Surface Ultrastructural Characteristics of the Hamster Oocyte and Its Investments during In Vivo Maturation

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Abstract: *Hamster cumulus-oocyte complexes (COCs) were recovered by dissection of antral follicles of ovaries for the germinal vesicle (GV) or metaphase I (MI) stages, and by flushing the oviduct for the metaphase II (MII) stage. The surface morphological characteristics of the oocyte and its investments at each time point were evaluated by scanning electron microscopy (SEM). Oocyte diameter was also measured during the SEM analysis. The cumulus cells had a compact structure with minimal intercellular spaces among them in the GV-stage oocytes. They became greatly expanded with increased intercellular spaces at the MI and MII stages. The zona pellucida (ZP) had an open mesh-like structure, and the size and number of mesh holes increased at the MII stage. The vitelline surface of oocytes at the GV stage was characterized by relatively sparse distribution of microvilli (MV). After maturation, the MV increased in density. The diameter of oocytes decreased significantly during maturation from 82 to 77 μm ($P<0.01$). Thus the hamster oocyte maturation processes were found to involve the expansion of cumulus cells, an increase in the mesh holes of the ZP and development of MV on the vitelline membrane.*

Key words: *Hamster oocyte, Cumulus, Zona pellucida, SEM ultrastructure.*

Mammalian oocytes normally stay arrested at the early meiotic prophase during follicular development. This arrest can be released by liberation of oocytes from their follicular environment, known as spontaneous maturation [1–3]. During meiotic maturation of mammalian oocytes, several cytological changes take place, including the breakdown of germinal vesicles (GVBD), chromatin condensation, microtubule reorganization (meiotic spindle formation), redistribution of cellular organelles, membrane polarization and first polar body emission. The process of oocyte maturation also includes biochemical changes within the cytoplasm, of which the production of maturation promoting factor is a notable example [4].

The cumulus-oocyte complexes (COCs) are maintained by a delicate cell-to-cell communication among the cumulus cells and with the oocyte [5–7]. The cumulus cells are linked to each other and the innermost layers of the cumulus cells form cytoplasmic processes penetrating through the ZP and maintaining contact with the vitelline membrane of the oocyte [7–14]. The cumulus-corona cell mass may be the cell complex acting as regulators of oocyte activity via the transzona junctional processes [6, 7, 11, 15, 16].

Many studies employing the scanning electron microscope (SEM) have shown the changes in the ZP and the vitelline membrane in the course of the penetration and fusion by the spermatozoon during fertilization in several species (see review by Hyttel *et al.* [17]; Talbot [18]), including hamster [19, 20]. But the relationships between these changes and oocyte maturation have been only occasionally investigated in mice [19, 21, 22] and in man [23–25]. Our previous study revealed that the cumulus cells, the ZP and the vitelline membrane of the bovine oocytes which matured *in vitro* and *in vivo* undergo some changes, along with the nuclear and cytoplasmic maturation [26, 27].

The objective of this work was to evaluate by SEM the surface morphology of the cumulus cells, the ZP and the vitelline membranes of immature and *in vivo* matured hamster oocytes. Changes in the diameter of these oocytes were also recorded for comparison during SEM observations.

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Materials and Methods

Recovery of oocytes

Golden hamsters (*Mesocricetus auratus*) were kept under conditions of constant temperature (21–23°C) and a cycle of 14-h light (0500h–1900h) and 10-h dark. They were given laboratory Chow (MF, Oriental Yeast Co. Ltd., Tokyo, Japan) and water *ad libitum*. Adult female hamsters 10–12 weeks old were stimulated by intraperitoneal injection of 30 i.u. pregnant mare's serum gonadotropin (PMSG, Teikoku-Zoki Pharmaceuticals, Tokyo, Japan). To obtain germinal vesicle (GV) stage oocytes, the COCs were collected by puncturing antral follicles 51 h after PMSG administration. Parallel cytological examination showed that approximately 90% (n= 71) of these oocytes were at the GV stage. To obtain metaphase I (MI) and metaphase II (MII) stage oocytes, PMSG injected hamsters were given 30 i.u. hCG (Mochida Pharmaceuticals, Tokyo, Japan) at 58 h after PMSG injection. Females were sacrificed either 5 h (for MI stage) or 17 h (for MII stage) after hCG administration. The COCs were collected by puncturing antral follicles for MI stage, and from the ampullar region of the oviducts just after ovulation for MII stage. Parallel cytological examination showed that 93% (n=74) and 97% (n=69) of the oocytes recovered were at the MI and MII stages, respectively. The oocytes recovered were randomly assigned to 3 groups: cumulus-intact, ZP-intact and ZP-free oocytes. The cumulus cells were dispersed by exposing the COCs to medium containing 0.1% hyaluronidase (Sigma) for 2–5 min. ZP was removed by immersing in warmed, acidified phosphate buffered solution (PBS, pH=2.5) for 40–60 sec and 0.5% pronase for 1–3 min.

SEM observations

Oocytes with or without their investments in each stage were fixed for 1 h in 3% glutaraldehyde and 0.5% paraformaldehyde in Hanks' balanced salt solution with 0.1% polyvinyl alcohol (HBSS). They were washed in 3 changes of HBSS and placed on small glass coverslips (6 × 6 mm) coated with 0.1% poly-L-lysine solution (Sigma). The oocytes on the coverslips were postfixed in 1% osmium tetroxide in HBSS for 1 h. After rinsing, the samples were incubated in 2% tannic acid solution for 2 h, rinsed and then reosmicated for 1 h in 1% osmium tetroxide in distilled water. The specimens were then dehydrated in a series of increasing concentrations of ethanol, critical point dried, and sputter coated with gold. Observations were made with a JSM5300 scanning electron microscope (JEOL, Japan) at an accelerating voltage of 10–20 kV. The diameter of ZP-free oocytes was also measured during observation by SEM.

Results

Fine structure of cumulus cells

SEM features of COCs at the GV stage displayed a compacted arrangement of spherical cumulus cells (Fig. 1). The cumulus cells possessed occasionally thin, thread-like cell processes, making contact with neighboring cells. At this stage, the cumulus cells of almost all oocytes (89%, 62/70) were covered sparsely with very short MV, and the others (11%) were covered with long MV.

At the MI stage, the shape of the cumulus cells was still spherical, but the intercellular spaces among them were more obvious (Fig. 2). The cumulus cells of most oocytes (80%, 43/54) were covered with long MV and occasionally covered with an extra cellular matrix, a mucus-like substance. The remaining 20% of the oocytes had sparsely distributed MV on the surface of cumulus cells as observed at the GV stage.

At the MII stage, the intercellular spaces among the cumulus cells became much wider (Fig. 3). Some of the cumulus cells had become enlarged and were often covered entirely with a mucus-like substance.

Fine structure of zona pellucida

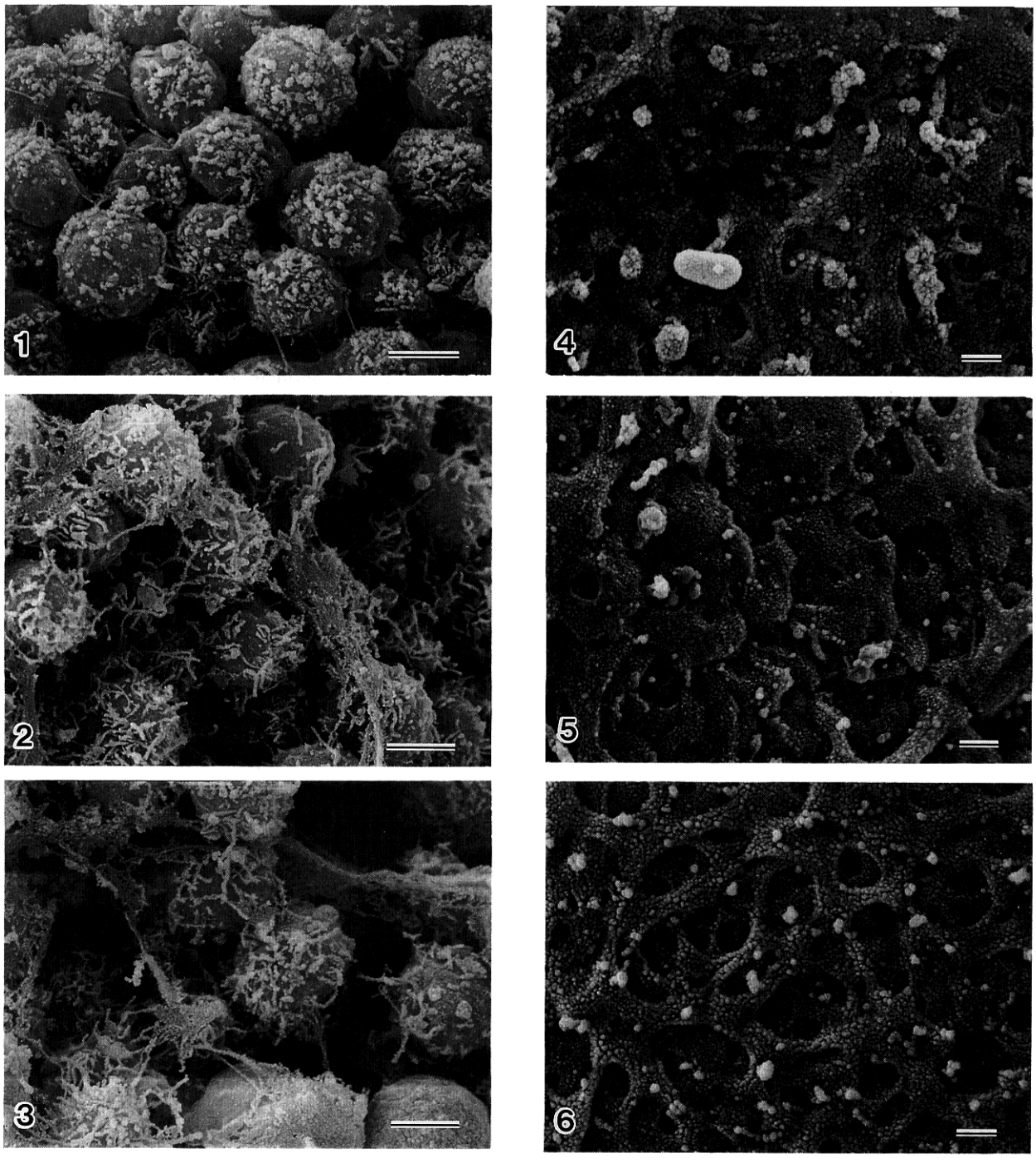
The ZP of the oocytes at the GV stage was characterized by a mesh-like structure with small openings (Fig. 4). Often large number of residuals of the cytoplasmic processes were found to be embedded in the meshes and holes of the ZP. They were probably the remaining

Characteristics of cumulus-oocyte complexes (COCs) during oocyte maturation.

Fig. 1. Surface features of a COC at the GV stage. The cumulus cells were spherical and on them very short MV were observed. Note an extensive network of thin cytoplasmic projections between the cumulus cells. Bar represents 5 μm.

Fig. 2. Surface features of a COC at the MI stage. MV have developed on the cumulus cells. Extra cellular matrix was frequently noted. Bar represents 5 μm.

Fig. 3. Surface features of a COC at the MII stage. The intercellular space among cumulus cells became wider than in the GV stage. Bar represents 5 μm.

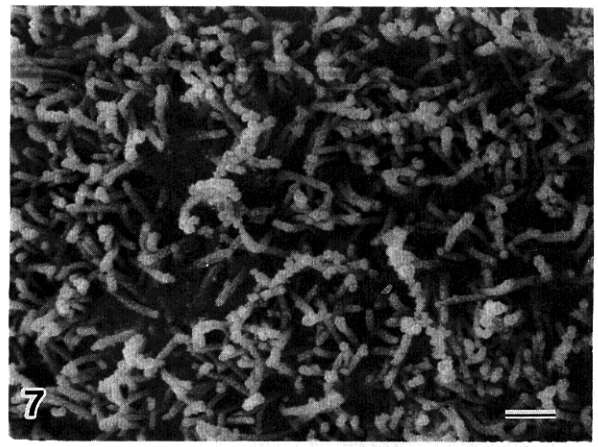


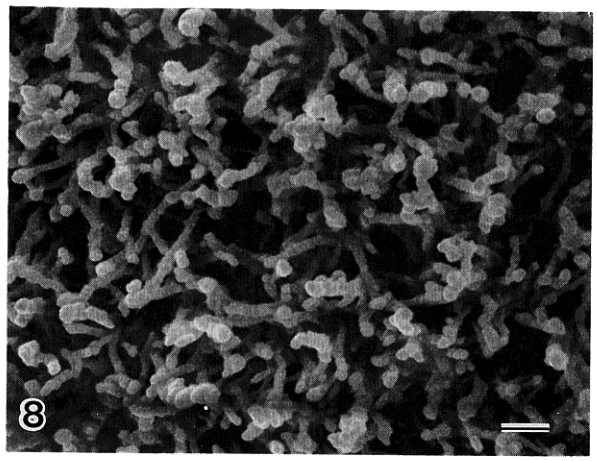
Characteristics of zona pellucida (ZP) during oocyte maturation.

Fig. 4. ZP surface of a GV-stage oocyte showing a mesh-like structure with small openings. The surface was relatively flat. Bar represents 1 μm.

Fig. 5. ZP surface of a MI-stage oocyte showing an increased number of mesh holes. The surface became undulating. Bar represents 1 μm.

Fig. 6. ZP surface of a MII-stage oocyte was characterized by the fibrous mesh structure. The mesh holes increased in number and in size. Bar represents 1 μm.





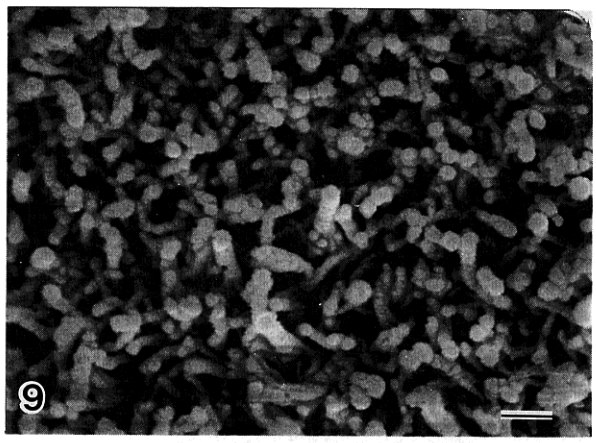


Table 1. Average diameters of hamster oocytes during maturation

Stage	n	Average ± SE (μm)	Range (μm)
GV	41	82.3 ± 3.5[a]	70.0–98.3
MI	38	76.9 ± 1.6[b]	69.0–86.0
MII	42	76.5 ± 2.0[b]	68.7–86.7

Means with different superscripts are different (P<0.01).

parts of the cumulus and corona-radiata cell processes, which normally extend through the ZP into the oocyte.

At the MI stage, the mesh holes of the ZP became larger, and the ZP surface became undulating (Fig. 5). The ZP of the mature oocytes at the MII stage was characterized by a fibrous network, with relatively wide meshes (Fig. 6). The mesh structure of the ZP appeared to diverge, showing a fine network of zona outer surface from the MI to the MII stage. The fine fibrous structure had been snapped in various places, resulting in the wider meshes on the ZP surface after maturation.

Fine structure of vitelline membrane

In 91% (62/68) of oocytes at the GV stage, the vitelline surface was sparsely covered with MV (Fig. 7), but the remaining 9% of oocytes were densely covered with MV. At the MI stage, the majority of the oocytes (94%, 49/52) were densely covered with well-developed MV (Fig. 8). The vitelline surface of the mature oocyte at the MII stage was characterized by dense distribution of MV (Fig. 9). Short, sprouting MV were often found among well-developed MV at this stage.

Size of oocytes

The analysis of variance showed significant differences among the GV, MI and MII oocyte groups (Table1, P<0.01). The diameter of the GV-stage oocytes was 82.3 ± 3.5 μm (mean ± SE), which decreased to 76.9 ± 1.6 μm at the MI stage and then 76.5 ± 2.0 μm at the MII stage.

Characteristics of vitelline membrane during oocyte maturation.

Fig. 7. Vitelline surface of a GV-stage oocyte covered with a sparse distribution of MV. Bar represents 1 μm.
Fig. 8. Vitelline surface of a MI-stage oocyte showing development of MV-predominant surface. Bar represents 1 μm.
Fig. 9. Vitelline surface of a MII-stage oocyte covered with a dense population of MV. Note 'sprouting' MV among long MV. Bar represents 1 μm.

Discussion

Surface characteristics of the cumulus cells and the zona pellucida

Our results on the cumulus cells demonstrated that surface morphology, intercellular spaces, and cell to cell communications changed dramatically during *in vivo* oocyte maturation in the hamster. Changes in the surface morphology of individual cumulus cells during maturation observed here are in agreement with reports on the mouse [28, 29] and man [23, 25]. In our previous SEM reports on cattle, the full expansion of cumulus cells was observed after oocyte maturation *in vivo* and *in vitro* [26, 27]. It is known that gonadotropic hormones induce expansion of cumulus cells in mice [16], pigs [30] and cattle [8, 31].

Our observations on changes in the ZP during maturation suggest a change in the maturation of the ZP fine structure. These observations were in agreement with a recent study on the mouse in which a correlation between the morphology of the ZP surface and the degree of oocyte maturity was reported [21]. Phillips and Shalgi [19] have examined the mouse and hamster ZP by SEM and showed that their ZP had numerous fenestrations, resulting in a sponge-like appearance and that no morphological changes were found in the ZP during fertilization, development of zygote or early cleavage, but the changes during maturation were not examined in their report. The present study clearly showed the dramatic changes in the ZP of hamster oocytes during maturation.

Surface characteristics of the vitelline membrane

The present observations on the vitelline membrane clearly showed that the hamster oocyte was characterized by a sparse distribution of MV at the GV stage and that the density of distribution appeared to increase during maturation. The surface characteristics of the vitelline membrane of the matured hamster oocyte are in agreement with the observations by Phillips and Shalgi [19]. Surface characteristics of the immature oocytes seemed to differ from species to species. Our previous reports showed that tongue-shaped cytoplasmic protrusions were frequently noted in immature bovine oocytes [26, 27] and slender, long MV of various density, from relatively dense to sparse distributions were observed in immature porcine oocytes (Suzuki, Jeong and Yang, unpublished observations). But the vitelline membranes of the matured oocytes were characterized by a well-developed, MV predominant surface in several species - cattle [26, 27], pigs (Suzuki, Jeong and Yang, unpublished observations), mice [19, 22, 28], rats [32], hamsters [19 and the present study] and in man [33].

Ultrastructural and immunocytochemical studies have shown that the cumulus cell processes pass through the ZP with gap junctions contacting the surface of the ooplasm [7–14]. These projections maintain the biochemical communication between the oocyte and cumulus cells [6, 7, 11, 15, 16].

It has been demonstrated that uncoupling between the bovine oocyte and cumulus cell projections occurred at 12 to18 h of culture where all junctional contact was disrupted [12, 34]. In addition, changes in locations of cytoplasmic organelles, such as mitochondria and some vesicles, have been reported to occur a few hours after the final loss of gap junctions *in vivo* [13, 35] and *in vitro* [12, 34]. Such organelle migrations may be linked with the loss of intercellular communication between the cumulus cells and the oocyte and the cytoskeletal changes in the cytoplasmic processes [8, 12].

Volumetric changes in oocytes

In the present study, the size of oocytes changed significantly during maturation, as determined by measurements of fixed oocytes during SEM observations. The formation and enlargement of the perivitelline space after oocyte maturation have been previously reported [13, 36–38]. The present results, together with others, showed a decrease in the size of the oocyte after maturation. This decrease in the size of the matured oocytes was probably due to physiological contractions in the cytoplasm of the oocyte rather than the release of the polar body. The increase in the size of the perivitelline space (decrease in the size of the cytoplasm) may aid in disconnecting the junctions between the oocyte and cumulus cell cytoplasmic projections under physiological conditions, but the biological significance of such a volumeric change in the ooplasm during maturation remains to be determined.

General discussion and conclusions

Full expansion of the cumulus cells, establishment of extensive mesh networks of the ZP surface and the development of the MV-predominant vitelline surface were obvious during hamster oocyte maturation. It has been hypothesized that the fact that wide intercellular spaces among the cumulus cells and many mesh openings in the ZP appear only after maturation is of evolutionary significance and should assist spermatozoa in becoming appropriately oriented to fertilize the oocyte. The MV-predominant surface pattern in ma-

tured oocytes may be a prerequisite for sperm penetration and fertilization, because actual contribution of the MV to gamete membrane fusion has been observed in the hamster [19, 20] and other species [36]. Uniform distribution of MV on the vitelline membrane was observed during early development of the embryos in the hamster [39] and cattle [40]. Recent studies have shown that the cytoskeleton, including microfilaments in the cytoplasm may play an important role during oocyte maturation [41, 42] and that the microfilaments are the basic structures of the cytoplasmic membrane [43]. Therefore further studies are needed to clarify the relationships between the changes in the surface morphology of the vitelline membrane and the cytoskeleton reorganization.

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