Granulosa cell-germ cell relationship in the developing rabbit ovary

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Granulosa cells play an important part in the nutrition of the oocyte during oogenesis (Raven, 1961). Anderson & Beams (1960) suggested that, in the primary follicle, nutritive material synthesized by the granulosa cells reaches the oocyte by diffusion across the zona pellucida. In the rabbit, follicle formation and elaboration of the zona pellucida begin at the end of the second week after birth (Gondos & Zamboni, 1967). Prior to this time germ cells and granulosa cells are clustered together within the ovarian cortex. Although participation of the granulosa cells in zona pellucida formation has been described in detail (Merker, 1961), the function of granulosa cells in the earlier stages of oogenesis is unclear. The following is an electron microscope study of the relationship between granulosa cells and germ cells in the prefollicular rabbit ovary.

MATERIAL AND METHODS

Ovaries were removed from New Zealand white rabbits, 1-15 days of age, and processed for electron microscope examination. Fixation was accomplished by one of the following techniques: (1) mincing of tissue fragments and immersion in 1% OsO₄ with salts added (Zetterqvist, 1956); (2) vascular perfusion with 2-5% glutaraldehyde in cacodylate buffer (Sabatini, Bensch & Barnett, 1963) and postfixation in osmic acid. All specimens were dehydrated in graded alcohols, embedded in Epon 812 and sectioned with an LKB ultramicrotome. For orientation purposes, sections 0.5-1 μ in thickness were stained with toluidine blue and examined by light microscopy. Ultrathin sections of suitable areas were stained with lead citrate and studied with a Hitachi HU-11 C electron microscope. Of the two fixation methods described, direct osmium immersion produced more satisfactory sections for light microscopy, while initial vascular perfusion resulted in superior preservation of cellular ultrastructure. Accordingly, the electron micrographs reproduced here are all prepared from sections of perfused ovaries.

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RESULTS

At the junctions between granulosa cells and germ cells, cell membranes are closely apposed (Fig. 1A, B). The width of the intercellular space measures 250–300 Å. Adjacent cell membranes follow the contour of one another in such a manner as to maintain an almost constant intercellular distance. This relationship is slightly altered by a widening of the intercellular space in areas where three cells come together. Granulosa cells and oogonia in division are closely related by tight application of adjacent cell membranes (Fig. 1C).

Surface contact between germ cells and granulosa cells is enhanced by the presence of interdigitating cytoplasmic projections arising from neighboring cells. These projections are shown at low magnification in Fig. 1A and at higher magnification in Fig. 2A and B. Extensive intermingling of projections often makes it difficult to determine which is the cell of origin. Fig. 2A shows projections arising from a granulosa cell, while Fig. 2B shows origin from a germ cell. The projections are long and slender, resembling microvilli in their shape, but with a less regular arrangement. They are folded and flattened to adapt to the narrow intercellular space. The long axis of a projection generally lies parallel to adjacent cell membranes. The projections are filled with ribonucleoprotein particles (ribosomes), the cytoplasm of each projection blending in with that of its cell body. Desmosome-like attachments between oocytes and granulosa cells are rarely seen in the prefollicular stage of development. However, condensations of adjacent cell membranes resembling desmosomes occasionally appear between interdigitating cytoplasmic projections.

Extending around the germ cells are prominent elongations of the granulosa cells. These elongations intervene between adjacent oocytes and between oocytes and basement membranes of the cortical clusters (Fig. 1B). Tips of granulosa cells extending in this manner make contact with one another to envelop individual oocytes in the process of follicle formation. The close relationship between granulosa cell extensions and adjacent oocytes is indicated by the direct apposition of cell membranes and the presence of numerous cytoplasmic projections (Fig. 1B).

**Figure 1**

Scale line represents 0.5 μ throughout

(A) Closely apposed germ cells (o) and granulosa cell (g) in ovarian cortex. Granulosa cell is distinguished by its dense cytoplasm and irregular nucleus. Cytoplasmic projections line granulosa cell-germ cell junction. Boundary between germ cells is regular except for intercellular bridge, at arrow.

(B) Granulosa cell elongation extends over surface of germ cell. Close intercellular relationship is maintained.

(C) Section shows dividing oogonium, with chromosomes (ch) oriented on mitotic spindle, and granulosa cell (nucleus at right). Cell membranes are directly applied to one another.
Invaginations of oocyte cell membranes by cytoplasmic projections of granulosa cells are frequently seen. One such invagination producing contact between granulosa cell membrane and oocyte nucleus is shown in Fig. 2C. Contact is facilitated by the peripheral location of the oocyte nucleus. The relationship thus established provides an extremely close association between cytoplasm of the granulosa cell and nuclear contents of the oocyte. The presence of numerous vesicles in the oocyte cytoplasm suggests the possibility of pinocytic exchange between the adjacent cells. The granulosa cell also shows abundant vesicle formation and a prominent Golgi complex next to the nucleus. In Fig. 2D, a deep, narrow indentation of the oocyte membrane is produced by a granulosa cell projection directed toward the oocyte nucleus. In this section contact is not demonstrated, but the distal portion of the projection approaches the border of the nucleus. A similar indentation is shown in Fig. 2E. In this case the cytoplasmic projection is sectioned obliquely producing the appearance of an inclusion in the oocyte cytoplasm.

Direct cytoplasmic continuity between germ cells and granulosa cells was not found. Intercellular bridges could frequently be seen between germ cells (see Fig. 1A), but extensive search for bridges communicating with granulosa cells yielded negative results.

**DISCUSSION**

The fine structural relationship between germ cells and granulosa cells in the developing rabbit ovary is similar to that described in the rat (Franchi, 1960), guinea-pig (Anderson & Beams, 1960), mouse (Odor & Blandau, 1969), hamster (Weakley, 1966) and human (Stegner & Wartenberg, 1963; Lanzavecchia & Mangioni, 1964). The close apposition of cell membranes of adjacent germ cells and granulosa cells and the presence of interdigitating cytoplasmic projections seem to be consistent features of mammalian oogenesis. The granulosa cell projections are similar to those seen in mature follicles, but in the latter the projections are more elongated, extending across the zona pellucida to make

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**FIGURE 2**

(A) Cell junction showing cytoplasmic projections of granulosa cell (g) extending along surface of germ cell (o).

(B) Projections arising from cytoplasm of germ cell (o) make contact with cell membrane of granulosa cell (g).

(C) Section shows deep invagination of germ cell membrane by cytoplasm of granulosa cell (nucleus at right). Arrow indicates point of contact between outer nuclear membrane of germ cell and cell membrane. Numerous large vesicles are seen in germ cell cytoplasm. Smaller vesicles are present in granulosa cell cytoplasm.

(D) Arrow indicates long, narrow granulosa cell projection directed toward germ cell nucleus, part of which is shown in lower right corner.

(E) Obliquely sectioned granulosa cell projection (arrow) simulating intracytoplasmic inclusion.
contact with microvilli on the oocyte surface (Baca & Zamboni, 1967). The function of cytoplasmic projections in the adult follicle has been considered to be the exchange of material between granulosa cell and oocyte (Hertig & Adams, 1967). It seems likely that a similar function applies in the developing ovary.

Granulosa cell projections extending deep into the cytoplasm of oocytes have been described in hamster (Weakley, 1966) and human (Baca & Zamboni, 1967) follicles, but were not seen in fetal and early postnatal mouse ovaries (Odor & Blandau, 1969). Extension of such projections to the point of nuclear contact was observed in the present study. An extremely close relationship was thus demonstrated between granulosa cells and germ cells at a time when germ cells are known to undergo active DNA synthesis (Peters, Levy & Crone, 1965). These findings suggest that the granulosa cells in the prefollicular rabbit ovary may have a role in the regulation of oocyte maturation. Flickinger & Fawcett (1967) proposed that Sertoli cells in the mouse testis have a combined function of germ-cell nutrition and co-ordination of cytological events during spermatogenesis. The close relationship between granulosa cells and germ cells during oogenesis has similarities to the relationship between Sertoli cells and testicular germ cells during spermatogenesis (Nicander, 1967). Evidence to indicate that Sertoli cells in the fetal guinea-pig may be capable of steroid biosynthesis was presented by Black & Christensen (1969). Since Sertoli cells are considered to be the male homologues of the granulosa cells (Witschi, 1951), it is of interest that granulosa cells in the neonatal rabbit exhibit a complex cytoplasmic organization suggesting capacity for synthetic and secretory activity (Gondos, 1969). During the period of follicular growth, granulosa cells have been observed to undergo rapid cell multiplication and actively synthesize protein (Björkman, 1962).

Oocytes and nurse cells in the developing ovaries of Drosophila and other invertebrates are connected by intercellular bridges (King, 1964). These bridges allow flow of nutrients from one cell to another during oocyte maturation (King & Mills, 1962). Although Kemp (1958) stated that he found intercellular connexions between germ cells and granulosa cells in developing mammalian ovaries, Franchi (1960) was unable to confirm these findings in the rat. Trujillo-Cenóz & Sotelo (1959) found no evidence of cytoplasmic continuity between projections of granulosa cells and oocytes in mature follicles of the rabbit, nor was any such continuity found in the prefollicular rabbit ovaries of the present study. The discrepancy between findings in invertebrates and vertebrates is not surprising. In Drosophila, 15 nurse cells and a single oocyte arise from a stem-line oogonium by four consecutive incomplete divisions, leaving the 16 cells interconnected (Brown & King, 1964). In all vertebrates that have been investigated, the supporting granulosa cells arise within the gonad, while primordial germ cells originate extragonadally (Franchi, Mandl & Zuckerman, 1962). Incomplete divisions of oogonia can give rise to interconnected germ cells in the ovaries of mammals (Gondos & Zamboni, 1969), but there is no evidence to suggest that granulosa cells can be formed in this manner.
SUMMARY
1. Ovaries of New Zealand white rabbits, 1–15 days of age, were studied with the electron microscope.
2. The relationship between granulosa cells and germ cells was characterized by close apposition of adjacent cell membranes, the presence of numerous interdigitating cytoplasmic projections and extension of granulosa cell projections deep into the cytoplasm of germ cells.
3. These observations indicate that, in addition to their role in germ cell nutrition and zona pellucida formation, granulosa cells of the developing ovary may have a direct influence on the maturation of germ cells.
4. Intercellular bridges, such as those connecting developing germ cells, were not found between germ cells and granulosa cells.

RÉSUMÉ
Les rapports entre les cellules germinales et la granulosa pendant le développement de l’ovaire de lapin
2. Les rapports entre les cellules de la granulosa et les cellules germinales sont caractérisés par une intime juxtaposition des membranes cellulaires adjacentes, par la présence de nombreux prolongements cytoplasmiques digités et la pénétration profonde des prolongements cellulaires de la granulosa dans le cytoplasme des cellules germinales.
3. Ces observations indiquent que, en plus de leur rôle dans la nutrition des cellules germinales et dans la formation de la zone pellucide, les cellules de la granulosa de l’ovaire en développement peuvent avoir une influence directe sur la maturation des cellules germinales.
4. Des ponts intercellulaires analogues à ceux qui établissent des connexions entre les cellules germinales, n’ont pas été observés entre les cellules germinales et les cellules de la granulosa.

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REFERENCES


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