Cellular interconnections in the young mouse ovary

Freeze-fracture study*

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Summary. Intercellular junctions in the young mouse ovary were examined by electron microscopy utilizing freeze-fracture and thin-sectioning techniques. Projections from the granulosa cells adjacent to the oocyte (GI) traverse the zona pellucida and form small gap junctions on the oocyte surface. On the P-face of these cells, the junctional aggregations are occasionally associated with linear strands of particles. In contrast, large gap junctional areas are frequently observed between the more peripherally located granulosa cells (GE) and are also present in the theca interna (TI) cell layer surrounding the follicles. Three types of tight junctional strands are discernible on the P-face of theca externa cells (TE): angularly zigzag strands consisting of intermittently distributed intramembranous particles on wide ridges, intermediate zigzag strands consisting of more continuously distributed particles, and wavy strands consisting of rather fused particles. Tight junctional strands are also present in the middle of grooves on the E-face of endothelial cells of blood vessels. In the germinal epithelial cell layer, tight junctional strands appear to be discrete and form a less anastomosing network.

Key words: Mouse ovary – Junctions – Freeze-fracture – Electron microscopy

Many previous workers have paid attention to the intercellular relationships in the mammalian ovary between oocytes and the innermost granulosa cells (GI) (Albertini and Anderson 1974; Szöllősi 1975; Anderson and Albertini 1976; Amsterdam et al. 1976; Gilula et al. 1978; Moore et al. 1980; Burghardt and Anderson 1981), between the more peripheral granulosa cells (GE) (Merk et al.
1973; Albertini and Anderson 1974; Albertini et al. 1975; Amsterdam et al. 1976; Burghardt and Anderson 1981) and between theca interna cells (TI) (Amsterdam et al. 1976; O'Shea et al. 1978; Burghardt and Anderson 1981). Little, however, is yet known of the freeze-fracture images of the cells outside the follicular epithelium (endothelium of blood vessels, theca externa cells (TE), including connective tissue cells and germinal epithelium), though the intercellular relationship between TI cells has previously been studied in adult animals (Amsterdam et al. 1976; O'Shea et al. 1978; Burghardt and Anderson 1981). Atretic oocytes are lost postnatally into the peritoneal cavity through the germinal epithelium in young animal ovaries (Jones and Krohn 1961; Peters 1969; Hiura and Fujita 1977).

The present investigation is an ultrastructural study of the cell junctions in young mice ovaries utilizing a freeze-fracture technique.

Materials and methods

Immature female ddY strain mice, from 1 to 21-days-old, were used. The animals were anesthetized by intraperitoneal injections of nembutal (pentobarbital sodium), and the ovaries dissected out and pre-fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2-4 h. Material was cut into small pieces and post-fixed in 2 % osmium tetroxide for 2 h. Thereafter, the tissue was dehydrated by graded ethanol series, and embedded in Epon 812. Sections were cut with glass knives on a LKB ultramicrotome. The sections for light microscopy were then stained with toluidine blue, and those for electron microscopy were stained with both uranyl acetate and lead citrate.

The procedure for lanthanum tracer study was as previously reported (Toshimori et al. 1979).

For the freeze-fracture technique, specimens fixed in 2.5 % glutaraldehyde were put into 30 % glycerol overnight for cryoprotecting, and then quickly frozen in liquid Freon 13 or 22 followed by liquid nitrogen. Thereafter, they were fractured in the FD-2A fracturing device (Eiko-engineering) or HFZ block type fracturing device (Hitachi company) at −110°C in $10^{-6}$–$10^{-7}$ Torr. The exposed fracture faces were shadowed with platinum-palladium and carbon while the specimens were rotated at 1 rps in the FD-2A device, or were shadowed while in the HFZ device. Replicas were cleaned with kitchen bleach (disodium hypochlorite) and washed with several changes of distilled water. Observations were made using JEOL 100B or 200CX electron microscopes at an accelerating voltage of 80–120 kV.

Results

The cell types in the young mouse ovary are shown in Fig. 1. In 3-days-old mice, numerous primary ovarian follicles, with a single layer of flattened granulosa cells, are located in the cortex, while the more developed follicles lie deeper in the stroma. External to the follicular epithelium are TI and TE cells (connective tissue), and the endothelium of blood vessels. In young animals (up to about 10-days-old), so-called “eliminating” atretic oocytes are frequently seen in the polymorphous germinal epithelium (ovarian peritoneal cells), but they are absent from the ovaries of older animals (Hiura and Fujita 1977).

The oocyte surface

Projections from the GI cells extend toward the oocyte surface through the zona pellucida, while short microvilli from the oocyte surface penetrate into the zona pellucida. The projections form gap junctional aggregations of about 20–100