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Oocyte ultrastructure in bovine primordial to early tertiary follicles

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Abstract The aim of the present study was to describe in detail the changes occurring in the cytoplasmic ultrastructure of the bovine oocyte from the onset of growth in the primordial follicle until the completion of growth in the tertiary follicle. Bovine oocytes from primordial, primary, secondary and early to mid-antral follicles were processed and analysed by light and transmission electron microscopy. The primordial follicular oocyte was characterized by numerous coated pits on the oolemma and the accumulation of free and organelle-related smooth (SER) and rough (RER) endoplasmic reticulum, round mitochondria and Golgi complexes around the nucleus, which was located slightly off centre. Up to the secondary follicular stage the oocyte displayed an increase in the number of microvilli, elongated mitochondria and Golgi complexes. During the secondary follicular stage, formation of the zona pellucida, development of gap junctions between the oocyte and the granulosa cells, formation of the cortical granules in the oocyte and reduction in the number of coated pits on the oolemma were seen. In the tertiary follicular oocyte up to 100 μm in diameter, the number of Golgi complexes and lipid droplets increased and the organelles were dislocated to the deep cortical region. During the final growth of the oocyte up to >120 μm, the organelles were dislocated further to the peripheral region, the extent of the free SER and RER compartments were reduced, the number of individual cortical granules increased, hooded mitochondria became abundant and the perivitelline space developed. In conclusion, the growth of the bovine oocyte is associated with the relocation and modulation of a number of cytoplasmic organelles as well as the development of oocyte specific structures such as the zona pellucida and cortical granules.

Key words Cattle · Ooplasm · Organelles · Growth

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Introduction

The growth phase of the mammalian oocyte occurs during the meiotic arrest in the prophase of the first meiotic division and is characterized by substantial RNA and protein synthetic activity. The intense synthetic activity of the growing oocyte is signalled by the modulation of various cytoplasmic organelles and the development of certain oocyte-specific elements such as the zona pellucida and cortical granules. The growth of the oocyte also results from the entry of proteins, lipids and carbohydrates from outside the cell. Some of these enter by endocytosis from surrounding extracellular fluids, others through direct cytoplasmic connections with the surrounding somatic cells. Most of the increase in oocyte mass that occurs during oogenesis involves the cytoplasm with its organelles and inclusions.

Investigations of bovine oocyte ultrastructure have, except for the work of Rüsse (1983), largely concentrated on the fully grown oocyte. The morphology of immature (de Loos et. al 1989; Van Blerkom et al. 1990), maturing (Flemming and Saacke 1972; Kruip et al. 1983; Hyttel et al. 1986a, 1987; de Loos et al. 1992), superovulated (Hyttel et al. 1986b; Assey et al. 1994b) and dominant versus subordinate (Assey et al. 1994a) bovine oocytes has been described in detail. Currently, there is considerable interest in the exploitation of bovine preantral follicles by isolation and culture (Hulshof et al.

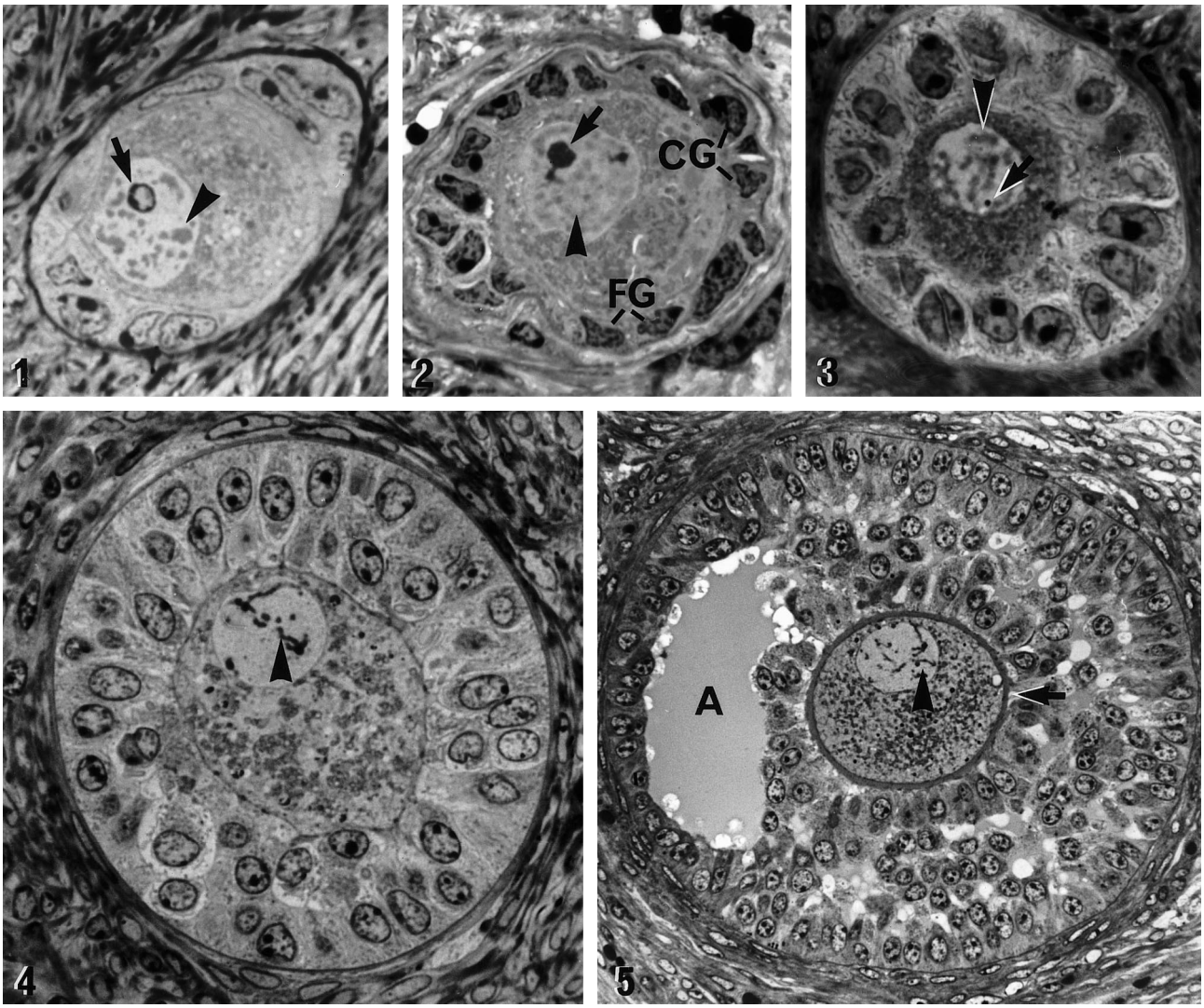


Fig. 1 Light micrograph showing a resting primordial follicle. Note the flattened granulosa cells, the oocyte containing an eccentrically located nucleus displaying patches of condensed chromatin (*arrowhead*) and a nucleolus (*arrow*). ×1120

Fig. 2 Light micrograph showing an activated primordial follicle. Note the flattened (*FG*) and cuboidal granulosa cells (*CG*), the oocyte containing an eccentrically located nucleus displaying patches of condensed chromatin (*arrowhead*) and a nucleolus (*arrow*). ×1040

Fig. 3 Light micrograph showing a primary follicle. Note the cuboidal granulosa cells, the oocyte containing an eccentrically located nucleus displaying patches of condensed chromatin (*arrowhead*) and a nucleolus (*arrow*). ×1000

Fig. 4 Light micrograph showing a secondary follicle. Note the bilayer of cuboidal granulosa cells, the oocyte containing an eccentrically located nucleus displaying patches of condensed chromatin (*arrowhead*). ×750

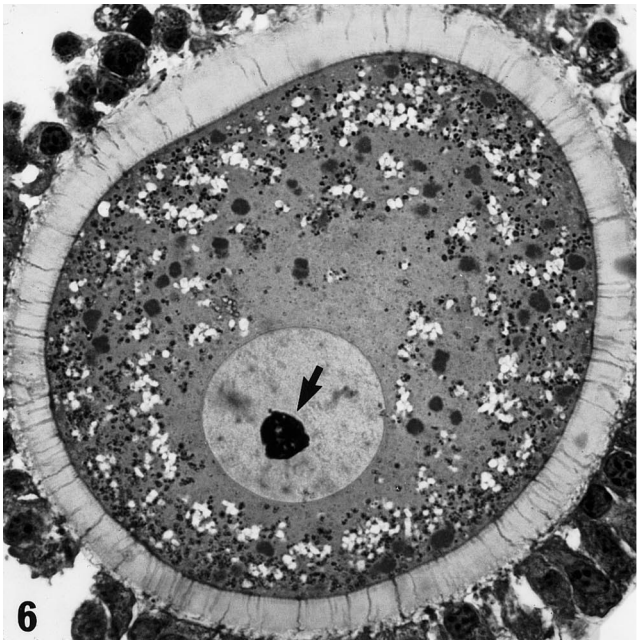
Fig. 5 Light micrograph showing an early tertiary follicle. Note the multiple layers of granulosa cells, the antral cavity (*A*), the oocyte (*arrow*) containing an eccentrically located nucleus displaying patches of condensed chromatin (*arrowhead*). ×370

1995; Telfer et al. 1996). Success in this field requires a complete understanding of their cell biology. Thus, our objective was to describe the morphological aspects of cytoplasmic development during the growth phase of the bovine oocyte as it occurs in the adult bovine ovary. The cytoplasmic ultrastructure of bovine oocytes from resting and activated primordial, primary, secondary and tertiary, i.e. antral, follicles, have been studied using light (LM) and transmission electron microscopy (TEM).

Materials and methods

Collection and processing of bovine ovarian tissue blocks with preantral and early antral follicles for light and transmission electron microscopy

Bovine ovaries collected at slaughter or ovariectomy were held at 32–37°C in phosphate buffered saline (PBS) supplemented with 200 IU/ml of penicillin and 200 μg/ml streptomycin. Following successive washing, ovaries were transferred to M199 Hepes containing 5% foetal calf serum (FCS) and 200 IU/ml penicillin and 200 μg streptomycin. Small pieces of ovarian cortex were dissected from the ovaries and were examined under a dissecting microscope in order to select blocks containing preantral follicles. The



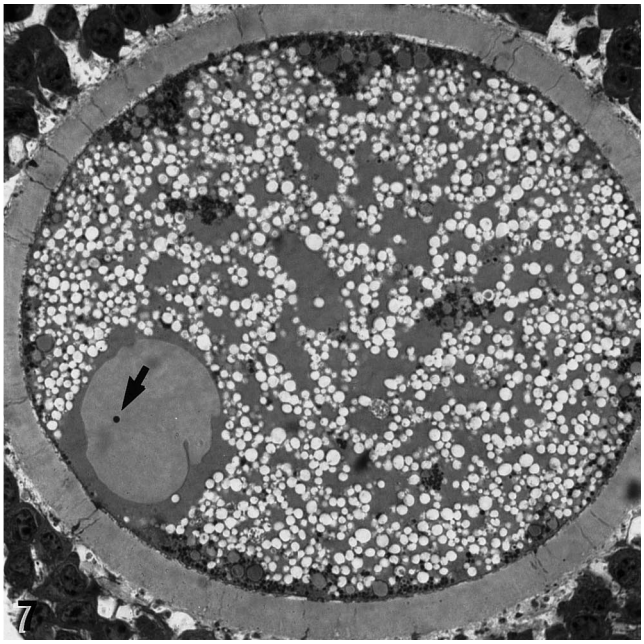


Fig. 6 Light micrograph of an oocyte <110 μm. Note the eccentrically located, round nucleus containing a nucleolus (*arrow*). ×470

Fig. 7 Light micrograph of an oocyte >110 μm. Note the peripherally located nucleus containing a nucleolus (*arrow*). ×500

ovarian tissue blocks were fixed in Karnovsky's fixative (2.5% glutaraldehyde and 0.75% paraformaldehyde in 0.1 M cacodylate buffer) for 60 min and post-fixed in 1% OsO_4 in 0.1 M cacodylate buffer for 60 min. The samples were then dehydrated by passing through an ethanol series, block-stained with uranyl, embedded in Epon and finally serially sectioned into semi-thin sections (2 μm). The sections were stained with Toluidine blue and examined at the LM level for the presence of preantral or early antral follicles containing an intact oocyte. The sections were further selected and those presenting an oocyte nucleus were reembedded and ultrathin (70 nm)-sectioned (Hyttel and Madsen 1987). The nucleus is generally located close to the mid sections of the follicles in preantral and early tertiary follicles, and therefore our observations were in general performed on the equatorial region of the oocytes. These sections were collected on copper grids, stained with uranyl acetate for 35 min at 42°C, washed repeatedly in distilled water and dried. They were subsequently stained with lead citrate for 12 min at room temperature, washed repeatedly, dried and examined by TEM (JEM-1200 EX, Jeol, Tokyo, Japan).

The follicles were divided into 5 classes: (1) resting primordial (Fig. 1), with a single layer of flattened granulosa cells (*n*=8); (2) activated primordial (Fig. 2), with a single layer of some flattened and some cuboidal granulosa cells (*n*=18); (3) primary (Fig. 3), with a single layer of cuboidal granulosa cells (*n*=18); (4) secondary (Fig. 4), with an incomplete or complete bilayer of cuboidal cells (*n*=9); (5) early tertiary (Fig. 5), with more than two layers of granulosa cells delineating one or several intercellular cavities (*n*=5).

Collection and processing of isolated bovine oocytes from later antral follicles for light and transmission electron microscopy

Bovine slaughterhouse ovaries, held at 32–37°C in physiological saline were returned to the laboratory within 3 h of collection. Cumulus-oocyte complexes (COCs) were recovered by slicing ovaries with a series of razor blades. Ovaries were completely submerged in PBS containing 10% foetal calf serum (FCS), (PBSS), during slicing. Recovered COCs with at least three layers of cumulus cells were washed twice in Hepes buffered TCM 199 medium supplemented with 10% FCS and 10 μg/ml heparin (H-8514, Sigma, St. Louis, MO, USA).

Following washing at 4°C, the COCs were mechanically pipetted using a fine bore pipette to partially denude the oocytes of cumulus cells and permit accurate measurement of the inside zona diameter of each oocyte. The oocytes were measured under an inverted microscope using a micrometer and were assigned to one of four groups according to their diameter: <100 μm (*n*=22, Fig. 6), 100–109 μm (*n*=20), 110–119 μm (*n*=29) and ≥120 μm (*n*=18, Fig. 7). These oocyte diameter groups correspond to a medium follicle size range (≤1–4 mm). The oocytes were fixed in Karnovsky's fixative as above, embedded individually in 4% agar, post-fixed and processed as above. LM sections were analysed and those presenting the mid-section of the oocyte and the oocyte nucleus were reembedded and ultrathin (70 nm)-sectioned (Hyttel and Madsen 1987) and further processed according to the procedures described above.

Analysis of the cytoplasmic ultrastructure

The location of the oocyte nucleus, the distribution and number of mitochondria, lipid droplets, vesicles and Golgi complexes were determined on equatorial sections of the oocytes. The location of the oocyte nucleus was described as: (1) central, i.e. located near or at the centre of the oocyte; (2) eccentric, i.e. located between the zona pellucida and the centre of the oocyte; (3) peripheral, i.e. located close to the zona pellucida. Organelle location was described as: (1) peripheral, i.e. located at the oocyte cortex; (2) deep cortical, i.e. confined to the area between the perinuclear region and the oocyte cortex; (3) perinuclear, i.e. in the area surrounding the nucleus; or (4) all over. The predominant mitochondrial morphological form was noted in each oocyte; where there were two or more forms predominating within the same oocyte, their distribution was noted as half or one third. The numbers of lipid droplets and Golgi complexes were counted on the equatorial section of each oocyte, and the range was noted for oocytes within each group. The number and size of ooplasmic vesicles were judged arbitrarily as: (1) many, i.e. the vesicles were so numerous that there were very few areas of cytoplasmic matrix; (2) moderate, i.e. the number of vesicles were plentiful, but small areas of cytoplasmic matrix were readily observed; (3) few, i.e. it was possible to see numerous areas of cytoplasmic matrix between the vesicles; (4)

Table 1 Ultrastructure of pre-antral through to mid-antral bovine follicular oocytes (*MV* microvilli, *SER* smooth endoplasmic reticulum, *RER* rough endoplasmic reticulum)

Structure	Follicle class					Oocyte Diameter mm			
	Resting primordial	Activated primordial	Primary	Secondary	Early antral	<100	100–109	110–119	>120
Granulosa/ oocyte junctions[a]	Adherens	Adherens	Adherens	Adherens	Gap/ adherens	Gap/ adherens	Gap/ adherens	Gap/ adherens	Gap/ adherens
Zona pellucida	None	None	Sparse	Few	All	All	All	All	All
MV2 Shape	Bent	Bent	Bent	Bent/erect	Erect	Erect	Bent/erect	Bent	Bent
Nucleus	Central/ eccentrical	Eccentrical	Eccentrical	Eccentrical	Eccentrical	Eccentrical	Eccentrical/ peripheral	Peripheral	Peripheral
Mitochondria location	Perinuclear	Perinuclear	Deep cortical	Deep cortical	All over	Deep cortical/	Deep / cortical peripheral	Peripheral	Peripheral
Dominant type	Round	Round	Round	Round	Round/ elongate	all over Round	Round/hood pleomorphic	Hood	Hood
Lipid number[b]	0–3	0–5	0 –3	0–15	5–20	10–20	10–20	10–30	10–40
Vesicle number[b]	Sparse	Sparse	Sparse	Few	Moderate	Moderate	Moderate	Many	Many
Free SER/RER	Extensive	Extensive	Extensive	Extensive	Extensive	Extensive	Moderate	Sparse	Very sparse
Golgi number[b]	1–4	1–4	1–3	1–2	2–6	2–9	4–7	4–12	5–8
Cortical granules	None	None	None	Few	All	All	All	All	All
Microtubles	–	–	–	–	+	+	±	–	–

[a] Junctions: adherens: zonula adherens-like junction; gap: gap junction

[b] Number: range of the number of structures counted on the equatorial section of each oocyte within the different follicle classes and oocyte diameter groups

sparse, i.e. they were almost invisible at the LM level, but TEM revealed a small number of them. The vesicles were described as small, medium or large in size.

To avoid repetition, where oocytes in different groups displayed the same ultrastructural features as the previous group they will not be described.

Results

An overview of the ultrastructural changes occuring in the growth phase of the bovine oocyte is presented in Table 1.

Preantral follicles

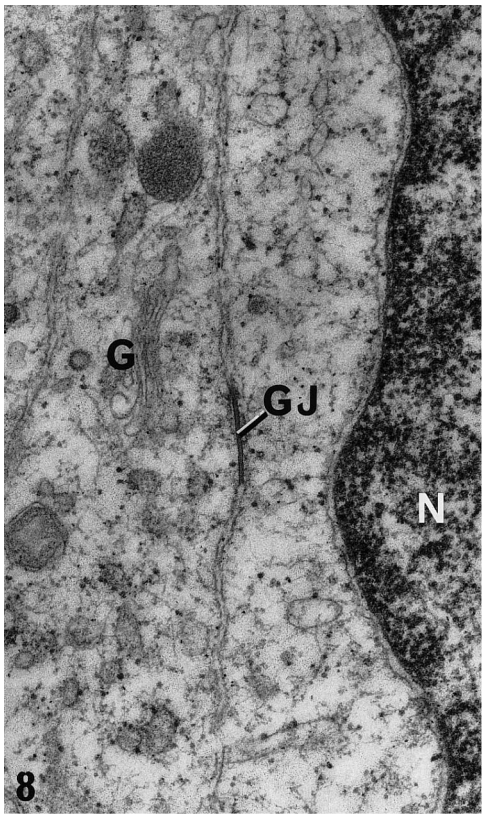
The ovoid to spherical oocyte of the *resting primordial follicle* was surrounded by 5–8 flattened granulosa cells at the equatorial section (Fig. 1). Gap (Fig. 8) and zonula adherens-like junctions were observed between adjacent granulosa cells. The oolemma was attached to the granulosa cells by zonula adherens-like junctions. In general, the oolemma formed numerous coated pits and coated vesicles were often noted in the cortical ooplasm (Fig. 9). The oolemma presented projections that penetrated in between adjacent granulosa cells and a few short bent microvilli lying parallel to the oocyte surface (Fig. 9). The oocyte nucleus occupied a central or eccentric position and the cytoplasmic organelles were concentrated in the perinuclear region. The mitochondria were predominantly round with peripheral longitudinal cristae (Fig. 12). However, a small number of elongated mitochondria with transverse cristae were observed in half of the oocytes, and in some cases mitochondria that appeared to be dividing in two were observed. Many of the mitochondria contained a large electron-dense granule (Fig. 9). Lipid droplets were sparse in this group. Both smooth (Fig. 12) and rough (Fig. 14) endoplasmic reticulum (SER and RER, respectively) were observed in all oocytes, either as isolated aggregations or complex

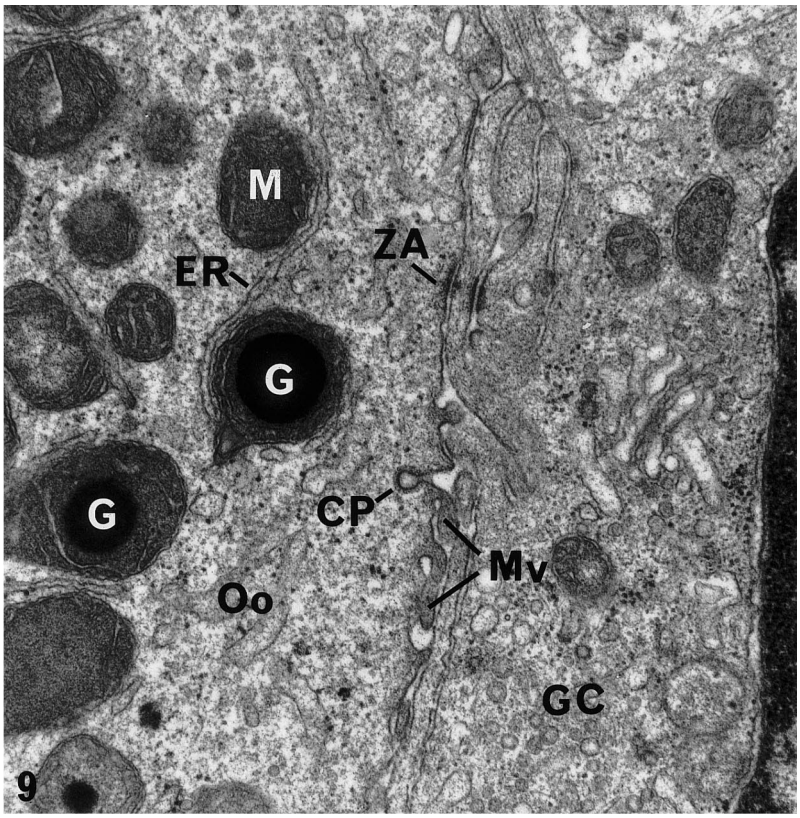
Fig. 8 Electron micrograph showing a detail from a primordial follicle. Note the gap junction (*GJ*) between two adjacent granulosa cell, the Golgi complex (*G*) and the granulosa cell nucleus (*N*). ×34,500

Fig. 9 Electron micrograph from a primordial follicle showing zonula adherens-like junctions (*ZA*) between the oocyte (*Oo*) and granulosa cell (*GC*) processes, the coated pit (*CP*) and microvilli (*Mv*) at the oolemma; note the mitochondria (*M*) containing granules (*G*) and the intimate contact between mitochondria and a tubule of endoplasmic reticulum (*ER*) within the oocyte. ×34,500

Fig. 10 Electron micrograph showing a detail from a secondary follicle. Patches of zona pellucida (*ZP*) material and a granulosa cell process ending in the process of invaginating the oolemma (*arrow*) are shown. ×9,200

Fig. 11 Electron micrograph from an early antral follicle showing zonula adherens-like (*ZA*) and gap (*G*) junctions between a granulosa cell and the oolemma. Note the erect microvilli (*Mv*) extending into the zona pellucida. ×42,000


G
GJ
N
8


M
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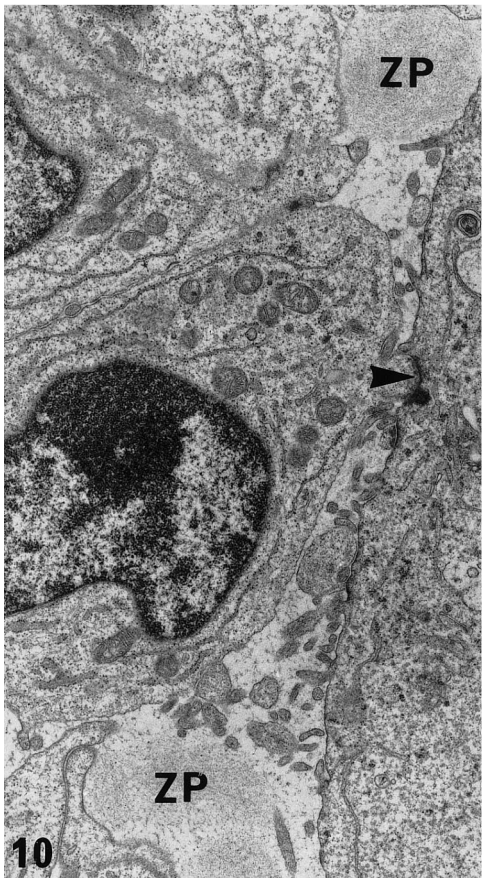
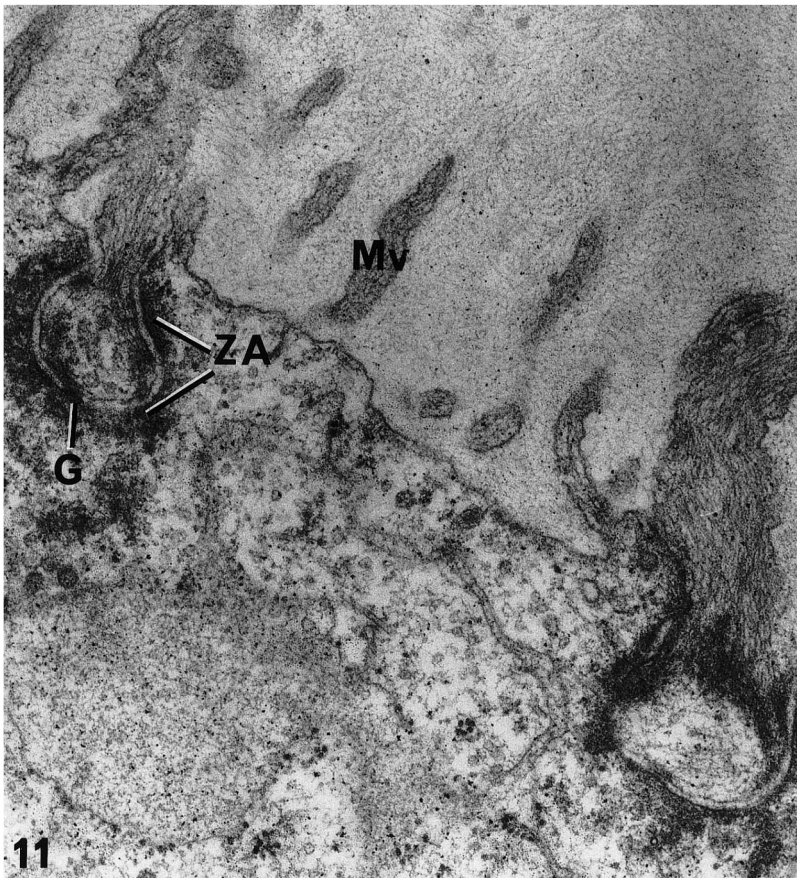
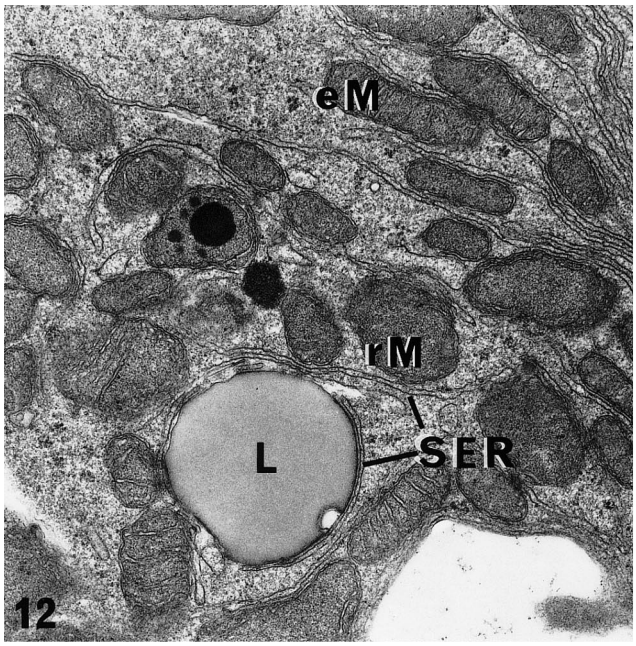
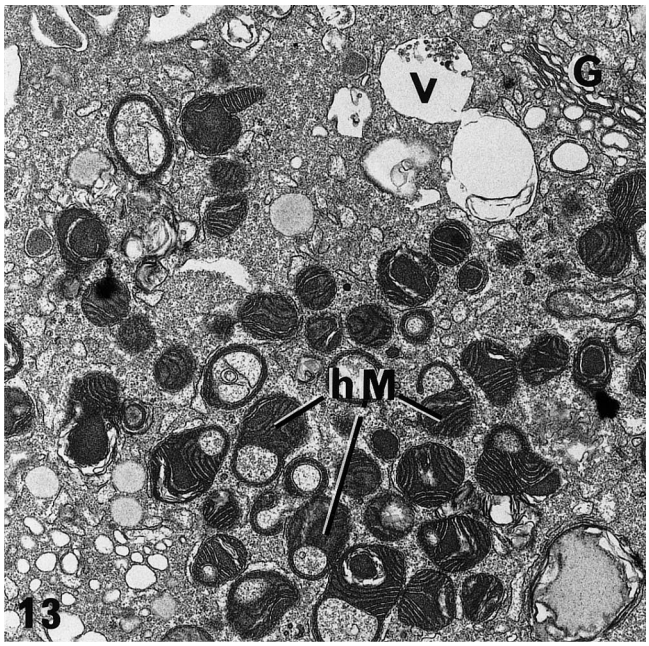
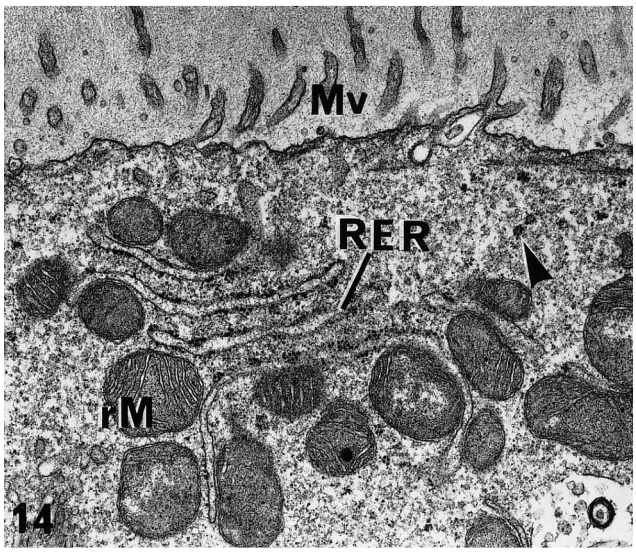
GC
9

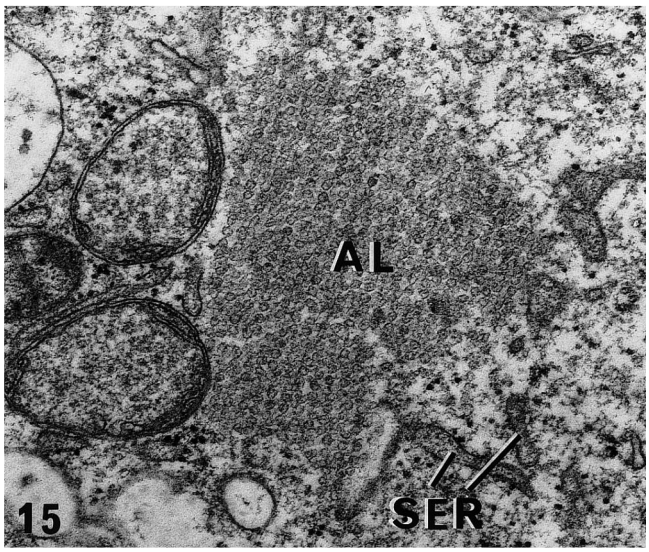

ZP
ZP
10


Mv
ZA
G
11


eM
rM
L
SER
12


V
G
hM
13


Mv
RER
rM
14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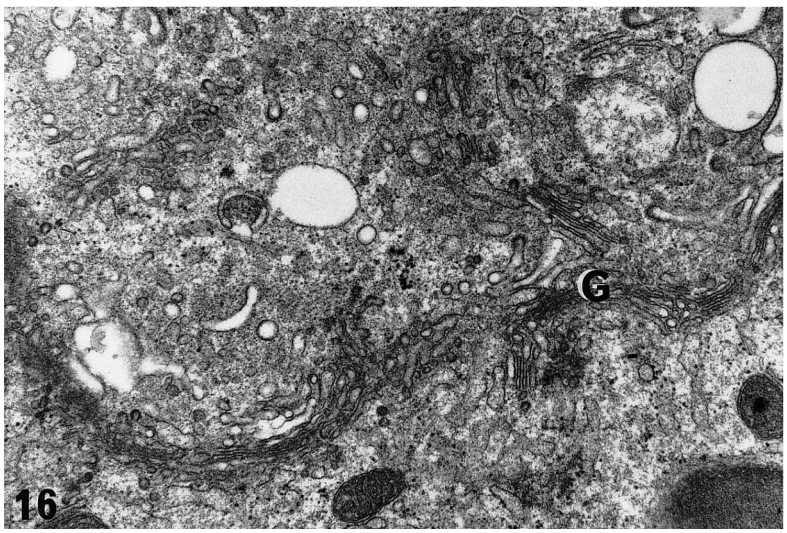
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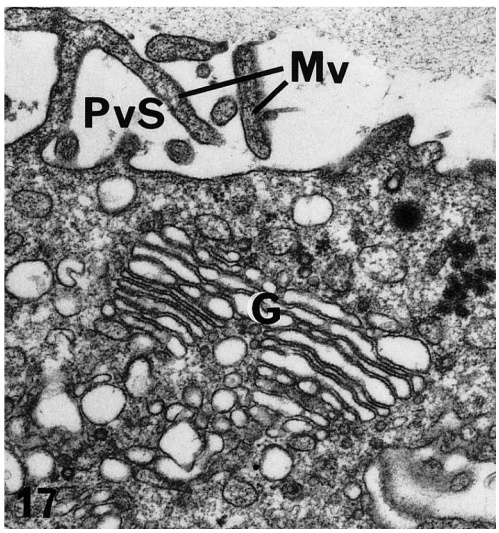

Mv
PvS
G
17

associations with mitochondria, lipid droplets and vesicles. The ER aggregations were often found in close spatial relation to the nucleus or free in the deep cortical ooplasm. A few small Golgi complexes (Fig. 16) were observed in the perinuclear or deep cortical region in all but one oocyte. Only a sparse number of small vesicles were observed and they were usually located among the organelles in the perinuclear region. Polyribosomes were observed on the surface of the RER and distributed throughout the ooplasm.

The *activated primordial follicles* were characterized by a single layer of a mixture of 5–14 cuboidal and flattened granulosa cells arranged around the oocyte at the equatorial section (Fig. 2). In general, most ultrastructural features of the ooplasm and its organelles and inclusions were similar to those described for the resting primordial follicles. There appeared to be a slight increase in the number of microvilli, and in a few cases they were erect. The nucleus was eccentrically located in most oocytes. The majority of the mitochondria were round, although elongated and dividing mitochondria had become more common.

The spherical oocyte of the *primary follicle* was surrounded by a single layer of 8–20 cuboidal granulosa cells at the equatorial section (Fig. 3). Two oocytes (n=18) appeared to show small patches of zona pellucida material between the oocyte and the surrounding granulosa cells (Fig. 10). The mitochondria had moved to the deep cortical region, and in some specimens the Golgi complexes had also migrated to this location. The number of polyribosomes appeared to have increased. One oocyte showed several large patches of annulate lamellae in the deep cortical region (Fig. 15). Interestingly, another oocyte displayed a cluster of cortical granule-like bodies of varying electron-density in the perinuclear region.

The *secondary follicles* were characterized by a partial or complete bilayer of cuboidal granulosa cells (Fig. 4). Patches of zona pellucida material were observed in half of the follicles and were usually associated with erect oocyte microvilli and the extension of the granulosa cell processes to the oolemma (Fig. 10). Zona pellucida formation was associated with the embedding of granulosa cell processes in the oolemma, forming gap junctions between the granulosa cells and the oocytes. Concomitantly, the frequency of coated pits at the oocyte surface was reduced. Elongated mitochondria were present in nearly all oocytes and in some specimens they prevailed. Although half of the oocytes did not display lipid droplets, there appeared to be an increase in their abundance within those that did. The number of small cytoplasmic vesicles appeared to increase. Occasionally, annulate lamellae were observed and a few oocytes displayed clusters of cortical granules in the deep cortical region.

The *tertiary follicles* were characterized by a multilayer of granulosa cells surrounding the oocyte with small cavities between the granulosa cells or a single small antral cavity (Fig. 5). All oocytes were surrounded by a zona pellucida, which was transversed by cumulus cell processes with endings that terminated in indentations of the oolemma (Fig. 11). Numerous erect microvilli extended into the zona pellucida and the number of coated pits appeared to have decreased. The mitochondria appeared to be distributed throughout the ooplasm. Both round and elongated mitochondria were equally abundant. Numerous lipid droplets were observed in all oocytes and the number of vesicles had increased. The number of Golgi complexes appeared to have increased and, furthermore, the individual cisternae had enlarged (Fig. 19). All oocytes displayed clusters of cortical granules either distributed all over the ooplasm or confined to the deep cortical region near the Golgi complexes. In one oocyte a large vesicle surrounded by cortical granule-like structures that appeared to be in the process of pinching off was observed (Fig. 18). In another, a multi-vesicular body that was surrounded by numerous cortical granule-like structures containing material of varying electron-density was noted. Arrays of microtubules were noted in the ooplasm.

Later stages of oocyte growth

The surface of *oocytes <100 μm* in diameter presented numerous erect microvilli that extended into the zona pellucida. In the majority of the oocytes the nucleus was eccentrically located. The mitochondria had relocated to the perinuclear to deep cortical region of the ooplasm in more than half of the oocytes. Round mitochondria were again the predominant form; the numbers of elongated mitochondria and mitochondrial granules had decreased. Hooded (Fig. 13) and pleomorphic (i.e. mitochondria which appeared subdivided into several lobules) mitochondria were observed for the first time. A moderate number of predominantly small vesicles were noted. Both SER and RER continued to be quite extensive, forming either isolated aggregations or organelle complexes as described earlier. The cortical granules were predominantly located in clusters in the deep cortical re-

Fig. 12 Electron micrograph from a primordial follicle showing elongated (*eM*) and round (*rM*) mitochondria in intimate contact with smooth endoplasmic reticulum (*SER*) and a lipid droplet (*L*). ×22,800

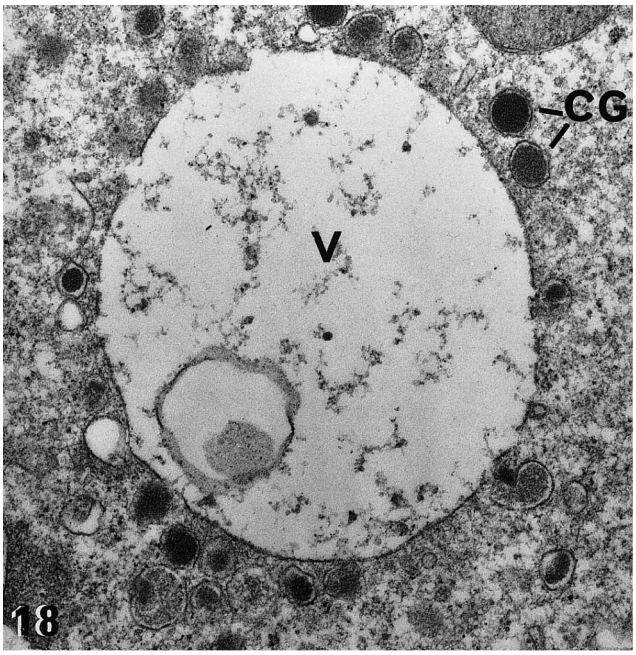
Fig. 13 Electron micrograph from an oocyte >110 μm showing hooded (*hM*), Golgi complex (*G*) and vesicles (*V*). ×10,400

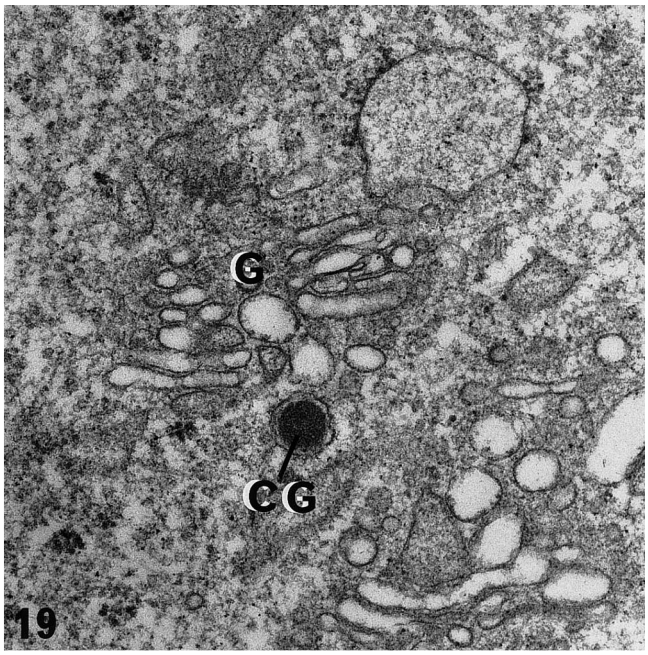
Fig. 14 Electron micrograph from an early antral follicle showing rough endoplasmic reticulum (*RER*) in intimate contact with round mitochondria (*rM*). Note the erect microvilli (*Mv*) extending into the zona pellucida. ×19,200

Fig. 15 Electron micrograph showing a detail from a secondary follicle. Note the large patch of annulate lamellae (*AL*) and the intimately associated, smooth endoplasmic reticulum (*SER*). ×38,000

Fig. 16 Electron micrograph from a primordial follicle showing several Golgi complexes (*G*). ×22,000

Fig. 17 Electron micrograph from an oocyte >110 μm showing a large Golgi complex (*G*); note the perivitelline space (*PvS*) and the bent microvilli (*Mv*). ×26,400





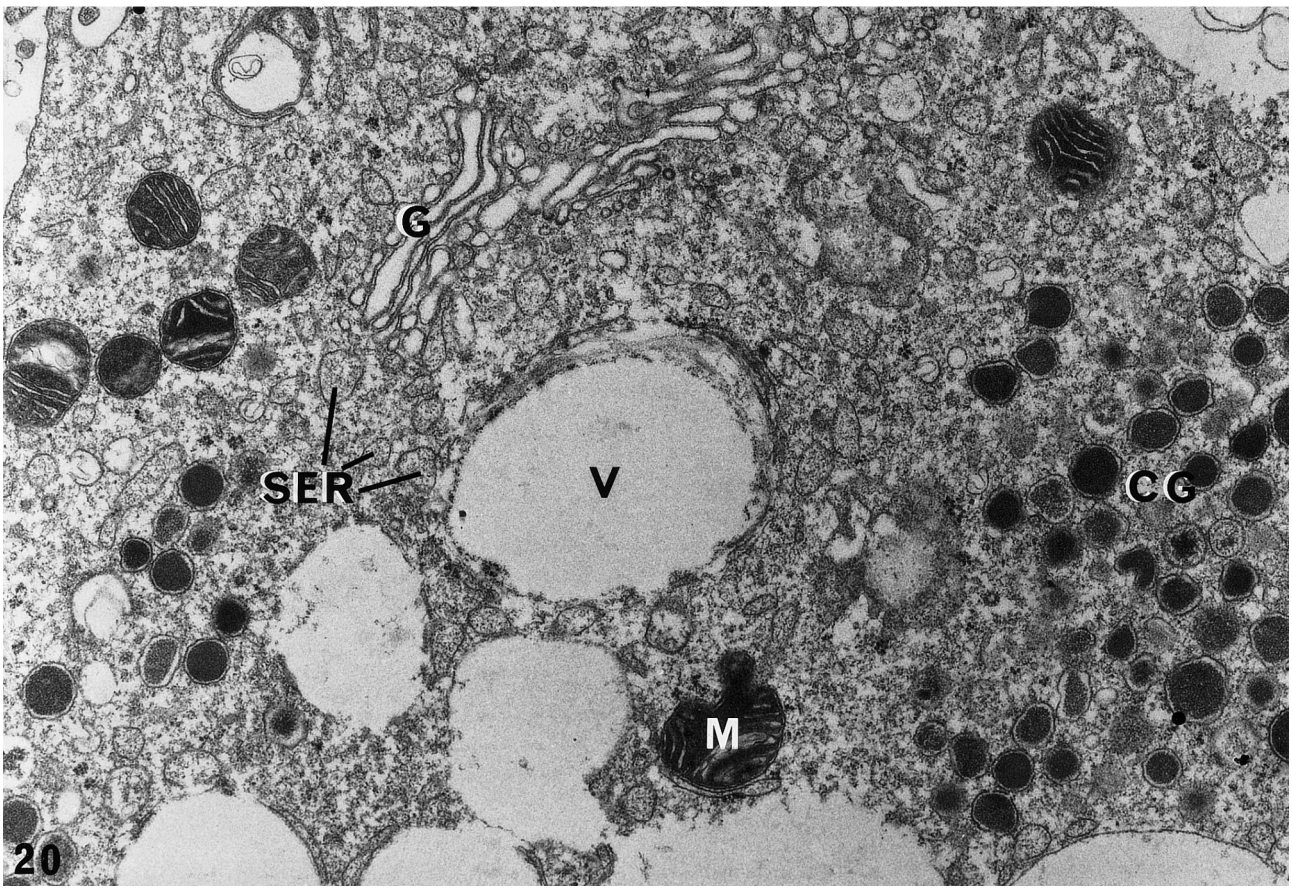


Fig. 18 Electron micrograph from an early antral follicle showing a large vesicle (*V*) surrounded by cortical granules (*CG*). ×32,000

Fig. 19 Electron micrograph from an early antral follicle showing a cortical granule (*CG*) in close proximity to a Golgi complex (*G*). ×39,000

Fig. 20 Electron micrograph from an oocyte >110 μm showing a cluster of cortical granules (*CG*); note the Golgi complex (*G*), mitochondria (*M*) and vesicles (*V*) in intimate association with smooth endoplasmic reticulum (*SER*). ×21,000

gion (Fig. 20) and arrays of microtubules were noted, in the ooplasm of most oocytes.

The increase in diameter of *oocytes 100–109 μm* was accompanied by perivitelline space (Fig. 17) formation in half of those examined. The development of the perivitelline space was associated with the liberation of the microvilli from the zona pellucida, resulting in bent microvilli lying parallel to the zona pellucida. Some oocytes, in the process of forming a perivitelline space, displayed both erect and bent microvilli. In approximately half of the oocytes the nucleus appeared to be peripherally located. Many of the oocytes also displayed peripherally located mitochondria. There was a further decrease in the numbers of elongated mitochondria and mitochondrial granules and an increase in the frequency of hooded mitochondria. In some oocytes the vesicles had increased in size. The free form of RER and SER, had become less extensive. There appeared to be an increase in the number of Golgi complexes present. Cortical granules were generally located in the peripheral area of the oocyte and the number of solitary granules had increased. Only a few oocytes showed arrays of microtubules.

The majority of *oocytes 110–119 μm* in diameter had completed perivitelline space formation and nuclear peripheral migration. The hooded form of mitochondria appeared to have been established as the predominant type and mitochondrial granules were scarce. There was an increase in the number and size of the vesicles and the number of Golgi complexes. Arrays of microtubules were no longer present.

All *oocytes ≥120 μm* displayed a perivitelline space. The mitochondria were either located at the periphery or evenly distributed. Mitochondrial granules had become very infrequent. The Golgi complexes and clusters of cortical granules were typically located at the oocyte periphery.

Discussion

The growth of the bovine primordial follicle to the preovulatory stage is manifested by the extensive proliferation and differentiation of the granulosa cells, antrum formation and an increase in oocyte diameter from approximately 30 to 120 μm. The factors which induce selection and initiation of the growth of some primordial follicles while others remain quiescent are still very much an enigma. The results from the present study and those of Rüsse (1983) indicate that the granulosa cells are the first to be activated. Their proliferation and restructuring are the first morphological features to be noted at primordial follicle activation in cattle.

We observed well-developed gap junctions between adjacent granulosa cells at all stages of follicular development. We were, however, unable to detect such junctions between the oocyte and granulosa cells until the formation of granulosa cell processes and the zona pellucida was initiated in the secondary follicles. On the other hand, in the primordial and primary follicles, the oolemma presented numerous coated pits and coated vesicles in the cortical ooplasm. Thus, it appears that granulosa-oocyte communication may be mediated through receptor-mediated endocytosis during the initial period of oocyte growth while it is redirected to a cell-to-cell coupling by gap junctions later. The granulosa-oocyte communication may be complex and serve both to maintain meiotic arrest and to stimulate oocyte growth. It could be speculated that the delayed development of gap junctional contact until during the secondary follicular stage may relate to the phenomenon that prior to this the oocyte is incompetent to resume meiosis and therefore the communication of signals for the induction of meiotic arrest is not required.

While maintaining the oocyte in meiotic arrest the follicle must also create the environment to allow oocyte growth and development. The cytoskeleton of the oocyte is believed to mediate several of the structural changes observed during oocyte growth, maturation and embryonic development. Structural proteins are required for the formation of this cytoskeleton. In the present study, tubulin, a structural protein, was observed in arrays of microtubules in the ooplasm during certain periods of oocyte growth. Similarly, the synthesis of actin (another structural protein) has been shown to constitute about 1% of total protein synthesis in the growing hamster oocyte (Racowsky 1985). In their review, Wassarman and Albertini (1994) list numerous proteins that are synthesized in rodent oocytes. Protein synthesis and post-translational modification are carried out by the RER and the Golgi complex, which according to the present study, appear to be plentiful during oocyte growth.

In the present study we observed that the mitochondria of the quiescent oocyte were predominantly round with longitudinal cristae. At the onset of oocyte growth, elongated mitochondria with numerous transverse cristae became more prevalent. Prior to the predominance of the hooded mitochondria, the round mitochondria resumed abundance for a period, suggesting that they may be a basal form from which all other forms arise. The establishment of the population of the hooded mitochondrial in oocytes >110 μm coincides with their development of competence to complete meiotic maturation (Fair et al. 1995).

At about the time when the secondary follicle is established the first traces of zona pellucida material were observed. In agreement with the findings of Rüsse (1983), we observed that the initial appearance of the zona pellucida coincides with the appearance of an increased number of microvilli on the oocyte surface and with the formation of granulosa cell process endings. The zona pellucida was formed in patches over the oocyte perimeter, and with the continuous production of material the oocyte became completely encased by the structure. The zona pellucida glycoproteins are synthesized in the RER and modified in the Golgi complex, where they are packed into vesicles that migrate to the oolemma for exocytosis (for review, Dunbar et al. 1994). The presence of extensive tubules of RER in the cortical

ooplasm and the enlargement, proliferation and peripheral migration of the Golgi complexes as noted in the present studies would appear to create the machinery for the production of zona pellucida material. However, actual exocytosis of zona pellucida material from the oocyte was not observed.

The observations of the present study appear to indicate that cortical granule formation, another essential task of the growing oocyte, is initiated in the secondary follicle and continues throughout oocyte growth. Although our observations do not clearly reveal the dynamics of cortical granule formation, numerous presumptive cortical granules were noted in close proximity to vesicular bodies, indicating that there may be more than one organelle involved in cortical granule formation. The progressive increase in vesicles, lipids, Golgi complexes and hooded mitochondria during oocyte growth and their peripheral migration most likely furnish the oocyte with the necessary equipment to carry out the range of protein synthesis (Sirard et al. 1989), post-translational modifications (Kastrop et al. 1991; Christmann et al. 1994) and extensive cytoplasmic reorganization required during meiosis and embryonic development.

In conclusion, the growth of the bovine oocyte from the primordial follicle to the fully grown stage involves the progressive increase in the numbers of mitochondria, Golgi complexes, lipid droplets and cytoplasmic vesicles, and a migration of these organelles to the oocyte periphery. Zona pellucida and cortical granule formation as well as gap-junctional coupling between the oocyte and the granulosa cells are initiated around the secondary follicular stage. During the later stages of oocyte growth (at an oocyte diameter >100 μm) the perivitelline space first appears, there is a decrease in the abundance of free SER and RER in the ooplasm, and the nucleus migrates from an eccentric to a peripheral location.

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