Ultrastructural Studies on Mitochondria of Preimplantation Rabbit Embryos

Soon O Chung, Young Hee Choi, Young Kun Deung

Department of Obstetrics & Gynecology, Yonsei University College of Medicine, Seoul, Korea

Moon Kyoo Kim

Department of Zoology, College of Liberal Arts and Sciences, Seoul National University, Seoul, Korea

ABSTRACT

The ultrastructural changes of mitochondria in the ovarian oocytes from Graafian follicles, the ovulated tubal ova, and the various stages of preimplantation rabbit embryos have been observed with an electron microscope.

From the ovarian oocytes to the 4-cell stage, mitochondria showed oval and round forms with a few cristae arranged concentrically and peripherally at the inner membrane. In 8-cell and 16-cell stages, mitochondria tended to change their forms to be elongated, and their sizes, and the outer membrane of the mitochondria had a tendency to become rough and irregular although there were few changes in the inner structure. In morula, some mitochondria began to show several transverse cristae proceeding into the matrix. Mitochondria rapidly increased in number at the late blastocyst stage. Matrix of mitochondria with transverse cristae found in the morula and in blastocyst stages was less dense than that of the earlier stages. The authors believe that the morphological changes of mitochondria during early embryonal development indicate the level of enzymatic activity at which this organelle is engaged in energy metabolism.

INTRODUCTION

The successful culture of rabbit ova from zygote to blastocyst has been accomplished by a number of investigators (Suzuki, 1966; Mauer et al., 1968; Kane and Foote, 1970; Brinster, 1970). The fact that the energy metabolites required by the preimplantation rabbit embryos undergo several changes during development in vitro is also evident from the results of investigations by Daniel(1967), Fridhandler(1968), and Brinster(1970). It is verified that the metabolism pattern also changes (Daniel, 1967; Fridhandler, 1961; Fridhandler et al., 1957), and that these changes in metabolism involve structural changes in the mitochondria during oogenesis and cleavage of the early embryos.
(Stern et al., 1971).

Recently, many authors have reported on the ultrastructure of mammalian oocytes and early embryos (Blanchette, 1961; Schlaefke and Enders, 1963; Zamboni and Mastroianni, 1966; Calarco and Brown, 1968; Hillman and Tasca, 1969; Stern et al., 1971). However, few papers have deeply discussed the correlation between the morphological changes of mitochondria and the energy metabolic alteration during cleavage of the rabbit embryos.

The purpose of the present experiment is to observe the structural changes of mitochondria of the oocyte and preimplantation stage of the rabbit embryos, in order to verify the correlation between the changes and energy metabolism.

**MATERIALS AND METHODS**

Domestic rabbits weighing 1.2–2.0 kg were used in this experiment. The ovarian oocytes were collected from Graafian follicles in the ovaries by the method of Donahue (1968).

For the collection of the ovulated tubal ova, the does were superovulated by a subcutaneous injection of 50 i. u. of PMS (pregnant mare’s serum gonadotrophin, Gestyl, Organon) per day for three days, followed, 24 hours later, by an intravenous injection of 100 i. u. of HCG (human chorionic gonadotrophin, Equinex, Ayerst). The tubal ova were harvested by flushing the fallopian tubes of the superovulated does with the culture medium (TC medium 199) 14 hours after final hormone injection. The same method as described above was employed for the collection of the fertilized ova and the various stages of preimplantation embryos, except the does were mated by a fertile buck just before injection of HCG.

The embryos were harvested by flushing the genital tracts at the appropriate time.

For electron microscopy, the oocytes and embryos were immediately prefixed for 30 min. in a 3% glutaraldehyde solution buffered (pH 7.4) with 0.1M phosphate buffer, and washed two times for 20 minutes each with phosphate buffer. The materials were then postfixed for 30 min. in a 1% osmium tetroxide buffered (pH 7.4) with 0.1M phosphate buffer. After double fixation the materials were rapidly dehydrated in ethanol series and prophenyl oxide, and embedded in Epon 812 (Luft, 1961). The thin sections were cut with Sorvall MT2 Porter-Blum ultramicrotome, and stained with saturated uranyl acetate (Watson, 1961) followed by lead citrate (Venable and Coggeshall, 1965), and examined under a Hitachi HU-11E-1 electron microscope operated at an accelerating voltage of 75KV.

**OBSERVATIONS**

In several sections of the rabbit embryos the plasma membrane contact between two blastomeres was relatively smooth. But, there were many ooplasmic processes protruding towards the space between blastomeres (Fig. 4). The organelles such as the nucleus, Golgi complex, endoplasmic reticulum and mitochondria, and inclusions such as the lipid droplets and ribosomes, were generally found in ooplasm. Mitochondria were randomly distributed in the ooplasm of blastomeres through out all embryonic stages. Mitochondria in the ovarian oocytes obtained from Graafian follicles, the ovulated unfertilized ova, the fertilized ova, and the 2- or 4-cell embryos, showed round or oval forms with a relatively unique size. The mitochondrial cristae were rarely developed and were
arranged circumferentially to the inner membrane (Fig. 1, 2, 3).

In 8-cell and 16-cell embryos, mitochondria showed some diversity in shape and size (Fig. 5). Some mitochondria were elongated, and the others rough and of various sizes. The surface of the mitochondria showed a tendency to become rough and irregular (Fig. 6). Some showed small intracrystal spaces arranged peripherally. Cristae were developing slightly, but were circumferential and concentrical to the inner membrane at this time. There were little differences in the inner structure when compared with that of the earlier stages. At these stages many mitochondria were associated with endoplasmic reticulum (Fig. 6).

In morula and early blastocyst stages some of the mitochondria became more developed with cristae arranged transversely in the matrix than that of the earlier stages, and the matrix was less dense. Other mitochondria still showed the primitive morphology noted in the earlier stages. Most of the mitochondria located near the Golgi complex were associated with the endoplasmic reticulum (Fig. 7, 8).

In mid and late blastocyst stages the mitochondria showing more elongated forms, were increased in number, and their cristae became more distinct and were arranged transversely in the matrix (Fig. 9, 10).

DISCUSSION

It has been known that mitochondria provides energy in the cell. Ample evidence that mitochondria are functionally organized at the molecular level has been accumulated since the works of Cooper and Lehninger (1956) and Davlin and Lehninger (1956). It is very interesting to verify the correlation between morphological changes of mitochondria and energy metabolic patterns concerned with the requirements for energy source during embryogenesis.

Anderson et al. (1970) observed in rabbit embryos that mitochondria, from the oocyte to all stages up to morula, have a simple and unique morphology. In the present studies, we found that the ovarian oocytes, ovulated ova, and embryos from the fertilized one-cell to the 4-cell stage showed mitochondria having a simple morphology, as the observation of Anderson et al. However, at the 8- or 16-cell stage morphological changes began to appear in the mitochondria. These changes are comparable to those seen at 4- or 8-cell stage of mouse embryos (Stern et al., 1971). It should be noticed that mitochondria at these stages were somewhat diverse in shape and size (Fig. 5) and that the outer membrane showed a tendency to become rough and irregular (Fig. 5, 6). In morula stage a portion of mitochondria showed several transverse cristae proceeding into the matrix (Fig. 7). As the morula developed toward the late blastocyst stage the number of mitochondria with transverse cristae increased gradually.

There are also some differences in vitro in metabolic patterns between mouse and rabbit preimplantation embryos. The former requires pyruvate and lactate to support cleavage up to 4- or 8-cell stage and beyond these stages, glucose is preferentially utilized by embryos (Brinster, 1965 a, b; Brinster and Thomson, 1966; Biggers et al., 1970). However, for rabbits, although carbohydrates are not essential, amino acids are necessary for continuing cleavage (Daniel and Krishman, 1967; Daniel and Olson, 1969; Mauer et al., 1968; Brinster, 1970). From these findings, it might be assumed that amino acids and endogenous nutrients could be utilized as en-
energy sources for cleavage in rabbit embryos. Stern et al. (1970) stated that the morphological changes of mitochondria in mouse embryos are interrelated with the changes in carbohydrate requirements. Even though the type of carbohydrate requirement is different between mouse and rabbit embryos, the changes of mitochondria during cleavage showed a similar pattern. That is, morphological changes of mitochondria begins to appear at 4- or 8-cell stages in mouse and at 8- or 16-cell stages in rabbit.

Daniel (1967) found that the 8-cell and 16-cell stages of rabbit embryos utilized 6-phosphogluconate, which does not support the earlier embryos. In addition to this, investigators (Brinster, 1968, 1969; Fridhandler, 1961) have found in the rabbit embryo that the ratio of CO₂ production from C₆ and C₃ labelled glucose for the preblastocyst stage was about 7-fold more than that for the blastocyst stage. These findings strongly suggest that a pentose shunt may be functional in the 8- or 16-cell stages in rabbit embryos hence this metabolic change might induce mitochondrial change as well, because, at these stages, primary changes in mitochondria can be seen at the space between outer and inner layer where adenylate kinase and nucleotide diphosphokinase are found. However, as yet, there has not been any data available to explain directly the mitochondrial changes in connection with such a physiological event. During the development from morula to blastocyst, mitochondria develop in form, and increase in number. Mitochondrial cristae increase and are arranged transversely in the matrix. Remarkable changes in the mitochondria at the blastocyst stage are concomi-

**REFERENCES**


Mitochondria of Preimplantation Rabbit Embryo


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Soon O Chung, Young Hee Choi, Young Kun Deung and Moon Kyoo Kim


Explanation of Figures

Fig. 1, 2, 3 and 4. From ovarian oocyte, tubal ovum, zygote, and 4-cell stage, respectively. Mitochondria (Mi) show round or oval forms, and have smooth outer membrane. Cristae are arranged concentrically and circumferentially to the inner membrane. The irregular ooplasmic processes (OP) proceed into the interspaces between the blastomerdes.

Fig. 5 and 6. From 8-cell and 16-cell stage, respectively. Mitochondria (Mi) show some modified forms and sizes: Elongated and various sized mitochondria, some with rough surfaces. Some mitochondria (Mi) are associated with endoplasmic reticulum (↑).

Fig. 7 and 8. From morula and early blastocyst, respectively. Mitochondria (Mi) show more diversity in shape and size than that of the earlier stage. Some mitochondria (Mi) contain several transverse cristae in the matrix and are closely associated with endoplasmic reticulum (↑).

Fig. 9 and 10. From mid and late blastocyst, respectively. Mitochondria (Mi) show more elongated forms. Cristae are transversely arranged in the matrix.

Abbreviation: Go, Golgi complex; IS, Interspace between the blastomerdes, Li, Lipid droplet; No, Nucleolus; Nu, Nucleus.
Fig. 5

Fig. 6
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Fig. 9

Fig. 10