CHAPTER 4

MORPHOLOGICAL STUDIES OF DEVELOPING GOAT OOCYTES IN VITRO

4.1. SUMMARY

The morphology of goat oocytes, and the structure and distribution of organelles within oocytes at various stages of incubation in vitro were studied. The oocytes which were obtained by slicing the ovaries were classified as cumulus-oocyte-complexes (COCs) and cumulus-free oocytes (CFOs). These oocytes were transferred in the TCM 199 medium and incubated in the CO_2 incubator for 0, 12, 15, 20, 25, 30, 35 and 40 hr. The finding showed that the goat oocyte is spherical in shape, 110 to 120 μm in diameter and consists of spherical mass of cytoplasm and surrounded by a thick transparent structure, zona pellucida. Between the cytoplasm and the zona pellucida is a fluid-filled space, the perivitelline space (ps). At 0 hr of incubation, the cumulus (cu) and corona cells (co) surrounding the zona pellucida were tightly packed. The zona pellucida had developed although the zonation was not evident. There are penetrations by cumulus cell processes, and these processes were also observed in the perivitelline space. Lipid bodies were present but no mitochondria were observed. At 14 hr, the cumulus

cells began to expand and loosen, however, the corona cells remained tightly packed. At 20 hr, both types of cells reached maximum expansion. First polar body could sometimes be seen in the perivitelline space after removal of cumulus cells. The zona pellucida had differentiated into thicker and thinner regions. Clusters of membrane-bound electron-transparent bodies were present in the perivitelline space. The mitochondria were fully developed, distributed evenly and usually in close proximity with dilated endoplasmic reticula. Cortical granules were distributed at the periphery. At 40 hr of incubation, the expansion and the loosening of the cumulus cells and the corona radiata was not different from those of 20 hr. The zona pellucida appeared thinner but with similar zonation pattern and larger lipid bodies, compared to those in the earlier stages. A number of mitochondria were hooded. In CFOs, the cumulus cells and the corona radiata were absent. At 0 hr, zonation within the zona pellucida was indistinct. Penetration of the zona pellucida was only by microvilli formed by folding of the oolemma. Very few vesicles and lipid bodies were observed. At 20 hr, the oolemma formed microvilli but no penetration of the zona pellucida was observed. The mitochondria were sparsely distributed and were not well developed and lacked cristae. At 40 hr, the zona pellucida was less compact toward the outside. The membrane-bound electron-transparent bodies were less numerous compared to the other group. Endoplasmic reticula were not dilated and with no associated

ribosome. The cortical granules were few and had no definite pattern of distribution. In COCs there was no notable differences in the ultrastructure of cytoplasmic organelles between 20 and 40 hr of incubation. The present observations not only suggest the superiority of COCs compared with those of CFOs, but also indicate that 20 hr incubation was sufficient for the attainment of full development of oocytes. This finding, together with that of chromosomal studies reported in Chapter 5, will become the basis for proceeding work on IVF reported in Chapter 6.

4.2. INTRODUCTION

Mammalian oocytes undergo development when cultured in the specific medium in vitro. This development is represented by nuclear and cytoplasmic organelles changes. From the point of view of nuclear changes, primary oocyte reach the diplotene stage of meiosis at approximately the time of birth (Brambell, 1956; Zuckermann, 1960). The germinal material of the oocyte is then arranged within the vesicular nucleus, the dictyate stage after which it remains arrested until few hours before ovulation. However, the primary oocytes are capable of resuming meiotic maturation spontaneously when removed from their follicles and cultured in vitro (Chang, 1955). Some of the important cytoplasmic organelle changes reported in sheep (Cran *et al.*, 1980) and bovine (Kruip *et al.*, 1983) were the disper-

sal of the mitochondrial band with cortical granules rested close to the oolemma. Development of goat oocytes in vitro has been studied (Kim *et al.*, 1984; Hanada, 1985; Song and Iritani, 1985; Younis *et al.*, 1991; De Smedt *et al.*, 1992) but no fine structural basis for the evaluation of such oocytes has been demonstrated. Among farm animals it appears that pig (Norberg, 1973), cattle (Fleming and Saacke, 1972; Brackett *et al.*, 1980; Kruip *et al.*, 1983) and sheep (Cran *et al.*, 1980) were the only animals in which the fine structure of their developing oocytes has ever been demonstrated.

The knowledge of cytoplasmic changes in goat oocyte is important for the understanding of the mechanism of subsequent IVF procedure in this animal. This is because it can indicate whether the developing oocytes were fertilizable or not. Therefore, the purpose of this chapter was to describe the morphological characteristics, particularly the ultrastructural changes that occur in COCs and CFOs at various stages of incubation in vitro.

4.3. MATERIALS AND METHODS

4.3.1. Oocytes Collection

A study on morphological characteristics was conducted in four replicates with a total number of 210 oocytes from the cumulus-oocytes complexes (COCs) and cumu-

lus-free oocytes (CFOs) groups. They were further divided into two groups of 105 each: Group 1 was for light microscopic study, and Group 2 for electron microscopic study. The oocytes were washed with washing medium as mentioned in Chapter 3.

4.3.2. Stereomicroscopic Studies

Out of 105 oocytes used for light microscopic study (Group 1), 50 oocytes were COCs and the remaining 65 were CFOs. For each replicate, the oocytes from each group were divided equally and kept in 4-well culture dish containing IVM medium as previously described (Chapter 3) and placed in an incubator containing a humidified 5% CO_2 in air at 39.0°C. The oocytes were examined under the stereomicroscope for normal and abnormal, general appearance, colour, compactness of cumulus cells, size and quality of oocytes. Under this microscope (20 x magnification) also, cumulus and corona cells expansion after 0, 12, 15, 20, 25, 30, 35 and 40 hr incubation was observed.

4.3.3. Inverted Microscopic Studies

Using this microscope (200x to 400x magnification), a more detailed gross structures were observed i. e. the number of layers of cumulus cells, polar body and nucleus.

4.3.4. Electron Microscopic Studies

Out of 105 oocytes used for electron microscope study (Group 2), 47 were COCs and the remaining 58 were CFOs. For each replicate, the oocytes from each group were divided equally and kept in 4-well culture dish containing IVM medium as previously described (Chapter 3) and placed in an incubator as before. All the oocytes were incubated for 0, 20 and 40 hr. At 0 hr, any 5 of the best COCs and 5 of the best CFOs were taken for electron microscopic study. At 20 and 40 hr, any 5 each of COCs with the best expanded cumulus cells and any 5 each of the best CFOs were taken for electron microscopic study.

The oocytes from each group at 0, 20 and 40 hr were first embedded in 1% (w/v) ion agar for easy handling. Agar blocks were then prefixed in 3% (v/v) glutaraldehyde with 0.5% (w/v) formaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, at room temperature for 40 minutes. After washing in buffer, the agar blocks were postfixed in 1% v/v osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.4, at room temperature for 40 minutes. Two percent (w/v) uranyl acetate in 0.005% acetic acid was used as an *en bloc* stain, i.e. by immersing the agar blocks in the stain for 40 min at room temperature. Washing with distilled water was followed by dehydration in graded ethanol series and replaced by acetone.

The agar blocks were embedded in Epon-Araldite resin (Mollenhauer, 1964). Ultrathin sections for transmission electron microscopy (TEM) were obtained using the Reichert Ultracut E ultramicrotome and stained with uranyl acetate and lead citrate and the ultrastructure of the oocytes were examined in a Philips CM12 transmission electron microscope at 80 kV.

4.4. RESULTS

4.4.1. Light Microscopic Studies

Cumulus-oocyte complex (COC) is spherical in shape, 110 to 120 μm in diameter, and consists of spherical mass of cytoplasm, the vitellus, bounded by a plasma membrane, the vitelline membrane, and further surrounded by a thick transparent structure, zona pellucida. Between the cytoplasm and the zona pellucida is a fluid-filled space, the perivitelline space. Outside the zona pellucida, there are packedly arranged corona cells followed by cumulus cells (Fig. 4.1; 4.2). The CFOs are of the same size as COCs, but with no cumulus and corona cells surrounding the zona pellucida (Fig. 4.3). There are some abnormal oocytes commonly observed such as oval-shaped oocytes and partly filled vitelline space by cytoplasm (Fig. 4.4a) and the presence of vesicles in the degenerated cytoplasm (Fig. 4.4b). The cumulus cells began to expand and showed the 'sun burst'

appearance 14 hr after incubation whereas the corona cells were still tightly packed (Fig. 4.5). At 20 hr both cumulus and corona cells were fully expanded and loosened (Fig. 4.6; 4.7). Sometimes there are oocytes which appeared normal but did not develop, judging from cumulus cells, even after 25 hr incubation (Fig. 4.8). In oocyte with fully expanded cumulus cells, when these cumulus cells were removed, some oocytes showed already extruded first polar body at the perivitelline space (Fig. 4.9).

4.4.2. Electron Microscopic Studies

4.4.2.1. Cumulus-Oocyte-Complexes

At 0 hr, the oocytes had fully developed zona pellucida of 6.0 to 7.8 μm thick although zonation within it was not evident (Figure 4.10; 4.11). Penetration by cumulus cell processes occurred throughout the zona pellucida and these processes, with diameters ranging 120 to 240 nm, were also observed in the perivitelline space. The oolemma was indistinct, and numerous vesicles of 0.25 to 1.20 μm in diameter were distributed throughout the cytoplasm. Lipid bodies, 1.7 to 2.8 μm in diameter were present, but no mitochondrion was observed, probably not in the plane of section.

At 20 hr, the zona pellucida had differentiated into a thicker and less fibrous inner region, and a thinner and

more fibrous outer region, with a total thickness of 1.5 to 15.0 μm. As in 0 hr oocytes, cumulus cell processes occurred throughout the zona pellucida as well as the perivitelline space (Fig. 4.12; 4.15; 4.16). The perivitelline space which was lined on the inside by the oolemma was more extensive and its thickness varied greatly (1.5 to 14.5 μm) from one region to another. Vesicles of 1.5 to 5.0 μm in diameter were present throughout the cytoplasm although occurrence of fusion between adjacent vesicles could not be ascertained owing to membrane disintegration. Fully developed mitochondria (0.42 to 1.0 μm in diameter) were distributed evenly in the cytoplasm, usually in close proximity with dilated endoplasmic reticula while electron-dense cortical granules had a peripheral distribution (Fig. 4.13; 4.14). Clusters of membrane-bound electron-transparent bodies occurred in some regions of the perivitelline space (Fig. 4.12; 4.19; 4.20). Similar bodies were also observed in smaller group throughout the cytoplasm, especially near the clusters of mitochondria. These fully developed mitochondria, a large proportion of which were hooded (Fig. 4.17; 4.18) could be observed throughout the cytoplasm although peripheral distribution was evidently preferred. Lipid bodies were relatively large, 2.1 to 4.6 μm in diameter, while the few vesicles present had disintegrated membranes. Most of the cortical granules were at the periphery, some were not membrane-bound and associated closely with invaginations of the oolemma (Fig. 4.19; 4.20).

At a later stage of incubation (40 hr) the zona pellucida appeared thinner (4.0 to 5.0 μm) but with similar zonation pattern and larger lipid bodies, 2.5 to 7.0 μm in diameter, compared to those in the earlier stages. Most of the vesicles had disintegrated membranes. Junction between the cumulus cell processes and the oolemma were observed (Fig. 4.21; 4.22). As at 20 hr, the mitochondria had an even distribution within the cytoplasm although they were always associated with endoplasmic reticula while cortical granules were mostly at the periphery (Fig. 4.21; 4.22; 4.23; 4.24). A number of mitochondria were hooded (Fig. 4.21; 4.22). Endoplasmic reticula were less dilated and some were in close proximity with the oolemma with no associated ribosomes. Membrane-bound electron-transparent bodies occurred in large clusters within the zona pellucida, with an electron transparent region towards the outside of clusters, but either singly or in groups of two or three throughout the cytoplasm (Fig. 4.23; 4.24).

4.4.2.2. Cumulus Free Oocytes

In these oocytes, cumulus and corona cells were absent. At 0 hr, zonation within the 5.7 to 6.8 μm thick zona pellucida was indistinct although gradual outward increase in its fibrillar nature was observed (Fig. 4.25). Penetration of the zona pellucida was only by microvilli formed by folding of the oolemma. Numerous mitochondria (0.32 to 0.96 μm in

diameter) occurred in groups and associated with dilated endoplasmic reticula throughout the cytoplasm, usually away from the nucleus. No cristae was observed within the mitochondria (Fig. 4.26; 4.27). Endoplasmic reticula were attached to ribosomes and no free ribosomes were observed. The nuclear membrane had numerous pores and seemed continuous with dilated endoplasmic reticula. Very few vesicles, with disintegrated membranes and lipid bodies, were observed.

Similar substructure and pattern of the zona pellucida, 3.0 to 3.8 μm thick were observed at 20 hours. The oolemma formed microvilli although no penetration of the zona pellucida was observed. Mitochondria were relatively sparse (Fig. 4.28) and not well developed, 0.34 to 1.4 μm in diameter, and fewer cristae were observed (Fig. 4.29). As in COC described previously, membrane-bound electron-transparent bodies occurs in large clusters, with electron-transparent periphery, within the zona pellucida (Fig. 4.29) and in smaller groups throughout the cytoplasm. Dilated endoplasmic reticula were not prominent and free ribosomes were absent. Very few relatively large vesicles, with disintegrated membranes, and membrane-bound cortical granules were observed particularly at the periphery.

The zona pellucida at 40 hr was 3.2 to 4.1 μm thick and appeared less compact towards the outside although it was fibrillar throughout (Fig. 4.30; 4.31). Similar clusters of

membrane-bound electron-transparent bodies occurred in the zona pellucida and the region between the oolemma and zona pellucida, both more numerous compared to oocytes described previously. These bodies were also observed throughout the cytoplasm in smaller clusters. Oolemma appeared intact and formed microvilli although they were not very prominent. Mitochondria (0.42 to 1.2 μm in diameter) were small in number, irregular in shape and had few or no cristae (Fig. 4.30; 4.31; 4.32). Numerous vesicles of various sizes, 1.0 to 8.0 μm in dimeter, with intact membranes and few lipid bodies were present. Endoplasmic reticula were not dilated as in other group of oocytes and with no associated ribosomes. Very few cortical granules with no definite pattern of distribution were observed.

4.5. DISCUSSION

4.5.1. Light Microscopic Studies

There was no apparent physical differences, particularly the cytoplasm and the zona pellucida surrounding it, and also the corona and cumulus cells between goat oocytes with other mammalian oocytes. The size of goat oocytes of 110 to 120 μm was within that of eutherian mammals (70-120 μm) noted by Austin and Short (1982). Among the eutherian mammals the oocytes varies little in size between species,

especially in comparison with different in body size (Hartman, 1929). Most of these species have oocytes with a vitelline diameter 120-150 μm but in a number of rodents such as rat, mouse and hamster only 70-75 μm.

Variation in size among mammalian oocytes is attributable mainly to the amount of yolk material (deutoplasm) in the vitellus (Austin and Bishop, 1957). The texture of vitelline substance varies greatly, depending on the different form assumed by the deutoplasm, and on the distribution of mitochondria. In the rat, mouse and hamster, the vitelline material is finely granular and contain little deutoplasm, while in sheep, bovine, goat and rabbit it contains much deutoplasm, though it is still granular in character (Austin and Bishop, 1957).

The ability of oocyte to mature is related to its size. Hirao *et al.* (1994) found that when the pig oocytes grown in vitro were liberated from the follicles and cultured for a further 48 hr in m-KRB-Ringer bicarbonate solution, 60% of the oocytes larger than 110 μm underwent germinal breakdown, whereas those of 90 to 100 μm only 6%.

The present study has found that the cumulus cells of goat oocytes began to expand 14 hr after incubation in the TCM 199 maturation medium. Comparison with similar studies

using the same species could not be done due to the lack of such information. Comparison with bovine oocytes, however, showed that cumulus cells of goat oocytes took 2 hr longer to begin to expand (Shamsuddin *et al.*, 1993). In goat, the cumulus cells expand fully 20 hr after incubation and remained unchanged thereafter. Again, this was 2 hr longer than the time taken for cumulus cells of bovine oocytes to fully expanded (Shamsuddin *et al.*, 1993). It should be noted however, that the evaluation of this criteria could differ between observers (Dandekar *et al.*, 1991). One way which could possibly minimize this dicrepancy is that in all studies, all oocytes should be evaluated by one individual. This was indeed the case in the present study.

The presence of first polar body (Pb1) in the in vitro cultured oocyte probably suggests that it attained a completion of the first maturation stage (Laufer *et al.*, 1984; Wahid *et al.*, 1990; Younis *et al.*, 1991). This study has shown that the first polar body appears as a stable structure at the perivitelline space. This study however did not establish as to when this polar body disappeared. In rabbit, guinea pig and hamster, the first polar body normally remains as a stable structure in the perivitelline space until the first cleavage (Bedford, 1971).

4.5.2. Electron Microscopic Studies

The purpose of this study was to characterize ultrastructurally the changes in cytoplasmic organelles of the developing goat oocytes in vitro. Although maturation process comprises of both cytoplasmic and nuclear changes, it was not the intention of this study to see whether the maturing process started with the germinal vesicle breakdown (GVBD) *per se*. As such, in this study, no relationship between GVBD and the cytoplasmic ultrastructural changes in developing oocytes can be made. Nevertheless cumulus cell expansion was used as a criterion to indicate developmental process since the presence of chromosome condensation seemed to be induced after the oocytes were recovered from follicles (Suss *et al.*, 1988) leading to GVBD. Correlation between the degree of expansion of cumulus and corona cells with oocyte maturation has been shown in human (Testart *et al.*, 1983; Dandekar *et al.*, 1991) and bovine (Shamsuddin *et al.*, 1993).

The role of cumulus cells in the acquisition of full developmental competence during oocyte maturation has been investigated (Sato *et al.*, 1977; Leibfried and First, 1979; Xu *et al.*, 1986). Leibfried and First (1976) and Dalhausen *et al.* (1981) showed that there was no maturation, or a low rate of maturation in bovine oocytes when the cumulus cells were removed before oocytes were matured in vitro. Critser

et al. (1986) found higher rate of embryonic development following IVF and culture of bovine cumulus-oocyte complexes matured in vitro compared with nude or corona-enclosed oocytes. These findings were consistent with the present observation on the ultrastructure of organelles in COC as compared with those without cumulus cells at different stages of development.

In both groups, zona pellucida had fully developed but zonation within it was not evident. In COC group, there were penetration by cumulus cell processes whereas in CFO group this penetration was only by microvilli formed probably due to the folding of the oolemma. In the former, this could be the route for uptake of nutrients through the plasma membrane, which were needed for the oocyte development processes. In the latter, the absence of this route probably reflected the incompetency of the oocytes to attain full maturity. It is possible also that CFOs were probably obtained from atretic follicles (Asakawa *et al.*, 1982) or small follicle (Richards and Midgley, 1976). In sheep, Cran *et al.* (1980) showed that the slender villi were only observed in oocytes with very small follicle, suggesting that the area available for uptake through the plasma membrane may be greatest in small follicles. Our finding also showed that mitochondria were usually in close proximity with endoplasmic reticulum and this was in agreement with other mammalian oocytes (Norrevang, 1986).

Localization of mitochondria at the periphery of the oocyte has been observed in cattle (Fleming and Saacke, 1972) and sheep (Cran *et al.*, 1980). The present finding in goat showed that these mitochondria were usually in close proximity with endoplasmic reticula, however, in cattle (Fleming and Saacke, 1972) dispersal mitochondria was associated with the formation of the perivitelline space. In sheep, although no association has been noted, the observation on the tight peripheral band in the earlier stage of development has also been reported (Cran *et al.*, 1980). The hooded appearance of mitochondria in COCs but not in the CFOs suggests the superiority of the COCs with regards to the development of the oocytes. Furthermore in CFOs, the mitochondria were relatively few, not well developed and have fewer cristae compared to COCs. Hooded appearance of mitochondria during late oestrus has been reported in sheep (Cran *et al* ., 1980) and in cattle (De Loose *et al.*, 1989; Senger and Saacke, 1970). Hooding of mitochondria increased their surface area and may provide a specific micro-environment to facilitate exchange of metabolic intermediates with the endoplamic reticulum (Cran *et al.*, 1980). The presence of intramitochondrial granules in a 2-cell bovine embryo demonstrated by Brackett *et al.* (1980) was not noted here. Intramitochondrial granules also have not been noted in oocytes or embryos of other species (Brackett *et al.*, 1980).

The present finding also showed that the endoplasmic reticula were dilated. While in goat some of these endoplasmic reticula were in close proximity with the oolemma with no associated ribosome, in sheep they were related to the presence of extended surface villi (Cran *et al.*, 1980). It is possible that it is the oolemma which made the extended surface villi. It is assumed that surface villi represent a temporary storage of products produced during the rapid growth phase of the oocytes.

Cortical granules in COC at 0 hr were numerous but dispersed randomly. At later stages of incubation (20 hr), they were not only numerous but also distributed at the periphery, just under the plasma membrane. In CFO, cortical granules were very few with no definite pattern of distribution was observed. This finding is in agreement with that in sheep that cortical granules were randomly scattered in small follicles but in the region of plasma membrane in the larger, whereas in late oestrus, these granules were localized immediately beneath the plasma membrane (Cran *et al.*, 1980). It appears then, that the late stage of cortical granules development found in goat was similar to that of sheep oocytes in late oestrus. Szollosi *et al.* (1978) in their studies in rabbit suggested that this is as a result of a loss of gap junctions between the foot of cumulus processes and the oocyte surface. Cran *et al.*, (1980) found a reduction in the relationship between the surrounding

granulosa cells and the oocytes as a result of degeneration of cumulus cells. However, different with that in sheep and rabbits, the present study showed that the gap junction between the foot of cumulus processes and the oocyte surface remain intact. The peripheral localization of cortical granules in developing oocytes have some bearing on their subsequent fertilization. As has been shown in hamster oocytes following the fusion of sperm and oocytes in hamster, cortical granule reaction occurred whereby the oocyte plasma membrane fused with the membrane of the cortical granules, releasing their enzymes into the perivitelline space which seeped into the zona pellucida (Brackett *et al.*, 1980; Wassarman, 1988b). These enzymes alter the zona pellucida's glycoprotein constituent, rendering it inactive as a sperm receptor (Wassarman, 1988b). Therefore it is tempting to suggest that the number and the distribution of these organelles play a major role in the development process.

In both groups of oocytes, regardless of the stages of development, clusters of membrane-bound electron-transparent bodies with associated electron-transparent boundary in isolated regions of the zona pellucida and the region between the oolemma and zona pellucida, causing invagination of oolemma in the latter case were observed (Fig. 4.19; 4.20). Cran *et al.* (1980) described similar structures in sheep as aggregation of particles or vesicles. However, these aggregations appeared to be larger in size. Kruip *et*

al. (1983) and De Loose *et al.* (1989) did not report such structures in bovine oocytes. Matthews and Martin (1971) had demonstrated the presence of annulate lamellae in human oocytes which consists of double membranes with periodic annuli occurring in regular order. Such lamellae were not observed in the present study of goat oocytes. However, the membrane-bound electron-transparent bodies appeared to have similar structures to the periodic annuli reported by Matthew and Martin (1971). The physiological significance of these bodies has yet to be determined particularly with regard to the development process since they were also found in the cumulus-free oocytes. The present finding also showed the presence of junctions between cumulus cell processes and oolemma. This finding is in agreement with that found in sheep (Cran *et al.*, 1980).

The results of the present study found that for COC, there was no notable differences in the ultrastructure of cytoplasmic organelles between 20 and 40 hr. On the other hand, there was no difference in the ultrastructure of CFO before and after 20 hr incubation. These observations not only suggested the superiority of the COC group of oocytes compared to those of CFO, but also indicated that 20 hr incubation was sufficient for the attainment of full development of oocytes. This possibility is in agreement with that of Shamsuddin *et al.* (1993) who found that an incubation period of 20 to 24 hr is sufficient for in vitro matu-

ration of bovine oocytes. Shamsuddin *et al.* (1993) has further shown that bovine oocytes incubated beyond 26 hr resulted in reduced maturation rate. Hyttel *et al.* (1986) in their studies on the ultrastructure of in vitro maturation of oocytes in cattle have also reported that such oocytes begin to degenerate 30 hr after incubation. These findings probably warrant further study of nuclear changes and subsequent in vitro fertilization of maturing oocytes cultured in vitro. These topics are reported in Chapters 5 and 6 respectively.

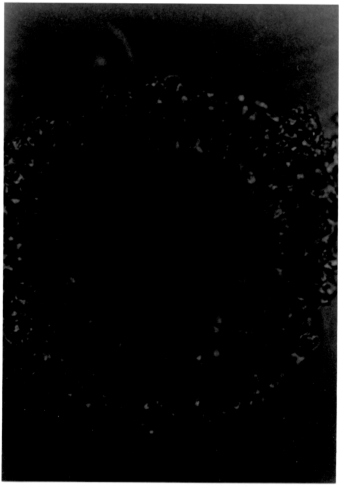

Figure 4.1. A Cumulus-oocyte-complex (COC), showing the compactness of cumulus cells (cu) and corona cells (co) surrounding the zona pellucida (zp). cy, cytoplasm. Scale bar: 20 μm.

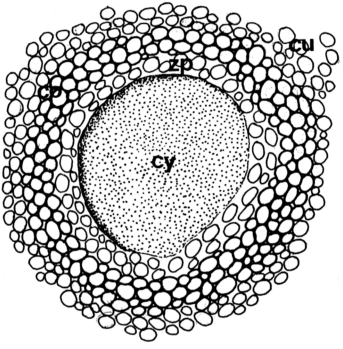

Figure 4.2. Schematic diagram of COC shown in Fig. 4.1 showing the compactness of cumulus cells (cu) and corona cells (co) surrounding the zona pellucida (zp). cy, cytoplasm.

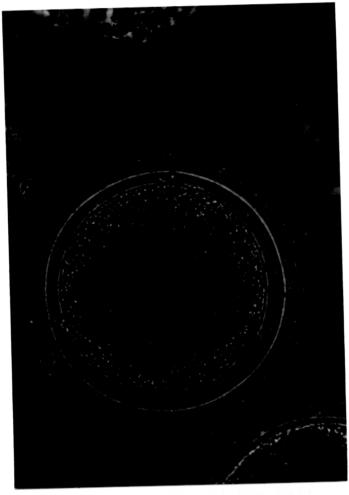

Figure 4.3. A cumulus-free oocyte (CFO), showing the absence of cumulus (cu) and corona cells (co). Cytoplasm (cy) is clearly seen. zp, zona pellucida. Scale bar: 15 μm.

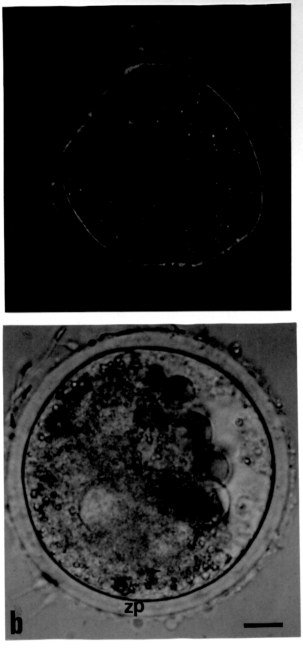

Figure 4.4. Some abnormal oocytes commonly found in goat. a, the oval-shaped oocyte and the partly filled perivitelline space (ps) with cytoplasm; b, oocyte with vessicles (v) in the degenerated cytoplasm (arrowhead). Scale bar: a) 20 μm; b) 15 μm.

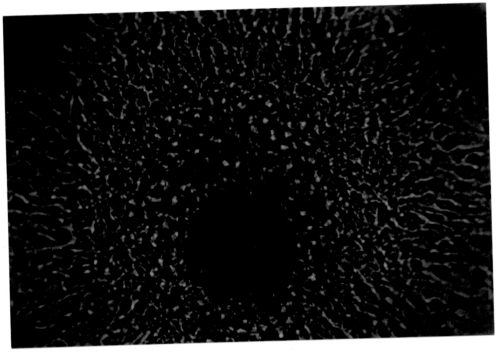

Figure 4.5. A developing goat COC 14 hr after incubation in the in vitro maturation (IVM) medium. Note the 'sun burst' appearance of cumulus cells (cu), but the corona cells (co) still tightly packed. Zona pellucida (zp) was not clearly seen. cy, cytoplasm; Scale bar: 40 μm.

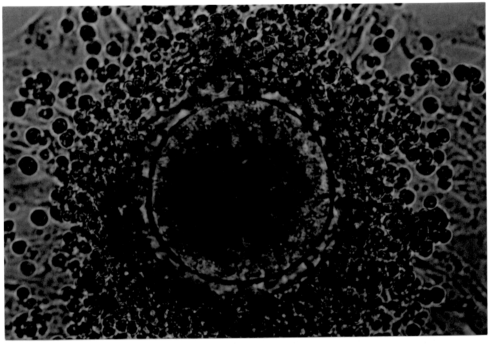

Figure 4.6. A developing COC 20 hr after incubation in the in vitro maturation (IVM) medium. Note that both cumulus (cu) and corona cells (co) were fully expanded and loosen. cy, cytoplasm; zp, zona pellucida. Scale bar: 25 μm.

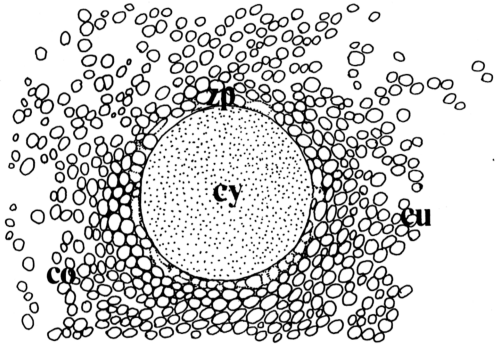

Figure 4.7. Schematic diagram of developing COC as shown in Fig. 4.6, showing the expansion and loosening of cumulus and corona cells.

Figure 4.8. A cumulus-oocyte complex (COC) which apparently normal but did not develop, judging from the lack of cumulus cell (cu) expansion and loosening, even after 25 hr of incubation in the in vitro maturation (IVM) medium. cy, cytoplasm; co, corona cells; zp, zona pellucida. Scale bar: 25 μm.

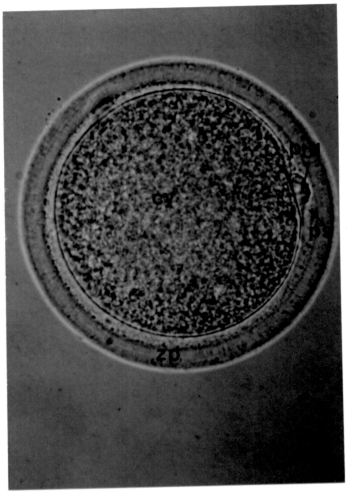

Figure 4.9. A developing goat oocyte with first polar body (Pb1) already extruded into perivitelline space (ps) about 20 hr after incubation in the in vitro maturation (IVM) medium. The cumulus cells have been removed. Scale bar: 15 μm.

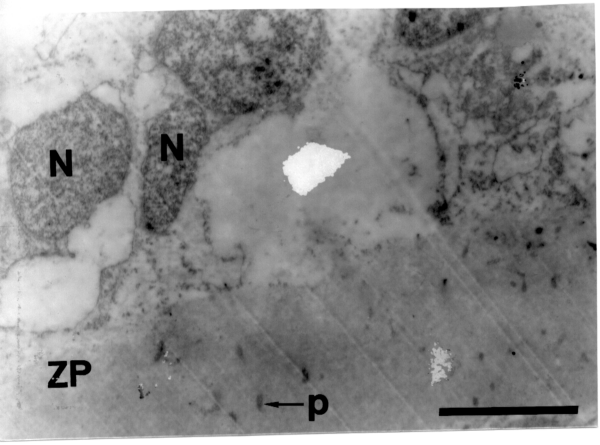

Figure 4. 10. Cumulus-oocyte complex (COC) incubated at 0 hr. Part of transverse section (TS) showing the zona pellucida (ZP) penetrated by cumulus cell processes (p). N is the nucleus of the cumulus cell. Scale bar: 5 μm.

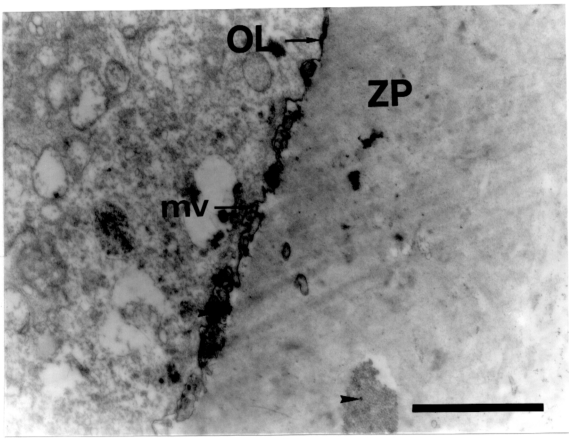

Figure 4.11. Cumulus-oocyte complex (COC) incubated at 0 hr. Part of transverse section (TS) showing microvilli (mv) and clusters of membrane-bound bodies (arrowhead) within zona pellucida and the region between oolemma and zona pellucida. Scale bar: 2 μm.

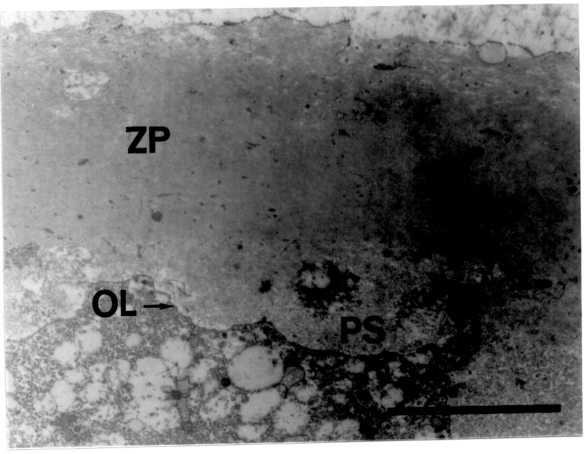

Figure 4. 12. Cumulus-oocyte complex (COC) incubated at 20 hr. Part of transverse section (TS) showing zonation within the zona pellucida and the perivitelline space (PS) lined on the inside oolemma (OL). Scale bar: 10 μm.

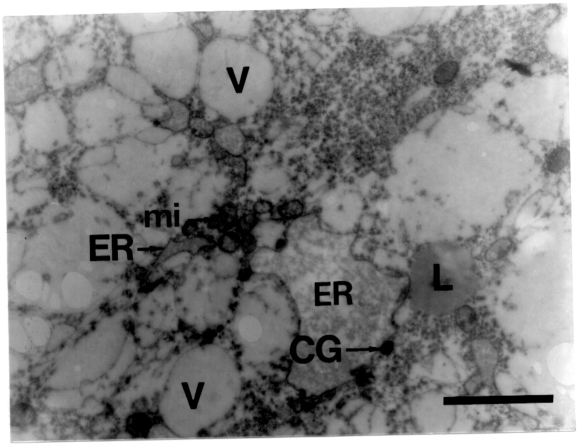

Figure 4.13. Higher magnification of part of section in Fig. 4. 12, showing mitochondria (mi), dilated endoplasmic reticulum (ER), vesicles with disintegrated membranes (V), cortical granules (CG) and lipid bodies (L). Scale bar: 3 μm.

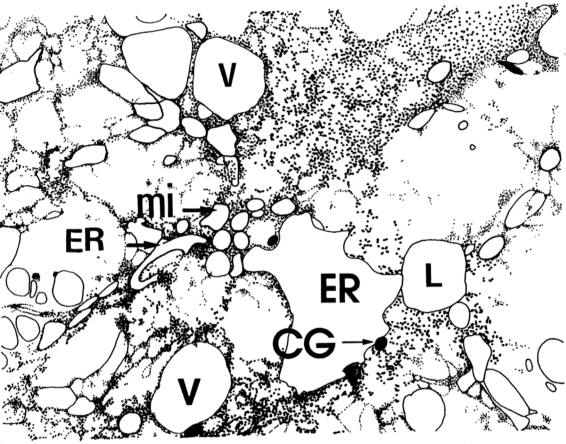

Figure 4.14. Schematic diagram of section shown in Fig. 4.13, showing mitochondria (mi), dilated endoplasmic reticulum (ER), vesicles with disintegrated membranes (V), cortical granules (CG) and lipid bodies (L).

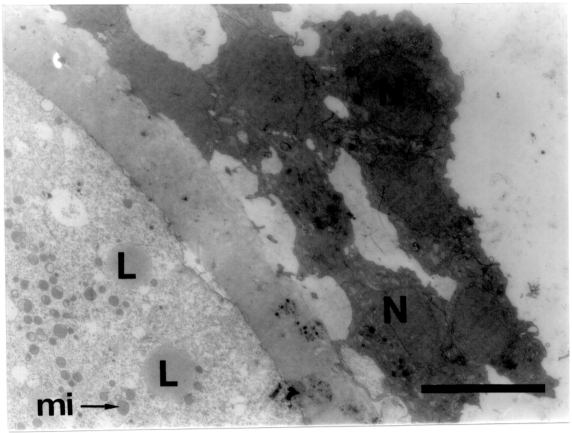

Figure 4.15. Cumulus-oocyte complex (COC) incubated at 20 hr. Part of transverse section (TS) showing cumulus cells, each with a nucleus (N), hooded mitochondria and lipid bodies (L). Scale bar: 10 μm.

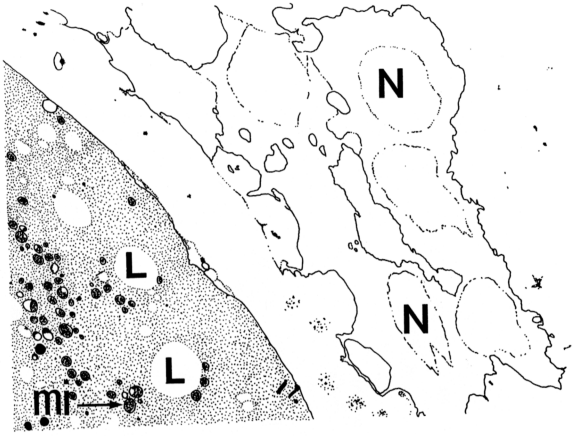

Figure 4.16. Schematic diagram of COC shown in Fig. 4.15, showing cumulus cells, each with a nucleus (N), hooded mitochondria (mi) and lipid bodies (L).

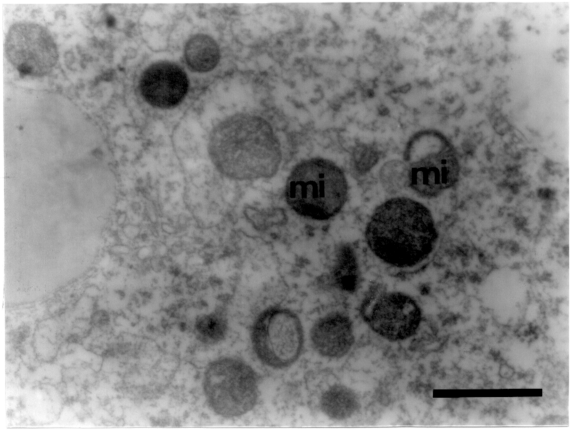

Figure 4.17. Cumulus-oocyte complex (COC) incubated at 20 hr. Sections showing hooded mitochondria (mi). Scale bar: 1 μm

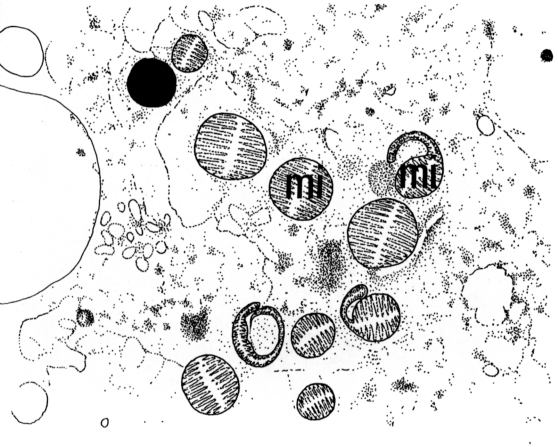

Figure 4.18 . Schematic diagram of COC shown in Fig. 4.17, showing hooded mitochondria (mi).

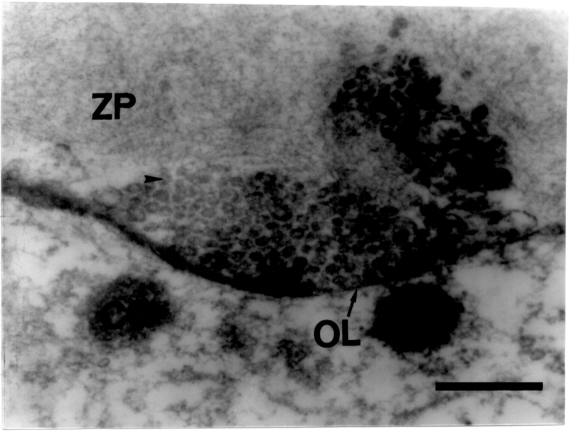

Figure 4.19. Membrane-bound bodies (arrowhead) enclosed between the invaginated oolemma (OL) and the fibrous zona pellucida (ZP) with associated non-membrane-bound cortical granules (CG). Scale bars: 0.3 μm.

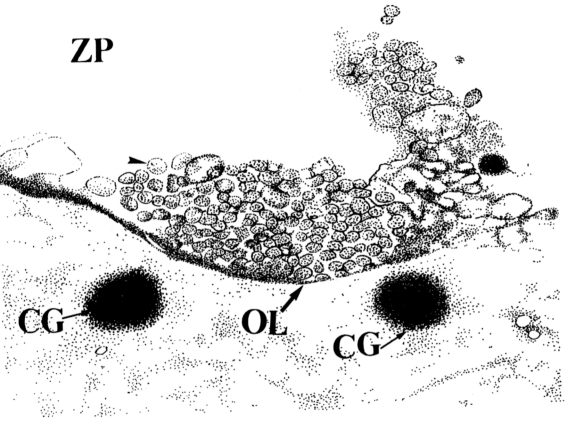

Figure 4.20 . Schematic diagram of section shown in Fig. 4.19, showing membrane-bound bodies (arrowhead) enclosed between oolemma (OL) and the fibrous zona pellucida (ZP) with asociated non-membrane-bound cortical granules (CG).

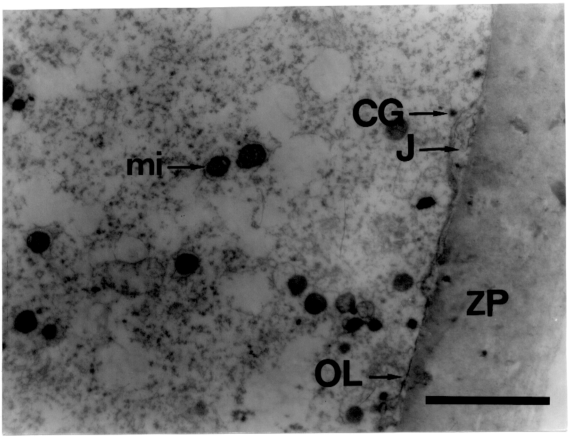

Figure 4. 21. Cumulus-oocyte complex (COC) incubated for 40 hr. Part of transverse section (TS) showing peripheral distribution of cortical granules (CG) and even distribution of mitochondria (mi). Note the close proximity of the cortical granules to the oolemma (OL), presence of junctions (J) bretween cumulus cell processes and oolemma. Scale bar: 4 μm.

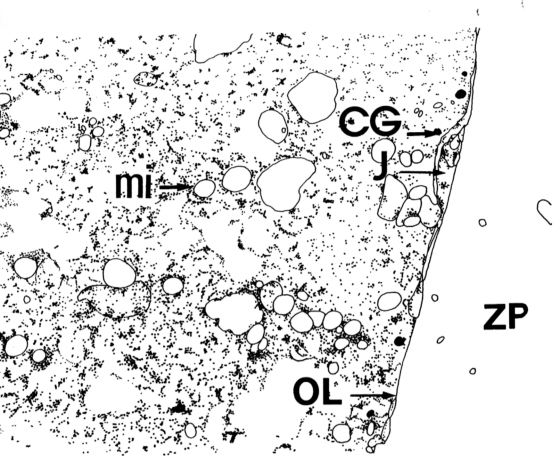

Figure 4.22. Schematic diagram of COC shown in Fig. 4. 21, showing peripheral distribution of cortical granules (CG) and even distribution of mitochondria (mi). Note the close proximity of the cortical granules to the oolemma (OL), presence of junction (J) between cumulus cell processes and oolemma.

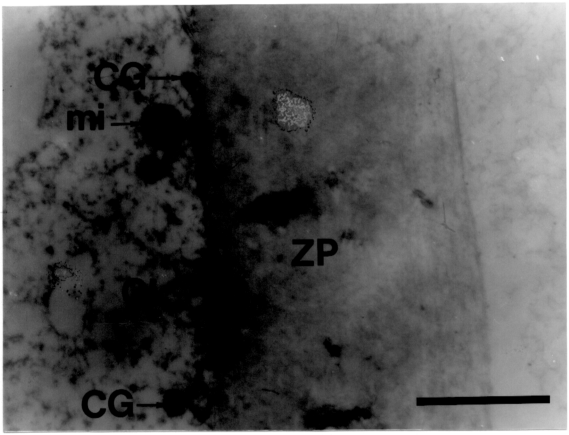

Figure 4.23. Cumulus-oocyte complex (COC) incubated for 40 hr. Part of transverse section (TS) showing clusters of membrane-bound bodies (arrowhead) within zona pellucida (ZP). Scale bar: 2 μm.

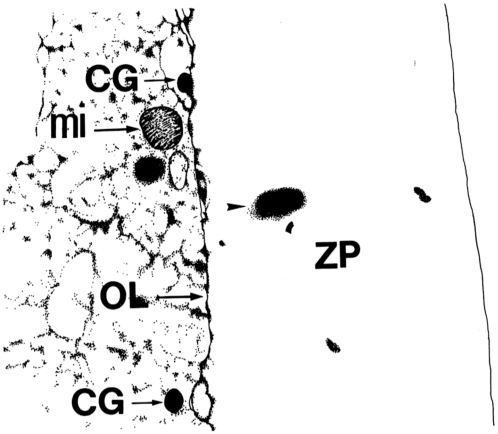

Figure 4.24. Schematic diagram of COC as shown in Figure 4.23, showing clusters of membrane-bound bodies (arrowhead) within zona pellucida (ZP).

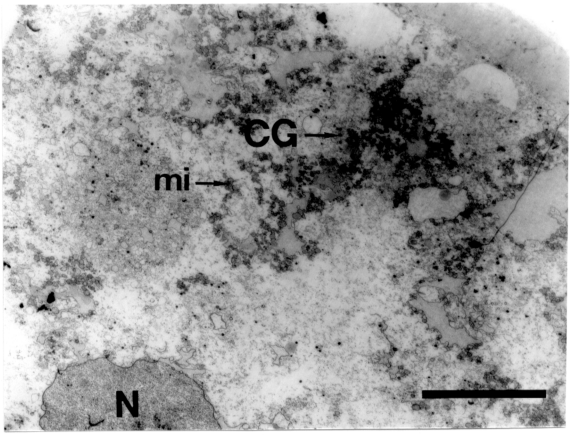

Figure 4.25. Cumulus-free oocyte (CFO) incubated at 0 hr. Transverse section (TS) showing distribution of mitochondria and cortical granules. N is the nucleus. Scale bar: 15 μm.

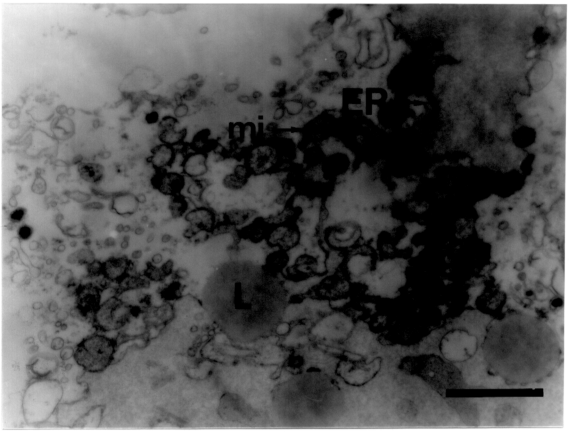

Figure 4.26. Higher magnification of part of section in Fig. 4.25, showing association of mitochondria and dilated endoplasmic reticula (ER). Scale bar: 2 μm.

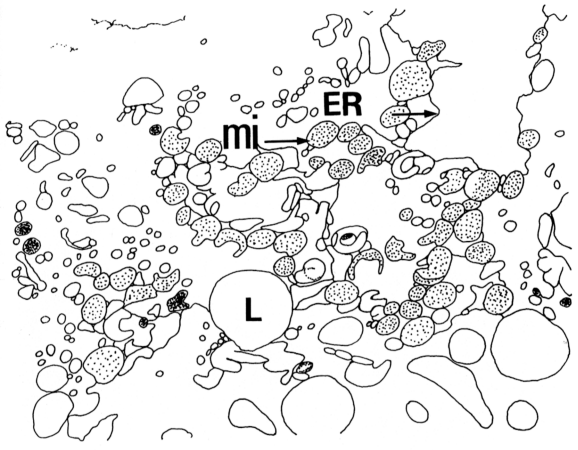

Figure 4.27. Schematic diagram of section shown in Fig. 4.26, showing association of mitochondria (mi) and endoplasmic reticula (ER).

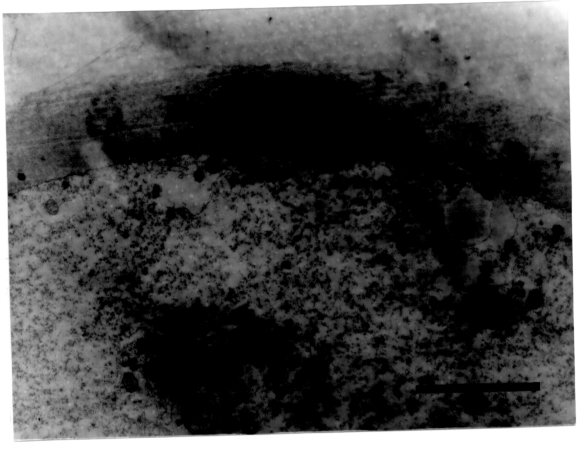

Figure 4.28. Cumulus-free oocytes (CFO) incubated at 20 hr. Part of transverse section (TS) showing the scarcity of organelles within the cytoplasm. Scale bar: 5 μm

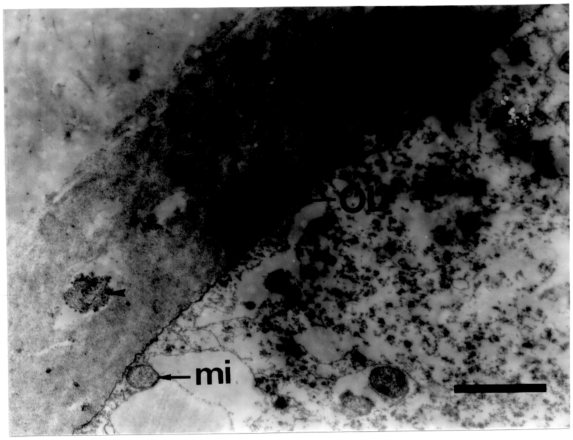

Figure 4.29. Cumulus-free oocytes (CFO) incubated at 20 hr. Note the presence of a cluster of membrane-bound bodies (arrowhead) within the zona pellucida (ZP). Scale bar: 2 μm.

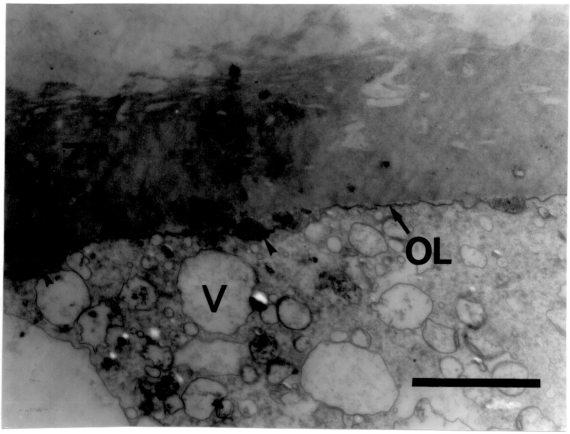

Figure 4.30. Cumulus-free oocytes (CFO) incubated at 40 hr. Part of transverse section (TS) showing clusters of membrane-bound bodies (arrowhead) enclosed between the invaginated oolemma (OL) and zona pellucida. Scale bar: 3 μm.

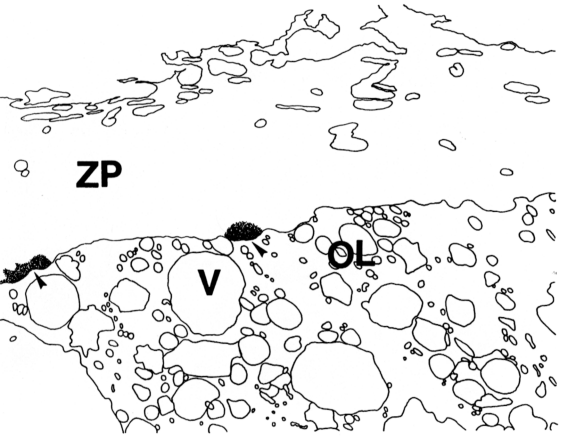

Figure 4.31. Schematic diagram of COC as shown in Fig. 4.30, showing clusters of membrane-bound bodies (arrowhead) enclosed between the invaginated oolemma (OL) and zona pellucida (zp).

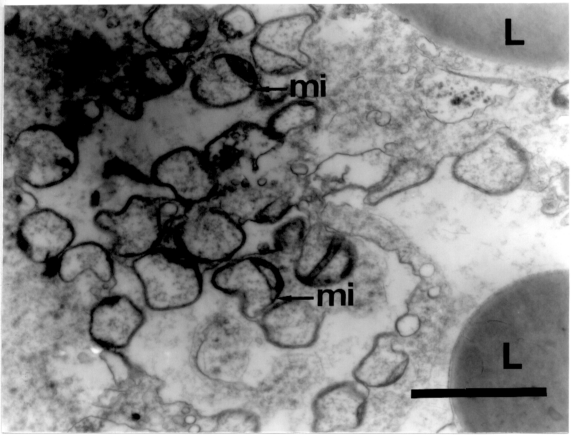

Figure 4.32. Cumulus-free oocytes (CFO) showing abnormally shaped mitochondria (mi) with few or no cristae. Scale bar: 2 μm.