The 2nd Joint Symposium of KSAR & JSAR

"Novel Approaches to Mammalian Oocytes by Young Scientists from East Asia"

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Abstracts from Japanese Speakers

General introduction “Novel Approaches to Mammalian Oocytes by Young Scientists from East Asia”

Takashi MIYANO

First successful in vitro fertilization of bovine and porcine oocytes were achieved in Japan in 1970s. Since then, the Japanese researchers have been leading the study of Animal Reproduction, especially in vitro maturation and fertilization of oocytes and in vitro culture of embryos. The progress has been accelerated by the introduction of the oocyte micromanipulation technology, such as ICSI and somatic cell nuclear transfer for producing cloned animals. Besides Japan, oocyte-manipulating technology in Korea has also been accelerated recently, and now the Korean Reproductive Biology has become the front-runner in this field. These technologies have been applied to animal reproduction as well as human infertility treatment. In the Reproductive Biology in Korea and Japan, some basic studies approaching mammalian oocytes have started, such as basic molecular and cellular mechanisms underling the oocyte maturation-fertilization and embryo development in mammals. The purposes of the seminar are to organize the new knowledge of Oocyte Science from both Korean and Japanese sides and to establish the cooperation between young scientists.

Shugoshin protects centromeric cohesion of sister chromatids

Tomoya KITAJIMA

Meiosis consists of two rounds of chromosome segregation following a single replication of the DNA to produce haploid gametes. Cohesion between sister chromatids is established during S phase, and homologous chromosomes are also physically linked by chiasmata, which are formed by recombination. At the anaphase onset of meiosis I, cohesion is destroyed along the chromosome arms, leading to resolving chiasmata, and thereby homologous chromosomes segregate to the opposite sides. However, at centromeres cohesion is protected to hold sister chromatids together until meiosis II. We identified the novel protein Sgo1 (shugoshin), which protects centromeric cohesion of sister chromatids in fission yeast meiosis. We also show that shugoshin homologues in human protect centromeric cohesion during mitotic pro- and prometaphase.

Mechanism of chromosome separation in mammalian oocyte maturation

Jibak LEE

Progression of anaphase (sister chromatid separation) in somatic cells requires anaphase-promoting complex/cyclosome (APC/C)-mediated degradation of Securin and Cyclin B. After degradation of Securin, Separase is activated and can cleave cohesin protein which connects sister chromatids until the onset of anaphase. In meiosis, however, homolog separation does not require the APC/C-mediated degradation in Xenopus laevis. Here I will introduce recent our study investigating the function of the essential APC/C activators, Cdc20 and Cdh1 in mouse oocytes. Further, I will also introduce our study on meiosis-specific cohesin Rec8 in mammalian oocytes.

Mechanism for establishment of sex-specific imprinting in mouse gametogenesis

Yayoi OBATA

Oocyte- and sperm-specific imprinting / DNA methylation, generated in mammalian gametogenesis is one of the requirements of a functional gamete. By now none of the specific mechanisms for sex-specific DNA methylation have been fully understood. To clarify this issue, bipotential germ cells were transferred into fully grown oocytes, and DNA methylation of imprinted genes was analysed in reconstituted oocytes after 5 days culture. The results showed that both Igf2r and H19 were methylated in oocytes, reconstituted by non-growing oocytes with absence of oocyte-specific imprinting. This suggests that non-growing oocytes have no blocking mechanism for H19 methylation.

The immunoglobulin superfamily protein “Izumo” is required for sperm to fuse with eggs

Naokazu INOUE

Representing the 60 trillion cells that build a human body, a sperm and an egg meet, recognize each other, and fuse to form a new generation of life. The factors involved in this important membrane fusion event, fertilization, have been sought for a long time. Recently, CD9 on the egg membrane was found to be essential for fusion, but sperm-related fusion factors remain unknown. Here, by using a fusion-inhibiting monoclonal antibody and gene cloning, we identify a mouse sperm fusion related antigen and show that the antigen is a novel immunoglobulin superfamily protein. We have termed the gene Izumo and produced a gene-disrupted mouse line. Izumo -/- mice were healthy but males were sterile. They produced normal-looking sperm that bound to and penetrated the zona pellucida but were incapable of fusing with eggs. Human sperm also contain Izumo and addition of the antibody against human Izumo left the sperm unable to fuse with zona-free hamster eggs.

Maternal contribution of the nucleolus to the zygote

Sugako OGUSHI

Oocytes and spermatozoa are equipped with complementary organelles and molecules necessary for the fertilization and further development. We found that the nucleolus in the oocyte is one of such organelle. Fully grown mammalian oocyte have large compact nucleoli, in contrast to the absence of nucleoli in spermatozoa. Since the oocyte nucleolus is transcriptionally inactive and disappears during maturation, the role of the nucleolus in maturation, subsequent fertilization and early development has not been elucidated. In the present study, pig oocytes at the germinal vesicle (GV)-stage were enucleolated (removal of the nucleolus by micromanipulation), and subjected to in vitro maturation followed by intracytoplasmic sperm injection (ICSI) or electro-stimulation to enable the pronucleus formation and early development. In summary, the prominent nucleoli in pig GV oocytes are not required for oocyte maturation and fertilization, however, they are indispensable for nucleolus formation in male and female pronuclei in the zygote. This confirms that the nucleolus in fertilized oocytes is of maternal origin.

Generation of Progeny from Embryonic Stem Cells by Microinsemination of Male Germ Cells from Chimeric Mice

Eiji Mizutani

Mice chimeric for embryonic stem (ES) cells have not always successfully produced ES-derived offspring. Here we show that the male gametes from ES cells could be selected in male chimeric mice testes by labeling donor ES cells or host blastocystes with GFP. Male GFP-expressing ES-derived germ cells occurred as colonies in the chimeric testes, where the seminiferous tubules were separated into green and non-green regions. When mature spermatozoa from green tubules were used for microinsemination, GFP-expressing offspring were efficiently obtained. Using a reverse study, we also obtained ES-derived progeny from GFP-negative ES cells in GFP-labeled host chimeras. Furthermore, we showed this approach could be accelerated by using round spermatids from the testes of 20-day-old chimeric mice. Thus, this technique allowed us to generate the ES cell-derived progeny even from the low contributed chimeric mice which can not produce ES-origin offspring by natural mating.