韓國家畜繁殖學會誌 20(4) : 423~426(1995)
Korean J. Animal Reprod.

Oogenesis and Oocyte Maturation in Mammals: A 1997 Perspective

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포유동물의 난 발생 및 난 성숙: 1997년 전망

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요 약

난 발생 및 난 성숙을 위한 난소내의 제어기능의 이해는 흥미로운 연구대상이다. 그러나, 난포에 의하여 지엽적으로 분비된 어떤 스테로이드 물질도 난 발생 및 난 제어를 위해 관여하지 않는 것으로 알려져 있다. 따라서, 난소내의 조절인자가 어떻게 난 발생 및 난 성숙을 위해 작용하는지를 구명하는 것은 난 발생 및 난 성숙을 위해 매우 중요한 연구과제라 할 수 있다. 본 총설에서는, 본 연구실의 최근의 업적을 중심으로 포유동물의 난 발생과 난 성숙을 위한 난소내의 조절인자에 대하여 정리하였다.

I. INTRODUCTION

Recently intraovarian control of oogenesis and oocyte maturation has become a subject of interest. Non steroidal substances secreted locally by the ovarian follicles have been implicated in the control of oogenesis and oocyte maturation. It is therefore desirable to see how these intraovarian regulators act to control oogenesis and oocyte maturation. In addition it is of interest to see what the chemical nature of these intraovarian regulators is. In the present talk recent advances in our study of intraovarian regulators of oogenesis and oocyte maturation in mammals are discussed.

1. Oocyte growth and capillary networks

Most of the germ cell population rests in a pool of non-growing small follicles in the mouse ovary. Only a small percentage of follicles are in the process of development in fetuses and newborns. In our observation, follicles containing oocytes 20~30μm in diameter came to be surrounded by apparent capillary networks, suggesting that the association of follicles with capillaries may influence the progress of growth of the oocytes[1]. Epidermal growth factor(EGF) is produced by interstitial cells in the ovary, and, in cell culture systems, EGF was shown to enhance the proliferation of vascular endothelial cells. EGF also effects neovascularization *in vivo*. EGF is soluble in tissue fluid, can be translocated in tissues and induces endothelial cells to proliferate and form capillaries. It was demonstrated that glycosaminoglycans (GAGs) from ovarian extracts potentiated the angiogenic activity of EGF in mice[2], suggesting the possibility that GAGs potentiate endogenous EGF in inducing angiogenesis, so that the tissue

specificity inducing angiogenesis may be due to the localization of ovarian components, including GAGs and EGF, to a specific region in ovaries allowing angiogenesis to occur. During follicular growth, GAGs accumulate in the follicle and outer layer of growing follicles containing oocytes more than 30μm in diameter in the ovaries of 3-day-old newborn mice. It may be that ovarian GAGs interact with EGF to induce vascularization and then accelerate the growth of oocytes in the follicles.

Ⅱ. PARACRINE REGULATION OF OOCYTE MATURATION

Isolated follicle-enclosed oocytes remain arrested in the dictyate stage. When released from the follicles, the oocytes undergo spontaneous maturation, indicating that a maturation-preventing factor within the follicles maintains the oocyte in the dictyate stage. Several maturation-preventing factors have been identified in follicular fluid and granulosa cells. Based on our findings, the following hypothesis is proposed to explain the mechanism of meiotic arrest and the resumption of meiosis[3]. Meiosis arresting factor (MAF) is located on the external surface and in the intercellular spaces of the granulosa cells. By direct contact between the granulosa cells and the cumulus-oocyte complex, MAF can sustain meiotic arrest. Gonadotropins stimulate the production and accumulation of GAGs in the intercellular space of the cumulus cells. The net result is a disruption of the gap junctions between the cumulus cells and the oocyte. GAGs can also prevent the action and flow of MAF on the oocyte by binding MAF. In this manner, the oocyte is protected from the arresting influence of the MAF and resume meiosis.

Ⅲ. CYTOPLASMIC REGULATORS OF OOCYTE MATURATION

During meiotic maturation, protein phosphorylation and dephosphorylation play key roles in a series of events such as nuclear envelope breakdown, chromosome condensation and cytoskeletal change. It is therefore important to investigate the regulation of protein kinase activation in elucidating the regulatory mechanisms for the induction of M-phase events. Mitogen-activated protein kinases (MAPKs), also termed extracellular signal-regulated kinases(ERKs), are intermediates in signal transduction pathways that are initiated by a variety of mitogens such as growth factors, tumor promoters and protooncogene products in mammalian cultured somatic cells. We have demonstrated for the first time that MAP kinases with molecular masses of 42kDa and 44kDa (ERK2 and ERK1, respectively) exist in porcine germinal vesicle (GV)-stage oocytes, and that their amounts do not change during oocyte maturation[4]. MAP kinase activity was low in GV-stage oocytes, its activation might occur after germinal vesicle breakdown (GVBD), and the high activity remained until metaphase Ⅱ, showing a transient slight decrease at the time of first polar body extrusion. These results indicate that the activation of MAP kinase is involed in the regulation of meiotic maturation in the pig.

We used c-mos knockout mouse oocytes and examined the role of Mos in mouse oocyte maturation, including whether Mos controls MAPK and MPF activity[5]. The kinetics of GVBD and the first polar body emission were similar in wild-type, heterozygous mutant and homozygous mutant mice. Activities of MPF were also not significantly different among the three gen-

otypes until the first polar body emission. In contrast, MAPK activity in c-mos knockout oocytes did not significantly fluctuate throughout maturation, and the oocytes had abnormal diffused spindles and loosely condensed chromosomes, although a clear increase in MAPK activities was observed after GVBD in wild-type and heterozygous mutant oocytes that had normal spindles and chromosomes. After the first polar body emission, 38% of c-mos knockout oocytes formed a pronucleus instead of undergoing second meiosis, indicating the crucial role of Mos in MPF reactivation after first meiosis. When oocytes that reached the second metaphase were fertilized or stimulated by ethanol, many c-mos knockout oocyted emitted a second polar body and progressed to the interphase, indicating that Mos deletion leads to compensatory factors that might not be degraded after fertilization or parthenogenetic activation. These results of ours indicate that Mos is located upstream of MAPK in mouse oocytes, but independent of MPF activity, and that Mos /MAPK is not essential for GVBD and first polar body emission, but is essential for maintaining condensation of chromosomes and a normal spindle by increasing MAPK activity. In addition, one of the most important roles of Mos may be to maintain high MPF activity after the first polar body emission to enable oocytes to progress from first meiosis to second meiosis and to correctly degrade after fertilization in order to deactivate MPF.

Ⅳ. POSSIBLE PHYSIOLOGICAL ROLES OF EXPANDED CUMULUS

The intercellular matrix of the expanded cumulus contains abundant GAGs, and the expansion of the cumulus is quite positively correlated with the production of the GAGs-HA by the cumulus-oocyte complex[6]. Our results suggest GAGs may play significant roles in oocyte viability[7]. Namely, porcine oocytes cultured for 72h or longer underwent, spontaneous fragmentation, and the percentage of fragmented oocytes increased thereafter.

The fragmented oocytes consisted of several "blastomeres" showing uneven distribution of DNA among the "blastomeres". Cytoplasmic bodies were also identified on the surface of fragmented oocytes and in the space among fragmented "blastomeres". DbcAMP at concentrations exceeding 50μM moticeably increased the induction of fragmentation. Porcine follicular fluid-GAGs added to the suspending medium at concentrations of 10μg'/ml or greater prevented the occurrence of dbcAMP-stimulated fragmentation of isolated porcine oocytes in a dose-dependent manner.

We clarified that the morphometrical features of oviductal wall changed locally under the influence of the cumulus-oocyte complexes, although no apparent differences were observed in the height of epithelial cells[8]. The height of the mucosal fold and muscle layer of the oviduct was less at the site facing the cumulus-oocyte complexes than in the adjacent portion where no cumulus-oocyte complexes were included, and the decrease in the height of the mucosal fold and muscle layer corresponded to the number of cumulus cells in cumulus-oocyte complexes that occupied the oviductal lumen. These results appear to indicate that cumulus cells surrounding the ovulated oocytes influence the stucture of the oviductal wall during passage through the oviduct, especially affecting the relaxation of the muscle coat surrounding the oviductal mucosa.

Ⅴ. REFERENCE

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