Electron Microscopic Studies of Mouse Oocytes and Two-cell Embryos exposed to Progesterone \textit{in Vitro}

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This experiment was undertaken in order to find out if there is any morphological change in oocytes and two-cell embryos whose development have been suppressed by progesterone for six hours \textit{in vitro}. It can be observed that some part of the outer side of nuclear membrane of the suppressed oocytes was damaged. The number of nuclear pores has decreased in suppressed oocytes and this suggests that progesterone might suppress the transport of intermediary metabolites between cytoplasm and nucleus. Sometimes, closely packed aggregates of parallel or irregular endoplasmic reticula were observed in suppressed oocytes. Microvilli of suppressed oocytes showed signs of degradation and the perivitelline space became apparent. Thus it is presumed that the egg membrane has constricted during cultivation under progesterone \textit{in vitro}. The other cell organelles such as mitochondria, multivesicular bodies, cortical granules and fibrillar lattices showed no difference in morphology between treated and control (intact) oocytes. In two-cell embryos, there was also no evident morphological change except for the fact that many vacuoles appeared clearly in suppressed embryonal cells. In brief, there was no fundamental morphological change in the oocytes and the embryonal cells exposed to progesterone for six hours even though it inhibits their development. The action of progesterone should be investigated thoroughly.

It has been known that some progestogens block early embryonal development when they are directly applied to the embryos \textit{in vitro} (Daniel and Levy, 1964, Whitten, 1967). It has also been found by us that progesterone in a medium (44 \(\mu\)g/ml) almost completely inhibits germinal vesicle break-down (GVBD) of the mouse oocytes \textit{in vitro} (Cho \textit{et al.}, 1974). Considering the fact that progesterone acts as an inducer of meiosis in amphibian oocytes (Schuetz, 1967, a,b; Masui, 1967), mammalian
oocytes seems to react in different way to progesterone than amphibian oocytes in vitro.

Some investigators (Daniel and Levy, 1964; Pratt and Daniel, 1966; Kirkpatrick, 1972) have studied the mechanism of this progesterone action on early embryos. Daniel and Levy (1966) found that rabbit embryos cleaved normally in a medium containing progesterone if excess amino acids were present in the medium and they also observed that 14C-progesterone given in the medium attached to the surface of the oocytes. From these results, they proposed that the progesterone in the medium changes permeability of the egg membrane and thus restricts the transport of the amino acids which are essential for the embryos to cleave, but no further study was performed on the progesterone affect on the physiological and morphological changes of embryos under influence of the hormone. However, it was presumed that inhibiting action of progesterone on GVBD of mouse oocytes was not likely to be related simply to permeability of egg membrane because resumption of meiosis did not take place in progesterone medium supplemented with excess energy sources or various mineral nutrition (Cho et al., 1974). We found that the oocytes did not allow penetration of 14C-progesterone given in the medium (unpublished). Based on these findings, it is assumed that the direct target of progesterone might be the egg membrane system.

The present experiments were conducted in order to find any morphological changes in the oocytes and two-cell embryos in the presence of progesterone and to clarify the mechanism of progesterone action.

MATERIAL AND METHOD

A-strain female mice were used in this experiment. The oocytes were obtained by puncturing the Graafian follicles under a dissecting microscope. Only those with an intact germinal vesicle (GV) and homogenous cytoplasm were used as material. A modified Krebs-Ringers bicarbonate salt solution (Biggers et al., 1971) was used as a basic culture medium. Collection of two-cell embryos were performed by the method introduced by Biggers et al. (1971). To obtain embryos, two-month old mice were superovulated by intraperitoneal injections of 10 IU pregnant mares serum gonadotrophin (PMS, Sigma) and 10 IU human chorionic gonadotrophin (HCG, Sigma), 44 to 48 hours apart between the two. The two-cell embryos were harvested in the basic medium after 48 hours of HCG injection by flushing the fallopian tubes of the mouse which had a vaginal plug. After collection, the oocytes and embryos were divided into two, control and experimental groups. Those of control group were not cultured and fixed right away and only those of experimental group were cultured as described below. Cultivation of the oocytes or embryos in the medium containing progesterone which is oil soluble was carried out in a microtube developed by Cho (1974). The detailed procedure for culture work was described in our previous work (Cho, 1974; Cho et al., 1974). The oocytes or two-cell embryos were cultured for six hours in the medium supplemented with 0.4% bovine serum albumin (BSA. Sigma) in the presence of progesterone. The concentration of progesterone was 50 µg/ml for oocytes and 20 µg/ml for two-cell embryos. All glassware used in culture work was sterilized by a hot air sterilizer and the culture media were filtered by a millipore filter before use.

For electron microscopy, the following procedures were carried out as described in previous work (Chung et al., 1973). The oocytes and embryos of control or experimental group were washed in phosphate buffer (pH
and immediately prefixed overnight in 1% glutaraldehyde and postfixed for 90 minutes in 1% osmium tetroxide in phosphate buffer (pH 7.4). After double fixation, the materials were rapidly dehydrated in a graded ethanol series (60~90%) and propylene oxide, and embedded in Epon 812 (Luft, 1961). The thin sections were cut with a Sorvall MT-2 Porter-Blum ultramicrotome and mounted on 200 mesh grids. The samples were stained doubly with saturated uranyl acetate (Watson, 1961) followed by lead citrate (Venable and Coggeshall, 1965) and observed with a Hitachi HU-11E electron microscope.

OBSERVATIONS

1. Oocytes

In both groups, the treated and the control, chromatin dots were dispersed homogeneously throughout the nucleus. The nuclear membrane was composed of two parts, an outer and inner membrane, but in some places in the suppressed oocytes, the outer part of the nuclear membrane was partly broken away (Fig. 5). Nuclear pores were distributed irregularly along the nuclear membrane in both groups, but more nuclear pores were observed in the control oocytes than in the suppressed oocytes. Frequently, many nuclear pores were located close together in a small portion of the control oocytes (Fig. 2). The cytoplasm of both groups contained numerous fibrillar arrays each consisting of five to ten parallel fibrils and at high magnification, the fibrils showed a faint periodicity (Figs. 2, 3, 6, 7). There were also many hemispherical arrays of fibrils and ribosomes were concentrated in this area (Figs. 2, 3, 6, 7). In the central portion of the cytoplasm of both groups of oocytes there were multiple Golgi clusters and these complexes had a round profile and consisted of closely packed vesicles and short tubules limited by a densely osmiophilic membrane. Peluso and Butcher (1974) found that in this area deposition of dense material followed by coalescence of a few vesicles gives rise to the formation of dense granules which are morphologically indistinguishable from cortical granules. The same phenomenon was found in our work. As it can be seen in Figs. 2 and 8, there were more cortical granules in the central region of the egg near the nucleus and especially in the vicinity of the multivesicular Golgi complex than in the cortical zone of the oocyte. Rough endoplasmic reticulum was present in meager amounts and was observed as irregular, short cisternae provided with a modest number of ribonucleoprotein particles and ergastoplasmic structures were frequently associated with mitochondria in both groups of oocytes (Figs. 3, 7). However, in suppressed oocytes, closely packed aggregates of parallel or irregular endoplasmic reticula were observed (Fig. 6). The mitochondria which were randomly distributed in the cytoplasm were spherical, slightly electron dense, and possessed cisternae which were circumferential and concentrical to the inner membrane but it frequently could be found that some cisternae were arranged transversely in the matrix. The mitochondrial matrix were sometimes very sparse and they looked vacant (Figs. 3, 4, 7) in both groups of the oocytes. There was a great difference in the morphology of the egg membrane between the two groups of the oocytes. In the control oocytes, numerous long slender microvilli were projected into the zona pellucida and the perivitelline space was very narrow (Fig. 1), but in the suppressed oocytes, the membrane had constricted and became relatively smooth and had less microvilli than the control ones. Between the egg membrane and zona pellucida, a clear perivitelline space was observed in the
suppressed oocytes (Fig. 5).

2. Two-cell embryos

In the nuclei of two-cell embryos, there were one to three nucleoli which were seen as spheroidal bodies made up of a very dense material in a highly compact state of aggregation. Electron dense chromatin masses were dispersed throughout the nucleus (Fig. 9). Because these chromatin masses were often associated with the inner nuclear membrane, it was impossible to distinguish nuclear pores from those in the nuclear membrane. In general, it was observed that cytoplasmic organelles of the embryonal cells were grouped more compactly than those of the oocytes. Fibrilla arrays of nucleoprotein complex were observed abundantly throughout the cytoplasm but the number of fibrils seemed to be less than that of the oocytes. It was also found that the periodicity of the fibrils became less distinctive in the cleaved cells than in the oocytes (Fig. 9). The treated two-cell embryos were accompanied by a number of vacuoles (Fig. 10). Microvilli of the embryonal cells were almost destroyed and the vitelline membrane was relatively smooth (Fig. 10). Golgi complexes no longer showed a multivesicular form and changed into a typical tubular form (Fig. 10). In brief, however, there were no fundamental morphological changes between the control and the treated groups of embryos.

DISCUSSION

Ever since Whitten (1957) reported that progesterone blocks the cleavage of early mouse embryos in vitro, several other investigators also have tried to find the mechanism of progesterone action. Pratt and Daniel (1966) found that progesterone acts directly on the cell membrane and changes the permeability of certain nutrients. However, Kirkpatrick (1972) studied incorporation of labeled lactate on embryonal cells and found that progesterone did not influence the permeability. As for the oocytes, it is evident that some progestogens inhibit germinal vesicle breakdown of mouse oocytes in vitro (Kwon and Chung, 1975). In this study, it was confirmed that progesterone given in vitro did not induce any fundamental change in morphology of the egg cells, even though some minor changes were found. As shown in Fig. 5, some part of outer nuclear membrane were damaged in treated oocytes and a smaller number of moderately dense particles were found in the suppressed oocytes on the nuclear pores than in the controls (Figs. 2, 8). However, since dibutyryl cyclic AMP (dbcAMP) also suppressed the GVBD (Cho et al., 1974), Choe (1975) found that the number of nuclear pores increased in oocytes in which the GVBD was suppressed by dbcAMP and proposed that the action of cyclic AMP is closely related to the nuclear membrane of the oocytes. It is difficult to explain the above contradictory facts. For progesterone, one possible suggestion is that progesterone in the medium suppressed the metabolism of oocytes to a certain extent so that further transport of intermediary metabolites between cytoplasm and nucleus also decreased. The partial destruction of outer nuclear membrane might be the early phase of GVBD which was soon blocked by progesterone. The microvilli of suppressed oocytes were markedly degenerated and perivitelline space became wide. These findings agreed with those of Choe (1975) and similar changes were observed by Suzuki (1974) in aged tubal ova of rabbits. It is presumed that in vitro, once follicular oocytes leave the Graafian follicle, the egg membrane starts to constrict and part of the microvilli are destroyed, but this phenomenon seems not to be related to permeability of the egg membrane.
The activity of cortical granule formation of multivesicular bodies was not influenced by progesterone in the oocytes (Figs. 2, 8). In two-cell embryos multivesicular bodies disappeared and Golgi complexes showed the typical tubular form. The morphology of two-cell embryos also did not change fundamentally under the influence of progesterone for six hours in vitro. Progesterone may induce some physiological change or inhibit some enzyme system which is essential for development or initiate its action through the adenyl cyclase system.

Further study should be performed on the effect of progesterone on macromolecule synthesis and the adenyl cyclase system of the oocytes or embryos in order to find the real mode of progesterone action.

REFERENCES


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Fig. 1. Control oocyte; Numerous microvilli (MV) are observed in egg membrane. There is no distinct perivitelline space (PvS) between zona pellucida (ZP) and egg membrane (↑). ×22,500.

Fig. 2. Control oocyte; Nuclear pores (NP) are concentrated at this region, multivesicular complex (MvB) with many cortical granules (CG) are observed and fibrilla lattice (FL) are dispersed throughout the cytoplasm. ×22,550.
Fig. 3. Control oocyte; Mitochondria (Mi) which are associated with endoplasmic reticulum (ER) are observed. Hemispherical arrays of fibrils (HaF) where ribosomes (Ri) are concentrated are observed. ×22,500.

Fig. 4. Control oocyte; Mitochondrial matrix are very sparse (Mi) and sometimes they looked vacant. ×22,500.
Fig. 5. Progesterone treated oocyte; Outer nuclear membranes are partly broken away (↑). A widened perivitelline space (Pvs) with the decreased occurrence of irregular microvilli (MV) is found. ×16,000.

Fig. 6. Progesterone treated oocyte: Aggregates of endoplasmic reticulum (ER) are found and hemispherical fibrilla lattice (FL) are also observed. ×30,000.
Fig. 7. Progesterone treated oocyte; Endoplasmic reticulum (ER) which are associated with mitochondria are found and it also shows that inner matrix of some mitochondria (Mi) are very sparse. ×30,000.

Fig. 8. Progesterone treated oocyte; Multivesicular body (MvB) with cortical granule (CG) are observed. Nuclear pores (↑) are distributed randomly throughout nuclear membrane (NM). ×16,000.
Fig. 9. Control two-cell embryo; Electron dense chromatin (Chr) masses are dispersed in nucleus and often associated with inner nuclear membrane (NM). Fibrilla arrays (FL) are dispersed abundantly throughout the cytoplasm. ×22,500.

Fig. 10. Progesterone treated embryo; A number of vacuoles (V) are observed. Cell membrane became relatively smooth and microvilli (MV) are almost destroyed. Abundant fibrilla arrays (FL) are also observed. Golgi complex (Go) showes a typical tubular form. ×40,000.