GAP JUNCTIONS BETWEEN THE OOCYTE AND COMPANION FOLLICLE CELLS IN THE MAMMALIAN OVARY

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The physical basis for communication between animal cells is thought largely to depend on the presence of gap junctions which are wide-spread among various vertebrate and invertebrate tissues (14, 20). Studies on cultured cell lines have indicated that, in addition to mediating electrical impulse propagation between neighboring cells (electrotonic coupling), gap junctions mediate certain metabolic aspects of cellular function by permitting the transcellular passage of "informational" cytoplasmic molecules between physically conjoined cells (metabolic cooperation) (10, 15, 23). Moreover, the morphological demonstration that gap junctions conjoin different cell types within tissues such as the vertebrate retina (19), an arachnid midgut (16), and the mammalian kidney (18) establishes a precedent for gap junction-mediated cooperation between distinct cell types in other tissues.

Recent physiological findings (3, 13) suggest that metabolic cooperativity between follicular-granulosa cells and oocytes within developing mammalian ovarian follicles may importantly regulate both the meiotic maturation of the egg and the transformation of the follicular epithelium into the corpus luteum. As an extension of our studies into the nature of coordinative cellular interactions in the ovarian follicle (2), we report here that gap junctions conjoin oocytes to companion follicular cells.

MATERIALS AND METHODS

Thin Section and Tracer Analysis

Ovaries from adult cycling female mice, rats, and rhesus monkeys (*Macaca mulatta*), from 1- and 5–6-day old rats and from estrous rabbits were fixed for freeze-fracture studies by immersion for 20 min at room temperature in 0.1 M cacodylate buffer (pH 7.4) containing 2% paraformaldehyde, 3% glutaraldehyde, and 5% sucrose. Thin-section and tracer samples were fixed for a total of 60 min. Final fixation in all cases was carried out on individual follicles which had been dissected free from surrounding stromal tissue (with the exception of the 5–6-day-old rat ovaries) and cut into 1-mm cubes. Better fixation of rabbit follicles from animals in estrous was achieved by omitting the paraformaldehyde from the primary fixative. Ionic lanthanum was used as an extracellular tracer as previously described (2). After several washes in buffer, the tissues were postfixed in 1% OsO_4 in 0.1 M cacodylate buffer at room temperature for 1 h, washed, dehydrated in a graded series of ethanol, and embedded in a mixture of Epon-Araldite (5). Thin sections were cut on a diamond knife, collected on 300-mesh copper grids, and examined in either a Philips 200 or 300 electron microscope, both unstained and after staining with uranyl acetate (25) and lead (21).

Freeze-Fracture Analysis

After primary fixation for 20 min, cubes of tissue were washed thoroughly, equilibrated for 90 min in 20% glycerol in 0.1 M cacodylate buffer, frozen on paper disks in liquid Freon 22, and stored in liquid nitrogen. A

Balzers apparatus (Balzers High Vacuum Corp. Santa Ana, Calif.) was used for freeze-fracturing and shadowing, which for the present study was operated at a stage temperature of −115°C and a vacuum pressure of 10^{-6} torr. Replicas were cleaned in Clorox, washed in distilled water, and mounted on 300-mesh copper grids before viewing in the electron microscope. All micrographs are mounted with the direction of shadowing from bottom to top. The terminology for complementary fracture faces suggested by Branton et al. (8) will be used in this paper.

RESULTS

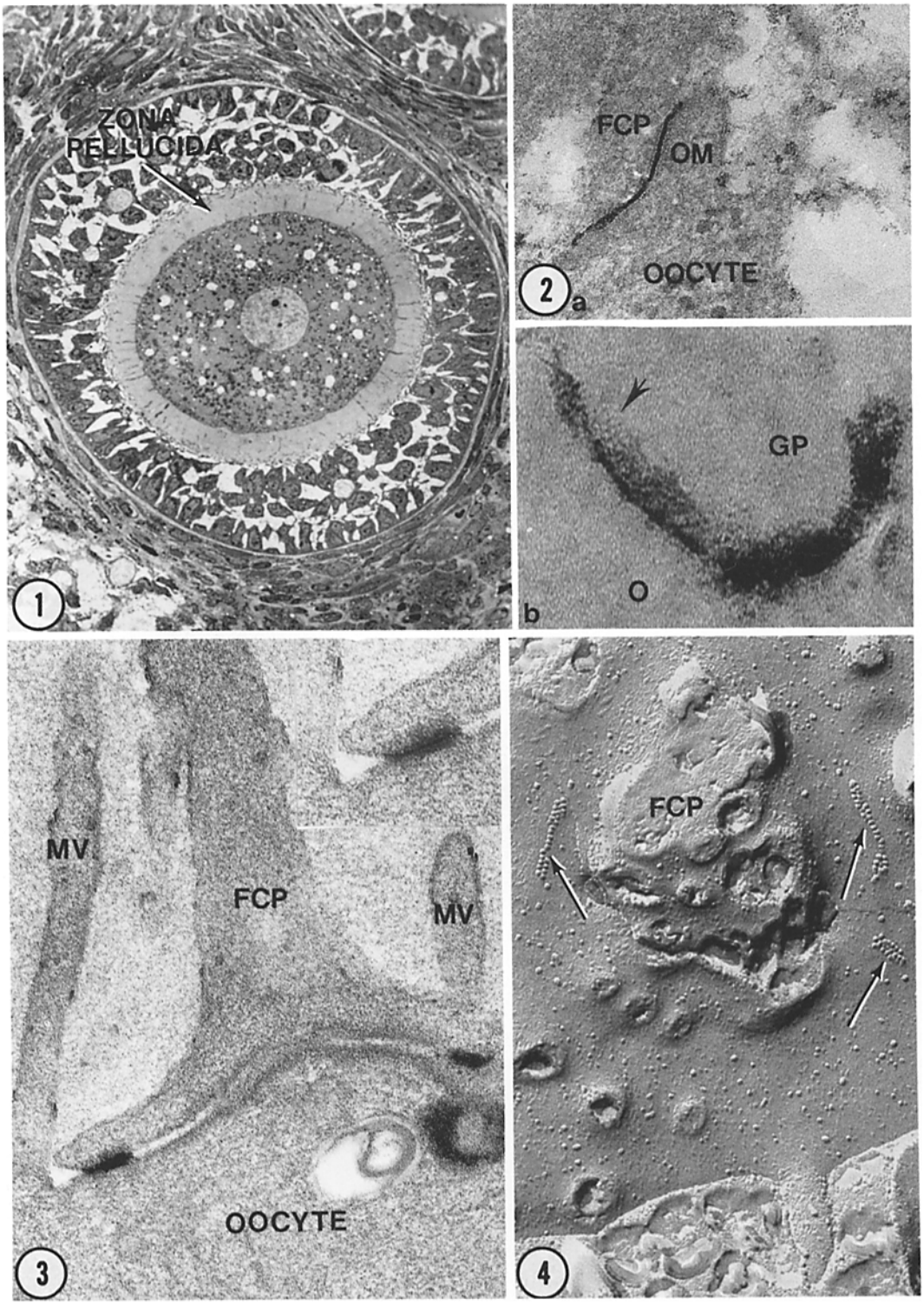
During preantral development of mammalian follicles from most species investigated thus far, the oocyte acquires an elaborate complex of surface microvilli which interdigitate at irregular intervals with variously shaped cytoplasmic processes from adjacent follicle cells. The follicle cell extensions traverse the zona pellucida (Fig. 1) and come to interact with the morphologically specialized oolemma in several ways depending on the species (Figs. 2*a*, 3). Immature rat ovaries (1- and 5–6-day-old) contain numerous primary follicles which, before the formation of the zona pellucida, reveal multiple focal contacts between blunt follicle cell projections and microvilli and nonmicrovillar aspects of the oolemma (Fig. 2*a*). Granulosa cell processes infrequently establish gap junctions with oocyte microvilli. More commonly, two types of gap junctional contacts are observed between follicle cell processes and the oolemma: (*a*) punctate contact of close membrane apposition and (*b*) macular contact. In both cases, the membranes of adjoining cells are separated by a space of 4–5 nm as seen in lanthanum-impregnated material (Figs. 2*a*, 3). *En face* views of this region of apposition reveal hexagonal lattice subunits outlined by the extracellular tracer lanthanum (Fig. 2*b*; arrow). Single granulosa cell processes often bifurcate and establish multiple points of junctional contact with the oolemma in a fashion reminiscent of synaptic *boutons* (Fig. 3). Desmosome-like connections coexist with gap junctions and have frequently been recognized between follicle cells and oocytes (2, 4).

Freeze-fracture images of the junctional associations described between oocytes and follicle cell extensions have consistently showed either single or multiple particulate aggregates on the P-face (Figs. 4, 5, 8) and complementary pitted differentiations on the E-face (Fig. 7). The P-face aggregates usually consist of 10–60 intramembrane particles arranged in single or multiple rows which rarely show lateral registering. Ovoid gap junctions, plaques or maculae are also found (Fig. 6). The particles comprising such aggregates at the oocyte surface are conspicuously smaller than nonjunctional particles, showing an average diameter of 7–8 nm: the constituent junctional particles are closely packed and display a packing periodicity of 8–9 nm. Surrounding the junctional aggregates are areas devoid of particles which are situated between tufts of microvilli. The junctional aggregates tend to occur in clusters which can be seen at points where the follicle cell processes impinge on the oolemma (Fig. 9).

DISCUSSION

Utilizing lanthanum tracer and freeze-fracture techniques, we have described gap junctional contacts between follicle cells and oocytes in ovarian follicles of several mammalian species. It was also demonstrated that these heterocellular connections (further distinguished by being between two ontogenetically different cell types) appear early in folliculogenesis, before deposition of the zona pellucida and formation of the antral cavity, indicating that functional membrane interactions between follicle cells and oocytes precede the widespread and rapid differentiation of gap junctions between granulosa cells which commence at the time of antrum formation (2). Unlike the granulosa cell gap junctions, the junctions observed at the surface of oocytes are extremely small in size, often consisting of 10–30 particles, and tend to form aggregates similar to those described between cone and rod cells in the vertebrate retina (19). While the junctions are small, they appear to be extremely abundant considering their frequency and the surface area of oocytes (for example, mouse ~75 μm in diameter; monkey ~150 μm in diameter). This fact alone suggests that the oocyte may be coupled to surrounding follicle cells during all stages of oogenesis and may thus not only participate in the preantral growth of the female gamete but also serve to regulate nuclear events associated with meiosis before and at the time of ovulation. In this connection it is interesting to point out that Pincus and Enzmann (17) stated that "... the associated follicle cells serve either to maintain the egg in a nutritional state wherein nuclear maturation is impossible, or that they actually supply to the ovum a substance or substances which directly inhibit nuclear maturation."

The extremely small size of individual oocyte gap junctions may explain why previous attempts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to characterize the nature of oocyte-follicle cell connections failed to reveal discrete junctions other than those of the macula adherens variety (26). The association of adhesion plaques with gap junctions is commonly observed in a variety of differentiating tissues (2, 12) and suggests that the adhesive forces established by *maculae adherentes* may serve to initiate and/or maintain functional communicative junctions between neighboring cells.

While the structural identification of gap junctions between oocytes and follicle cells implies the existence of a functional interaction between these two different cell types, we feel that certain recent observations on the physiological regulation of oocyte and granulosa cell functions strengthen the idea that a mutual interaction is fundamental to the control of folliculogenesis. For example, essential metabolic substrates utilized by oocytes under in vitro conditions often require the presence of associated cumulus cells (7, 11, 17). Similarly, it has long been recognized that in the mouse ovary, oocytes arrest at the diplotene (dictyate) stage of prophase of the first meiotic division before birth and remain in this stage until just before ovulation when the oocyte and the cumulus of companion follicle cells detach from the epithelial wall of the follicle. Oocytes are induced to resume meiosis by hormonal stimuli in vivo (6) or by physically releasing oocytes into a suitable culture medium (7). Although the factors regulating meiotic maturation remain obscure, cyclic adenosine monophosphate (cAMP) and its dibutyryl derivative have recently been shown to reversibly inhibit the spontaneous maturation of mouse oocytes in vitro (9, 24). Since cAMP is known to influence cell cycle events (1), and since molecules of comparable size have been reported to permeate gap junctions in other tissues (22), it is conceivable that a crucial step in the maintenance of meiotic arrest within the follicle is the ability of follicle-cell-oocyte gap junctions to transmit informational molecules between the two cell types.

A clear definition of the functional importance of gap junctions conjoining oocytes with follicle cells will first require the demonstration that these two ontogenetically different cell types are indeed electrically coupled and/or capable of exchanging intracellular substances. The observations reported here provide structural evidence for the existence of communicative pathways that may be essential to the regulation of oocyte-follicle cell function during folliculogenesis and ovulation.

SUMMARY

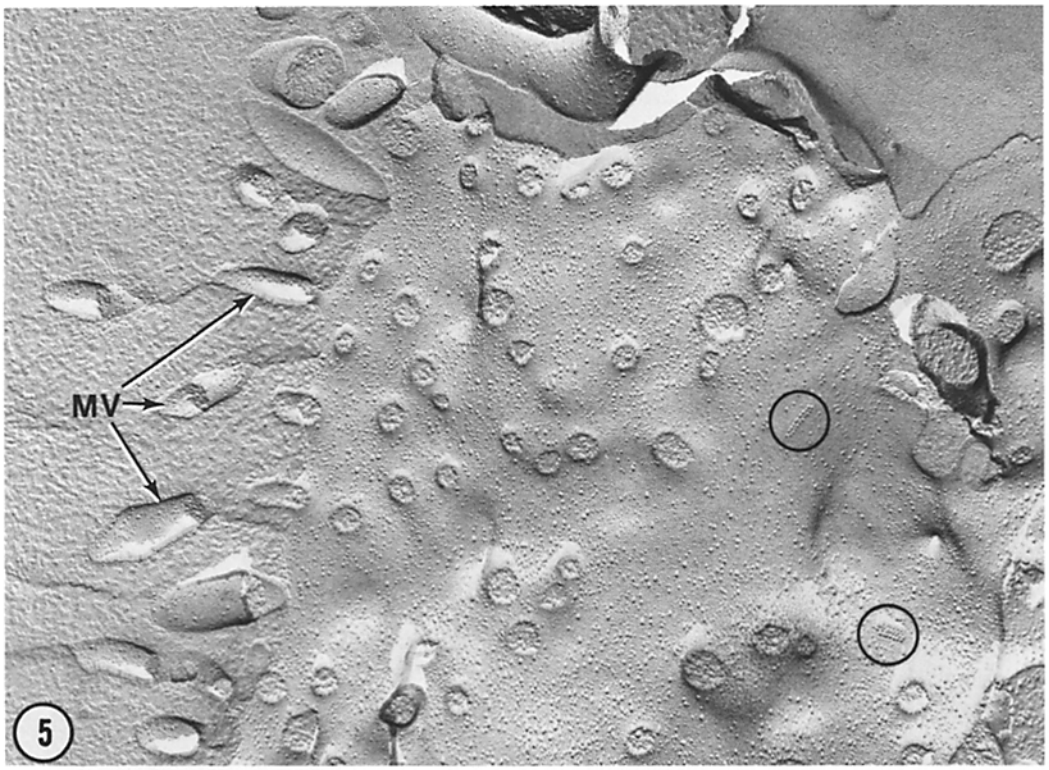
Tracer and freeze-fracture electron microscopy of the ovaries of neonatal rat and adult mouse, rat, rabbit, and primate have revealed the presence of gap junctions between follicle cells and oocytes. The junctional connections are found at the ends of follicle cell projections which traverse the zona pellucida and terminate upon microvilli and evenly contoured nonmicrovillar regions of the oolemma. Gap junctions are often seen associated with a macula adherens type of junction. The gap junctions occasionally consist of minute ovoid plaques, but more frequently appear as rectilinear single- or multiple-row aggregates of particles on the P-face or pits on the E-face. The functional significance of follicle cell-oocyte gap junctions is

FIGURE 1 A photomicrograph of a young oocyte showing processes transversing the zona pellucida. Toluidine blue stain. Rabbit. × 500.

FIGURE 2 (*a*) Follicle cell process (*FCP*) and oocyte microvillus (*OM*) making a macular junctional contact. From material impregnated with lanthanum. × 120,000. (*b*) An *en face* section of a gap junction revealing the hexagonal ordering of subunits (arrow). (*GP*) granulosa cell process; (*O*) oocyte. Rat. × 210,000.

FIGURE 3 An electron micrograph of a bifurcated follicle cell process (*FCP*) from lanthanum-impregnated material establishing gap junctional contact (also see *inset*) with the oocyte. *MV*-microvilli. *Macaca mulatta*. × 70,700; *Inset* × 140,000.

FIGURE 4 Freeze-fracture replica of the surface of a rabbit oocyte. Note three rectilinear gap junctional aggregates (arrows) surrounding a follicle cell process (*FCP*) cleaved during specimen preparation. × 85,000.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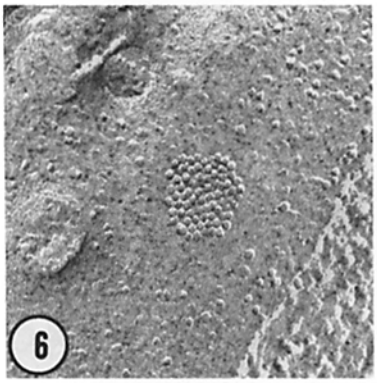
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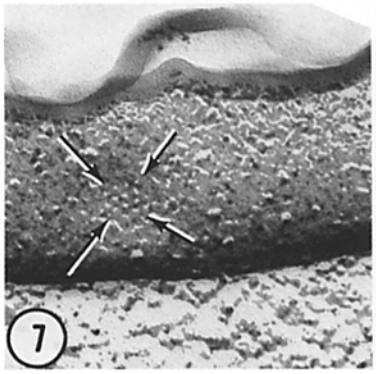
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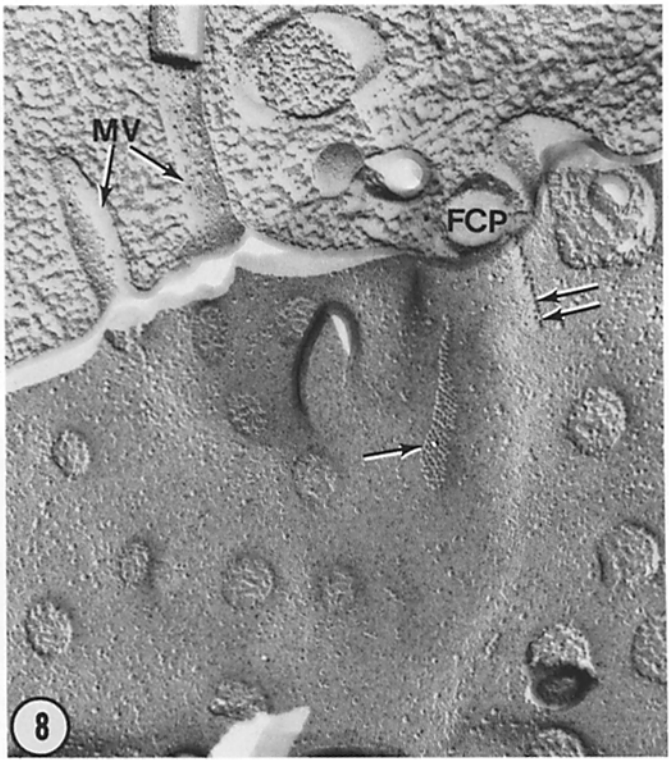
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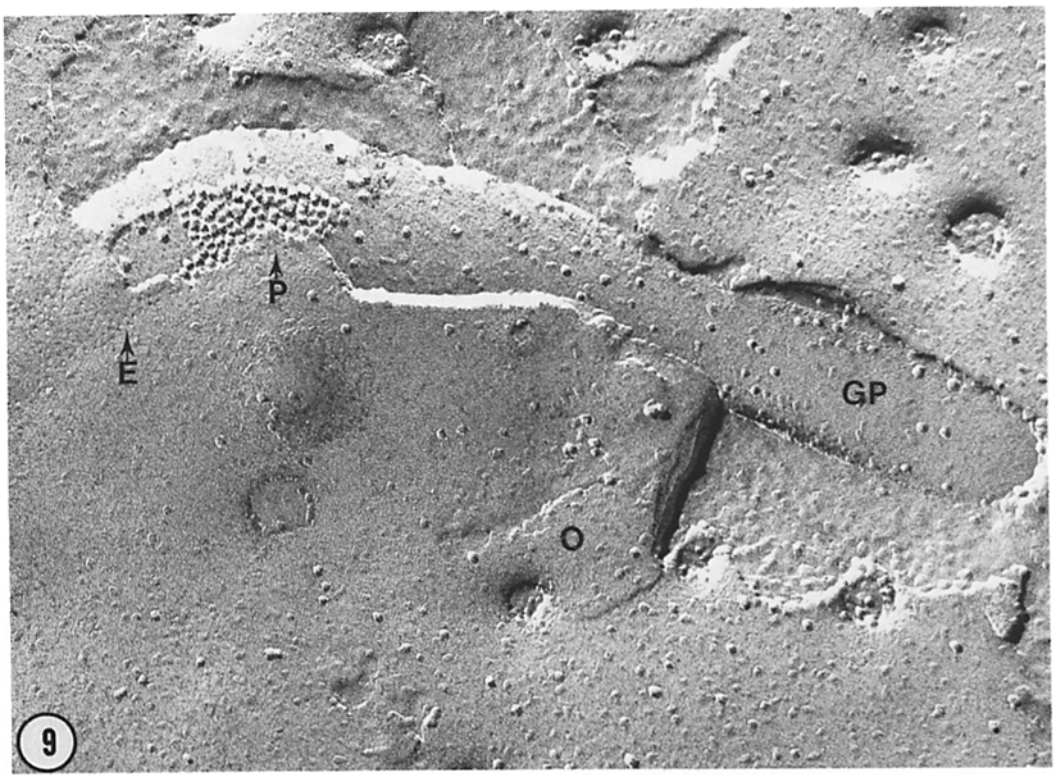

FIGURE 9 Freeze-fracture replica of a mouse oocyte depicting a gap junction between the oocyte (*O*) and a granulosa cell process (*GP*). E-face; P-face. × 165,000.

FIGURE 5 Relatively low magnification of a freeze-fracture replica of rat oocyte P-face, showing several minute gap junctions (see parentheses). × 60,000.

FIGURE 6 Freeze-fracture replica of a discoid junctional plaque (P-face) showing regular particle packing. Rat. × 165,000.

FIGURE 7 Freeze-fracture replica of a hexagon-shaped plaque of E-face. Rat. × 210,000.

FIGURE 8 Freeze-fracture replica of a rat oocyte showing junctions composed of a single-particle row (double short arrows) and a rectilinear aggregate (single arrow). Note that the single-particle row terminates at a follicle cell (*FCP*) contact with the oocyte. *MC*-microvilli. × 100,000.

discussed with respect to the regulation of meiosis and luteinization.

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