz., atretic and preatretic oocytes. Atretic oocytes showed extensive vacuolization throughout the ooplasm, few or no microvilli on the cell membrane, and a condensed ground substance. Those oocytes which were obtained from small follicles and were atretic had probably degenerated early in the cycle, while preatretic immature oocytes, showing numerous vacuoles but otherwise a well-preserved ooplasm, might have commenced degeneration shortly before collection. If this is true, approximately 20% of the immature oocytes had commenced degeneration shortly before collection. This is in agreement with earlier observations (23,24). Since preovulatory oocytes cultured for 2 to 3 days were either normal or atretic, it is possible that degeneration of these latter oocytes had started already prior to collection. This view is strengthened by the fact that one preovulatory oocyte (clinically classified) was shown by electron microscopy to be atretic at collection. Furthermore, another preovulatory oocyte (clinically classified) was preatretic and possessed a GV at recovery. Light microscopy is thus not always adequate for judging the condition of ova surrounded by cumulus cells. Early degeneration of preovulatory oocytes might, to some extent, explain why not all seemingly normal oocytes (clinically classified) become fertilized and cleave.

Some clinical conclusions can be drawn from the results. For instance, immature oocytes which showed a normal appearance in the light microscope had commenced atresia in approximately 20% of the oocytes, according to TEM. Thus, four of five such oocytes might have the ability to mature in vitro. Among the preovulatory oocytes, one of four was atretic after 2 to 3 days of culture. However, degeneration might have started already prior to collection. The clinical classification of oocytes by their cumulus mass can be erroneous, since a preovulatory cumulus mass can harbor an atretic oocyte and a degenerating oocyte possessing a GV. Also, the interpretation of pronuclei and polar bodies/cytoplasmic fragments can be erroneous in the light microscope. Thus, these structures can be large MV complexes or corona cells trapped in the perivitelline space, respectively. Consequently, not all oocytes in which pronuclei-like and polar body-like structures are observed are necessarily fertilized oocytes.

REFERENCES

1. Fishel SB, Edwards RG, Walters DE: Follicular steroids as a prognosticator of successful fertilization of human oocytes in vitro. J Endocrinol 1983;99:335–344
2. Sundström P, Nilsson O, Liedholm P: Cleavage rate and morphology of early human embryos obtained after artificial fertilization and culture. Acta Obstet Gynecol Scand 1981;60:109–120.
3. Wramsby H, Kullander S, Liedholm P, Rannevik G, Sundström P, Thorell J: The success rate of in vitro fertilization of human oocytes in relation to the concentrations of different hormones in follicular fluid and peripheral plasma. Fertil Steril 1981;36:448–453
4. Tsafriri A, Bar-Ami S, Lindner HR: Control of the development of meiotic competence and of oocyte maturation in mammals. *In* Fertilization of the Human Egg in Vitro, HM Beier, HR Lindner (eds). Berlin, Springer-Verlag, 1983, pp 3–17

5. Edwards RG: Conception in the Human Female. London, Academic Press, 1980
6. Speroff L, Glass RH, Kase NG: Clinical Gynecologic Endocrinology & Infertility, 3rd ed. Baltimore/London, Williams & Wilkins, 1983, p 455
7. Stegner H-E, Wartenberg H: Elektronmikroskopische Untersuchungen an Eizellen des Menschen in verschiedenen Stadien der Oogenese. Arch Gynäkol 1963;199:151–172
8. Hertig AT, Adams EC: Studies on the human oocyte and its follicle. J Cell Biol 1967;34:647–675
9. Hertig AT: The primary oocyte: Some observations on the fine structure of Balbiani's vitelline body and the origin of the annulate lamellae. Am J Anat 1968;122:107–138
10. Baker TG, Franchi LL: The fine structure of oogonia and oocytes in human ovaries. J Cell Sci 1967;2:213–224
11. Baca M, Zamboni L: The fine structure of human follicular oocytes. J Ultrastruct Res 1967;19:354–381
12. Santhananthan AH, Lopata A: Ultrastructure of human eggs: Aspirated preovulatory mature ova prior to fertilization. Micron 1980;11:469–470
13. Noriega C, Oberti C: Studies of the human unfertilized tubal ovum. Fertil Steril 1973;24:595–601
14. Zamboni L, Thompson RS, Moore Smith D: Fine morphology of human oocyte maturation in vitro. Biol Reprod 1972;7:425–457
15. Zamboni L, Moore Smith D, Thompson RS: Migration of follicle cells through the zona pellucida and their sequestration by human oocytes in vitro. J Exp Zool 1972;181:319–340
16. Suzuki S, Kitai H, Tojo R, Seki K, Oba M, Fujiwara T, Ilzuka R: Ultrastructure and some biologic properties of human oocytes and granulosa cells cultured in vitro. Fertil Steril 1981;35:142–148
17. Santhananthan AH, Trounson AO, Wood C, Leeton JF: Ultrastructural observations on the penetration of human sperm into the zona pellucida of the human egg in vitro. J Androl 1982;3:356–364
18. Santhananthan AH, Trounson AO: Ultrastructural observations on cortical granules in human follicular oocytes cultured in vitro. Gamate Res 1982;5:191–198
19. Hopkins CR: Structure and Function of Cells. London, Saunders, 1978.
20. Cardasis CA, Schuel H, Herman L: Ultrastructural localization of calcium in unfertilized sea-urchin eggs. J Cell Sci 1978;31:101–115
21. Lawrence TS, Ginzberg RD, Gilula NB, Beers WH: Hormonally induced cell shape changes in cultured rat ovarian granulosa cells. J Cell Biol 1979;80:21–36
22. Aitken RJ, Kelly RW: Analysis of the direct effects of prostaglandins on human sperm function. J Reprod Fertil 1985;73:139–146
23. Breitenecker G, Friedrich F, Kemeter P: Further investigations on the maturation and degeneration of human ovarian follicles and their oocytes. Fertil Steril 1978;29:336–341
24. Veeck LL, Wortham JWE, Witmyer J, Sandow BA, Acosta AA, Garcia JE, Jones GS, Jones HW: Maturation and fertilization of morphologically immature human oocytes in a program of in vitro fertilization. Fertil Steril 1983;39:594–602