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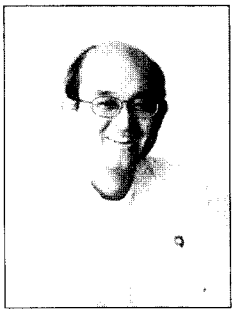
Yoshiharu Morimoto

CEO & Chairman, IVF NAMBA CLINIC, IVF JAPAN



2005	CEO & Chairman, IVF JAPAN
2003	CEO & Chairman, IVF NAMBA CLINIC
1999	President, Sunkaky Medical Corporation
1998	Director, IVF OSAKA CLINIC
1993-present	Associate Professor, Department of Obstetrics and Gynecology, Kansai Medical University
1990	Director, Morimoto Kinen-Kenshin Clinic
1988	Board Certified (Ob / Gyn Specialist) by Japan Society of obstetrics and Gynecology
1987	Director, Department of Obstetrics and Gynecology, Kawachi General Hospital
1987	Ph.D. (Doctor of Medical Science) from Kansai Medical University
1983	Director, Morimoto Womens Hospital
1979-1983	Postgraduate student of Kansai Medical University
1977-1979	Resident, Department of Obstetrics and Gynecology, Kansai Medical University
1977	Passed the Examination of National Board, Japan License No. 237040
1977	Graduated from Kansai Medical University, Moriguchi City, Osaka, Japan

In vitro Maturation of Oocyte and its Ultrastructure

Yoshiharu Morimoto, M.D., Ph.D.
IVF NAMBA CLINIC, IVF JAPAN

Introduction

In polycystic ovary syndrome, it should be strongly recommended not to stimulate ovaries. It is because it may cause severe side effects such as ovarian hyperstimulation syndrome and multiple gestation. *In vitro* maturation technique (IVM) is a wonderful alternative to prevent those miserable sufferings. In order to improve the technique toward better result, the understanding of the oocyte maturation, especially of cytoplasm, is indispensable. In this presentation, what is the maturation process of human oocyte, what is going on during maturing process from the standpoint of ultrastructure are described.

Oocyte maturation

The maturation process in animal and human oocyte has been investigated and described for more than decades. Firstly, in the fourth week of embryonic development human primordial germ cells migrate to the coelomic epithelium of the gonadal ridges and develop to oogonia. Oogonia forms and exists as a manner of nest. The first follicles are recruited by the action of neurotrophic factors such as brain derived neurotrophic factor (BDNF), nerve growth factor and neurotrophins (NT). On the way of its process, one layer of follicular cells covers oocyte and follicle development comments. For the transformation Kit system and anti-mullerian hormone has important roles on the process.

Oocyte maturation process is divided into two categories `such as nuclear and cytoplasmic maturation. Furthermore genetic approach to this phenomenon has been tried and recently the concept of genetic and epigenetic maturation has been proposed.

Nuclear maturation

Meiosis is a reducing process of chromosomes from diploid to haploid in gametes. At the time of migration to the coelomic epithelium of the gonadal ridges, primordial germ cell undergoes mitotic division. The first meiotic division is a transition from oogonia to oocytes. The completion of nuclear maturation occurs with germinal vesicle breakdown by the action of maturation promoting factor and finishes at extrusion of polar body.

Cytoplasmic maturation

Cytoplasmic maturation of oocyte may be an essential phenomenon for the oocyte to acquire

competence to fertilize and cleave. Oocyte undergoes its maturation in harmonization with maturation of nucleus and cytoplasm. A plenty of mRNA including maternal genes are accumulated in the cytoplasm.

Ultrastructure of the ooplasm in maturing process

Immature and matured oocytes were donated by PCO patients aged from 27 to 33 years who underwent IVM program in the two centers of infertility treatments in Osaka after informed consent. Oocytes were cultured in IVM medium NG 1.3 (Medicult, Denmark), 10% patient serum, 100 IU/L human chorionic gonadotropin and 75 IU/L follicle stimulating hormone under the atmosphere of 5% CO_2, 5% O_2 and 90% N_2. Those oocytes were fixed and ultrathin sectioned and observed by electron microscope at 0, 6, 12 and 24 hours of culture.

Immature oocyte which is still in GV stage cultured *in vitro* for 6 hours showed that granulosa cells surrounding oocyte looked very active and included a plenty of lipid droplet. Mitochondria were dispersed in cytoplasm and cortical granules were prominent on the edge of the plasmalemma. After 24 hours of *in vitro* culture, the ultrastructure showed remarkable change. In the nucleus, germinal vesicle was broken down and cortical granules disappeared on the edge of the cell. More noteworthy character is that of mitochondria. They have migrated over into the center of the cell, increased in number and aggregated. Furthermore microvilli on the surface of the oocyte showed morphological remarkable development.

Genetics and Epigenetics on oocyte maturation

According to the Ovarian Kaleidoscope Database, there is 280 oocyte related genes and 398 granulosa cell related genes. Moreover 89 genes are common both in oocyte and granulosa cells. And more number of genes are being discovered day after day. Not many of them have been studied to be specified for oocyte maturation. Some genes such as mos gene are related with nuclear maturation and others are identified in cytoplasmic maturation. Epigenetic modification may be more essential for oocyte maturation. It may not only influence on gamete-specific imprint on the genome, but regulate gene expression on the maturation process.

Conclusion

The IVM procedure has been developed recently worldwide, especially applicated for the treatment of polycystic ovary syndrome. However satisfying and stable clinical success has not yet been achieved. In order to improve it, it is indispensable to know the process of the maturation of oocyte, especially in cytoplasm. Oocyte cytoplasmic maturation can be expressed as the accumulation of messenger RNA, but its each function of genes is not adequately clarified. Despite during the short period of 24 hours, the cell construction has dramatically changed in its ultrastructure. The

remarkable development of microvilli on the surface of the oocyte indicates the active communication from the inside to the outside through plasma membrane. The purpose of the oocyte maturation is defined as preparation for fertilization and cleavage. The movement and aggregation of the activated mitochondria indicates the competence acquirement in preparation for coming big events by charging up cell activity. In order to identify maturation process, more study on genetic expression and epigenetic modification should be essential.

Reference

1. Eppig J E, et al. Regulation of mammalian oocyte maturation The Ovary. Leung PCK and Adashi EY p.113 CA, U.S.A, 2004.
2. Trounson A et al. Oocyte maturation Hum Reprod., 13:52-62, 1998.
3. Gougeon A. Ovarina follicular growth in humans:ovarian ageing and population of growing follicles. Maturitas 30:137-142, 1998.