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Maturation of the Rat Cumulus-Oocyte Complex: Structure and Function

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ABSTRACT The cumulus cells that surround the mammalian oocyte become dispersed following the preovulatory surge of the pituitary gonadotropin, luteinizing hormone (LH). We have examined cumulus-oocyte complexes of PMSG-primed immature rats before and at 1, 2, 3, 4, 6, and 8 hr after injection of human chorionic gonadotropin (hCG), which acts on the rat ovary like the pituitary gonadotropin. Associations between projections of the cumulus cells and the oocyte were analyzed in thin sections. We observed that some cumulus projections were greatly enlarged where they associate with the oocyte. These enlarged regions were filled with numerous small vesicles. Gap junctions between cumulus cell projections and the oocytes were small. We quantitated the number and size of gap junctions between cumulus cells. The number of small gap junctions (<1 μM) between cumulus cells did not change significantly over the 8-hr period after hCG administration. Larger gap junctions, however, showed a general downward trend beginning after the third hour post hCG. Light microscopic observations of plastic sections revealed that dispersion of the cumulus oophorus is not observed until after 4 hr post-hCG, but between 4 and 8 hr after gonadotropin administration the cumulus becomes markedly dispersed. In the majority of the oocytes in these complexes the germinal vesicle (GV) displayed some irregularity in shape at 2 hr post-hCG, although absence of the GV was not observed until later.

Our observations suggest a new means of communication in the cumulus-oocyte complex by the vesicle-filled enlargements of the cumulus cell projections at the oocyte surface. They further indicate that the decrease in metabolic coupling observed in rat cumulus-oocyte complexes soon after exposure to LH is not associated with a change in number and size of the gap junctions between the cumulus cells. We suggest that it is either the disruption of the gap junctions at the region of contact of the cumulus cell projections with the oocyte surface or the operation of a gating mechanism that blocks the junctional channels without affecting their morphological appearance that is responsible for uncoupling of the oocyte from the cumulus cells.

Key Words: Gap junctions, Germinal vesicle, Oocyte maturation

INTRODUCTION

The cells of the innermost layer of the cumulus oophorus, the corona radiata, are associated with the oocyte by long cytoplasmic projections that traverse the zona pellucida and intermingle with numerous microvilli from the oocyte surface (Paladino, 1890; Yamada et al., 1957; Sotelo and Porter, 1959; Anderson and Beams, 1960; Odor, 1960; Tardini et al., 1960; Björkman, 1962; Zamboni, 1974; Dekel et al., 1976, 1978; Albertini, 1984). Gap junctions present in the regions of contact between the cumulus cell projections and the oolemma (Szöllösi, 1975; Amsterdam et al., 1976; Anderson and Albertini, 1976; Gilula et al., 1978; Larsen et al., 1987) provide the means for metabolic coupling in the cumulus-oocyte complex (Cross, 1973; Wassarman and Letourneau, 1976; Moor et al., 1980; Heller and Canellakis, 1981; Eppig, 1982; Brower and Schultz, 1982; Colonna and Mangia, 1983; Racowsky and Satterle, 1985; Racowsky and Baldwin, 1989). Interconnection by means of gap junctions have also been described between adjacent cumulus as well as granulosa cells (Björkman, 1962; Merck et al., 1972; Anderson and Albertini, 1976; Zamboni, 1974; Szöllösi, 1975, Gilula et al., 1978; Fletcher, 1979). These specific structures have been observed between closely opposed cell membranes and at the regions of contact of microvilli and cell membranes.

The extensive network of communication established between the somatic cellular components of the follicle and the female gamete is probably of major functional importance. A likely physiological role for this type of heterologous cell communication is nutritional; follicle cells directly provide nutrients that are necessary for oocyte growth (Eppig, 1979; Heller and Canellakis, 1981; Brower and Schultz, 1982). Junctional communication within the follicle could also mediate transmission of regulatory signals that are responsible for control of the meiotic status of the follicular oocyte (Sherizly et al., 1988; Racowsky, 1984; Racowsky and Baldwin, 1989).

In female mammals meiosis of germ cells is initiated before parturition. At birth, all the oocytes are arrested at the dictyotene stage of the meiotic prophase and it is

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not until after puberty that the preovulatory LH surge stimulates oocytes to resume meiosis. In these oocytes the nuclear membranes break down and the chromosomes form a metaphase plate. These changes are often referred to as oocyte maturation. The large meiotic prophase nucleus is referred to as the germinal vesicle (GV) and the dissolution of the meiotic nucleus as germinal vesicle breakdown (GVB). Oocyte maturation can also be induced by exogenous administration of hCG. Both these gonadotropins, LH and hCG, uncouple the cumulus-oocyte complex (Gilula et al., 1978; Dekel et al., 1981; Racowsky and Baldwin, 1989; Dekel, 1986; Sherizly et al., 1988).

In 1935 Pincus and Enzmann initially reported that oocytes resume meiosis spontaneously upon their removal from the ovarian follicle. These investigators proposed that the somatic cells of the follicle produce a factor that maintains the oocyte in meiotic arrest. Later studies provided further evidence in support of the idea that the association between the oocyte and the follicular cells plays a central role in the regulation of oocyte maturation (Dekel, 1986). We have used microscopic analysis of semithin and thin sections to further explore the nature of the associations between the cumulus cells, as well as between cumulus cells and the oocyte, and to examine the response of the rat oocyte and its vestments to hCG.

MATERIALS AND METHODS

Microscopy

Twenty-six day old Wistar-derived female rats were injected subcutaneously with 15 IU of pregnant mare serum gonadotropin (Intervet International B.V., Boxmeer, Holland). Forty-eight to fifty-two hr later animals were either killed or injected intraperitoneally with 4 IU of human chorionic gonadotropin (hCG) (Ikapharm, Israel). Animals were killed by cervical dislocation before or at 1, 2, 3, 4, 6, and 8 hr after hCG injection and their ovaries removed and placed in Leibovitz's L-15 medium (GIBCO, Grand Island, NY). The large follicles were ruptured and the cumulus-oocyte complexes immediately placed in 3% glutaraldehyde in 0.2 M phosphate buffer at pH 7.4. All follicles were removed within 10 min of death of the animal. After fixation for up to 1 week cumulus-oocyte complexes were rinsed in phosphate buffer, postfixed in 1% O_sO_4 in 0.1 M phosphate buffer dehydrated in alcohol to propylene oxide and embedded in EPON 812. One micron sections were stained with 1% toluidine blue in 1% sodium borohydrate. Sections were photographed with a Zeiss Photomicroscope II using a 1.4 NA planapo objective lens. Thin sections were cut on a Reichart OmU3 ultramicrotome and stained in 3% aqueous uranyl acetate at 45°C for 1 hr. Sections were examined and photographed with a Philips 300 microscope.

Quantitation of Junctions Between Cumulus Cells

Our methods are based on those used by White (1984) and Coons and Espey (1977). Sections of each cumulus-oocyte complex were first examined in the electron microscope. Complexes with many cumulus cells containing numerous lysosomes, focal cytoplasmic degradation, or pycnotic nuclei were regarded as degenerating and were not examined further. Gap junctions were quantitated directly from the TEM viewing screen. To do this a grid (G200HS Ted Pella, Inc.) was viewed at very low magnification (SC with objective aperture removed) and a section was selected. Fifty cells in which the nucleus had been sectioned were examined and scored. In a few of the sections there were less than fifty cumulus cells with nuclei in the section. In these cases parallel sections were used. To quantitate junctions, sections were viewed at a magnification of 4,700. Cells were scored only if they were transected through the nucleus and no portion of the cell was over a grid bar. The cell was viewed through the 10× binocular microscope and the number of gap junctions were counted. The size of the junctions was determined as being small (less than 1 μ), medium (1–2 μ) and large (larger than 2 μ) by comparison to a drawing. We quantitated junctions for six cumuli before hCG injection and six at each time period of 1, 2, 3, 4, 6, and 8 hr after hCG injection.

RESULTS

Light Microscopy

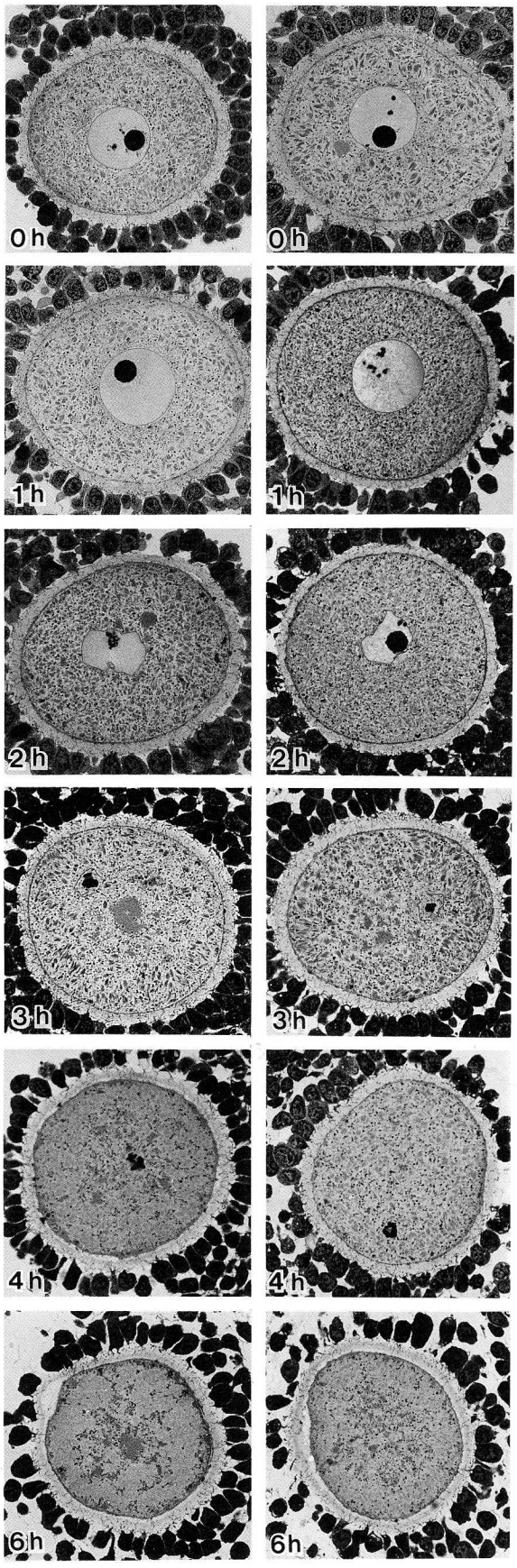
The time of germinal vesicle breakdown is generally determined by quantitating the percentage of oocytes without germinal vesicles. Because of the increased resolution which one can obtain with semithin sections as compared to viewing whole oocytes, we examined 1 μ-thick toluidine blue stained sections in an attempt to detect early stages of germinal vesicle breakdown.

At 0 and 1 hr, the germinal vesicles were large and circular (Fig. 1). However, at 2 hr most oocytes contained nuclei with irregular shapes (Fig. 1). By 3 hr only remnants of the germinal vesicles were observed in most oocytes, and metaphase chromosomes were observed by 4 hr (Fig. 1). Although no obvious changes in cumulus cells were observed at up to 4 hr, by 6 hr the cumulus cells were found to be considerably dispersed (Fig. 1).

Quantitation of Gap Junctions

Two days after PMSG injection the oocyte with its large germinal vesicle is surrounded by a cumulus composed of closely associated cells. The rat cumulus does not have a distinct corona radiata as is present in the human and rabbit cumulus (Figs. 2 and 3). Even in low-magnification micrographs gap junctions are clearly visible between neighboring cumulus cells (Fig. 3). At high magnification these junctions display the typical ultrastructure of gap junctions (Figs. 4 and 5). Similar gap junctions have previously been reported in the cumuli oophori of other mammalian species (Björkman, 1962; Zamboni, 1974; Anderson and Albertini, 1976; Szöllösi, 1975; Gilula et al., 1978).

The number of junctions per cell was quantitated as described in the Methods section. These data are pre-



sented in Table 1 and summarized in Table 2. The data were analyzed by analysis of variance using time as the grouping factor. When justified by the results of ANOVA, multiple pair-wise comparisons were made and significance levels adjusted for post hoc comparisons. There was no significant difference in the number of small (> 1 μm), medium (1–3 μm), or large (> 3 μm) junctions per cumulus cell over the first 2 hr. Thereafter, there was a general downward trend for the medium and large junctions with significant decreases at 6 ($P < 0.01$) and 8 hr ($P < 0.001$) for medium size junctions, 3, 4 (P, 0.01) and at 6 and 8 hr ($P < 0.001$) for large junctions when compared to their respective t=0 groups. In contrast, for the small junctions (< 1 μm) there were no significant changes at any time, although there was a trend toward lower values in the number of total junctions with time beginning at 4 hr; this was significant only at 8 hr ($P < 0.001$).

Electron Microscopy

The breakdown of the germinal vesicle is a very elaborate process. In the light microscope most germinal vesicles observed at 2 hr post-hCG were irregular in shape. In the electron microscope these nuclei were observed to have complex shapes (Fig. 2). Similar irregularly shaped nuclei have been reported in other mammalian species during GV breakdown (Szöllösi et al., 1972, 1978; Hyttel et al., 1987) and first cleavage (Longo and Anderson, 1969).

Cumulus-Oocyte Associations

In mammals long microvilli extend from the cumulus cells that surround the surface of the zona pellucida. These microvilli pass through the zona pellucida and form gap junctions where they associate with the oocyte surface. This has been observed in ovarian oocytes of various sizes in several mammalian species (Anderson and Albertini, 1976; Szöllösi, 1975). Gilula et al. (1978) examined cumulus cell association in only the small nonpreovulatory follicles of rats after PMSG and hCG. They also observed numerous gap junctions between cumulus cells and oocytes. We examined thin sections of cumulus-oocyte complexes isolated from large central follicles 0 to 4 hr after hCG treatment. We chose to examine large complexes since it is these that will mature and ovulate in response to the gonadotropin surge.

In thin section the associations appear complex and varied. Along most of the surface oocyte microvilli are small and evenly distributed. However, we observe areas where numerous microvilli protrude from the surface. Often these microvilli interdigitate with cu-

Fig. 1. Photomicrographs of representative oocytes at the time of hCG injection and 1, 2, 3, 4, and 6 hr after injection of hCG. Although the GV is intact at 2 hr post-hCG the irregular shape indicates that GVB has been initiated. Such oocytes might be considered to have intact GVs when viewed in whole oocytes. × 350.

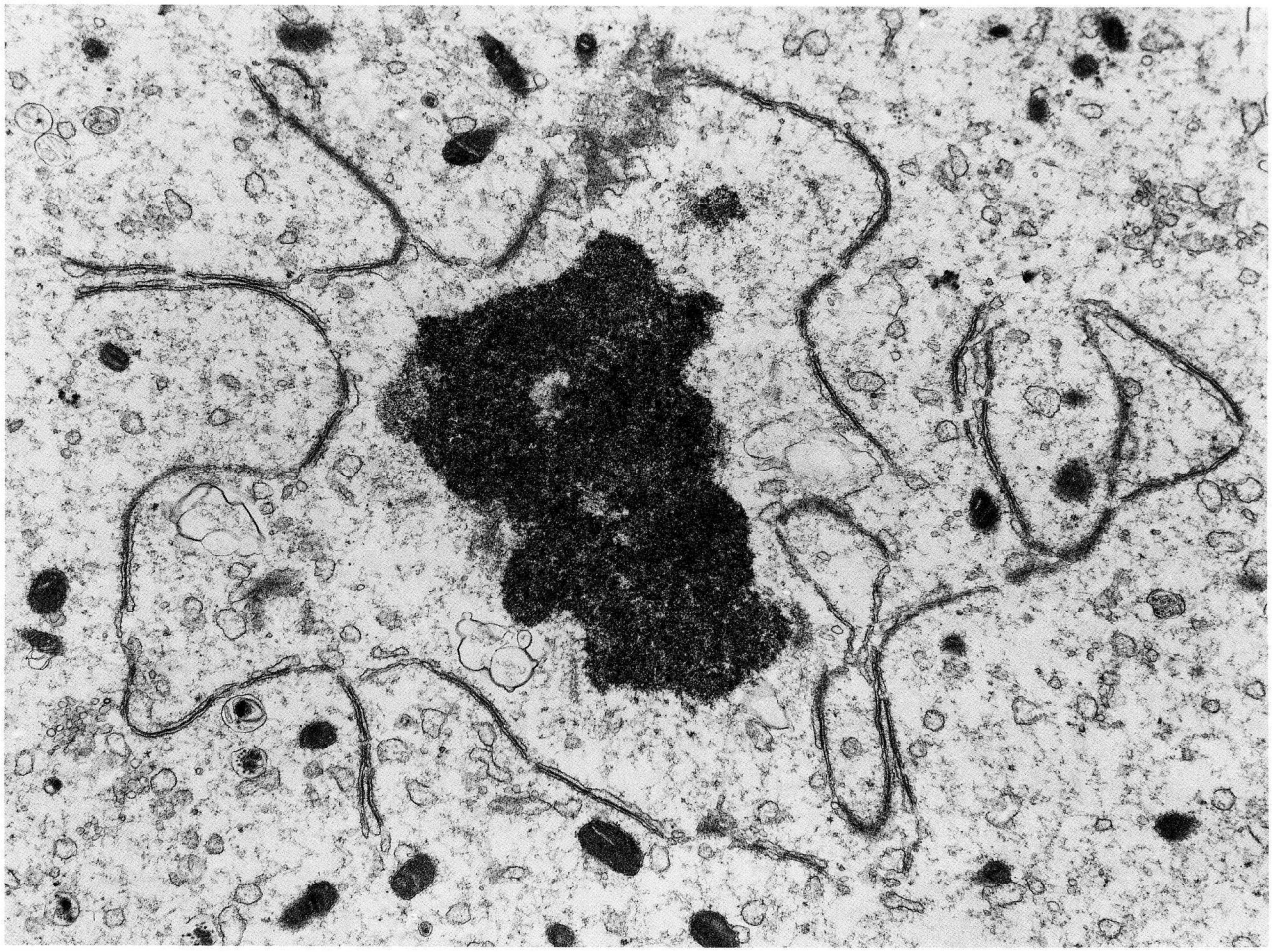


Fig. 2. GVB is a lengthy process during which the GVs become crenulated into complex shapes. × 19,000.

mulus cell microvilli (Figs. 6 and 7). Associations between cumulus cell microvilli and the oocytes are far less prevalent than have been reported in oocytes isolated from small follicles (Anderson and Albertini, 1976; Gilula et al., 1978). Very small gap junctions are sometimes observed where cumulus cell microvilli associate with the oocyte surface (Figs. 7 and 8). Some cumulus cell microvilli are unusual in that they are very enlarged where they associate with the oolemma. The cytoplasm of these microvilli is characterized by numerous vesicles. The vesicles have a diameter of 0.1 to 0.2 μ (Figs. 8 and 9).

DISCUSSION

The present theories for regulation of oocyte maturation suggest that its association with the cumulus cells is essential for maintenance of meiotic arrest. The follicular fluid oocyte maturation inhibitor (OMI) will not affect oocytes in the absence of cumulus cells (Dekel et al., 1979), and the supply of the inhibitory cAMP is also dependent on the integrity of the oocyte-cumulus/granulosa compartment (Dekel, 1986; Sherizly et al., 1988; Racowsky and Baldwin, 1989). Motivated by these ideas, we studied in detail the interactions of the oocyte with the cumulus cells. We found that the cumulus cell projections form different types of associations with the oolemma. Some projections extend for a long distance along the oolemma while others associate with tufts of oocyte microvilli. Occasionally small gap junctions are observed between the cumulus cell projections and the oolemma. Still other projections have vesicle-filled, expanded ends at the oocyte surface. Vesicles are known to transmit signaling molecules at neural junctions and they may have similar functions in the cumulus-oocyte complex. Furthermore, it could be possible that the different morphological types of associations between cumulus cells and the oocytes serve different functions.

According to the model proposed by our studies (Dekel, 1986), which has been supported by Racowsky

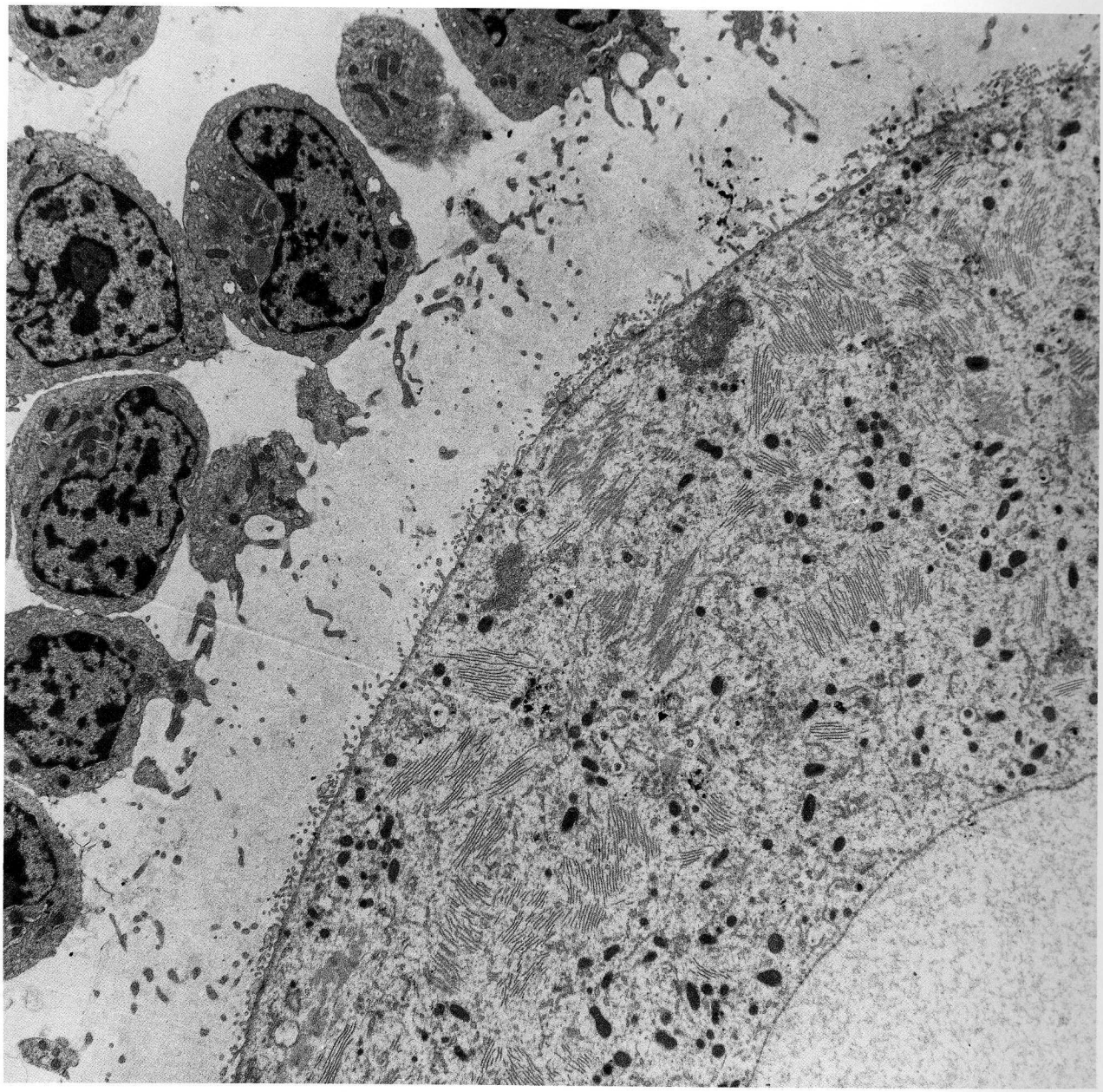
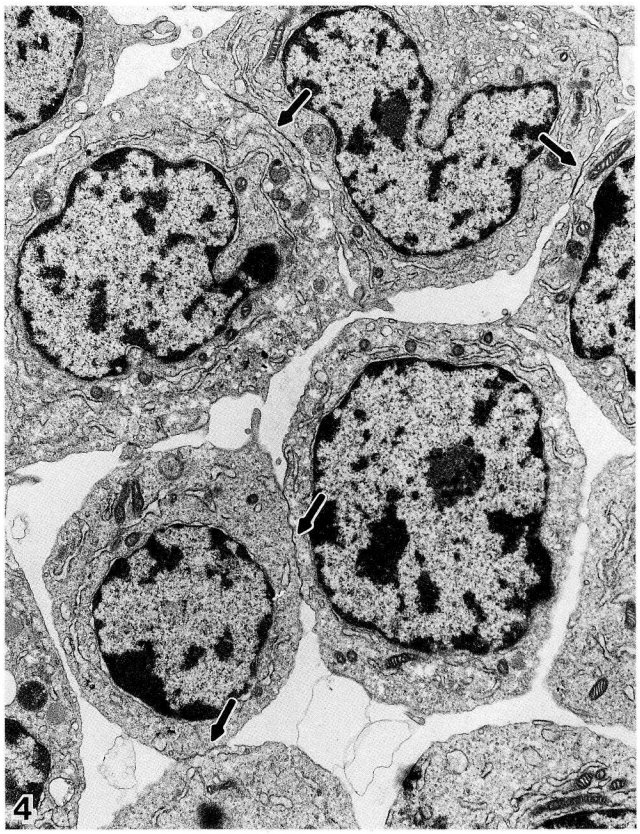


Fig. 3. Micrograph showing a portion of a GV (lower right) in a rat oocyte 48 hr after injection of PMSG. The zona pellucida surrounding the oocyte is typically polarized such that the surface facing away from the oocyte is more irregular in shape than the surface adjacent to the perivitteline space. Oocytes are characterized by short microvilli of similar lengths. Long microvilli are occasionally observed in the zona pellucida and associated with the surface of the oolemma. × 3,500.



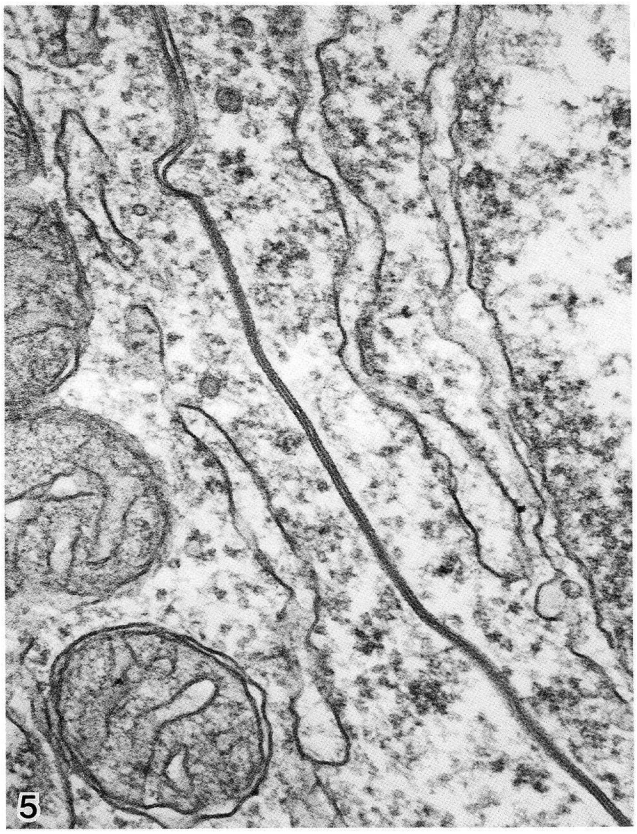


Fig. 4. A region of the cumulus oophorus (2 hr after injection of hCG). Numerous gap junctions (arrows) are observed between neighboring cells of the cumulus oophorus. × 5,000.
Fig. 5. Gap junction between two cumulus cells. × 82,000.

and Baldwin (1989) in their recent report, it is the alternation between established communication and uncoupling in the cumulus-oocyte system which controls the meiotic status of the oocyte. Essential to the causal relationship between the breakdown of communication and the reinitiation of meiosis is the temporal relationship between these events.

Morphological analysis by light microscopy for the presence or absence of the GV is commonly used to assess the meiotic status of the oocyte. Using light microscopy in a previous study with the same strain of rats, we have reported that GVB is observed in 50% of the oocytes at 3.2 hr after injection of hCG (Dekel et al., 1979). Using thin sections in the present study, we have observed that it is at 2 hr after injection of hCG that most GVs display irregular shapes. Thus, the nuclear changes in the oocyte appear to take place before the actual disappearance of the GV. At the time we observed no change in the number or size of the cumulus cell gap junctions. We did observe a gradual reduction in the number of large gap junctions at 3 hr. Larsen et al. (1986) found a decrease in the total gap junctional area of the rat cumulus that occurs at about the same time (between 2 and 3 hr after hCG administration). Furthermore, in a more recent study these investigators (Larsen et al., 1987) have demonstrated that the heterologous gap junctions between the oocyte surface and the cumulus cell projections are disrupted even after exposure to the hormone. Taken together, these studies seem to contradict our previous findings that metabolic coupling in rat cumulus-oocyte complexes significantly drops soon after hCG administration unless gap junctions can lose these functions while maintaining normal morphology. Lowenstein has presented evidence for a hormonally regulated gating mechanism, possibly operated by phosphorylation-dephosphorylation of the gap junction proteins (Lowenstein, 1985). If this is the case, even if morphological examination reveals that gap junctions do not disappear soon after hormonal stimulation, it is possible that metabolic coupling in this system has been interrupted. We conclude, therefore, that the structural demonstration of gap junctions may not necessarily mean that communication has been maintained.

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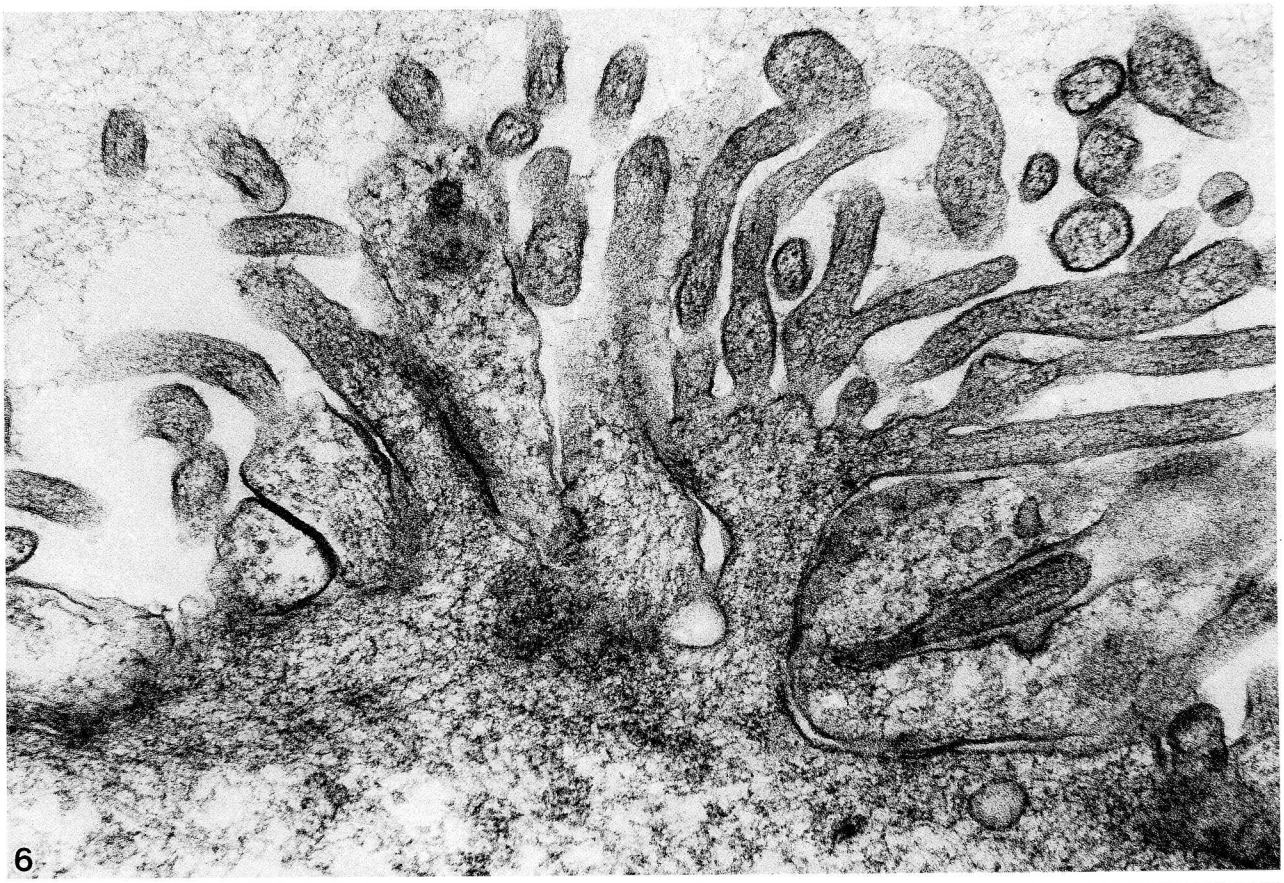
TABLE 1. Average Number of Small, Medium and Large Gap Junctions Per Cumulus Cell as Measured in Thin Sections

	Rat number	Total	<1μ	1-3 μ	>3 μ	GV
0 hr	1	0.96	0.28	0.46	0.12	Present
	2	1.14	0.48	0.44	0.22	Present
	3	1.42	0.74	0.54	0.14	Present
	4	1.08	0.18	0.74	0.16	Present
	5	1.54	0.72	0.64	0.18	Not observed
	6	1.52	0.66	0.52	0.26	Present
1 hr	7	1.52	0.62	0.84	0.06	Present
	8	1.22	0.70	0.40	0.12	Present
	9	1.70	0.80	0.62	0.28	Present
	10	1.12	0.28	0.66	0.18	Present
	11	1.66	0.88	0.58	0.20	Not observed
	12	1.30	0.58	0.70	0.02	Present
2 hr	13	0.96	0.42	0.40	0.14	Present
	14	0.66	0.44	0.16	0.06	Irregular
	15	1.58	0.66	0.68	0.24	Irregular
	16	1.24	0.62	0.40	0.22	Not observed
	17	1.14	0.26	0.70	0.18	Present
	18	1.34	0.58	0.56	0.20	Irregular
3 hr	19	1.28	0.64	0.58	0.06	Irregular
	20	1.02	0.40	0.52	0.10	Irregular
	21	1.38	0.66	0.56	0.16	Not observed
	22	1.20	0.74	0.42	0.04	Not observed
	23	1.20	0.62	0.36	0.12	Irregular
	24	1.18	0.70	0.46	0.02	Present
4 hr	25	0.78	0.34	0.44	0.00	Not observed
	26	0.68	0.18	0.42	0.08	Not observed
	27	1.10	0.62	0.36	0.12	Irregular
	28	1.10	0.62	0.36	0.12	Not observed
	29	0.56	0.44	0.12	0.00	Not observed
	30	1.10	0.80	0.28	0.02	Irregular
6 hr	31	1.26	1.02	0.20	0.04	Not observed
	32	0.80	0.48	0.28	0.04	Not observed
	33	0.54	0.46	0.08	0.00	Not observed
	34	1.24	0.74	0.50	0.00	Not observed
	35	0.86	0.80	0.06	0.00	Not observed
	36	0.44	0.32	0.12	0.00	Not observed
8 hr	37	0.22	0.20	0.02	0.00	Not observed
	38	0.50	0.38	0.12	0.00	Not observed
	39	0.40	0.34	0.06	0.00	Not observed
	40	0.34	0.22	0.08	0.04	Not observed
	41	0.44	0.32	0.12	0.00	Not observed
	42	0.60	0.40	0.16	0.04	Not observed

TABLE 2. Summary of Data Presented in Table 1

Time after hCG (hr)	Total	<1 μ	1-3 μ	>3 μ
0	1.27 ± 0.09	0.51 ± 0.04	0.55 ± 0.04	0.18 ± 0.02
1	1.42 ± 0.09	0.64 ± 0.07	0.63 ± 0.05	0.14 ± 0.03
2	1.15 ± 0.12	0.49 ± 0.05	0.48 ± 0.07	0.17 ± 0.02
3	1.19 ± 0.05	0.62 ± 0.04	0.48 ± 0.03	0.08 ± 0.02
4	0.88 ± 0.09	0.49 ± 0.08	0.34 ± 0.05	0.04 ± 0.02
6	0.85 ± 0.13	0.63 ± 0.10	0.20 ± 0.06	0.01 ± 0.007
8	0.45 ± 0.05	0.31 ± 0.03	0.09 ± 0.02	0.01 ± 0.007



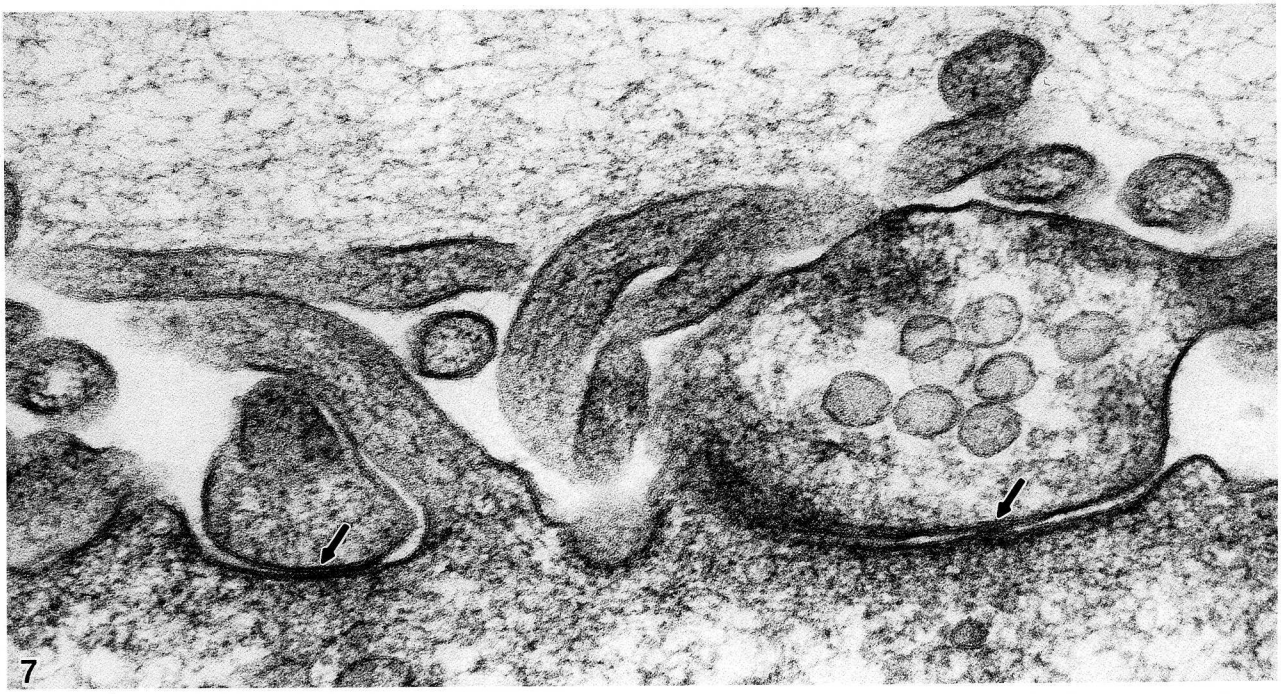
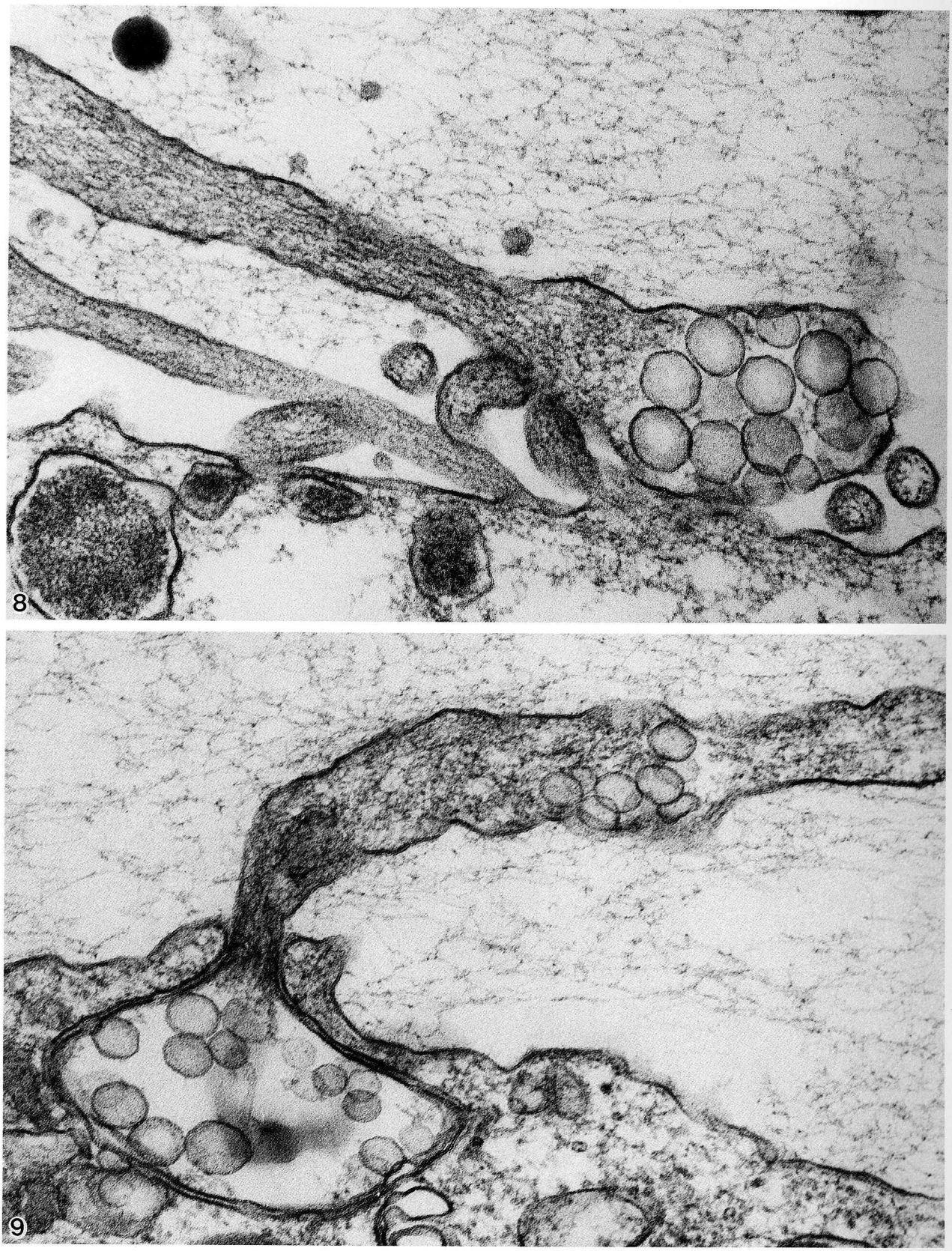


Fig. 6. Tufts of oocyte microvilli are frequently observed on the oocyte surface. Cumulus and oocyte microvilli interdigitate in these regions. × 83,000.

Fig. 7. Small gap junctions are sometimes observed where cumulus cell microvilli associate with the oolemma (arrows). × 126,000.



Figs. 8 and 9. Many cumulus cell microvilli have enlarged tips containing vesicles. Such enlarged ends are either associated with the cell membrane of the oocyte (Fig. 8) or protrude into the oocyte (Fig. 9). × 96,000.

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REFERENCES

Albertini DF (1984): Novel morphological approaches for the study of oocyte maturation. Biol Repord 30:13–28.

Amsterdam A, Josephs R, Lieberman ME, Lindner HR (1976): Organization of intermembrane particles in freeze-cleaved gap junctions of rat Graafian folicles: Optical diffraction analysis. J Cell Sci 21:93–105.

Anderson E, Beams HW (1960): Cytologic observations on the fine structure of the guinea pig ovary with special reference to the oogonium, primary oocyte and associated follicle cells. J Ultrastruct Res 3:432–446.

Anderson E, Albertini PF (1976): Gap junctions between the oocyte and the companion follicle cells in the mammalian ovary. J Cell Biol 71:680–686.

Björkman N (1962): A study of the ultrastructure of the granulosa cells of the rat ovary. Acta Anat 51:125–147.

Brower PT, Schultz RM (1982): Intercellular communication between granulosa cells and mouse oocytes: Existence and possible nutritional role during oocyte growth. Dev Biol 90:144–153.

Colonna R, Mangia F (1983): Mechanism of amino acid uptake in cumulus-enclosed mouse oocytes. Biol Reprod 28:797–803.

Coons LW, Espey LL (1977): Quantitation of nexus junctions in the granulosa cell layer of rabbit ovarian follicles during ovulation. J Cell Biol 74:321–325.

Cross PC (1973): Role of cumulus cells and serum in mouse oocyte maturation in vitro. J Reprod Fertil 34:241–245.

Dekel N, Dabush S, Messer Y (1976): Effect of LH on the ultrastructure of rat cumulus cells. Proc 6th Europ Congr Electron Microscopy, pp 595–597.

Dekel N, Kraicer PF, Phillips DM, Ramon S, Segal SJ (1978): Cellular association in the rat oocyte-cumulus cell complex: Morphology and ovulatory changes. Gamete Res 1:47–57.

Dekel N, Hillensjö T, Kraicer PF (1979): Maturational effects of gonadotropins on the cumulus-oocyte complex of the rat. Biol Reprod 20:191–197.

Dekel N, Lawrence TS, Gilula NB, Beers WH (1981): Modulation of cell to cell communication in the cumulus-oocyte complex and the regulation of oocyte maturation by LH. Dev Biol 86:356–362.

Dekel N (1986): Hormonal control of ovulation. In G. Litwack (eds.): "Biochemical Actions of Hormones," Vol. 13. New York: Academic Press, pp 57–83.

Eppig JJ (1979): A comparison between oocyte growth in co-culture with granulosa cells and oocytes with granulosa cell-oocyte junctional contact maintained in vitro. J Exp Zool 209:345–353.

Eppig JJ (1982): The relationship between cumulus cell-oocyte coupling, oocyte meiotic maturation and cumulus expansion. Dev Biol 89:268–272.

Fletcher WH (1979): Intercellular junctions in ovarian follicles: A possible functional role in follicle development. In W.A. Sadler (eds): "Ovarian Follicular Development and Function." New York: Raven Press, pp 113–120.

Gilula NB, Epstein MC, Beers WH (1978): Cell-to-cell communication and ovulation: A study of the cumulus-oocyte complex. J Cell Biol 78:58–75.

Heller JS, Canellakis ES (1981): Cellular control of ornithine decarboxylase activity by its antizme. J Cell Physiol 107:209–217.

Hyttel P, Xu KP, Smith S, Callesen H, Greve T (1987): Ultrastructure of the final nuclear maturation of bovine oocyte in vitro. Anat Embryol 176:35–40.

Larsen WJ, Wert SE, Brunner GD (1986): A dramatic loss of cumulus cell gap junctions is correlated with germinal vesicle breakdown in rat oocytes. Dev Biol 113:517–521.

Larsen WJ, Wert SE, Brunner GD (1987): Differential modulation of follicle cell gap junction populations at ovulation. Dev Biol 122:61–71.

Longo FJ, Anderson E (1969): Cytological events leading to the formation of the two-cell embryo. J Ultrastr Res 29:86–118.

Lowenstein WR (1985): Regulation of cell-to-cell communication by phosphorylation. Biochem Soc Symp 50:43–58.

Merck FB, Botticelli CR, Albrioght JJ (1972): An intercellular response to estrogen by granulosa cells in the rat ovary, an electron microscope study. Endocrinology 90:992–1007.

Moor RM, Smith MW, Dawson RMC (1980): Measurement of intercellular coupling between oocytes and cumulus cells using intracellular markers. Exp Cell Res 126:15–29.

Odor LD (1960): Electron microscopic studies on ovarian oocytes and unfertilized tubal ova in the rat. J Biophys Biochem Cytol 7:567–574.

Paladino G (1980): Il ponte intercellulare tra l'uovo ovarico e la cellula follicolare e la formazion della zona pellucida. Anat Anz 15:254–259.

Pincus G, Enzmann EV (1935): The comparative behavior of mammalian eggs in vivo and in vitro. I. The activation of ovarian eggs. J Exp Med 62:665–675.

Racowsky C (1984): Effect of forskolin on the spontaneous maturation and cyclic AMP content of rat oocyte-cumulus complexes. J Reprod Fertil 72:107–116.

Racowsky C, Satterle RA (1985): Metabolic fluorescent dye and electrical coupling between hamster oocytes and cumulus cells during meiotic maturation in vivo and in vitro. Dev Biol 108:191–202.

Racowsky C, Baldwin KW (1989): In vitro and in vivo studies reveal that hamster oocyte meiotic arrest is maintained only transiently by follicular fluid but persistently by membrane/cumulus granulosa cell contact. Dev Biol 134:297–306.

Sherizly I, Galiani D, Dekel N (1988): Regulation of oocyte maturation: Communication in the rat cumulus-oocyte complex. Hum Reprod 3:761–766.

Sotelo JR, Porter KR (1959): An electron microscope study of the rat ovum. J Biophys Biochem Cytol 5:327–341.

Szöllösi D, Calarco P, Donahue RP (1972): Absence of centrioles in the first and second meiotic spindles of mouse oocytes. J Cell Sci 2:251–541.

Szöllösa D (1975): Ultrastructural aspects of oocyte maturation and fertilization in mammals. In C. Thibault (eds): "La fecondation." Paris: Masson et Cie, pp 13–35.

Szöllösi J, Falkay GY, Sas M (1978): Mesterolone treatment of patients with pathospermia. Int Urol Nephrol 10:251–256.

Tardini A, Vitali-Mozza L, Manzani FE (1960): Ultrastrutture dell'ovocitoumano maturo. 1. Rapporti fra cellule dela corona radiata, pellucida a ed ovoplasma. Arch De Vecch Anat Path 33:281–305.

Wassarman PM, Letourneau GE (1976): RNA synthesis in fully grown mouse ooctyes. Nature 261:73–74.

White FH, Thompson DA, Gohari K (1984): Ultrastructural morphometry of gap junctions during differentiation of stratified squamous epithelium. J Cell Sci 69:67–85.

Yamada E, Muta T, Motamura A, Kaga H (1957): The fine structure of the oocyte in the mouse ovary studied with electron-microscope. Kurune Med J 4:148–153.

Zamboni L (1974): Fine morphology of the follicle wall and follicle cell-oocyte association. Biol Reprod 10:125–149.