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<Review>

Nuclear and Cytoplasmic Dynamics in Mammalian Oocytes during Sexual and Asexual Developments

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포유동물 난자의 유성 및 무성 발생과정 동안 핵 및 세포질의 변화

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ABSTRACT: At fertilization, sperm penetrates into oocyte, male and female pronuclei are fused together, and mitotic division follows. However, little information is available on the interactive roles and dynamic processes between cytoplasmic and nuclear components during the pronuclear formation, migration and cell division. The assisted reproductive technologies such as, intracytoplasmic sperm injection (ICSI) and round spermatid injection(ROSI) could provides new treatments for the male infertility as well as tools for the study of basic mechanism during fertilization. Nuclear transfer can also provide a mechanism on the interactive roles between nucleus and cytoplasm since the process includes nuclear reprogrammming of differentiated cells in the enucleated oocytes. Recently, I have investigated developmental processes in porcine oocytes following fertilization, parthenogenesis, ICSI, ROSI and nuclear transfer using indirect immunocytochemical and electron microscopic studies. The results could provide an insight into biological questions related with epigenesis as well as strategies for the enhancement of embryology in general such as ICSI and nuclear transfer.

Key words: Fertilization, Parthenogenesis, ICSI, ROSI, Nuclear transfer.

요 약: 수정에 의한 배 발생은 정자가 난자 내로 침입하여 정자와 난자의 반수체 핵질이 융합되고 이어 유사분열로 이어지는 과정에서 시작된다. 하지만 수정 및 초기 배 발생 동안 자웅 핵질과 난 세포질 구성 요소 상호간의 작용기전에 관해서는 명확히 알려져 있지 않은 부분이 많다. 수정보조기법인 세포질 내 정자 직접 주입법의 개발은 남성불임치료에 혁신적인 기술로 자리잡고 있을 뿐만 아니라 포유동물의 수정과정을 이해하는데 많은 도움을 주고 있다. 핵치환에 의한 복제동물 생산기법도 분화된 핵이 난 세포질 내에서 재 분화 (reprogramming) 하여 발생하는 유일한 과정으로 세포질 구성요소들의 상호작용과 발생 조절 기능을 이해하는데 도움을 준다. 최근 몇 년간 돼지 난자 세포질에 정자 및 원형정자 직접주입, 세포질 이식, 세포질 융합, 및 핵치환 한 후 난자의 발생과정을 간접 면역형광 분석법과 주사 전자현미경으로 조사하였다. 이러한 연구를 통해 체외수정, 세포질 이식 및 정자직접 주입법 등과 같은 임상치료기술 과 핵치환에 의한 복제동물 생산 기법의 개선에 필요한 기초자료를 얻을 수 있었고, 포유동물 난자의 후생적 발생과정 (epigenesis)에 관해 공부할 수 있었다.

INTRODUCTION

At fertilization, male and female nuclei undergo dramatic rearrangements which are necessary for the union of paternal and maternal genomes. Changes in nuclear structure include male and female pronuclear formation, pronuclear movements, intermixing of paternal and maternal genome and mitotic processes for the completion of fertilization processes. Recently, various assisted reproductive technologies and nuclear transfer techniques have been developed in the area of embryology in general. The assisted reproductive technology includes intracytoplasmic sperm injection (ICSI), round spermatid (ROSI) or round spermatid nucleus injection (ROSNI) and cytoplasm transfer. More intensive studies on the interactive dynamics between nucleus and cytoplasm components are required to improve assistant reproductive and nuclear transfer techniques. Recently, I have investigated developmental processes in poricne oocytes following fertilization, parthenogenesis, ICSI, ROSI and

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nuclear transfer. The results obtained could provide insight into biological questions related with epigenesis as well as strategies for the enhancement of embryology in general such as ICSI and nuclear transfer.

FERTILIZATION AND PARTHENOGENESIS

1. Microtublues

Microtubules, homologous polymers of α-, β-, and γ-tubulin, are dynamics and intrinsically polar filaments. The organization of microtubules is controlled by centrosomes located at the spindle poles and at kinetochores on chromosomes. Obsevations made during fertilization in bovine, porcine and human, rabbit showed that an aster of microtubules is seen adjacent to the incorporated sperm head (Schatten, 1994). Kim et al (1996c) observed that the sperm aster enlarged during sperm decondensation and extended throughout the cytoplasm at the time of pronuclear apposition. Treatment with nocodazole inhibited pronuclear migration, suggesting a role of microtubules during pronuclear apposition in the pig. In most animals, the microtubules of the sperm aster have been shown to be responsible for moving the male and female pronuclei from the inner face of the oocyte cortex in the egg cytoplasm (Schatten, 1994). However, in the mouse numerous cytoplasmic microtubule foci have been observed in the cytoplasm (Schatten et al., 1985; Maro et al., 1985). During pronuclear development, these microtubule containing asters increased in size as the cytoplasm became filled with a microtubule matrix. Although the pattern of microtubule configurations during mouse fertilization is atypical, microtubule activity is required to achieve pronuclear union. The pronuclei are embedded within microtubule matrix, and by a process involving both assembly and disassembly the male and female pronuclei are moved into apposition at the cell center.

2. Microfilaments

The distribution of microfilaments has been studied in mammalian ova. In mature mouse (Maro et al., 1984) and rat (Zernicka-Goetz et al., 1993) oocytes, microfilaments are located mainly in the cell cortex overlying the meiotic spindle. This domain, rich in microfilaments, seems to be responsible for the maintenance of the meiotic spindle and chromosomes in a peripheral position (Webb et al., 1986). Maro et al. (1984) found that following fertilization of mouse oocytes, this domain disappeared and microfilaments were concentrated around pronuclei. Recently, Kim et al. (1996a; 2000b) demonstrated that, in mature porcine and bovine oocytes, two domains (a thick and a thin microfilament domain) exist in the oocyte cortex. Chromosomes were located in the thick microfilament domain of the cortex, which may be important for polar body extrusion and normal development during fertilization. Kim et al (1997a) demonstrated that in the porcine oocyte, microfilaments concentrated to the both male and female chromatin following sperm penetration. In aged porcine oocytes, the metaphase chromatin was frequently located outside of the microfilament rich domain (Kim et al., 1996c). The migration of chromatin from the dense microfilament domain may be the major cause of formation of two female pronuclei without polar body extrusion in aged eggs, since polar body extrusion is dependent upon organization of the microfilaments (Maro et al., 1986) Van Blerkom & Bell (1986) have shown that in the mouse the chromosomes gain the capacity to modify the organization of microfilaments in their vincity during oocyte maturation. Following sperm penetration, a rapid series of increases of intracellular calcium ions has been observed in many species. Battaglia & Gaddum-Rosse (1986) showed that an elevation in calcium ions in the rat egg cytosol was directly or indirectly associated with changes in the actin cytoskeleton. Therefore, following sperm penetration or parthenogenetic activation elevation of calcium ions may affect cortical microfilament assembly. Kim et al.(1996e) also demonstrated abnormal patterns of microfilament organization in porcine oocytes following *in vitro* maturation compared with oocytes matured *in vivo*.

3. Endoplasmic Recticulum

The distribution of the endoplasmic recticulum during oocyte maturation and fertilization is of particular interest because the endoplasmic recticulum (ER) release Ca^{2+} at fertilization, and because the ability to release Ca^{2+} develops during oocyte maturation (Chiba et al., 1990). In addition, components involved in the meiotic cell cycle may be associated with the ER (Duesbery & Masui, 1993). ER has recently been visualized in live sea urching eggs by confocal microscopy after injection of an oil drop saturated with the fluorescent lipophilic dye, DiI

(Jaffe & Terasaki, 1994). It is known that DiI diffuse from the oil drop into the continuous membrane of the ER, but does not label other cytoplasmic components. Recent studies using DiI dye showed ER of the immature oocyte interior is composed of interconnected membrane sheets.

4. Mitochondria

The distribution of mitochondria in the cytoplasm reflects some events of oocyte maturation and early embryogenesis as well as being correlated with reorganization of microtubular networks in electrofused somatic cells. I have examined mitochondrial distribution in mouse oocytes during embryogenesis and fusion of blastomeres of homogeneous and heterogenous species by fluoresence microscope for visualization of the functional mitochondria. Vital staining of embryos showed that mitochondria were dispersed throughout the cytoplasm with a ring-like (around the nucleus) or spot-like (over the metaphase plate) concentration in the blastomeres. During mammalian fertilization, sperm mitochondria enter the oocyte, but they are lost soon after fertilization or during early embryogenesis (Hiraoka & Hirao, 1988). Interestingly, in mice, the elimination of paternal mtDNA appeared to be occurred following fertilization between individuals of same species, but not in the interspecific hybrids (Glysten et al., 1991; Kaneda et al., 1995). Similar researches in the human cells also showed species-specific function of mitochondria. While mtDNA from gorillas, chimpanzees and pigmy chimpanzees could substitute for human mtDNA to support respiration and mitochondrial protein synthesis, but that of remote species, such as orangutans could not. These results suggest that cytoplasm has a species-specific mechanism that recognizes and eliminates foreign mitochondria, on the basis of nuclear DNA encoded proteins, not on the mtDNA itself (Cummins, 1998).

5. Cortical Granules

During sperm penetration, cortical granules fuse with the overlying oolemma and release their contents into the perivitelline space. The cortical granule exudate appears to block polyspermy by changing the properties of oolema and zona pellucida. In pig oocytes, cortical granule exocytosis has been noted because polyspermy is a notable problem in this species following IVM/IVF (Yoshida et al., 1993; Kim et al., 1996a,b,c). During oocyte maturation, centrifugal movement and an increase in the number of cortical granules following ovulation appear to be a common feature in mammals (Cran & Cheng, 1985; Yoshida et al., 1993; Kim et al., 1996d). Quantitive examination revealed that sperm penetration induces localized cortical granule reaction between the germinal vesicle and metaphase I (MI) stage, whereas a normal wave of global CG loss from the site of sperm entry develops between MI and MII (Ducibella & Buetow, 1994). However, studies on the sperm penetrability and morphological changes of male and female pronuclei following sperm penetration in pig oocytes maturing *in vitro* have been reported (Wang et al., 1997).

Kim et al (1997c) demonstrated that propranolol, a β-adrenergic receptor blocker, induced an abnormally high incidence of polyspermic penetration, which could be reversed by epinephrine. Incomplete cortical reaction was also observed in the propranolol-treated oocytes following sperm penetration, suggesting the involvement of the adrenergic system in complete cortical granule reaction in the pig. This result is similar to the finding of Nicorta & Schatten (1997), who observed an increase in polyspermy and atypical cortical reaction in propranolol-treated sea urchin oocytes following insemination.

Relationship between actin filaments and cortical granule have been demonstrated. In sheep, treatment of matured oocytes with the microfilament inhibitor, cytochalasin D, caused profound internal structural changes to the cytoplasm of the cortical granules and, in some regions, induced a loss of contact with the plasma membrane. In unfertilized sea urchin oocytes a network of filamentous actin along the inner surface of the plasma membrane seems to function to facilitate cortical granule positioning for exocytosis. Recently, Kim et al. (1996e) have imaged the integrated dynamics of cortical granules and microfilaments during maturation and fertilization. Inhibition of microfilament assembly prevented cortical granule movement, suggesting that the cortical microfilament assembly is linked with the distribution and exocytosis of cortical granules during fertilization. Greater understanding of cortical granule distribution and exocytosis during maturation and fertilization will improve abnormally high incidence of polyspermy following IVM/IVF in the pig.

6. Oocyte Polarity

Oocytes polarity has long been recognized in lower species; the animal pole contains nuclear material while vegetable pole contains yolk. Recently similar cytoplasmic polarity can be detected in mouse and human oocytes. Antczak & Van Blerkom (1997) suggested that two hemispheres can be detected in mammalian oocytes on the distribution of leptin and STAT 3. Their study showed leptin and STAT 3 are localized in the near of the first polar body. However, the functional importance of polarity in mammals is not yet fully understood. A variety of micromanipulation techniques in assisted reproductive technologies now used in human IVF and in embryology in general, but little information is available on this subjected.

INTRACYTOPLSAMIC SPERM INJECTION (ICSI)

Recent advance in assisted reproductive technologies, such as intracytoplasmic sperm injection (ICSI) or round spermatid injection (ROSI) would provide exciting opportunities for the male infertility. When a spermatozoon or Triton X-100 treated porcine sperm head was injected into porcine oocytes, the oocyte was activated, whereas the sperm tail did not induce activation. Injection of either a trypsin treated or NaOH treated sperm head failed to induce activation. A male pronucleus was formed in the activated oocytes following injection of Triton X-100 treated sperm head. Neither a trypsin nor NaOH treated sperm head was decondensed. Following ICSI, the sperm aster was organized from the neck of spermatozoon, and filled the whole cytoplasm. In contrast, the sperm aster was not organized following isolated sperm head injection. Instead, microtubules were organized from the oocyte cortex and then filled the whole cytoplasm in all cases in normally fertilized oocytes (n=35). This organization is similar to what has been shown previously in the parthenogenetically activated oocytes (Kim et al., 1996 b,c; 1997 a,b,c) or in the oocytes following round spermatid injection (Lee et al., 1998).

At 20 to 24 h after spermatozoon or isolated sperm head injection, the incidence of pronuclear apposition, mitosis and two cell division was considered as normal fertilized. During pronuclear movement the sperm aster filled the whole cytoplasm following ICSI, suggesting their role for the pronuclear apposition. In contrast, following sperm head injection, microtubules organized from maternal sources filled the whole cytoplasm, which seems to move male and female chromatin. After pronuclear apposition the microtubules were less detectable in the cytoplasm in the oocytes following ICSI or isolated sperm head injection.

Kim et al (1999) showed that intracytoplasmic sperm injection of foreign species such as bovine, mouse or human activated porcine oocytes. Some oocytes (23%) were activated in the oocytes following sham injection, probably due to parthenogenetic stimulation. The incidence of activation and pronuclear formation was not different in oocytes following injection of porcine, bovine, mouse or human spermatozoa. Pronuclear apposition was observed in all oocytes following injection of porcine, bovine, mouse or human sperm. Following porcine sperm injection, the microtubular aster was organized from the neck of spermatozoon, and filled the whole cytoplasm as shown earlier. Maternal derived microtubules were organized from the cortex to the center of all oocytes, which have male and female pronuclei.

At 6 h following round spermatid injection, the microtubules were organized from the oocyte cortex and then filled whole oocyte cytoplasm in all case of normally fertilized oocytes (Lee et al., 1998). This organization is similar to what has been shown previously in the parthenogenetically activated oocytes. During pronuclear movement, the maternal derived microtubules filled whole cytoplasm, which seems to move both male and female pronuclei. After pronuclei movement, the microtubules are less detectable in the cytoplasm. These results suggested that self organization mechanism for microtubule assembly would be presented in the cytoplasm, which possibly arrange chromatin in proper position during mitosis. Taken together, the cell cytoplasm may have the ability to organize the appropriate microtubules for the chromatin dynamics during pronuclear apposition or mitosis, although it is poorly understood.

NUCLEAR TRANSFER

Cloned animals can be commonly produced at present by transfer of somatic cell nucleus into enucleated oocytes. Following nuclear transfer, nuclear reprogramming is prerequisite to clonal development of reconstructed embryos. The nuclear

reprogramming processes appeared to be result in exchange maternal cytoplasmic factors with the introduced nucleus. Chromatin condensation, nuclear swelling and DNA synthesis are well known morphological changes in reconstructed oocytes following nuclear transfer. Recently Kim et al (2000a) determined nuclear and microtubule dynamics in porcine oocytes following nuclear transfer with porcine cumulus fibroblast cells using indirect immunocytochemistry and transmission electron microscopy. In addition, DNA synthesis was also determined in the reconstructed oocytes during nuclear remodeling. Porcine cumulus cells into enucleated oocytes was condensed and formed transient spindle between 3 and 6 h and transformed into pronuclear like structure and swelled between 6 and 12 h. Self-assembled microtubules were organized from the oocyte cortex, and these seem to move the chromatin to the appropriate position. At 3h following fusion, interchromatin-like granules and some cytoplasmic components, such as mitochondria and golgi complexes of donor cells were seen. The nuclear precursor body, which is composed of densely packed fibular material, was observed at 9 h suggesting G1 phase of the eggs. At 18 h following nuclear transfer, vacuolated nuclear precursor body was seen in the center of nucleolar precursor body. Some oocytes contained intermingled filamentous components and intranucleolar granules, which seems to be in processes of S or G2 phase. DNA synthesis was initiated at 9 h and completed at 18 h following fusion. These results suggest that nuclear remodeling and reprogramming are occurred within 24 h in porcine oocytes following nuclear transfer with somatic cells.

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